Spectral Ratio of Reflectance for Estimating Chlorophyll Content of Leaf*

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Nondestructive method for estimating chlorophyll content of leaves has been established by means of the difference of attenuances at the wavelengths 670 and 750 nm⁵⁾. A handy chlorophyllometer¹⁴⁾ based on this method was developed, and has been used not only for plant researches but also for nutritional diagnosis of crop plants growing in the fields^{6,7,15)}. However, the chlorophyllometer is not very efficient when it is used to estimate the mean value of chlorophyll concentrations in a vegetation canopy, because it needs to take many readings with individual leaves at different sites inside the canopy.

For estimating the greenness of rice plant canopy, the use of a color chart designed on the basis of colorimetry has been practiced^{11,12)}. This method is quite simple but the reproducibility of measured values is not satisfactory because they are rated by human vision.

Remote sensing based on the fact that radiation reflected from green leaves has a characteristic spectrum, which is clearly distinguishable from the spectrum reflected by soil, has been developed. In the spectral signature analysis by remote sensing, the combination of red and near infrared wavebands is widely used for estimating the biomass and the productivity of vegetation^{3,4,8)}. However, it is not clear whether those two wavebands can be applied to estimate chlorophyll concentration itself of a vegetation canopy or not.

This paper aims to find a reliable index for estimating chlorophyll content of a leaf by

light reflected from the leaf, as the first step to develop a remote sensing method for the nutritional diagnosis of field crops.

Materials and Methods

Leaves of rice (*Oryza sativa* L., cv. Nipponbare) grown in a natural light-growth chamber were used at the heading stage, and those of wheat (*Triticum aestivum* L., cv. Norin No. 61) and barley (*Hordeum vulgare* L., cv. Ehimehadaka No. 1) grown in the field were used at the booting stage. Leaf blades of each plant species were selected so that the deepness of green color distributed as widely as possible. Leaf piece of 2-cm length was cut from the position at about one third from the tip of the blade and subjected to measurement of the reflectance spectrum, chlorophyllometer reading and chlorophyll content. The number of leaf pieces used was 20 for rice, 10 each for wheat and barley, and some for non-chlorophyllous leaves.

Hemispherical reflectance spectra of adaxial surface of the leaf pieces were recorded against the reference of a white surface by Hitachi 320 spectrophotometer with an integrating sphere attached. Relative chlorophyll contents of the leaf pieces were estimated with Fuji GM-1 chlorophyllometer¹⁴⁾. Chlorophyll contents of the leaf pieces were analysed with the 80% acetone extracts according to MACKINNEY-ARNON equation²⁾.

Results

Hemispherical reflectance spectra of selected leaves are shown in Fig. 1. In the green leaves, it is clear that in the waveband 500 to 650 nm the reflectance decreases regularly with the increase of chlorophyll content per unit leaf area (Chl/LA), but in the

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other wavebands the reflectance has no definite relation with Chl/LA, particularly in the leaves containing relatively high Chl/LA. In the waveband above 750 nm the reflectance reaches the maximum and is kept constant in all the leaves.

The reflectance in the white and yellow-white leaves, which contain a small amount of carotenoids but no chlorophyll, is almost constant in the wavelengths above 500 nm. While the reflectance in the yellow leaf, which contains a large amount of carotenoids but no chlorophyll, lowers sharply in the wavelengths shorter than 550 nm. The reflectance of the frosted black paper is very low and spectrally independent.

From these facts, two constrastive wavebands relating Chl/LA were well-defined; one is chlorophyll-dependent visible band (V, 500–650 nm) and another, chlorophyll-independent near infrared band (IR, above 750 nm).

Correlation of difference or ratio of reflectances in the IR and the V bands with Chl/LA was investigated. The reflectance at 800 nm (ρ_{800}) was used for the IR band and each of reflectance at 510 to 680 nm (ρ_{λ}) was used for the V band, respectively (Fig. 2). Correlation coefficients of $\rho_{800} - \rho_{\lambda}$ with Chl/LA are the highest ($r = 0.920$

for the whole) when the wavelength 550 nm was used as λ but considerably low in both above and below 550 nm, excepting barley leaves showing very high coefficients throughout the wavelengths 510 to 680 nm.

On the other hand, correlation coefficients of $\rho_{800}/\rho_{\lambda}$ with Chl/LA are remarkably high and constant at least from $\lambda 530$ to $\lambda 630$ for all the crops used, and they decrease more or less at $\lambda 680$ which corresponds to the absorption peak due to chlorophyll and at $\lambda 510$ which is absorbed not only by chlorophyll but also by carotenoids in intact leaves as given in Fig. 1. In either $\rho_{800} - \rho_{\lambda}$ or $\rho_{800}/\rho_{\lambda}$, the highest correlation coefficient was obtained at $\lambda 550$, which provides the peak reflectance in the V band, for the samples of the individual crops and the whole.

The correlation of ρ_{800}/ρ_{550} with Chl/LA is illustrated in Fig. 3. The coefficients for rice, wheat, barley and the whole were 0.986, 0.928, 0.991 and 0.970, respectively, and all the plots lay on or around a single straight line. In Fig. 3, the ordinate has been expressed in $\rho_{800}/\rho_{550} - 1$ to provide the value proportional to Chl/LA, referring to the white leaf shown in Fig. 1. However, regression formula indicates that a factor of 0.36 should be added to 1 for the perfect correc-

Fig.1. Hemispherical reflectance spectra of leaves with various contents of chlorophyll. 1, white leaf; 2, yellow-white leaf; 3, yellow leaf; 4–6, rice leaves; 7–9, wheat leaves; 10, frosted black paper, respectively. Parenthesized numerals indicate chlorophyll contents per unit leaf area (mg dm^{-2}).

Fig.2. Correlation coefficients of the differences or ratios of leaf reflectances at 800 nm and those at each wavelength of the visible band with chlorophyll contents per unit area of the leaves.

Fig.3. Correlation of the ratios of leaf reflectances at 800 nm to those at 550 nm with chlorophyll contents per unit area of the leaves.

tion. The magnitude of this factor may correspond to the lowered degree of reflectance at 550 nm due to the absorption of carotenoids, which are contained in leaves regardless of chlorophyll content. The ratios of ρ_{800}/ρ_{550} also has a close correlation with the readings of the chlorophyllometer (Fig. 4).

Discussion

This study clearly indicates that the ratio of hemispherical reflectance of light in the IR band above 750 nm to that in the V band within 530 to 630 nm may be the most reliable index to estimate Chl/LA of a leaf for the three gramineous crops.

It has been demonstrated that the hemispherical reflectance of an individual leaf is insufficient to describe the remotely sensed bidirectional reflectance of a vegetation canopy^{1,9,10}. This is because a vegetation canopy is not a large leaf but is composed of a mosaic of leaves, other plant structures, background and shade³. However, TUCKER and MAXWELL¹³ showed that simple grass canopies with a high leaf area index have reflectance properties similar to those of a leaf.

Therefore, the index found in this study will probably be applicable for remotely sensing chlorophyll of a vegetation canopy of crops under daylight, provided that the field is sufficiently covered by the vegetation and

Fig.4. Correlation of the ratios of leaf reflectances at 800 nm to those at 550 nm with chlorophyllometer readings of the leaves.

the sensing direction is chosen so that the reflected light incident on the sensor comes exclusively from the leaf canopy. Moreover, the index seems to be advantageous to obtain reproducible values because it is based on ratioing of reflectances in the two wavebands.

Summary

This study was made to find an index for estimating chlorophyll content (Chl) of a leaf by means of reflected light with rice, wheat and barley leaves.

Two well-discriminating wavebands were defined from hemispherical reflectance (ρ) spectra of the leaves; one is Chl-dependent visible band (V, 500–650 nm) and another Chl-independent near infrared band (IR, above 750 nm). When the wavelengths 800 and 550 nm were chosen for IR and V bands, respectively, the highest correlation with Chl per unit leaf area (Chl/LA) was found in either difference ($\rho_{800} - \rho_{550}$) or ratio (ρ_{800}/ρ_{550}) of reflectances. The correlation with Chl/LA was generally higher in the ratios than in the differences of reflectances and the coefficients were almost constant in the ratios when the wavelengths 530 to 630 nm were used for V band.

The ratios of ρ_{800}/ρ_{550} also had a very close correlation with the readings of a chlorophyllometer.

These facts clearly demonstrate that the ratio of hemispherical reflectance in the wavelengths above 750 nm to that of 530–630 nm may be the most reliable index to estimate Chl/LA of a leaf for at least the three gramineous crops.

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* In Japanese with English summary.

** In Japanese, the title was translated by the present author.

〔和 文 摘 要〕

葉の葉緑素含量推定に有効な反射率の二波長間の比について

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葉の葉緑素含量を反射光を用いて非破壊的に推定するのに役だつ指標を明らかにするため、イネ、コムギおよびオオムギの葉について研究を行った。

葉の半球反射率スペクトルは、葉緑素に依存する可視域(V, 500~650 nm)と葉緑素に無関係な近赤外域(IR, 750 nm 以上)の二つの十分離れた帯域に分けられた。IR を800 nm, V を550 nm とした場合に、両帯域における反射率(ρ)の差($\rho_{800} - \rho_{550}$)および比(ρ_{800}/ρ_{550})とも単位葉面積当たりの葉緑素含量(Chl/LA)に対する相関が最も高く、相関係数(r)は全試料葉を含めてそれぞれ0.920および0.970であった。Chl/LAに対する相関は一般に反射率の比の方が差よりも高く、V帯域の波長(λ)を変化させた場合の反射率の比 $\rho_{800}/\rho_{\lambda}$ とChl/LAとの間の r は、 λ が530~630 nmの範囲ではほぼ一定の高い値(0.9以上)を示すことが供試各作物および全体について認められた。

また、 ρ_{800}/ρ_{550} の値は、吸光法に基づく葉緑素計の示度との間にもきわめて高い相関($r=0.968$)を示した。

以上の結果から、波長約530~630 nm間の反射率に対する750 nm以上のIR反射率の比は、少なくとも供試したイネ科作物葉のChl/LAを推定するための指標として最もすぐれていることが明らかになった。