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Relating photosynthetic performance to leaf greenness in litchi: A comparison among genotypes

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ABSTRACT

In this study, leaf greenness (determined with a SPAD meter), photosynthesis, and chlorophyll fluorescence were analyzed on mature sun leaves in different litchi (*Litchi chinensis* Sonn.) cultivars to obtain an understanding of the relationship between leaf greenness and photosynthetic characteristics. Leaf greenness differed significantly among the 21 cultivars tested and was poorly correlated with N concentration, suggesting variations in leaf greenness among genotypes were unlikely a result of N differences. Four cultivars with different greenness, 'Heiye' (HY, SPAD 54–58), 'Feizixiao' (FZ, SPAD 49–52), 'Nuomici' (NM, SPAD 43–47) and 'Baili' (BL, SPAD 35–38) were selected for relating leaf greenness to photosynthesis. SPAD values were highly correlated with chlorophyll concentrations. Cultivars with darker leaves tended to have lower chlorophyll a/chlorophyll b ratios (C_a/C_b) than those with lighter leaves. Chloroplasts in dark green HY and FZ had more appressed lamellae, and denser grana and stroma than light green NM and BL. HY had also a higher maximum photosynthetic rate (P_{max}) than the other cultivars tested. P_{max}, light saturation point (LSP) and light compensation point (LCP) displayed significant linear correlations with SPAD values. Apparent quantum efficiency (AQE) seemed not related with leaf greenness, while dark respiration rate (R_d) displayed a strong negative correlation with it. Maximal and minimal yields of fluorescence (F_m and F_o) showed good positive correlations with SPAD values, whereas maximal photochemical efficiency of PSII (F_v/F_m) was not significantly different among cultivars and was poorly correlated with SPAD values. Effective quantum yield of photosystem II (Φ_{PSII}), photochemical quenching coefficient (q_P), and non-photochemical quenching coefficient (q_N) were poorly correlated with SPAD values. Φ_{PSII} decreased with the increase of light exposure, indicating an increased proportion of closed PSII reaction centers under higher light. The slope of this decrease was the lowest in NM and highest in BL and seemed not to be related to SPAD values. Hence, AQE, F_v/F_m, q_P, q_N and responsiveness of closure of the PSII reaction center under high light are independent of leaf greenness. Yet, darker green leaves contribute to better light energy capture and conversion, CO_2 fixation, and ability to utilize both strong and weak light.

1. Introduction

Leaf greenness, which reflects chlorophyll concentration and can be nondestructively quantified with a SPAD-502 (Minolta, Inc., Osaka, Japan) chlorophyll meter (Dwyer et al., 1991; Kapotis et al., 2003), may provide valuable insights into the physiological performance of leaves (Kapotis et al., 2003). Light adaptability of plants is closely related to leaf greenness. Compared with sun-loving plants, shade-loving plants generally have darker green leaves with higher concentrations of chlorophylls, larger chloroplasts, and a higher thylakoid/grana ratio, enabling better capacity for quantum capture under low irradiance (Boardman, 1977). Leaves of sun-loving plants also respond to shade by increasing leaf chlorophyll concentration (Olsen et al., 2002; Nemali and van Iersel, 2004; Jeong et al., 2007). Leaf greenness is also a sensitive indicator of nutritional status. Typically, it reflects N status because N comprises part of the chemical structure of chlorophyll molecules as well as photosynthetic proteins, which account for more than half of the N in leaves (Evans, 1989). Based on a linear correlation between leaf greenness and nitrogen content, which has been found in a wide range of crops, SPAD values have been widely used to indicate N status in plants (Blackmer and Schepers, 1995; Neilsen et al., 1995; Bondada and Syvertsen, 2003; Chang and Robison, 2003; Bonneville and Fyles, 2006; Papasavvas et al., 2008). Apart from N deficiency, many other stress conditions lead to loss of leaf greenness in plants,

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such as drought (Fanizza et al., 1991), salt stress (Atlassi et al., 2009), heat (Rosyara et al., 2010), high light (Olsen et al., 2002) and ozone (Neufeld et al., 2006).

Photosynthesis, one of the most fundamental metabolic processes in plants, is directly related to the abundance of chlorophylls, which absorb light energy that drives carbon-fixing reactions. Hence, leaf greenness may be closely related to photosynthetic performance. A strong correlation between SPAD values and photosynthesis was observed in olive (Boussadia et al., 2011), soybean (Ma et al., 1995), *Amaranthus vlitus* (Kapotis et al., 2003) and *Beta vulgaris* (Papasavvas et al., 2008). However, despite the darker greenness, shade leaves have a lower photosynthetic capacity than sun leaves due to poorer leaf structure as reflected by thinner lamina (Jiang et al., 2011; Pantin et al., 2011). Excessive N supply may lead to an increase in leaf greenness but not necessarily an increase in photosynthesis (Bondada and Syvertsen, 2003). Farquhar et al. (1989) suggested that in high-radiation environments, high chlorophyll content could be an indicator of low photo-inhibition of photosynthesis.

Litchi (*Litchi chinensis* Sonn.) is an important subtropical woody fruit tree cultivated widely in regions with warm subtropical climate across the world. Originated in south China, litchi dominates wild forests and is part of the overstory canopy in Hainan province (Huang et al., 2005), indicating that it is a typical sun-loving species. There are over 200 litchi cultivars or lines with distinguishing characteristics and delicate differences in various traits (Wu, 1998). One of the differences is leaf greenness, which is the basis of some cultivar names. For example, cvs. 'Baiye' and 'Baili', meaning "white leaves" and "white litchi tree" respectively, were named for their light green leaf colour, whereas 'Heiye', literally meaning "black leaf", has dark green leaves. We hypothesized that genetically-based greenness of litchi leaves is related to nitrogen concentration and photosynthetic performance.

In this study, we investigated the differences in nitrogen concentration, chloroplast ultrastructure, photosynthesis and chlorophyll fluorescence parameters among litchi cultivars. The relationship between these variables and leaf greenness in different cultivars growing in the same orchard under the same management, which excluded variations beyond genetic background, was also examined in order to obtain an understanding of the physiological variables related to leaf greenness in litchi. This understanding should provide valuable reference for using leaf greenness (SPAD values) as an indicator for selecting genotypes with high photosynthetic performance.

2. Materials and methods

2.1. Plant materials

The study was conducted on litchi trees growing in the experimental orchard at South China Agricultural University in Guangzhou, China (23°N 113° W). Three 10-year-old trees of each of 21 litchi cultivars (see Table 1) were used for leaf greenness (SPAD) measurements. These trees were under standard management with regular irrigation and fertilization (Zhang et al., 2010). They were pruned between late June and early July after harvest by heading back the last season's shoot to 3 or 4 nodes. Irrigation and fertilization regimes are shown in Table 2. SPAD values were measured on ten exposed fully mature leaves at different canopy positions on each tree using an SPAD-502 chlorophyll meter (Minolta, Inc., Osaka, Japan) in early November 2009, when the buds were in quiescent status. Based on the SPAD values and cluster analysis to segregate groups according to SPAD values, four cultivars: 'Heiye' (HY), 'Feizixiao' (FZ), 'Nuomici' (NM) and 'Baili' (BL), each representative of a different one of the 4 groups, were selected for studying chlorophyll content and composition, nitrogen content, photosynthetic characteristics and chlorophyll fluorescence.

Table 1
Names and abbreviations of the 21 litchi cultivars used for leaf greenness measurements.

Cultivar name	Abbreviation
1. Heiye	HY
2. Fenghua	FH
3. Chenzi	CZ
4. Lanzhu	LZ
5. Huaizhi	HZ
6. Congxing	CX
7. Guiwei	GW
8. Feizixiao	FZ
9. Hehuadahongli	HH
10. Songjiaxiang	SJ
11. Kulin	KL
12. Zhumuru	ZM
13. Xiafanzi	XF
14. Nuomici	NM
15. Siliangguo	SG
16. Zhuangyuanhong	ZY
17. Wuheli	WH
18. Ziniangxi	ZN
19. Shuilin	SL
20. Xuehuaizi	XH
21. Baili	BL

2.2. Chlorophyll concentrations

For measurement of chlorophyll concentrations, six leaf disks (totaling 1.96 cm^2) from fully matured leaves of HY, FZ, NM and BL were collected and suspended in 10 mL of 80% acetone and kept overnight in darkness. The absorbance of the extract was determined at 663 and 645 nm with a UV2550 spectrophotometer (Shimadzu, Kyoto, Japan). The chlorophyll concentrations were calculated according to Arnon (1949): $C_a = 12.7A_{663} - 2.69A_{645}$; $C_b = 22.9A_{645} - 24.68A_{663}$; $C_t = C_a + C_b$, where C_a represents chlorophyll a, C_b represents chlorophyll b and C_t represents total chlorophylls.

2.3. Nitrogen analysis

Twenty fully matured leaflets were taken randomly from each tree and their areas and SPAD values were individually measured using a portable SPAD-502 chlorophyll meter (Minolta Camera Co.

Table 2
Fertilization and irrigation regimes in the litchi orchard of South China Agricultural University. One mm of rainfall within a week will reduce per tree irrigation volume by 30 L in the week (Zhang et al., 2010).

Time of application	N	P_2O_5	K_2O
Fertilization (kg per tree)			
Preflowering application: early February to early March	0.15	0.05	0.3
Application during fruiting: May to June	0.18	0.10	0.60
Preharvest application: mid June to late June	0.18	0.02	0.12
Postharvest application: August to September	0.09	0.03	0.18
Irrigation (L per week per tree)			
Late October to early January before panicle emergence	200		
Late January to March after panicle emergence and throughout flowering	600		
April to June	800		
August to October	600		