Remote Sensing Reviews, 1990, Vol. 5(1), pp. 207–213
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Printed in the United Kingdom

12. Leaf Chlorophyll Content

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I. INTRODUCTION

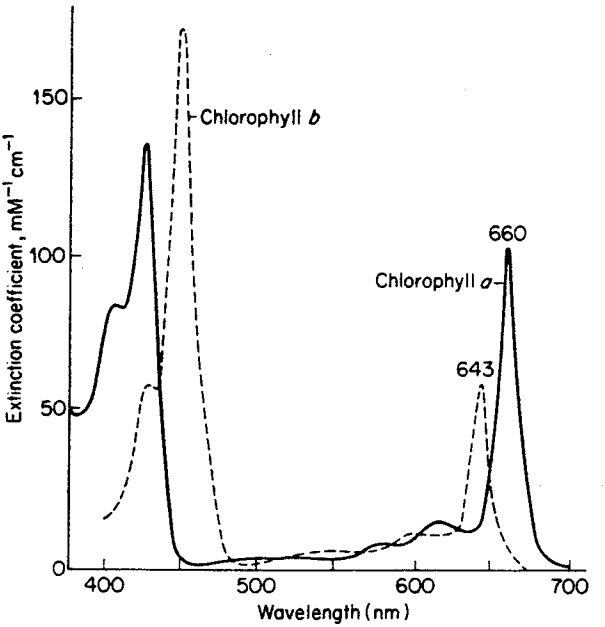
Chlorophyll is the pigment that gives plants their characteristic green co Chlorophyll pigment occupies a unique role in the physiology, productivity economy of green plants. Quantity of chlorophyll per unit area is an indicato photosynthetic capacity of a plant. Amount of chlorophyll in leaf tissu influenced by nutrient availability and environmental stresses such as drou salinity, cold and heat etc. Therefore, it has been of special interest to p scientists to quantify chlorophyll contents in leaves.

Higher plants contain primarily two types of chlorophylls, namely chloroph and chlorophyll b. Chlorophyll a is bluish green and chlorophyll b is yello green. The contents of chlorophyll a are usually three times higher than chl phyll b in the leaf tissue. The molecular formula for chlorophyll a is C_{55} H_{72} N Mg and chlorophyll b is C_{55} H_{70} N_4 O_6 Mg. The chlorophyll molecule contai porphyrin 'head' and a phytol 'tail'. In the cell chlorophyll is sandwiched betw protein and lipid layers of the chloroplast lamellae. The porphyrin part is boun the protein while the phytol 'tail' extends into the lipid layer. Chlorophylls insoluble in water but soluble in organic solvents.

It was first demonstrated over 60 years ago that chlorophylls have uni optical absorption properties. The absorption maxima of chlorophyll a chlorophyll b in ether are, respectively, at 660 and 643 nm (Figure 1). In acet the peaks are at 663 and 645 nm, and in ethanol the peaks are at 665 and 649 Using acetone extracts Arnon (1949) gave the following equation for estimatio chlorophyll content.

$$C = 20.2\,A_{645} + 8.02\,A_{663} \quad \ldots .$$

where C is the total chlorophyll contents in mg/liter of acetone extract, A_{645} A_{663} are the absorptions of the extract at 645 and 663 nm. Since the two absorp



URE 1 Absorption spectra of chlorophylls extracted in ether (source: Hall and Rao, 1987).

ter Bruinsma (1961) showed that for acetone extracts the correct equation used uld be

$$C = A_{652}/36 \tag{3}$$

ethanol extracts the two curves intersect at the wavelength of 654 nm. From se measurements Wintermans and DeMots (1965) devised the following ation for ethanol extracts.

$$C = A_{654}/39.8 \tag{4}$$

his chapter outlines the procedure used in the estimation of chlorophyll tents from the measurements of absorption of chlorophyll extracts. A cussion and brief account of other methods is also presented.

EXTRACTION METHOD (Destructive)

f chlorophyll content is most routinely determined by spectrophotometry of ue extracts. Most widely used extractants are ethanol, dimethyl ether, dimethyl mamide, acetone and methanol. Currently 96% ethanol is preferred over other

genizing is usually not necessary; incubating the leaf slice in 95% ethanc night is generally sufficient to completely extract chlorophyll. However, in leaves, especially those with thick cuticles, often vigorous homogeniz required for chlorophyll extraction. In hard to extract leaf tissues, fre homogenization is combined with maceration in the presence of silica to down the tissue for complete extraction.

Step 2. Removal of Debris: To remove the debris, the homogenate is then or centrifuged. Filtration procedure requires that filtrate be rinsed several t completely remove chlorophyll from debris. This is a bit cumbersome and in excessive dilution of the sample. Thus, whenever possible, centrifug preferred over filtration.

Step 3. Reading the Absorbance: Cleaned filtrate (homogenate) is brou known volume. After thoroughly mixing a part of the filtrate is taken in a (cell) with 1 cm light path and the absorbance is read at 654 nr spectrophotometer. The absorbance of 96% ehtanol taken in the same cu used as a blank or reference for these measurements.

Step 4. Calculation of Chlorophyll Contents: The total chlorophyll conte be calculated from the absorbance readings using the following e (Wintermans and De Mots, 1965).

$$C = 1000 \times A_{654nm}/39.8$$

where C = chlorophyoll contents in μg/ml

Knowing the total volume of the filtrate and the weight of the tis chlorophyll content in μg/g leaf tissue can be calculated.

Example:

One g of leaf was homogenized and extracted in 96% ethanol. The final vo the clear filtrate was 25 ml. The absorbance of the filtrate at 654 nm was 0.9 chlorophyll content (C) was then calculated as following using equations

$$\begin{aligned} C &= 1000 \times 0.955/39.8\ \mu\text{g/ml of filtrate} \\ &= 23.995\ \mu\text{g/ml} \\ &= 23.995 \times 25\ \mu\text{g in total 25 ml of filtrate} \\ &= 599.87\ \mu\text{g in 25 ml of filtrate and 1 g leaf tissue} \\ &= 0.6\ \text{mg/g fresh wt of leaf tissue} \end{aligned}$$

Comments:

Clearly the extraction procedure is destructive. This precludes the monit chlorophyll on the same tissue with time. Often, in heavily cutinized leaf tis procedure can be tedious and slow. Nevertheless, this is the most accurate

NDESTRUCTIVE METHODS

eo Image Analysis

ly microcomputer-assisted video image analysis has been used to structively measure chlorophyll contents of whole leaves (Spomer *et al.*, In this method a video image of an attached leaf is captured and digitized. lorophyll concentration is estimated from densimetric changes (grey level the assumption being that the greater the chlorophyll concentration within a of a leaf, the darker that area is in the image. These authors were able to strate very good correlation between this densimetric measurement and phyll content measured on the same leaf by extraction procedure.

ents:

analysis has the potential of a nondestructive and a noninvasive procedure ntitative estimate of leaf chlorophyll contents. The method appears to have ity to detect differences in chlorophyll concentration between treatments ect changes in chlorophyll concentration by a treatment such as environ- or biological stress (salinity, drought, temperature, diseases, etc.). er, very careful calibration is required to account for differences in ny (leaf thickness) or other pigmentation. This is a serious limitation of this ure because many factors that influence chlorophyll concentration can also ce other pigmentation or even influence leaf anatomy, including leaf ess. Image analysis has not yet been tested widely. Its future usefulness will otedly require studies with different species.

asurement of Chlorophyll Fluorescence

chlorophyll absorbs light, part of the absorbed light is emitted as cence (a red light) which can be measured with a spectrofluorometer. This cence is known to be associated with chlorophyll and the yield of cence is influenced in a very complex manner by the events that are directly rectly related to photosynthesis (Krause and Weis, 1984). Thus fluorescence es a measure of the efficiency of the photosynthesis and has been proposed ect "health" of the photosynthetic machinery and even "plant health". ly several companies have developed instruments that are portable and can d to measure fluorescence, e.g. (i) plant stress meter by P.K. Morgan, nents Inc., Andover, Massachusetts 01810 (Phone 508 470-0473), (ii). roductivity fluorometer Model SF-10 by Richard Branker Research Ltd. 27 Street, Ottawa, Canada K1S3Y7 (Phone 613 233-1621), (iii) Hansatec neter distributed in the US by Decagon Devices, Inc, Box 835, Pullman,

initiated research on the development of an operational instrument that coul used for remote sensing plant response to stress. The instrument is targeted to l a range of 10–100 m with an adjustable field of view 5–20 mrad. Details ca obtained from NEDINSCO B.V., t.a.v. Drs. L.A.J.M. Mertens, Postbus 5900AA Venlo, Netherlands.

Comments:

Whereas the chlorophyll fluorescence is indicative of functioning of ph synthetic machinery, it does not measure the chlorophyll content. For exa many studies have documented the use of room temperature chloro fluorescence measurement to evaluate photosynthetic integrity following envi mental stress (for details see Krause and Weis, 1984).

C. Measurement of Leaf Transmittance

Light incident on a leaf surface is either reflected, absorbed or transmitted. F time to time several attempts have been made to quantify leaf chlorophyll con from the measurement of leaf reflectance or leaf transmittance (Hardwick Baker, 1973; Macnicol *et al.*, 1976; Hardacre *et al.*, 1984; Yadava, 1 Reflectance is influenced by water and dust on leaf surfaces. Thus reflect methods have been limited to dry, dust free and relatively glossy leaf surf Initially the methods utilized cumbersome and delicate commercial reflectan absorption photometers. The use of such instruments was limited to labora condition. More recently portable photometers have been described whic cold light source and heat insensitive detectors. In these instruments light-emi diode (LED) is used as a light source and light-dependent resistor is used detector.

Hardacre *et al.*, (1984) described a portable photometer for the measureme chlorophyll in intact leaves. The instrument consisted of a spring loaded piece containing the light source and detector assemblies. The compo together (including display, power supply and amplifier) weight about 1.2 Kg leaf sample is clamped between the spring-loaded jaws of the photometer. meter reading is related to the chlorophyll content using a quadratic equa However at high chlorophyll contents a "scaling factor" needs to be deter (different for different species) that is multiplied to the values obtained fro quadratic equations. The authors claim that chlorophyll contents coul predicted with an accuracy of better than $\pm 6\%$. Examination of the data giv the paper shows considerable variability. For example, at a meter reading of 1 leaf chlorophyll content varied from 23 to 40 μg chlorophyll/cm^2. This instru is currently marketed as chlorophyll Meter by Design Electronics Limited, Box 898, Palmerston North, New Zealand, Ph. (063) 85 702.

Minolta Corporation is marketing a chlorophyll Meter (SPAD-501) whi

ificant correlation was found between SPAD readings and chlorophyll centration determined by extraction procedure. However, there was consider- e variability among the replicates. For details on the instrument contact olta Corporation, 101 Williams Drive, Ramsey NJ 07446 (Phone 825–4000).

nments:

ge variations among replicates remains a problem with these methods. This y be, in part, due to measurement of a relatively small leaf area. For example, AD-501 measures only 12.57 mm^2 of leaf surface. The reading depends on the thickness and the maximum thickness that can be used with this meter is mm. Large variations in the readings can occur due to changes in leaf thick- s, leaf anatomy, presence or absence of leaf veins in the area being measured due to presence of other pigments. For example, Hardacre *et al.* (1984) nowledge significant overestimation of chlorophyll concentration in the sence of high level of red pigment (anthocyanin). In addition very careful bration between the leaf chlorophyll contents (estimated by extraction cedure) and the photometer readings are required to get reliable results.

SUMMARY

chlorophyll pigment occupies a unique role in the economy of green plants. ntity of chlorophyll is an indicator of photosynthetic capacity of a plant and quantity is influenced dramatically by biotic and abiotic stresses. A brief ount of various methods used for the estimation of chlorophyll contents, is sented in this chapter. The standard and most commonly used procedure olves extraction of chlorophyll in an organic solvent and subsequent ntification by spectrophotometry. The nondestructive procedures includes eo image analysis and the measurement of leaf fluorescence and leaf smittance. Relative advantages and limitations of these methods are sented.

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