Using a chlorophyll meter to estimate tea leaf chlorophyll and nitrogen contents

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Abstract

A chlorophyll meter can conveniently estimate foliar chlorophyll and nitrogen (N) contents in many species. The objective of this study was to evaluate the utility of a chlorophyll meter to inform nitrogen fertilization rates of tea (*Camellia sinensis* L.) at different places and times. Plot-scale (5×20 m) experiments with three replications were conducted in 2010. Each plot was treated with 248 kg pure N ha^{-1} per year as urea. At the same time, a field-scale (32 ha) experiment, with the same fertilization rate, was conducted at the same plantation. Tea leaves were sampled in August and November and analyzed with a chlorophyll meter *in situ* and in the lab. The chlorophyll and nitrogen contents and chlorophyll meter (SPAD) readings of tea leaves in August were greater than in November, and plot-scale values were greater than field-scale values. SPAD readings could estimate the chlorophyll content of tea leaves regardless of temporal and spatial considerations. However, space and time must be considered when using SPAD readings to estimate the nitrogen content of tea leaves.

Keywords: chlorophyll indicator, nitrogen nutrition, nitrogen partitioning, SPAD reading, tea leaves.

1. Introduction

Nitrogen (N) is the major nutrient affecting tea growth, yield, and quality. Applying N fertilizer can substantially increase the production of new shoots and the content of functional compounds, such as amino acids (Venkatesan and Ganapathy, 2004; Kamau *et al.*, 2008). The northwest of Zhejiang Province is one of the most intensively managed tea-growing areas in China. To maximize yield, local tea growers often use great quantities of N fertilizers three to four times a year. Excessive N is quickly lost from tea fields in runoff, which not only lowers the fertilization efficiency, but also causes environmental pollution (Jaynes *et al.*, 2001; Zvomuya *et al.*, 2003). Therefore, timely and appropriate nitrogen application would be important to improving yield and quality as well as reducing environmental pollution (Errebhi *et al.*, 1998; Costa *et al.*, 2001). However, a rapid method to evaluate the N status of tea plants is lacking.

Conventional methods of evaluating crop N status are time-consuming and complicated. A chlorophyll (Chl) meter is a small and portable instrument that can non-destructively and rapidly evaluate crop N status. Such meters have been used to estimate foliar Chl and N content in various species, such as rice (Huang *et al.*, 2008), wheat (Le Bail *et al.*, 2005), beans (Abdelhamid *et al.*, 2003), and fruit trees (Schaper and Chacko, 1991). However, the literature does not address whether a Chl meter can evaluate Chl and/or N contents in tea plants.

A Chl meter estimates *in situ* relative foliar Chl levels by measuring absorption at 650 and 940 nm wavelengths. Photosynthetic proteins account for more than half of the N in a crop leaf, and crop foliar Chl content is significantly correlated with foliar N content (Evans, 1989). Therefore, the SPAD-502 chlorophyll meter (SPAD) reading should reflect foliar N status (Denuit *et al.*, 2002; Le Bail *et al.*, 2005). The relationship between SPAD readings and foliar Chl or N for other crops has been intensively studied and is known to be affected by plant variety (Hoel, 2002), growing conditions (Campbell *et al.*, 1990), sampling point (Chapman and Barreto, 1997), leaf age (Jinwen *et al.*, 2009), and other factors. However, most previous studies were conducted using microscale plot trials, and little information is available at the field scale. Furthermore, we know of no research on how the relationships between Chl and N contents and SPAD readings change in space and time.

The aims of this study were to investigate: (1) whether SPAD readings could indicate Chl and/or N contents; (2) temporal and spatial differences in Chl and N contents and in SPAD readings; and (3) correlations in space and time between the SPAD and the actual foliar Chl and N contents as a measure of the utility of SPAD readings for assessing the Chl and N status of tea leaves.

2. Materials and Methods

2.1 Site and experimental treatments

The experiments were conducted at Hanghzhou experimental tea farm, in Zhejiang province, China (119°53′ E, 30°23′ N; elevation: 20 m). The tea plants used in the experiments were forty years old (*Camellia sinensis* L. cv. "Jiukeng"). Plot- (5×20 m^2) and field-scale (32 ha) experiments were conducted simultaneously. The plot-scale experiments were replicated three times. Tea plants in each plot were treated with 248 kg pure N ha^{-1} per year as urea, with 124 kg pure N ha^{-1} basally applied on May 1 and on October 1, 2010. At the same time, a field-scale experiment was conducted

with the same fertilizer treatment. All other farm management practices were conducted in the same way.

2.2 Sampling and measurements

Tea leaves were sampled on August 1 and November 1 using a grid sampling method. A 2.5 × 2.5 m grid was established in the plot experiment, and the field experiment used an 80 × 80 m grid that was determined using a global positioning system. There were 16 sampling points in each plot and 50 sampling points in the field. At every point, a composite sample was obtained from 20 fully expanded, current-year mature tea leaves (the fourth or fifth leaf from the apex on each of 20 randomly-sampled shoots per plant) within a 1-m radius of each grid point. A SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan), was used *in situ* to estimate chlorophyll content. The SPAD reading of each tea leaf was obtained at three locations: (a) about one-third of the leaf length from the petiole, (b) at the midpoint of leaf, and (c) about one-third of the leaf length from the apex (Lin *et al.*, 2010). At each location, a measurement was taken on both sides of the leaf vein. The six SPAD readings were averaged to provide a single reading per leaf.

Immediately after the SPAD measurements, the sampled leaves were removed from the plant and taken to the lab in an insulated box full of ice. They were either analyzed immediately or frozen (-20°C) until testing. Five fresh leaves from each sampling point were extracted in 96% (v/v) ethanol and the absorbance was measured at 649 and 665 nm with a spectrophotometer (Fritschi and Ray, 2007). Chlorophyll contents were determined using the method of Arnon (1949).

For foliar nitrogen determination, tea leaves were dried at 105°C for 30 minutes to rapidly stop the ac-

tivity of polyphenol oxidase and then at 70°C until dry weight was constant. Dried tea samples were ground, and foliar N content was determined by the Dumas combustion method (Rapid N cube, Elementar Analysensysteme, Hanau, Germany) (Jung *et al.*, 2003). Three replicates were taken for each measurement.

2.3 Data analysis

SPSS v.17 (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. Two-way analysis of variance (ANOVA) was used to assess temporal and spatial differences. All statistical analyses were considered significant when *P*-values were <0.05.

3. Results

3.1 Temporal variation in chlorophyll and nitrogen contents and SPAD readings

There were significant temporal differences in the Chl *a*, Chl *b*, and N contents of tea leaves, whereas no significant temporal differences were observed in the actual (laboratory-measured) foliar total Chl contents and SPAD readings (Table 1). Foliar Chl *b* and N contents in August were significantly higher than in November, while foliar Chl *a* contents in August were significantly lower than in November. Chl, Chl *a*, and Chl *b* contents and Chl/N ratio were significantly positively correlated with SPAD readings (P<0.01), while the Chl *a/b* ratio was significantly negatively correlated with SPAD readings in August and in November (Table 2). In general, the relationships between foliar Chl, Chl/N ratio, and SPAD readings were stronger in August than in November, with the correlation coefficients decreasing from August to November.

Table 1. SPAD readings and chlorophyll and nitrogen contents measured in tea leaves at plot and field scales in August and November, 2010. There were 16 sampling points at the plot scale (100 m²) and 50 sampling points at the field scale (32 ha). Results from a two-way ANOVA are also shown.

Foliar indexes	Plot scale (n=16)		Field scale (n=50)		P		
	August	November	August	November	Temporal (T)	Spatial (S)	T×S
SPAD reading	63.04±4.29	61.08±5.21	62.78±4.83	60.92±5.96	ns	ns	ns
CV (%)	6.81	8.53	7.69	9.78			
Chl (mg g⁻¹)	2.69±0.36	2.67±0.38	2.62±0.42	2.58±0.39	ns	ns	ns
CV (%)	13.38	14.23	16.03	15.12			
Chl *a* (mg g⁻¹)	1.97±0.22	2.01±0.25	1.63±0.20	1.75±0.22	<0.05	<0.001	ns
CV (%)	11.17	12.47	12.09	12.44			
Chl *b* (mg g⁻¹)	0.72±0.13	0.66±0.16	0.99±0.24	0.83±0.19	<0.001	<0.001	ns
CV (%)	18.03	24.08	24.57	23.08			
N (%)	3.81±0.29	3.33±0.30	3.42±0.33	3.17±0.29	<0.001	<0.001	ns
CV (%)	7.61	9.07	9.54	9.14			

Note: Data are means of replicates ± standard deviation. SPAD readings, chlorophyll meter readings; Chl, Chl *a*, and Chl *b*, foliar mass-based chlorophyll, chlorophyll *a*, and chlorophyll *b* content, respectively; N, foliar mass-based nitrogen content; CV, coefficient of variation; SPAD readings have no units; ns, nonsignificant.

Table 2. Correlation coefficients among indexes measured in tea leaves at plot and field scales in August and November, 2010. There were 16 sampling points at the plot scale (100 m²) and 50 sampling points at the field scale (32 ha).

Scales	Month		SPAD readings	N	Chl	Chl a	Chl b	Chl a/b
Plot scale (n=16)	August	N	0.53*					
		Chl	0.92**	0.61**				
		Chl *a*	0.90**	0.62**	0.99**			
		Chl *b*	0.90**	0.57*	0.97**	0.93**		
		Chl *a/b*	-0.78**	-0.36	-0.81**	-0.72**	-0.91**	
		Chl/N	0.84**	0.2	0.90**	0.88**	0.88**	-0.80**
	November	N	0.45					
		Chl	0.90**	0.48*				
		Chl *a*	0.90**	0.53*	0.99**			
		Chl *b*	0.86**	0.35	0.96**	0.91**		
		Chl *a/b*	-0.54*	-0.05	-0.67**	-0.56*	-0.84**	
		Chl/N	0.83**	0.18	0.95**	0.91**	0.95**	-0.76**

Continued...

Scales	Month		SPAD readings	N	Chl	Chl a	Chl b	Chl a/b
Field scale (n=50)	August	N	0.21					
		Chl	0.88**	0.37**				
		Chl a	0.80**	0.36**	0.94**			
		Chl b	0.86**	0.35*	0.96**	0.82**		
		Chl a/b	-0.81**	-0.30*	-0.80**	-0.59**	-0.90**	
		Chl/N	0.81**	-0.22	0.82**	0.77**	0.80**	-0.67**
	November	N	-0.14					
		Chl	0.84**	-0.1				
		Chl a	0.80**	-0.07	0.96**			
		Chl b	0.80**	-0.12	0.95**	0.82**		
		Chl a/b	-0.64**	0.11	-0.74**	-0.53**	-0.90**	
		Chl/N	0.74**	-0.61**	0.84**	0.78**	0.82**	-0.66**

Note: N, foliar mass-based nitrogen content (%); Chl, Chl *a*, Chl *b*, foliar mass-based chlorophyll, chlorophyll *a* and chlorophyll *b* content (mg g^{-1}); Chl *a/b*, the ratio of chlorophyll *a* and *b* mass-based content; Chl/N, the ratio of chlorophyll and nitrogen mass-based content (mg g^{-1}); SPAD readings and Chl *a/b* have no unit. * Significant at the 0.05 probability level, ** Significant at the 0.01 probability level.

3.2 Spatial variation in chlorophyll and nitrogen contents and SPAD readings

There were significant spatial differences in the Chl *a*, Chl *b*, and N contents of tea leaves (P<0.001), but no significant differences in foliar Chl contents and SPAD readings were observed in space (Table 1). There were no significant temporal × spatial interactions for any of the foliar indexes. The coefficients of variation (CV) of foliar Chl and N contents and SPAD readings in field-grown plants were greater than in plot-grown tea trees, which meant that these values fluctuated more when the spatial scale increased (Table 1). Foliar Chl *a* and N contents in the plots were significantly higher than in the field, while foliar Chl *b* contents in the plots were significantly lower than in the field. In general, the correlations between Chl, Chl *a*, and Chl *b* contents, Chl/N ratio, and SPAD readings

of tea leaves were greater in the plots than in the field; the correlation coefficients decreased with increasing spatial scale (Table 2).

3.3 Foliar Chl contents and SPAD readings

There were strong curvilinear relationships between total foliar Chl contents and SPAD readings (Figure 1), with coefficients of determination ranging from 0.719 to 0.853 (P < 0.001). The slopes increased at greater SPAD values. In both months (August and November) and at both scales (plot and field), foliar Chl *a* accounted for most of the Chl content, ranging from 62.21% to 75.28% (Table 1). The relationships between foliar Chl *a* and Chl *b* contents and SPAD readings were similar to those between total Chl contents and SPAD readings.
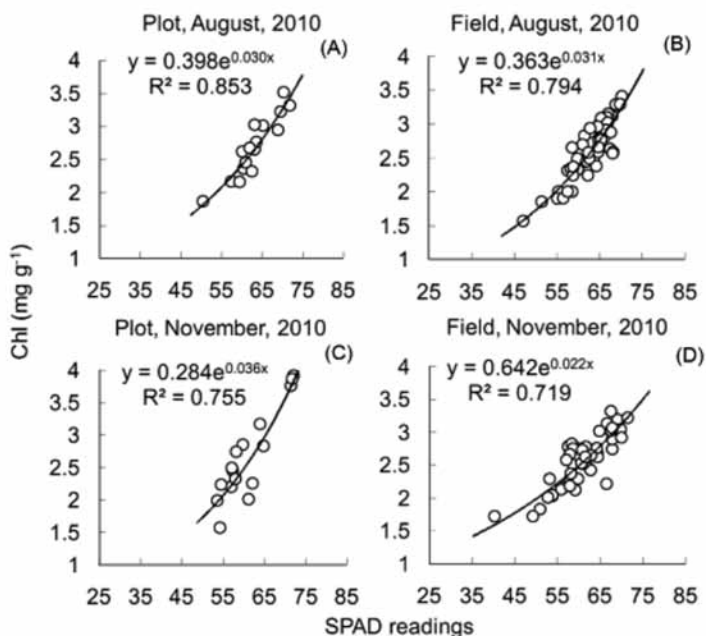
Figure 1. Relationships of foliar SPAD readings with chlorophyll (Chl) content in August (A and B) and November (C and D), 2010, at plot (A and C) and field (B and D) scales. There were 16 sampling points at plot scale (100 m^2) and 50 sampling points at field scale (32 ha).

3.4 Foliar N contents and SPAD readings

Nitrogen contents of tea leaves were significantly positively correlated with SPAD readings in the plots in August (p<0.05) but not in November. Foliar N content and SPAD readings were not significantly correlated at the field scale (Table 2).

3.5 Chl/N and SPAD readings

There were significant correlations between foliar N and Chl contents at the plot scale in both August (P<0.01) and November (p<0.05), but at the field scale they were only significantly correlated in August (P<0.01). Similar to the relationships between foliar Chl and SPAD readings, there was a strong exponential relationship between Chl/N and SPAD readings (Figure 2), with coefficients of determination ranging from 0.595 to 0.723 (p < 0.001).
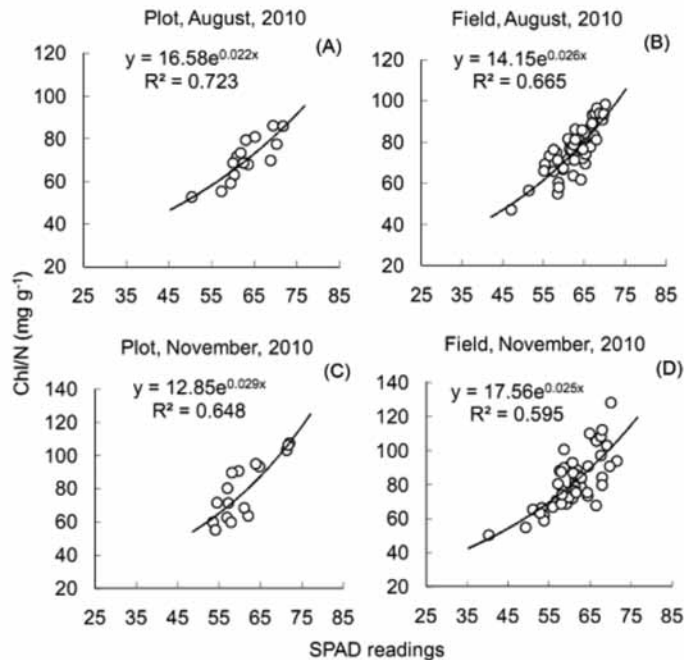
Figure 2. Relationships between foliar SPAD readings and chlorophyll/nitrogen (Chl/N) ratio in August (A and B) and November (C and D), 2010, at plot (A and C) and field (B and D) scales. There were 16 sampling points in plot scale (100 m^2) and 50 sampling points at field scale (32 ha).

4. Discussion

The relationships between Chl and SPAD readings were well described by an exponential function. This result was similar to previously-reported studies in other species (Marenco *et al.*, 2009; Uddling *et al.*, 2007). Temporal and spatial variation did not significantly affect foliar Chl contents, SPAD readings, or their relationships. These results indicated that SPAD readings could be used to estimate the relative Chl contents of tea leaves at different times and spatial scales.

The relationships between N contents and SPAD readings of tea leaves were not strong at the field level, nor in November at the plot level. This effect may

have been due in part to heterogeneity in leaf thickness and foliar N distribution within a leaf (Chang and Robison, 2003). Additionally, nutrients other than N also affect Chl concentrations, which could further affect the applicability of SPAD readings to assess N nutrition status (Fritschi and Ray, 2007). Furthermore, nutritional status of other elements (Valenzuela *et al.*, 1994), plant characteristics, environmental factors (Wang *et al.*, 2004), and insect damage might all affect leaf Chl and N contents and therefore foliar SPAD readings.

The Chl/N ratio indicates the proportion of N allocated to Chl at the expense of other macromolecules, such as rubisco, other proteins, nucleic acids, free amino acids, and secondary metabolites (Martin

et al., 2007; Rozendaal *et al.*, 2006). Plants alter how they partition N within the photosynthetic apparatus depending on growth conditions, e.g., the light environment (Kitaoka and Koike, 2004). In shaded conditions, more leaf N is allocated to Chl to enhance light capture, whereas, in sunny conditions, more leaf N is invested in Rubisco to increase carbon fixation (Kimura *et al.*, 1998). Evergreen plants like tea undergo substantial changes in light and temperature conditions over the course of a year, and these changes may affect nitrogen partitioning (Muller *et al.*, 2005). Therefore, these factors may cause temporal and spatial variation in the relationship between foliar N contents and SPAD readings. Additional research will be required to clarify how the relationship between N content and SPAD readings of tea leaves changes in space and time.

5. Conclusions

In conclusion, SPAD readings could estimate foliar Chl contents of tea plants in space and time. However, spatial and temporal differences must be considered when using SPAD readings to directly estimate foliar N contents of tea plants. SPAD readings could be used to optimize fertilizer application in tea plantations if a stable relationship between foliar N content and SPAD readings could be identified. Further research is required to explore the relationship between foliar N contents and SPAD readings for tea plants.

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