Chlorophyll Fluorescence: a Non-Destructive and Rapid Tool to Select Chinese Fir Clones Best Adapted to a Particular Region

LI JIANG[1], BO JIANG[2], ZONGGEN SHEN[1], SHENCHAO CHEN[1], ZHECHEN QI[3], JIADONG CHANG[3], NITIN MANTRI[4] AND HONGFEI LU[1, 3]*

[1]College of Chemistry and Life Science, Zhejiang Normal University, 688 Yingbin Road, Jinhua, 321004, China
[2]Department of Biological and Food Engineering,
Changshu Institute of Technology, 99 Sanhuan Road, Changshu, 215500, China
[3]College of Life Sciences, Zhejiang Sci-Tech University, 2 Xiasha Road, Hang zhou, 310018, China
[4]School of Applied Sciences, Health Innovations Research Institute, RMIT University,
Melbourne, Victoria, 3000, Australia
Corresponding author: Hongfei Lu
Tel.: +86-13566997763 E-mail:luhongfei0164@163.com
Author: Li Jiang Tel.: +86-18757699705 E-mail: 992858598@qq.com

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Abstract

Forestry management requires rapid and nondestructive methods to detect the optimal clones or cultivars or species suitable for particular geographical location. We compared the leaf size, plant height, chlorophyll content and chlorophyll fluorescence of four Chinese fir (*Cunninghamia lanceolata*) clones to understand the differences between their ecophysiological characteristics and provide recommendations for cultivation. The results demonstrate that plant height of clone 13 was significantly higher ($P < 0.05$) than clone 3. Further, the content of chlorophyll a (Chl a), chlorophyll b (Chl b) and Chl a+ Chl b of clones 39 and 3 were significantly higher ($P < 0.05$) than the other clones, respectively. Compared to clones 39 and 3, clones 13 and 6 had higher Chl a/Chl b ratio. Furthermore, photochemical energy conversion (Yield) and electron transport rate (ETR) of clones 13 and 6 decreased slowly, and this corresponded well with lower non-photochemical quenching (NPQ) and its coefficient (qN) of clones 13 and 6 with increasing PAR. These results indicate that the clones 13 and 6 are suitable for high light environment but clones 39 and 3 are acclimatized to low light environment. In conclusion, this suggests that a deeper understanding of chlorophyll fluorescence, Chl b and Chl a/Chl b would improve our ability to select appropriate clones or cultivars or species for a particular location.

Key words: Chinese fir; plant height; chlorophyll content; chlorophyll fluorescence; light use efficiency.

Introduction

Chinese fir (*Cunninghamia lanceolata*), belonging to the gymnosperm, is an evergreen conifer with excellent timber quality (Farjon and Garcia 2003). It is widely planted in southern China with a planting history spanning more than 1000 years (Wu et al. 2011). Due to increased demand of its timber for domestic and industrial building and furniture, Chinese fir plantations have been rapidly expanding since the 1980s (Zhang et al. 2004) with an estimated plantation area in China of 9.11 million hectares (Wu et al. 2011). In addition, it has been reported that the main essential oil of Chinese fir has significant antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella typhi* (Ye et al. 2005). Although it is regarded as the most important timber tree species, its productivity is largely affected by poor establishment (Si et al. 2003). Due to rapid growth of human population, and associated demand for timber, planting Chinese fir has become a common practice of forest management (Zhang 1993).

Different clones or cultivars of the same species produce significantly different yields when cultivated at same site. This can be attributed to the influence of climate, soil

nutrients, and water (Novoa and Loomis 1981, Yang et al. 2013). It is relatively easy to control water/nutrient supply through irrigation and fertilization. In contrast, light intensity (one of the most important plant growth requirements) is more difficult to control (Wang et al. 2007). Under high irradiance, the photosynthetic apparatus absorbs excessive light energy, resulting in the inactivation or impairment of the chlorophyll (Chl) containing reaction centers of the chloroplasts (Bertaminia et al. 2006). As a consequence, photosynthetic activity is reduced by photoinhibition (Osmond 1994). In contrast, under low irradiance, insufficient ATP is produced to allow for carbon fixation and carbohydrate biosynthesis. This leads to reduced plant growth. Therefore, there is a crucial need to identify clones or cultivars that can grow vigorously in native conditions.

Leaves with higher Chl b content can absorb more light energy in weak light environment (Kramer and Kozlowski 1960). To identify precisely clones or cultivars suitable for a particular location, it is desirable to conduct photosynthetic capacity under controlled conditions (Medrano et al. 2009). The estimation of Chl b content of leaves using Chl fluorescence technique allows rapid and nondestructive assessment of their suitability in a particular environment. However, there was little information about the Chl fluorescence of different Chinese fir clones. Therefore, the aim of this study was to evaluate the Chl content and Chl fluorescence of four Chinese fir clones to help estimation of their suitability for planting under the same conditions.

Materials and methods

Plants and growth conditions

Four disease-free clones of Chinese fir with a height of 11 cm were collected from Kaihua Forest Farm. Every clone was five strains, respectively. The cutting seedlings of *Cunninghamia lanceolata* (clone 39, clone 3, and clone 13) and *C. lanceolata* cv. Glauca (clone 6) were planted in the Zhejiang Normal University botanical garden in September 2012. Plants were cultivated on a hillside in a mixture of peat, sand, and humic soil (1:2:1). Plants were grown in orthogonal design under the same light environment (800-2500 umol m^{-2} s^{-1} in the daytime). Local production practices such as fertilization regime was followed. The growth of plants was evaluated after six months of planting (March 2013). The tender leaves that were just matured of the lateral branch were collected for analyses of leaf length, leaf width, leaf area, chlorophyll (Chl) content and Chl fluorescence.

Leaf analysis and plant height

The growth characteristics of the plants including leaf parameters and plant height were evaluated after the cut seedlings were planted for six months. Leaves were scanned with a Xatbed graphics scanner and the images analyzed with WinFOLIA (Shen et al. 2007). Plant height was determined using image analysis software (HarFA, Harmonic and Fractal Image Analyzer 5.4, freeware at http://www.fch.vutbr.cz/lectures/imagesci/) as described by Pandolfi et al. (2009). A digital camera (EOS 50D, Canon), set at a resolution of 1.5 million dpi, was used to acquire images.

Chl contents

Leaves were collected for determination of Chl content including Chl a and Chl b. Chl pigments were extracted by grinding leaves in 80 % acetone in the dark at room temperature. Absorbance of the resulting extracts was measured at three wavelengths: 663.6, 646.6 and 470 nm. Chlorophylls levels were expressed as mg/g FM from the equation given by Porra (2009) and Dai (2009).

Chl Fluorescence Measurements

A MINIPAM (pulse-amplitude modulation) fluorometer (WALZ, Effeltrich, Germany) was used in this study as reported by Zheng et al. (2010) and Jiang et al. (2013). The photochemical energy conversion in photosystem II (Yield), electron transport rate (ETR), photochemical quenching (qP), non-photochemical quenching (NPQ) and its coefficient (qN) of Chl fluorescence were obtained over a range of PAR values from 0 to 1,200 μmol m^{-2} s^{-1}. The leaves were dark-adapted for nearly 30 minutes prior to measurements.

Data analysis

Each experiment was repeated five times. Data was expressed as mean ± *SE* (standard error). Statistical significance between mean values was assessed by Duncan's multiple range tests ($P \leq 0.05$ was considered as significant). Data were analyzed by SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL).

Results

Leaf analysis and plant height comparison

The leaf parameters and plant height of the four Chinese fir clones are showed in Figure 1 after six months of growth. Leaf length was not significantly different in the four clones. Clone 13 had significantly wider leaves ($P < 0.05$) than clone 3, but leaves of clone 6 and 39 were not significantly ($P > 0.05$) different. Further, the leaf area of clone 6 and 13 were significantly larger than that of the other two clones, respectively. There was no significant difference between the leaf area of clone 6 and 13. The plant height of clones 6 and 13 were not significantly different ($P > 0.05$), although the plant height of clone 13 was the highest of all four clones. Interestingly, the plant height of clones 13 and 6 were significantly taller ($P < 0.05$) than clone 3.

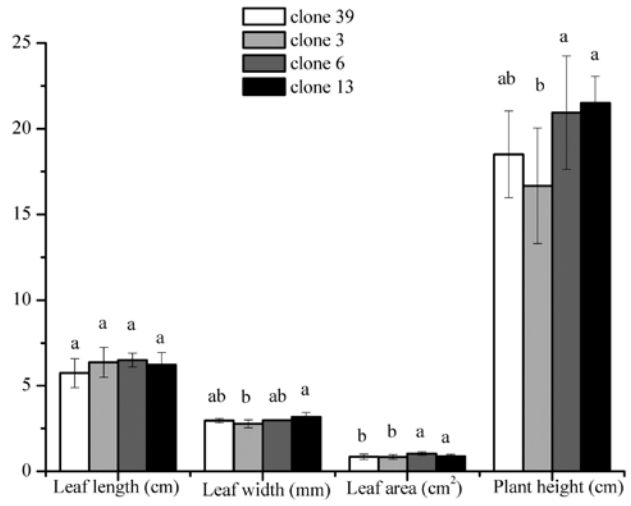

Figure 1. Leaf length, leaf area, leaf width and plant height of the four Chinese fir clones at the age of six months. Values are means ± *SE*

Chl contents

The Chl content of the leaves of the four clones was analyzed (Figure 2). Clones 39 and 3 had significantly higher Chl a, Chl b, Chl a + b content ($P < 0.05$) than the other two clones, respectively (Figure 2). The Chl a, Chl b, Chl a + b content of clone13 was lower than the other three clones, but it had higher Chl a / Chl b ratio value than the other three clones (Figure 2). The lowest Chl a, Chl b and total Chl content and the highest Chl a / Chl b ratio were observed in clone 13.

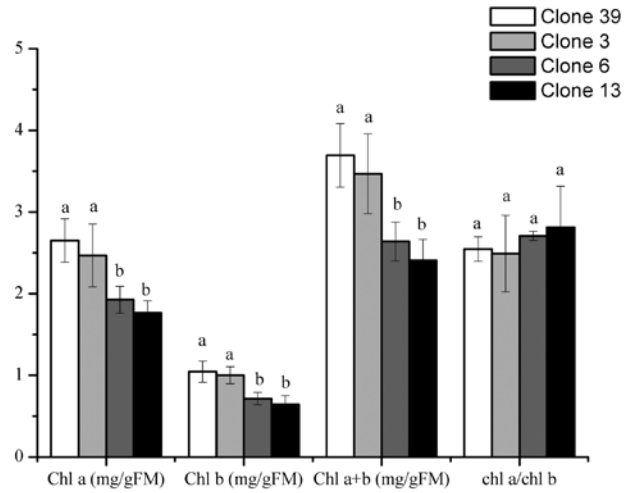

Figure 2. Comparison of chlorophyll (Chl) content in leaves of the four Chinese fir clones . Values are means ± *SE*

Chl fluorescence

The changes of effective quantum yield of Yield were determined. Though the Yield of the four clones decreased with increasing PAR, the Yield of clone 13 and 6 decreased slower than the other clones (Figure 3a). Clone 3 had the highest Yield when the PAR was between 0 and 225 μmol m^{-2} s^{-1}. However, the Yield of clone 3 was lower than the other three clones between 300 and 1,200 μmol m^{-2} s^{-1}. The Yield of clone 13 was the highest between 300 and 1,200 μmol m^{-2} s^{-1}, followed by clone 6.

ETR increased rapidly as PAR increased, and the ETR of clones 13 and 6 increased faster than the other two clones (Figure 3b). The ETR value of clone 3 was at its highest when the value of PAR was between 0 and 300 μmol m^{-2} s^{-1}, and the ETR of clone 13 was the highest at PAR between 300 and 1200 μmol m^{-2} s^{-1}. The ETR of clone 6 was lower than this of clone 39 at PAR between 0 and 300 μmol m^{-2} s^{-1} but it was higher than this of clone 39 at PAR between 300 and 1200 μmol m^{-2} s^{-1}.

The qP in clone 13 was the highest when its PAR was between 600 and 1200 μmol m^{-2} s^{-1}, while values of clone 3 were the lowest when its PAR was between 225 and 1200 μmol m^{-2} s^{-1} (Figure 3c). NPQ and qN also changed in response to light irradiance at different levels. NPQ (Figure 3d) and qN (Figure 3e) increased positively with increasing PAR. There is no significance of NPQ of four clones between 0 and 150 μmol m^{-2} s^{-1}. The comparison of NPQ showed a trend (39-clone > 3-clone> 13-clone > 6-clone) between 225 and 1,200 μmol m^{-2} s^{-1}. The qN of clone 39 was higher than of other three clones at PAR between 150 and 1,200 μmol m^{-2} s^{-1}. Further, these values were higher in clone 3 than in other two clones (clone 13 and 6) between 225 and 600 μmol m^{-2} s^{-1}.

Correlation of plant heightwith Chl content and Chl fluorescence

The Pearson correlations of plant height with Chl content and Chl fluorescence parameters during the light intensity of 800 μmol m^{-2} s^{-1} are shown in Table 1. Interestingly, we found that the correlation of Chl a with Chl b was much high ($P < 0.01$). Chl fluorescence parameters (Yield and ETR) were negatively correlated with Chl b ($P < 0.01$), but being positively correlated with Chl a/Chl b ratio and plant height, respectively ($P < 0.05$) (Table 1). In addition, there was good correlation among Yield, ETR, Chl b, Chl a/Chl b ratio, plant height for the four Chinese fir clones, respectively. Clones 39 and 3 had significantly higher Chl b content ($P < 0.05$) than the other two clones, respectively. So the different photosynthesis physiological index of the four clones may change at same irradiance.

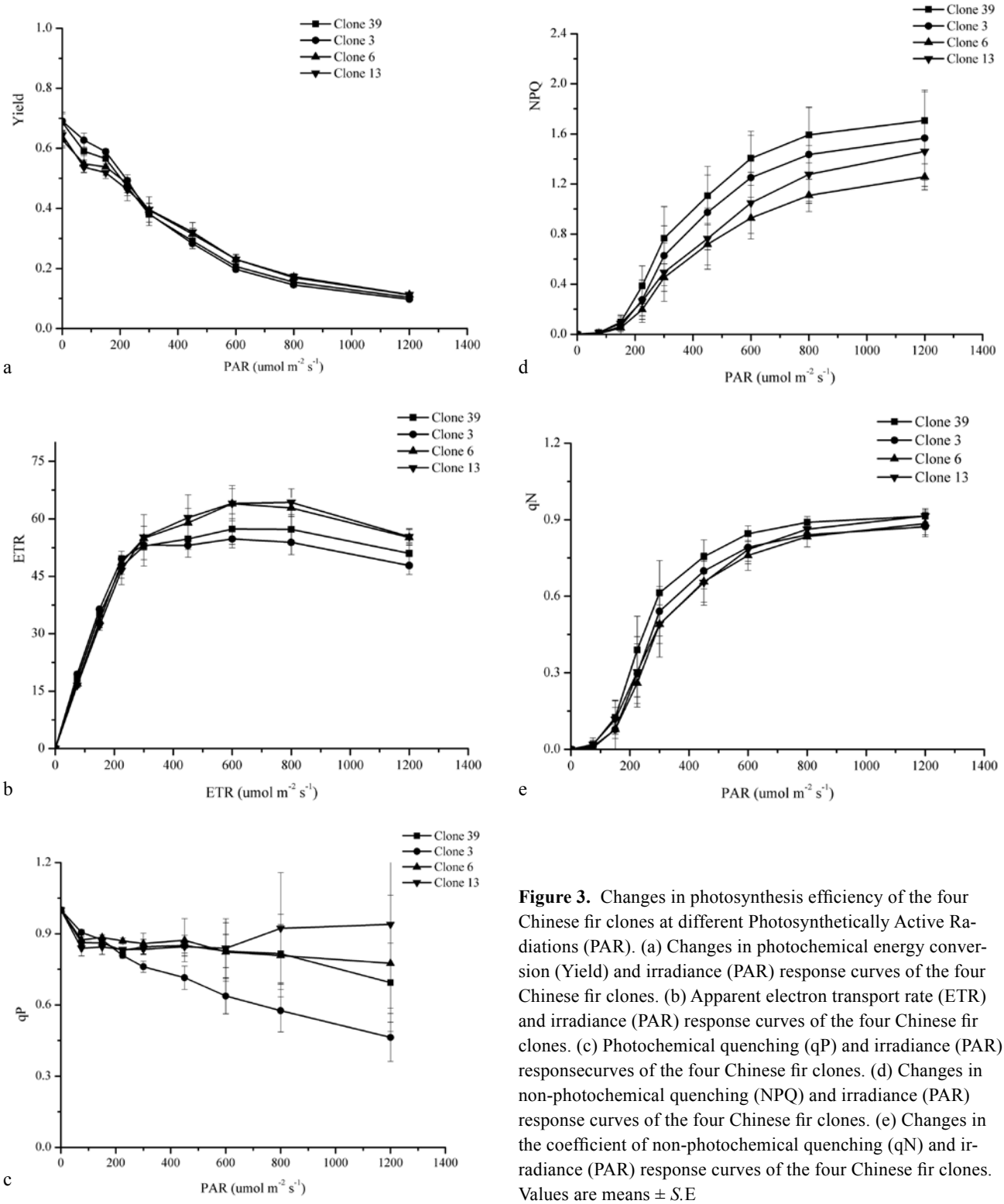

Figure 3. Changes in photosynthesis efficiency of the four Chinese fir clones at different Photosynthetically Active Radiations (PAR). (a) Changes in photochemical energy conversion (Yield) and irradiance (PAR) response curves of the four Chinese fir clones. (b) Apparent electron transport rate (ETR) and irradiance (PAR) response curves of the four Chinese fir clones. (c) Photochemical quenching (qP) and irradiance (PAR) responsecurves of the four Chinese fir clones. (d) Changes in non-photochemical quenching (NPQ) and irradiance (PAR) response curves of the four Chinese fir clones. (e) Changes in the coefficient of non-photochemical quenching (qN) and irradiance (PAR) response curves of the four Chinese fir clones. Values are means ± *S*.E

Table 1. The Pearson correlations between pigment content (Chl a, Chl b and Chl a+Chl b), pigment parameters (Chl a/Chl b ratio) and chlorophyll fluorescence parameters (Yield, ETR, qp, qN and NPQ) at 800 μmol m^{-2} s^{-1}

	Chl a	Chl b	Chl a+Chl b	Chl a/Chl b		ETR		qN
Chl b	0.848**							
Chla+Chl b	0.967**	0.914**						
Chl a/Chlb	-0.166	-0.635**	-0.333					
Yield	-0.667**	-0.869**	-0.750**	0.605*				
ETR	-0.666**	-0.869**	-0.749**	0.606*	1.000**			
qp	-0.211	-0.197	-0.206	-0.048	0.382	0.382		
qN	0.336	0.331	0.321	-0.214	-0.237	-0.235	0.661**	
NPQ	0.549*	0.476	0.495	-0.129	-0.509*	-0.507*	0.120	0.784**

**P<0.01; *P<0.05

Discussion and conclusions

Light saturation rate of photosynthesis depends on leaf traits such as effective leaf size (Koike 1988). Leaf area of clones 6 and 13 was the largest (Figure 1) and it increased the photosynthetic capacity of their leaves. The Chinese fir is used for its wood. Therefore, it is critical to ensure that the plants maintain high growth rate. Our results indicated that at the age of six months, the height of clones 13 and 6 were higher than of the other clones (Figure 1), which was in accordance with their large leaf size, Yield and ETR.

Chl content is closely associated with photosynthetic rate of plants in the field (Sang et al., 2011; Wang and Yuan, 2001). Chl a/ Chl b ratio is more directly involved in determining photosynthetic activity (Givnish 1988). Further, Chl b is synthesized from Chl a by chlorophyllide a oxygenase and the conversion of Chl a to Chl b is photochemical rather than biochemical process (Tanaka and Tanaka 2005). The results demonstrate that the content of Chl b in clones 39 and 3 were significantly higher (P < 0.05) than in the other two clones. However, the Chl a/Chl b ratio of clones 39 and 3 was lower than this of the other two clones. Chl b can increase the relative content of concentrated pigment protein to absorb and make use of more scattered light and transmitted light so that leaves in the weak light environment can absorb more light energy for photosynthesis (Kramer and Kozlowski 1960). This suggests that clones 39 and 3 are more suitable for growing under low light intensity than the other two clones.

Light energy absorbed by Chl is transformed into Chl fluorescence (Maxwell and Johnson 2000). Chl fluorescence gives a valuable insight into the exploitation of excitation energy by PSII and indirectly by the other protein complexes of the thylakoid membranes (Roháček 2002). The occurrence of photoinhibition was highlighted by the significant decline of Yield that is a measure of the total photochemical efficiency of PSII under photosynthetic steady-state conditions (Zlatev 2009). In this experiment, the differences in the values of Yield indicate that the four clones had significant differences in the electron transport activity of PSII when plants are grown under same conditions. Under stronger illumination, the quantum yield of PSII was high in clones 13 and 6 and low in the clones 39 and 3.

The ETR value represents the relative quantity of electrons passing through PSII during steady-state photosynthesis (Tezara et al. 2003). Thus, ETR value reduction means loss of capture efficiency/excitation of Chl. The ETR values of clones 39 and 3 reduced significantly between 600 μmol m^{-2} s^{-1} and 1200 μmol m^{-2} s^{-1}. Reductions in ETR may be due to the inefficiency of capturing excitation (Flowers et al. 2007). Photochemical quenching (qP) depends on the proportion of reaction centers, which are photochemically "open", rather than on the efficiency with which an absorbed photon can reach a reaction centre (Maxwell and Johnson 2000). High qP is advantageous for the separation of electric charge in the reaction center, and is beneficial to electron transport and PSII yield (Guo et al. 2006, Mao et al. 2007). Non-photochemical quenching (NPQ) and its coefficient (qN) can represent the energy, which cannot be utilized to transport photosynthetic electrons being dissipated harmlessly as heat energy from PSII antennae (Muller et al. 2001; Vasil'ev et al. 1998, Veres et al. 2006). The lower NPQ of clones 13 and 6 indicates that these plants effectively reduced the irradiance heat and efficiently utilized the energy absorbed by antenna pigments in PSII. The higher NPQ of clones 39 and 3 suggested that the energy absorbed in the physiological range of irradiances was much higher than photochemical utilization, which caused inhibition of photosynthesis (Vasil'ev et al. 1998).

Yield and ETR were negatively correlated with Chl b, but being positively correlated with Chl a/Chl b ratio and plant height. Chl a/Chl b ratio is directly involved in determining photosynthesis (Givnish 1988). Thereby Chl fluorescence gives a valuable insight into the exploitation of excitation energy by PSII and indirectly by the other protein complexes of the thylakoid membrane. Chl fluorescence supports a possible non-destructive method to evaluate the chemical changes and provide a valuable insight into the exploitation of excitation energy by PSII (Roháček 2002). The Yield, ETR and qP values, and the NPQ value combined with leaf morphology (leaf size) and plant height, and the ratio of Chl a/Chl b ratio of the clones grown under the same condition in this experiment suggested that clones 39 and 3 are suitable for low light conditions but the clones 13 and 6 are adapted to grow under high light conditions. In the high light conditions, clones 39 and 3 produce destructive oxidative molecules resulting in damage to the photosynthetic apparatus via photoinhibition (Krause 1988, Aro et al. 1993).Traditionally, foresters mainly select clones or cultivars or species subjectively based on surface features that may substantially diverge from their expectations because of ontogenetic and adaptable features of the clones or cultivars or species. However, understanding the variability of photosynthesis and associated growth rate of clones or cultivars or species is important when predicting which clones or cultivars or species are suitable for particular geographical region. In this research, light irradiance levels significantly affected the growth of Chinese fir clones. High light is concluded to be the suitable condition for clones 13 and 6 that had higher photosynthesis productivity. Further, clones 39 and 3 were more adapted for low light conditions and high light greatly reduced their photosynthesis ability and productivity. Chl fluorescence can be used as a measure of photosynthesis and status of photosynthetic apparatus thereby providing a quantitative index to select appropriate varieties for a particular location. Therefore, this technique is important for selecting appropriate cultivars for forest management due to its sensitivity, convenience, and non-destructive characteristics.

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