PHOTOSYNTHETICA 46 (4): 634-636, 2008

BRIEF COMMUNICATION

# An evaluation of non-destructive methods to estimate total chlorophyll content

D. CASSOL[*], F.S.P. DE SILVA[*], A.R. FALQUETO[*], and M.A. BACARIN[*,+]

*Laboratório de Metabolismo Vegetal, Depto. de Botânica, Instituto de Biologia,Universidade Federal de Pelotas, 96010-900 Pelotas/RS, Brazil*[*]

## Abstract

The portable chlorophyll (Chl) meter (*CL-01*, *Hansatech*) has been successfully used for a rapid and direct estimation of total Chl content in the leaves of some crops. We compared *CL-01* meter readings (Chl value) and Chl contents in leaves of *Zea mays*, *Cucumis sativus*, *Raphanus sativus,* and *Ceiba speciosa*. Chl index was linearly and positively correlated to Chl content in all the species.

*Additional key words*: *Ceiba*; *Cucumis*; *Raphanus*; *Zea*.

Plants contain chlorophylls (Chls) *a* and *b*. The two compounds have different solubilities in organic solvents, and somewhat different, though overlapping, electronic spectra in the visible region. Traditionally, chemical methods of determination have required Chl extraction by a solvent, followed by the spectrophotometric determination of absorbance by the Chl solution, and conversion from absorbance to concentration using standard equations (Arnon 1949, Lichtenthaler 1987, Ritchie 2008). In the standard method of Chl determination, extraction requires destructive sampling and is relatively time consuming (Richardson *et al*. 2002). More recently, non-destructive optical methods, based on the absorbance and/or reflectance of radiation by the intact leaf have been developed. Optical methods yield a 'Chl index' value that expresses relative Chl content but not absolute Chl content per unit leaf area or per mass of leaf tissue. These newer methods are non-destructive, very quick, and can be used in the field (Markwell *et al.* 1995, Hawkins *et al.* 2007).

Many papers show the application of "Chl value" to the estimation of leaf Chl content (Richardson *et al.* 2002, Uddling *et al.* 2007), but some have failed to show the applicability of the index across different studies, plant species, or stresses. Neufeld *et al.* (2006) working with ozone-affected leaves of cutleaf coneflower (*Rudbeckia laciniata* var. *digitata*) observed that when relatively uninjured leaves were measured, SPAD meter readings were linearly related to total Chl content. However, when leaves with foliar injury were added, it was no longer possible to use the same equation to obtain Chl estimations for both classes of leaves.

The objective of this work was to evaluate the existence of relation between the Chl content and "Chl value" measured by hand-held Chl meter *CL-01* in maize (*Zea mays*), cucumber (*Cucumis sativus*), radish (*Raphanus sativus*), and floss-silk tree (*Ceiba speciosa*). Plants were grown from seed in a glasshouse. Approximately 50 leaf samples of the each species, spanning as wide a range of Chl contents as possible, from very pale yellow to very dark green leaves, were used. One hand-held Chl meter, the *CL-01* (*Hansatech*, King's Lynn, Norfolk, England) was used. This equipment provides a convenient, low cost method of measuring the relative Chl content of a leaf sample using dual wavelength optical absorbance (620 and 940 nm) measurements from leaf samples. Five separate measurements were made on each leaf and we used the arithmetic mean of these measurements for all subsequent analyses, and the results were expressed in "Chl value". In the same position we measured Chl content spectrophotometrically after extraction with 80 % acetone (m/v) (Arnon 1949). The Chl was extracted from five disks (each 5.0 $cm^2$; approx. 150 mg fresh mass, FM) from each leaf sample. Absorbances of both blank and sample were measured at 647 and 663 nm (Lichtenthater 1987). The Chl concen-

*Received* 15 May 2008, *accepted* 6 August 2008.
[+]Corresponding author; fax: + 55 53 3275 7169, e-mail: marcos.baccarin@gmail.com
*Acknowledgements*: This work was supported by funding from CNPq.

Table 1. Specific leaf area (SLA) [$m^2\ kg^{-1}$], chlorophyll (Chl) value estimated by *CL-01*, and Chl content determined spectrophotometrically [$mg\ kg^{-2}$(FM) or $mg\ m^{-2}$(leaf area].

| Species | | *n* | SLA | Chl value | Chl per fresh mass | Chl per area |
|---|---|---|---|---|---|---|
| Maize | $C_4$ | 67 | 7.3±1.0 | 4.33±2.38 | 1243±565.78 | 164.7±75.6 |
| Cucumber | $C_3$ | 50 | 6.1±0.6 | 6.34±2.27 | 730±161.73 | 158.9±41.4 |
| Radish | $C_3$ | 46 | 4.1±0.4 | 4.45±1.86 | 724±237.34 | 179.7±58.3 |
| Floss-silk tree | $C_3$ | 56 | 6.1±0.5 | 7.72±3.50 | 1001±348.95 | 160.9±45.7 |
| Total | | | | 5.64±2.81 | 969±420 | 170.4±5.67 |

Table 2. Equations and coefficients of determination ($r^2$-value) for the linear regression lines between chlorophyll (Chl) content (Y) and Chl value (x).

| | [mg(Chl) $kg^{-1}$(FM)] | $r^2$ | [mg(Chl) $m^{-2}$(area)] | $r^2$ |
|---|---|---|---|---|
| Maize | Y=360.98+203.63 x | 0.74 | Y=4.88+2.81 x | 0.81 |
| Cucumbers | Y=357.87+ 55.74 x | 0.64 | Y=5.10+1.74 x | 0.73 |
| Radish | Y=305.15+ 94.24 x | 0.65 | Y=6.80+2.51 x | 0.64 |
| Floss-silk tree | Y=338.68+ 85.99 x | 0.74 | Y=7.66+1.10 x | 0.70 |
| All data set | Y=514.92+ 77.98 x | 0.27 | Y=9.57+1.27 x | 0.41 |

tration was then converted to leaf Chl content per leaf area or FM. To arrive at a ranking of the best calibration equation we used generally first-order polynomial equations and the correlation coefficients to assess the goodness-of-fit of the calibration equation.

The "Chl value" ranged from 4.33 and 7.72 for maize and floss-silk tree, and the mean value of 5.64 comprised all tested plant species (Table 1). The Chl content of radish and maize per FM varied from 724 to 1 243 mg $kg^{-1}$(FM) and per leaf area from 158.9 to 179.7 mg $m^{-2}$, respectively. The anatomical characteristics of the leaves, *e.g.* specific leaf area (SLA), can interfere with the analysis of the data. Nevertheless, the leaf Chl content expressed by soil plant analytical development (SPAD) Chl meter reading varied significantly among peanut genotypes with wide genetic variation for the SLA (Sheshshayee *et al.* 2006).

The non-uniform distribution of Chl molecules within the leaf is influenced by structural organization of grana in chloroplast, amounts of chloroplasts within cell, and cells within tissue; these patterns may differ strongly among species (Fukshansky *et al.* 1993) and/or by non-uniform distribution of radiation across the leaf surface and differential scattering and reflection of radiation (Uddling *et al.* 2007). This fact can alter the relationship between Chl content and "Chl value" for the studied plant species.

The linear relationship between leaf content measured by spectrophotometer and "chlorophyll value' for each species is shown in Table 2. Most studies in the literature that quantify this relationship employ linear regression (Cate and Perkins 2003, Madeira *et al.* 2003, Wang *et al.* 2004, 2005), but some studies report non-linear relationship between Chl content and "chlorophyll value" (SPAD) (Markwell *et al.* 1995, Richardson *et al.* 2002, Uddling *et al.* 2007).

The coefficients of determination ($r^2$-value) for linear equations were high and significant ($p$<0.001), both per unit leaf area or FM ($r^2$>0.60) for all species, but the highest values were estimated in maize. When we compared the expression per leaf area or FM, the last $r^2$-value was higher for maize and cucumber. But, when we used all data, the correlation coefficient dropped to 0.41 and 0.27, respectively, both per unit leaf area or per FM. When we compared all species, the angular coefficients of the linear equations were different. This may be explained by anatomical characteristics of the leaves of these species, or even patterns of heterogeneous distribution of Chls in leaf, which can interfere in properties of absorption and reflection of radiation used in the determination of the values of Chl.

Uddling *et al.* (2007) suggest that SPAD calibration curves should generally be parameterized as non-linear equations, and they hope that the relationships between content of Chl and SPAD can facilitate the interpretation of Chl meter calibrations in relation to optical properties of leaves. They concluded that the effect of non-uniformly distributed Chl is likely to be more important in explaining the non-linearity in the empirical relationships.

## References


Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Cate, T.M., Perkins, T.D.: Chlorophyll content monitoring in sugar maple (*Acer saccharum*). – Tree Physiol. **23**: 1077-1079, 2003.

Fukshansky, L., von Remisowsky, A.M., McClendon, J., Ritterbusch, A., Richter, T., Mohr, H.: Absorption spectra of leaves corrected for scattering and distributional error – a radiative transfer and absorption statistics treatment. – Photochem. Photobiol. **57**: 538-555, 1993.

Hawkins, J.A., Sawyer, J.E., Barker, D.W., Lundvall, J.P.: Using relative chlorophyll meter values to determine nitrogen application rates for corn. – Agron. J. **99**: 1034-1040, 2007.

Lichtenthaler, H.K.: Chlorophylls and carotenoids: pigments of photosynthetic membranes. – In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol. **148.** Pp. 350-382. Academic Press, San Diego – New York – Berkeley – Boston – London – Sydney – Tokyo – Toronto 1987.

Madeira, A.C., Ferreira, A., de Varennes, A., Vieira, M.I.: SPAD meter versus tristimulus colorimeter to estimate chlorophyll content and leaf color in sweet pepper. – Commun. Soil Sci. Plant Anal. **34**: 2461-2470, 2003.

Markwell, J., Osterman, J.C., Mitchell, J.L.: Calibration of the Minolta SPAD-502 leaf chlorophyll meter. – Photosynth. Res. **46**: 467-472, 1995

Neufeld, H.S., Chappelka, A.H., Somers, G.L., Burkey, K.O., Davison, A.W., Finkelstein, P.L.: Visible foliar injury caused by ozone alters the relationship between SPAD meter readings and chlorophyll concentrations in cutleaf coneflower. − Photosynth. Res. **87**: 281-286, 2006.

Richardson, A.D., Duigan, S.P., Berlyn, G.P.: An evaluation of noninvasive methods to estimate foliar chlorophyll content. – New Phytol. **153**: 185-194, 2002.

Ritchie, R.J.: Universal chlorophyll equations for estimating chlorophylls *a*, *b*, *c*, and *d* and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. – Photosynthetica **46**: 115-126, 2008.

Sheshshayee, M.S., Bindumadhava, H., Rachaputi, N.R., Prasad, T.G., Udayakumar, M., Wright, G.C., Nigam, S.N.: Leaf chlorophyll concentration relates to transpiration efficiency in peanut. – Ann. appl. Biol. **148**: 7-15, 2006.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., Pleijel, H.: Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. – Photosynth. Res. **91**: 37-46, 2007.

Wang, Q.B., Chen, M.J., Li, Y.C.: Nondestructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. – J. Plant Nutr. **27**: 557-569, 2004.

Wang, Q.B., Chen, J.J., Stamps, R.H., Li, Y.C.: Correlation of visual quality grading and SPAD reading of green-leaved foliage plants. – J. Plant Nutr. **28**: 1215-1225, 2005.