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A simple method for large scale estimation of leaf chlorophyll content in *Hevea brasiliensis* using a chlorophyll meter

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Abstract

Measurement of chlorophyll content of leaves by the conventional laboratory method involves discriminate sampling, weighing, extraction and spectrophotometric measurements that become laborious and time consuming especially when large number of samples is processed at a time. Nowadays, hand-held chlorophyll content meters are widely used for rapid and non-destructive estimation of chlorophyll content. Chlorophyll content in *Hevea* leaves was estimated spectrophotometrically by conventional extraction method and CCI values (chlorophyll content index) were recorded using a chlorophyll content meter, CCM - 200 (Opti-Sciences, USA) and regression models were developed. Significant positive linear relations between CCI and actual leaf chlorophyll content estimated by conventional extraction method were obtained. Separate equations were derived for determining chlorophyll content on unit fresh weight basis and unit leaf area basis. The method provides a rapid, accurate and non-destructive estimation of chlorophyll content of large number of leaves making the measurements simple and easy compared to conventional spectrophotometric assay.

Keywords: chlorophyll estimation, chlorophyll content meter, *Heave brasiliensis*

Chlorophylls are responsible for the capture of solar energy in the form of visible light during photosynthesis and therefore estimation of chlorophyll content assumes vital significance in physiological, ecological, pathological and agro-forestry studies. In higher plants, chlorophyll mainly consists of chlorophyll *a* and chlorophyll *b*. Chlorophyll *a* is the major pigment and chlorophyll *b* is the accessory pigment present in a ratio of approximately 3:1 (Gross 1991) and it can vary according to prevailing irradiance conditions (Litchenthaler *et al.* 1981, Anderson 1986). In unfavorable stress conditions the chlorophyll content in leaves tend to decrease and in low light situations it considerably increases (Boardman 1977). Chlorophyll content is a good indicator of plant health. The methods normally used in determination of chlorophyll content are destructive. Conventionally, chlorophyll estimation involves weighing, grinding, centrifugation and spectrophotometric determination of leaf extracts (Arnon 1949, Porra *et al.* 1989, Litchenthaler 1987). However, these methods are slow, tedious and time consuming. It is difficult to process a large number of leaf samples at a time. Recently new portable chlorophyll meters based on dual wavelength absorption were developed for rapid, non-destructive estimation of chlorophyll in intact leaves (Markwell *et al.* 1995, Biber 2007, Richardson *et al.* 2002, Udding *et al.* 2007). Further remote estimation of chlorophyll using reflection indices based on reflectance and absorption spectra in visible and near infra-red ranges was also attempted (Gitelson and Merzlyak 1997, Merzlyak *et al.* 2003).

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Portable, hand-held chlorophyll content meters were widely used for rapid estimation of chlorophyll and in large number of plants significant correlations were derived between chlorophyll content Index and the actual chlorophyll content estimated by extraction method. So far no attempts were made to use these techniques for rapid estimation of chlorophyll content in rubber plants. *Hevea* is a perennial tree with dark green to pale colored leaves cultivated both in traditional and non-traditional localities. The trees are subjected to various types of natural and anthropogenic stresses in filed and assessment of chlorophyll content will therefore be a good means for detecting the physiological status of plants. Therefore the present study was planned with an objective to determine the possible relationship of chlorophyll content index measured using CCM-200 chlorophyll meter (Opti-Sciences, USA) and chlorophyll content in *Hevea* leaves estimated spectrophotometrically.

Materials and methods

Budded plants of one year old *Hevea brasileinsis* were used for this study. The chlorophyll content indices (CCI) of young and mature leaves from three different clones RRII 105, RRIM 600 and GT 1 were determined using the chlorophyll content meter- CCM 200 (Opti-Sciences, USA). The instrument measured the index values from an area of 1 cm^2 and the mean of three readings was taken from one sample. Similar measurements were taken from 20 plants each from clones RRII 105, RRIM 600 and GT1. After reading CCI values the same leaves were collected for estimation of total chlorophyll, chlorophyll a and chlorophyll b. Fresh leaf discs (size =1 cm^2) were

Clone		CC Index	Chlorophyll content (mg/g f.w't)		
			T. Chl.	Chl. *a*	Chl. *B*
a). Chlorophyll content on leaf weight basis (mg/g f.w't)					
RRII 105	Y	20.98±0.5	1.83±0.03	1.32±0.02	0.50±0.01
	M	77.64±3.0	3.31±0.03	2.11±0.01	1.19±0.03
RRIM 600	Y	18.20±1.0	1.79±0.06	1.27±0.04	0.52±0.02
	M	69.09±4.0	2.96±0.09	1.91±0.04	1.06±0.04
GT1	Y	21.00±0.4	1.81±0.02	1.30±0.02	0.51±0.01
	M	72.60±3.0	3.81±0.06	2.02±0.01	1.17±0.05
b). Chlorophyll content on leaf area basis (mg/cm²)					
RRII 105	Y	20.98±0.5	0.030±0.0006	0.022±0.0004	0.008±0.0002
	M	77.64±3.0	0.060±0.0009	0.038±0.0002	0.021±0.0007
RRIM 600	Y	18.20±1.0	0.029±0.0001	0.020±0.0008	0.008±0.0003
	M	69.09±4.0	0.057±0.0004	0.037±0.0001	0.020±0.0004
GT1	Y	21.00±0.4	0.031±0.0006	0.022±0.0004	0.008±0.0002
	M	72.60±3.0	0.058±0.0004	0.036±0.0004	0.021±0.0009

Table 1. CC index and actual chlorophyll content determined spectrophotometrically (Y=young leaves, M=mature leaves).

punched from the leaves and the weights were recorded immediately. After measuring the area of leaf, the discs were soon transferred to beakers containing a mixture of DMSO and acetone in 1:1 proportion for chlorophyll estimation (Arnon, 1949). The samples were kept overnight in the solution in dark for extracting the chlorophyll pigment. The extracts were transferred to curettes and the optical density was measured at 663 and 645 nm in a Shimadzu UV-240 spectrophotometer. The total chlorophyll content, chlorophyll *a* and chlorophyll *b* were estimated. The chlorophyll contents for unit leaf area (mg/cm²) and unit leaf fresh weight (mg/g) were calculated. For a direct comparison of CCI with conventional spectrophotometric measurements all values of estimated chlorophyll content (mg/g) were plotted against the CCI values for regression analysis. Similar comparisons were made for chlorophyll determined on a unit leaf area (mg/cm²) basis also.

Results and Discussion

Chlorophyll content indices varied significantly in different leaves and stages of maturity and clones. In young leaves of *Hevea* the chlorophyll content indices varied from 15.6 to 23. In mature leaves it was in the range of 56.5 to 90 CCI. Mean chlorophyll content indices of three clones ranged from 18- 21 in young leaves. Compared to mature leaves the clonal variations were not evident in young leaves (Table 1). In mature leaves the mean index varied from 69 for clone RRIM 600 to 77.6 in clone RRII 105. Chlorophyll content estimated by extraction method too differed significantly between clones. Leaves of clone RRII 105 estimated higher chlorophyll content than other clones. Mean chlorophyll content in clones ranged between 2.9 - 3.3 mg/ gram fresh weight of leaf tissue. Similarly the chlorophyll content in mg/cm² tissue was also calculated for all three clones (Table 2). Differences in chlorophyll content per unit area were more evident in mature leaves than in young leaves. Chlorophyll content per unit area was also higher in clone RRII 105. All clones showed significantly higher chlorophyll *a* content and less chlorophyll *b* content in their leaves.

The chlorophyll content indices showed significant positive relationship with chlorophyll content estimated *via* the extraction method. Regression equations were developed for direct estimation of actual chlorophyll

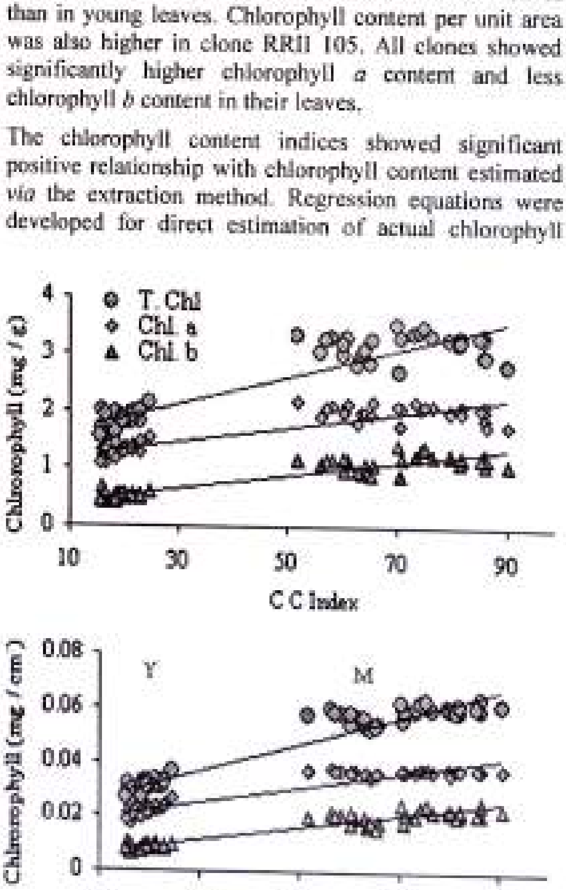

Fig. 1. Scatter plot of chlorophyll content index and actual chlorophyll in young (Y) and mature (M) leaves

Table 2. Regression equations for calculation of chlorophyll contents (x=CC Index)

a). On leaf weight basis (mg/g.f.w't).

Regression equations	
Total Chlorophyll (mg/g)	
$y = 0.0238x + 1.3802$	$R^2 = 0.87$
Chlorophyll a (mg/g)	
$y = 0.0127x + 1.0719$	$R^2 = 0.84$
Chlorophyll b (mg/g)	
$y = 0.0111x + 0.3086$	$R^2 = 0.85$

b). On leaf area basis (mg/cm²).

Regression equations	
Total Chlorophyll (mg/cm²)	
$y = 0.0005x + 0.0210$	$R^2 = 0.94$
Chlorophyll a (mg/cm²)	
$y = 0.0003x + 0.0167$	$R^2 = 0.93$
Chlorophyll b (mg/cm²)	
$y = 0.0002x + 0.0044$	$R^2 = 0.91$

content both on a unit weight or area basis using the chlorophyll content index values (Tables 3, 4). A stronger relationship exhibited for CCI values *versus* chlorophyll content per unit cm^2 area ($r^2 = 0.94$) compared to unit gram weight ($r^2 = 0.87$) of leaf. Using these equations the total chlorophyll, chlorophyll *a* and chlorophyll *b* contents were calculated from CCI for different clones studied. Similar relationships between chlorophyll content and CCI were reported in many other species (Yadava 1986, Schaper and Chacko 1991, Yamamoto *et al.* 2002, Wang *et al.* 2004). The present study revealed the potential of this technique as an alternative tool for estimation of chlorophyll content in intact rubber leaves of varying stages of maturity in different clones. The size, orientation and surface characters exert little effect on chlorophyll measurements as the *Hevea* leaves are flat and uniform across the surfaces. The flat and smooth surfaces serve better at reflecting and transmitting the LED light to the detector in CCM-200 meter (Biber, 2007). The leaf characteristics of *Hevea* plants enable an added advantage for accurate determination of chlorophyll content directly from CCI values. Chlorophyll content indices could be effectively utilized for routine measurements of chlorophyll in large number of *Hevea* plants.

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