

Full Length Research Paper

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.*

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Abbreviations: REEs, Rare earth elements; LRM, light rare earth elements mine; NM, non mining.

dichotoma in normal growth conditions were preliminary studied (Wang et al., 2005, 2006). In places where *D. dichotoma* grow, photoinhibition, a common phenomenon, occurs because the plant photosystem cannot dissipate excited energy in time (Demmig-Adams and Adams, 1992, 1996). Plant usually use xanthophyll cycle to quench extra energy.

However, the possible roles of REEs in *D. dichotoma* and effects of photoinhibition on *D. dichotoma* are still not well explained. In this study, the contents of REEs as well as pigments compositions in lamina of *D. dichotoma* from LREEs mine (LRM) and non-mining (NM) area in Longnan county of Jiangxi Province, China, were measured, and the REEs effects on photosynthetic characteristics of ferns under photoinhibition conditions were also discussed.

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 1 439.8 to 1 515.6 mm, annual mean relative humidity of 76 to 79% and annual sunshine time of 1 863.1 to 1 909.9 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 to pass a 100-mesh sieve. These samples were dissolved by HNO₃/HClO₄/H₂O₂ ashing and before determination by ICP-MS, 1 cm³ of de-ionized water was added. The ICP-MS was conducted according to Jarvis (1997).

Chlorophyll and xanthophyll contents measurements

Chlorophyll (Chl) was extracted with 80% ice-cold acetone from 0.1 g samples of fresh leaves. The extract was measured spectrophotometrically at 475, 645, and 663 nm. Specific Chl contents were determined according to Lichtenthaler (1987) with a DU800 spectrophotometer (Beckman, Coulter, USA). Xanthophyll cycle pigments were separated and quantified by high performance liquid chromatography (HPLC) (Thayer and Bjorkman, 1990). The samples were extracted in ice-cold 100% acetone and the pigment extracts were filtered through a 0.45 μm membrane filter before assay with LC-MS2010 (Shimadzu, Tokyo, Japan).

Isolation of chloroplasts

Chloroplasts were prepared according to Wang et al. (2006). The fern lamina were thoroughly washed by deionized water, then 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⁻¹ 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 test 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 1 494.45 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 Figure1 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

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

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

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

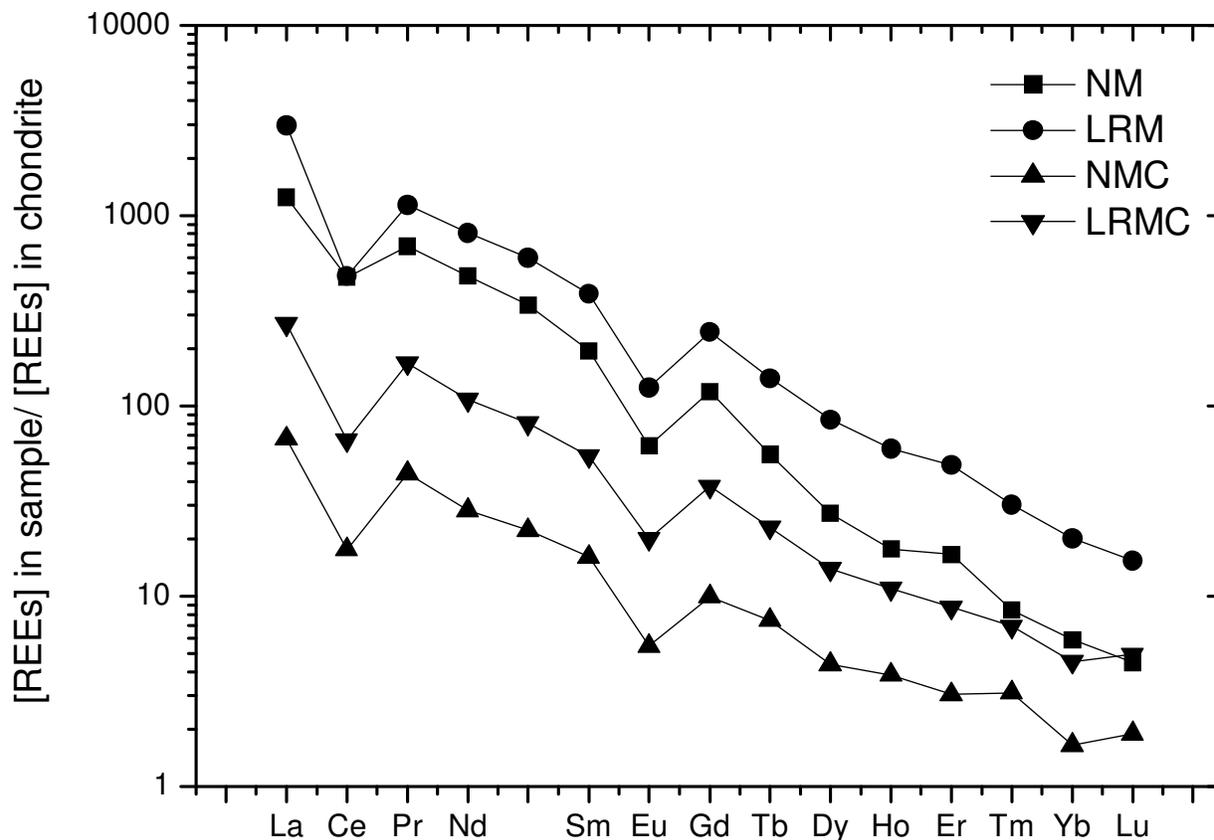
**Figure 1.** REEs distribution patterns of in lamina and chloroplast of *D. dichotoma* in NM and LRM.

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 ($\mu\text{mol}\cdot\text{m}^{-2}$).

Type	Place	Chl a+b	β -car	Chl a/b
Immature lamina	NM	272.80 \pm 10.30	9.15 \pm 0.34	2.33 \pm 0.08
	LRM	140.00 \pm 2.61**	5.82 \pm 0.67**	2.42 \pm 0.06
Mature lamina	NM	167.00 \pm 6.52	2.40 \pm 0.21	2.14 \pm 0.05
	LRM	218.30 \pm 10.64**	13.98 \pm 0.95**	2.11 \pm 0.03

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

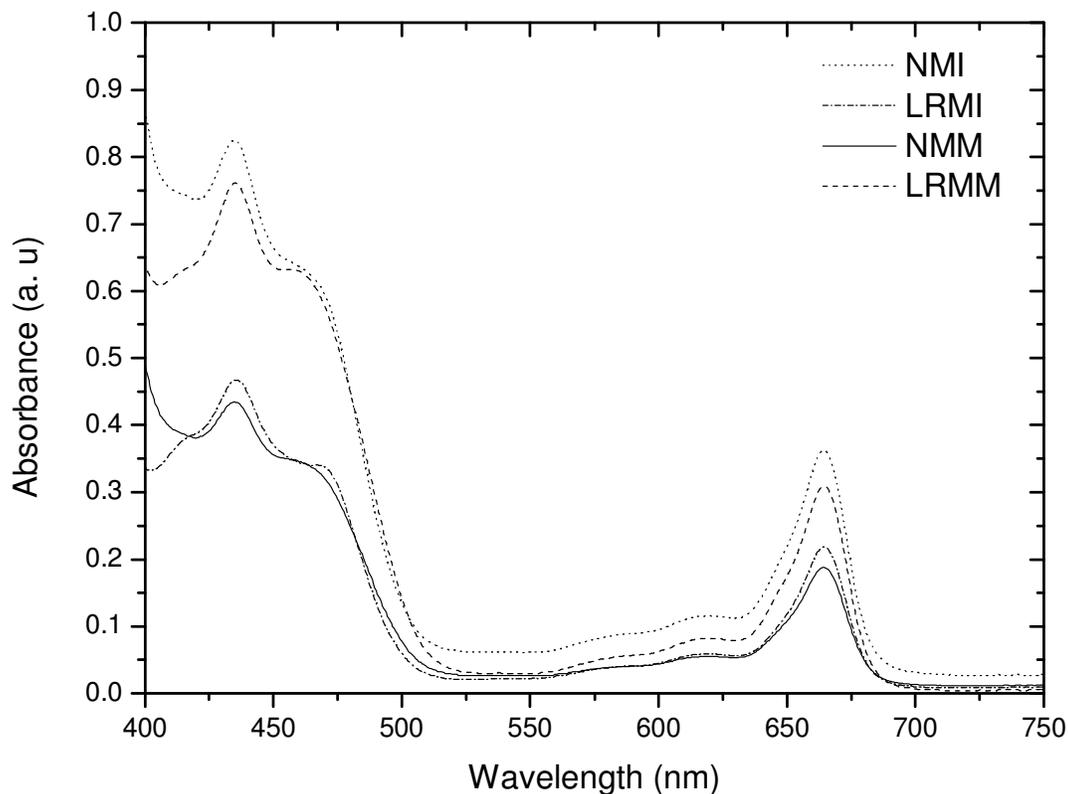


Figure 2. The room temperature (298K) absorption spectrum of immature and mature lamina chlorophyll in *D. dichotoma* in two places. LRMI, immature lamina in LRM; NMI, immature lamina in NM; LRMM, mature lamina in LRM; NMM, mature lamina in NM.

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 I_s/I_q 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 I_s/I_q 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 (F_v/F_m) 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 (Φ_{PS2}), the efficiency of excitation energy trapped by open PS2 reaction centers in the light-adapted state (F'_v/F'_m), and photochemical quenching (q_P) were increased at low light intensities (100 to 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$),

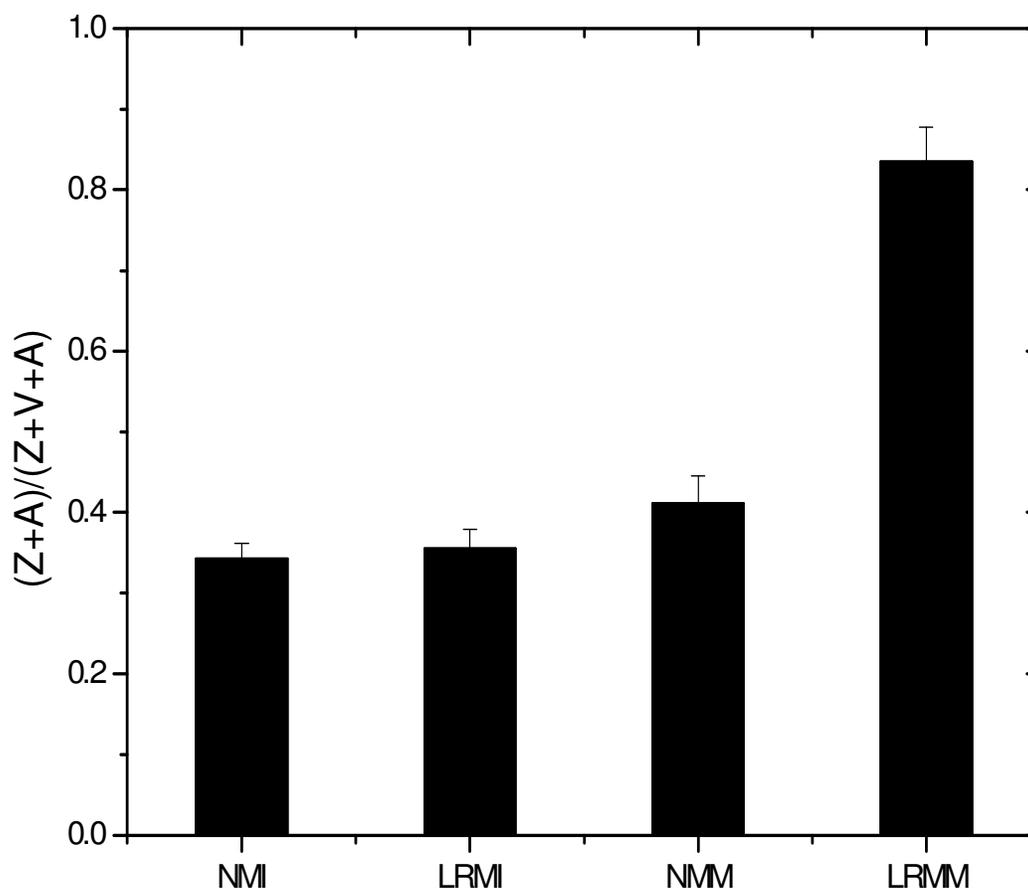


Figure 3. The (Z+A)/(Z+A+V) ratios of immature and mature lamina chlorophyll in *D. dichotoma* in two places.

while decrease at photoinhibition light ($1,000 \mu\text{molm}^{-2}\text{s}^{-1}$) 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

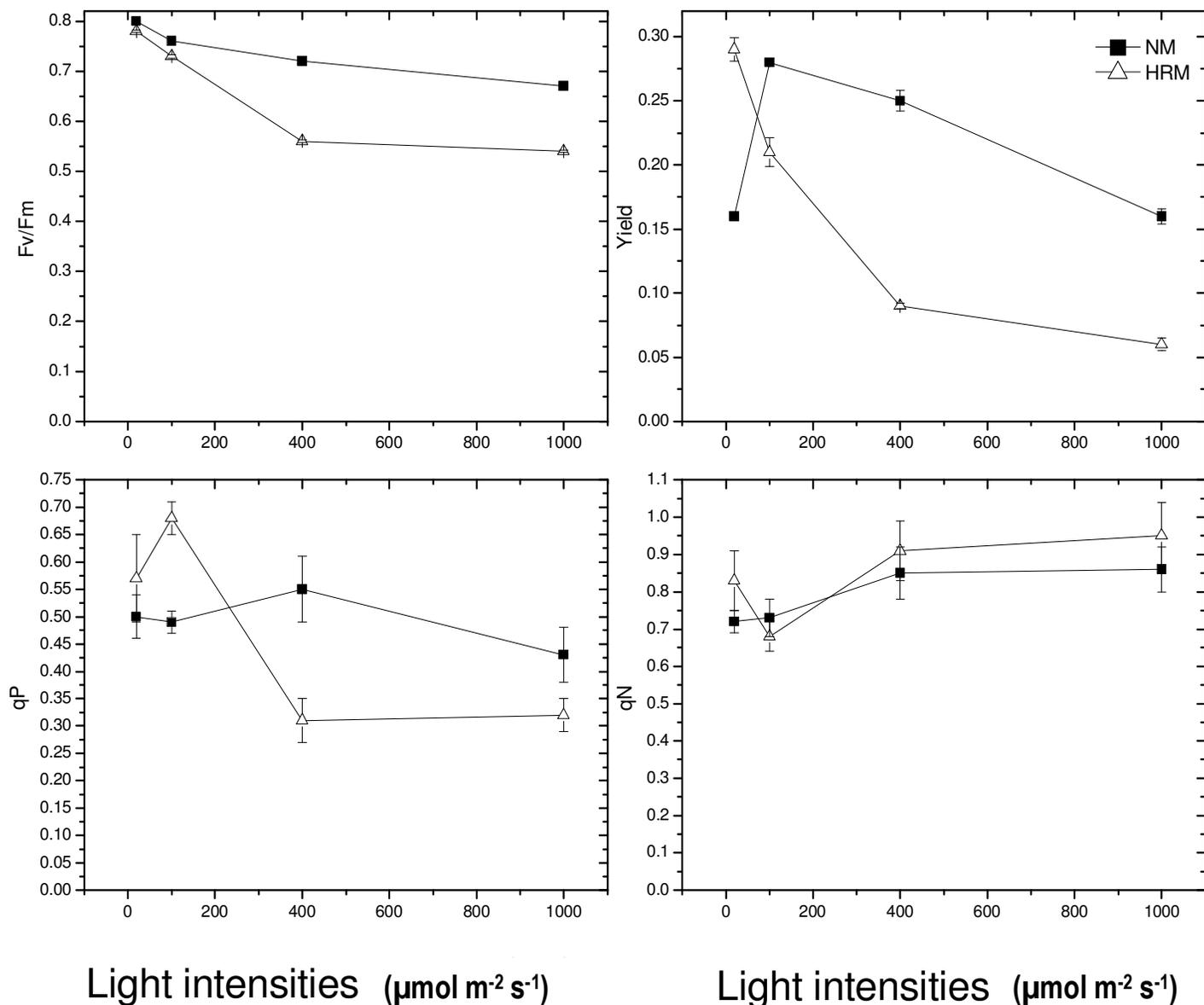


Figure 4. The Fv/Fm, Yield, qP and qN fluorescence parameters of mature lamina in *D. dichotoma* in two places under difference light intensities.

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 (Φ_{PSII}) 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

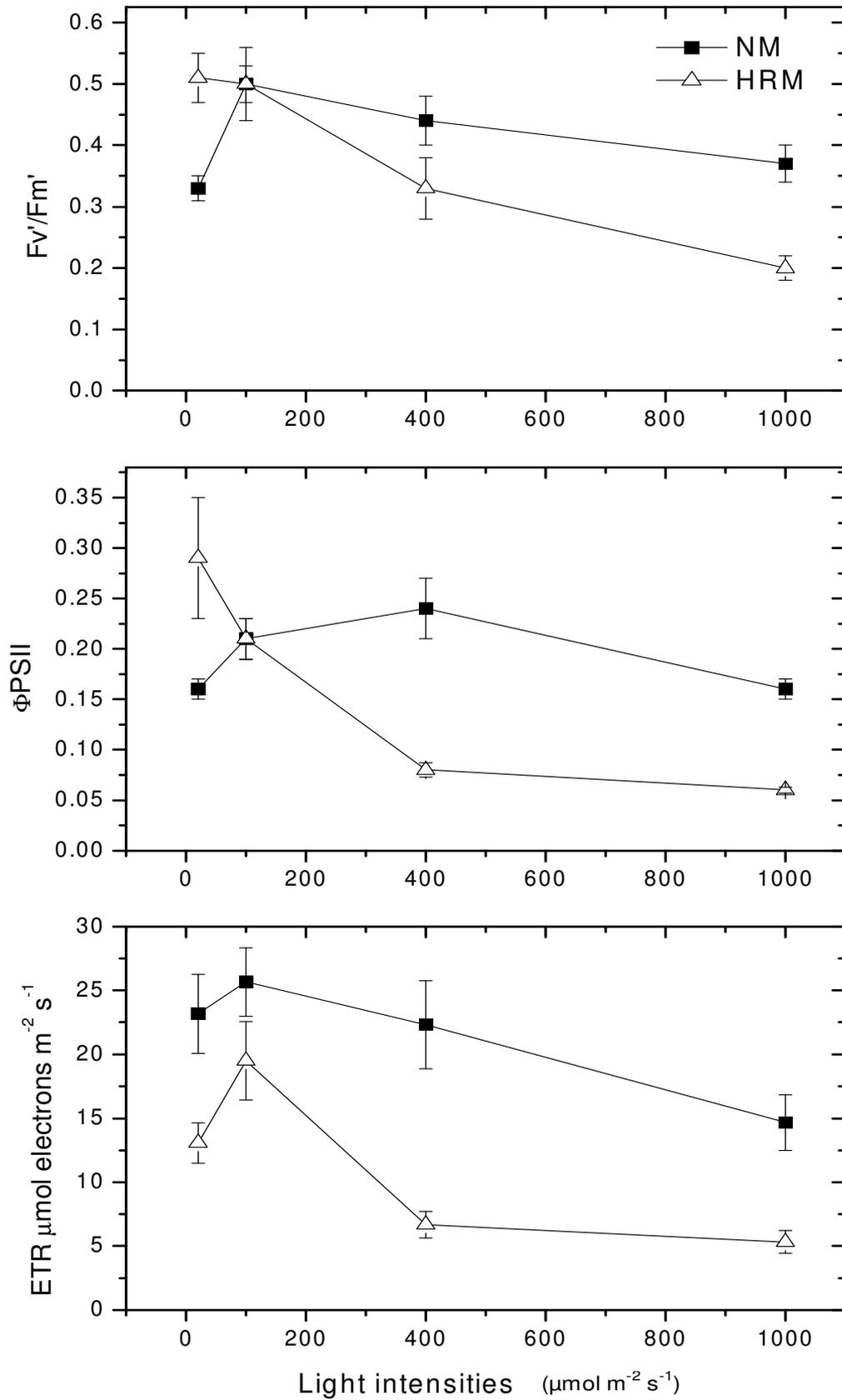


Figure 5. The F_v/F_m' , Φ_{PSII} and ETR fluorescence parameters of mature lamina in *D. dichotoma* in two places under difference light intensities.

high concentrations of REEs on their photosynthetic characteristics.

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