Photosystem 2 photochemistry and pigment composition of *Dicranopteris dichotoma* Bernh under different irradiances

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The distribution of rare earth elements (REEs) in the lamina and chloroplast of fern *Dicranopteris dichotoma* Bernh from a light rare earth elements mine (LRM) and a non-mining (NM) area in Longnan county of Jiangxi province, China, were investigated by means of inductively coupled plasma-mass spectrometry (ICP-MS). The photosynthetic characteristics and pigment composition in immature and mature leaves of *D. dichotoma* were studied by chlorophyll (Chl) a fluorescence kinetics and high performance liquid chromatography (HPLC). Results show that contents of REEs in the lamina and chloroplast of *D. dichotoma* in LRM were higher than those ferns in NM. By comparing with *D. dichotoma* from NM area, the efficiency of photosystem 2 photochemistry and electron transport rate were significantly enhanced in mature lamina of the plant from LRM because *D. dichotoma* could change its xanthophyll cycle content to avoid the damaging effect of high REEs content. However, high irradiance decreased the photosystem 2 photochemistry efficiency in lamina from ferns in LRM suggested that large amount of REEs reduce the capacities to avoid photo damage in *D. dichotoma*.

Key words: *Dicranopteris dichotoma*, photoinhibition, rare earth element, xanthophylls.

INTRODUCTION

Rare earth elements (REEs), comprising lanthanides (Ln) and yttrium (Y), can be divided into the light rare earth elements (LREEs) and heavy rare earth elements (HREEs) group according to their atomic mass. The REEs at low concentrations (usually less than 0.5 mmol/L) can improve plant photosynthetic efficiency, crop quality and plant resistance to disease and stress (Hu et al., 2002; Liu et al., 2008; Zhang and Chen, 2007). In contrast, the REEs at high concentrations inhibit growth of plants (Chu et al., 2000). The mechanisms underlying different effects to plants were partly because dose effect (Huang and Zhou, 2006) and partly because that REEs can modulate plant photosynthesis by K⁺, Na⁺, or Ca²⁺, ribulose-1,5-bisphosphate carboxylase/oxygenase (Chen et al., 2000), oxidative damage and redox system (Gao et al., 2005) and indolylacetic acid (Shen and Zhang, 1994). REEs also help plants survival from stress conditions such as ultraviolet-B radiation, drought and acid rain (Liang et al., 2005).

The fern *Dicranopteris dichotoma* Bernh., which belongs to *Gleicheniaceae*, is a hyper-accumulator of REEs and can be used in phytoremediation of REEs pollution (Ichihashi et al., 1992) in mining area of China. High contents of REEs were found in root, stem and lamina of this species. The REEs binding protein (Wang et al., 2003), polysaccharides (Wang et al., 1997), nucleic acids (Wang et al., 1999) and chlorophyll (Hong et al., 1999) were distributed in the lamina of the plant. Other component such as oxygenated phenolic derivatives were also identified in *D. dichotoma* (Li et al., 2006, 2007). The photosynthetic characterizations of *D.
**MATERIALS AND METHODS**

Longnan County is located at 114°56′ to 114°58′E, 24°41′ to 24°52′N. The climate of Longnan County is warm and moist, with an annual mean temperature of 18.5 to 19.0°C, annual mean frost-free period of 272 to 287 days, annual rainfall of 1439.8 to 1515.6 mm, annual mean relative humidity of 76 to 79% and annual sunshine time of 1863.1 to 1909.9 h. The pH value in soil at 20 cm depth is 3.92 to 4.80. *D. dichotoma* samples were collected from LRM and NM of Longnan County in Jiangxi Province, China, respectively. The completely expended lamina with dark green color (hereafter abbreviated mature) and fist-type lamina with light green color (hereafter abbreviated immature) was used for the following assay.

**REEs determination in *D. dichotoma***

For each station, *D. dichotoma* samples were randomly collected, lamina were detached from petiole and mixed together. Samples were thoroughly washed with deionized water, then dried at 65°C and ground in dark room at 4°C for 20 s with a blender in a medium containing 0.33 M Sorbitol, 50 mM MES, 10 mM NaCl, 2 mM MgCl₂, 2 mM EDTA Na₂, 0.5 mM KH₂PO₄, 2 mM Na iso-ascorbate per liter and 0.20% (W/W) bovine serum albumin (BSA) (pH 6.1). The slurry was filtered through 500, 195 and 20 μM nylon mesh and centrifuged at 300 × g for 3 min. The pellets were re-suspended in the grinding medium and centrifuged at 5,000 × g for 7 min to collect the chloroplasts. The isolated chloroplasts were then washed with the grinding medium and resuspended in the buffer containing the same contents as the grinding medium except replacing MES with 25 mM L-Hepes-NaOH (pH 7.6). The final chloroplast concentration was higher than 1 mg/ml Chl and stored in refrigerator at -80°C before use.

**Modulated Chl fluorescence**

Chlorophyll fluorescence was measured in attached leaves with a PAM-2500 portable fluorometer (Walz, Effeltrich, Germany) connected to a notebook computer with data acquisition software (DA-2000) (Wang et al., 2011).

**Photoinhibition treatment**

Irradiance of 100, 200 and 1,000 μmolm⁻²s⁻¹ was provided by a 1,000 W tungsten bulb. A water tank with recycled water was used between radiation source and samples to absorb heat.

**Statistical analysis**

All data were analyzed on SPSS analytical software package (version 18.0) and one-way ANOVA with Duncan text was used to assess P<0.05 (probability level). Figures were drawn by Origin data analysis and graphing software, OriginPro8 (Version8E, Origin Lab Corporation, Massachusetts, USA). All of the measurements were performed 6 times, and the means and calculated standard deviations (SD) are reported.

**RESULTS**

**REEs contents in lamina and chloroplast**

REEs concentrations in lamina and chloroplast of *D. dichotoma* in two places of LRM and NM are shown in Table 1. The concentrations of ΣREEs in lamina was 1,494.5 mg/kg dry weight in NM area, while in LRM, the values was 2,648.79 mg/kg dry weight in NM area, while in LRM, the values was 2,648.79 mg/kg, respectively. The LR/HR ratios were 24.28 and 17.2, respectively. To depict REEs abundance variations in lamina and chloroplasts, the chondrite-normalized REEs patterns in two places are shown in Figure 1 using of a set of chondrite normalizing values. The chondrite-normalized REEs patterns were given as the logarithm of the normalized abundance versus atomic number. The Figure 1 shows that *D. dichotoma* in two places had similar distribution patterns in lamina and chloroplasts. The total contents of HREEs were lower than LREEs contents in two places.

The immature and mature lamina with fixed area were cut and Table 2 showed the total Chl (a+b), β-Car and Chl a/b ratio in two places. The total Chl content and β-Car in mature lamina of *D. dichotoma* in the LRM was higher than those in NM, while in immature lamina the total Chl content and β-Car was lower than those in NM (P<0.01). The Chl a/b ratio in immature and mature leaves were thoroughly washed with deionized water, then dried at 65°C and ground in dark room at 4°C for 20 s with a blender in a medium containing 0.33 M Sorbitol, 50 mM MES, 10 mM NaCl, 2 mM MgCl₂, 2 mM EDTA Na₂, 0.5 mM KH₂PO₄, 2 mM Na iso-ascorbate per liter and 0.20% (W/W) bovine serum albumin (BSA) (pH 6.1). The slurry was filtered through 500, 195 and 20 μM nylon mesh and centrifuged at 300 × g for 3 min. The pellets were re-suspended in the grinding medium and centrifuged at 5,000 × g for 7 min to collect the chloroplasts. The isolated chloroplasts were then washed with the grinding medium and resuspended in the buffer containing the same contents as the grinding medium except replacing MES with 25 mM L-Hepes-NaOH (pH 7.6). The final chloroplast concentration was higher than 1 mg/ml Chl and stored in refrigerator at -80°C before use.

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Table 1. REEs concentration (mg/kg dry weight) in lamina and chloroplast of *D. dichotoma*.

<table>
<thead>
<tr>
<th>REEs</th>
<th>NM</th>
<th>Chloroplast</th>
<th>LRM</th>
<th>Chloroplast</th>
</tr>
</thead>
<tbody>
<tr>
<td>La</td>
<td>458.53</td>
<td>24.62</td>
<td>1095.80</td>
<td>98.88</td>
</tr>
<tr>
<td>Ce</td>
<td>451.98</td>
<td>16.80</td>
<td>461.40</td>
<td>63.28</td>
</tr>
<tr>
<td>Pr</td>
<td>94.64</td>
<td>6.04</td>
<td>155.62</td>
<td>22.90</td>
</tr>
<tr>
<td>Nd</td>
<td>342.59</td>
<td>20.07</td>
<td>577.94</td>
<td>76.58</td>
</tr>
<tr>
<td>Sm</td>
<td>45.08</td>
<td>3.72</td>
<td>89.77</td>
<td>12.58</td>
</tr>
<tr>
<td>Eu</td>
<td>5.36</td>
<td>0.48</td>
<td>10.84</td>
<td>1.74</td>
</tr>
<tr>
<td>Gd</td>
<td>36.42</td>
<td>3.05</td>
<td>74.79</td>
<td>11.59</td>
</tr>
<tr>
<td>Tb</td>
<td>3.23</td>
<td>0.43</td>
<td>8.08</td>
<td>1.33</td>
</tr>
<tr>
<td>Dy</td>
<td>10.38</td>
<td>1.67</td>
<td>32.24</td>
<td>5.29</td>
</tr>
<tr>
<td>Ho</td>
<td>1.51</td>
<td>0.33</td>
<td>5.07</td>
<td>0.93</td>
</tr>
<tr>
<td>Er</td>
<td>4.13</td>
<td>0.76</td>
<td>12.21</td>
<td>2.16</td>
</tr>
<tr>
<td>Tm</td>
<td>0.30</td>
<td>0.11</td>
<td>1.08</td>
<td>0.25</td>
</tr>
<tr>
<td>Yb</td>
<td>1.46</td>
<td>0.41</td>
<td>5.00</td>
<td>1.13</td>
</tr>
<tr>
<td>Lu</td>
<td>0.17</td>
<td>0.07</td>
<td>0.59</td>
<td>0.18</td>
</tr>
<tr>
<td>Y</td>
<td>38.68</td>
<td>8.61</td>
<td>118.34</td>
<td>29.35</td>
</tr>
<tr>
<td>ΣREEs</td>
<td>1494.45</td>
<td>78.55</td>
<td>2648.79</td>
<td>298.82</td>
</tr>
<tr>
<td>LR/HR</td>
<td>24.28</td>
<td>10.50</td>
<td>17.20</td>
<td>12.07</td>
</tr>
<tr>
<td>δCe</td>
<td>0.51</td>
<td>0.32</td>
<td>0.26</td>
<td>0.31</td>
</tr>
<tr>
<td>δEu</td>
<td>0.40</td>
<td>0.43</td>
<td>0.40</td>
<td>0.44</td>
</tr>
</tbody>
</table>

REEs, rare earth elements; NM, non-mining; LRM, light rare earth elements mine.

Figure 1. REEs distribution patterns of in lamina and chloroplast of *D. dichotoma* in NM and LRM.
Table 2. The Chl a+b, β-car contents and Chl a/b ratio of immature and mature lamina of *D. dichotoma* in two places (µmol·m⁻²).

<table>
<thead>
<tr>
<th>Type</th>
<th>Place</th>
<th>Chl a+b</th>
<th>β-car</th>
<th>Chl a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature lamina</td>
<td>NM</td>
<td>272.80±10.30</td>
<td>9.15±0.34</td>
<td>2.33±0.08</td>
</tr>
<tr>
<td></td>
<td>LRM</td>
<td>140.00±2.61**</td>
<td>5.82±0.67**</td>
<td>2.42±0.06</td>
</tr>
<tr>
<td>Mature lamina</td>
<td>NM</td>
<td>167.00±6.52</td>
<td>2.40±0.21</td>
<td>2.14±0.05</td>
</tr>
<tr>
<td></td>
<td>LRM</td>
<td>218.30±10.64***</td>
<td>13.98±0.95**</td>
<td>2.11±0.03</td>
</tr>
</tbody>
</table>

**Means significant level, *P*<0.01.

![Figure 2.](image)

**Figure 2.** The room temperature (298K) absorption spectrum of immature and mature lamina chlorophyll in *D. dichotoma* in two places. LRMI, immature lamina in LRM; NMI, immature lamina in NM; LRMM, mature lamina in LRM; NMM, mature lamina in NM.

showed little difference, thus suggesting that the chlorophyll composition remained stable. More also, the peaks in Soret band and Q band in immature lamina in LRM (LRMI) and NM (NMI) were 435, 434.5, 664.0 and 664.0 nm, respectively and the Is/Iq ratio in LRM (LRMM) and NM (NMM) were 2.28 and 2.14, respectively. While the peaks in Soret band and Q band in mature lamina in LRM and NM were 434.5, 434.5, 664.0 and 664.5 nm, respectively and the Is/Iq ratio in LRM and NM were 2.46 and 2.31, respectively. The absorption of unit chlorophyll from ferns in LRM was therefore higher than that in NM both in immature and mature lamina.

As shown in Table 3 and Figure 3, the xanthophyll cycle pigments in two places were significantly different (*P*<0.01). The xanthophyll cycle pigments in ferns from LRM were higher than those in NM. Although, the (Z+A) / (Z+A+V) ratios in immature lamina showed little difference, the ratios in mature lamina in LRM were higher than those in NM. Furthermore, Figure 4 shows the light-induced Chl a fluorescence kinetic parameters of the mature lamina from NM and LRM under different irradiance. Although, the maximal efficiency of PS2 photochemistry (Fv/Fm) was only little different in the ferns in two places, they showed decreased patterns along with the increase of light intensities. Other parameters such as the actual photochemical efficiency of PS2 (ΦPS2), the efficiency of excitation energy trapped by open PS2 reaction centers in the light-adapted state (F'v/F'm), and photochemical quenching (qP) were increased at low light intensities (100 to 200 µmolm⁻²s⁻¹),
while decrease at photoinhibition light (1,000 µmol m⁻² s⁻¹) was observed. Meanwhile, the non-photochemical quenching (qN) which reflects the process competing with PS2 photochemistry for absorbed excitation energy showed increase patterns along with the increase of light intensities in the lamina. The qN values in ferns from LRM which were higher than those in NM suggested that high REEs increase the effects of photoinhibition.

DISCUSSION

The hyperaccumulation of REEs in all the parts of *D. dichotoma*, especially high in root and lamina were well studied and the results suggested that the absorption of REE was not only determined by environment, but also by its own characters (Wang et al., 2005). The LREEs were easily transported to the lamina of *D. dichotoma* than HREEs, hence in lamina, more LREEs, especially La and Ce were accumulated in *D. dichotoma* in LRM and NM (Table 1). However, very little concentrations of REEs were found in chloroplast indicating that only small RREs have direct effects on photosynthetic apparatus. On the other hand, high concentrations of REEs altered the pigments compositions in different type lamina of *D. dichotoma* in two places (Figure 2). Combined with the changes of absorptions of unit chlorophyll, it was safely confirmed that *D. dichotoma* changed its physiological characterization such as DNA, chlorophyll and oxygenated phenolic derivatives for tolerance of high concentrations of REEs (Wang et al., 1999; Hong et al., 1999; Li et al., 2006). Until now, the functions of these components in *D. dichotoma* are largely unknown.

The findings of the effect of REEs concentrations and type on photosynthetic activities of *D. dichotoma* provided new sight to explaining the hyperaccumulation mechanisms of REEs by *D. dichotoma*. High concentrations of REEs do have great harmful effects on crops and vegetables, so only few species can grow on REEs mining area. Usually, light RREs changed PS II activity, while heavy REEs changed the activity of PS I in *D. dichotoma*. The presence of REEs influenced the normal photosynthetic characterizations which in turn triggered another important excited energy quenching pathways, the xanthophyll cycle (Demmig-Adams and Adams, 1996). The high amounts of β-Car associated with the

![Figure 3](image-url)  
*Figure 3.* The (Z+A)/(Z+V+A) ratios of immature and mature lamina chlorophyll in *D. dichotoma* in two places.
adoption of *D. dichotoma* in REEs mine. The significant difference of \((Z+A) / (Z+A+V)\) ratio under normal irradiance in the two places confirmed the important roles of xanthophyll cycle in hyperaccumulation of REEs (Figure 3). These results will explain why the efficiency of excitation energy trapped by PS2 reaction centre (F'v/F'm), the quantum yield of primary photochemical reaction (Yield) and the efficiency of photon energy utilization of PS2 (ΦPSII) are remarkably better in *D. dichotoma* from LRM than those from NM under low irradiances (Figures 4 and 5). However, these compensating effects are only effective at low irradiance; high light intensities remarkable decrease the PSII photochemistry of *D. dichotoma* in LRM.

**CONCLUSION**

The strategies plants use to cope with high concentrations of toxic metal were to deposit them (Küpper et al., 1999, 2001) and change their physiological characters (Lasat, 2002). Similarly, the mechanism of hyper-accumulation of REEs by *D. dichotoma* was to fix REEs in the lamina and chloroplasts, as well as alter their physiological characters such as use of β-Car and xanthophyll cycle pigments to avoid the direct effect of
Figure 5. The Fv/Fm’, ΦPSII and ETR fluorescence parameters of mature lamina in *D. dichotoma* in two places under difference light intensities.
high concentrations of REEs on their photosynthetic characteristics.

REFERENCES


