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Analysis and Estimation of Chlorophyll Using Non-Destructive Methods

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ABSTRACT: Photosynthesis is a process through which plants produces food for themselves. Chlorophyll is the most important content that is required for the photosynthesis process as well as one of the most important biochemical parameters of plants and is usually an indicator of plants nutritional status, photosynthetic capacity and the health status of plants; that is why, it is an important information parameter in research on crop quality monitoring, ecosystem productivity estimation, carbon cycles, etc. In this paper we have studied non-destructive method to determine chlorophyll content and concentration in different plants. Reflectance measurement makes it possible to quickly and non-destructively assess, the chlorophyll content in leaves. Chlorophyll is a pigment that has a clear impact on the spectral responses of plants, mainly in the visible spectrum portion.

KEYWORDS: Chlorophyll, Spectral Reflectance, FieldSpec, Biochemical, Carotenoids.

I. INTRODUCTION

Plants cover more than 70% of the worldwide ground surface and are among the most important resources on the Earth. Their distributions are also strongly and nearly related to human movement. Photosynthesis is the procedure of capturing sunlight energy and transforming it to carbohydrate energy, in the presence of chlorophyll using water and carbon dioxide.

Chlorophyll (Ch) is a key biochemical component in the molecular apparatus that is responsible for photosynthesis in which the energy from sunlight is used to produce oxygen. Chlorophyll a is one of the most large form of chlorophyll within photosynthetic organisms and, for the most part, gives plants their green color. The different forms of chlorophyl coded as b, c, and d, which enlarge the overall fluorescent signal. In all photosynthetic organisms the chlorophyll a, is present but vary in concentrations. Chlorophyll concentration strongly associated with the photosynthetic potential of a plant and subsequently to physiological and metabolic status of the plant. Photosynthesis is the largest-scale synthetic process on earth. There are different kinds of photosynthetic pigments as, chlorophylls, carotenoids, and phycobilins in plant leaves. The chlorophylls are considered as the key factor because the photo-chemical reactions take place only at the trapped chlorophyll molecules. Light absorbed by chlorophyll stimulate electrons in the molecules, enabling them to be transferred to other molecules for glucose production and thus empower vegetation growth. Chlorophyll content can directly determine photosynthetic potential and primary production. Foliar chlorophyll concentration has always been one of the important issues of research using remote sensing techniques for vegetation. Typical reflectance of vegetation in the visible–infrared region will increase as water deficit occurs. As leaves dehydrate or vegetation suffers water stress, leaf water potential becomes increasingly negative and the rate of photosynthesis is reduced because water deficit can cause chlorophyll abasement and thus significantly decreases foliar chlorophyll concentration specifically, the magnesium ion (Mg2C) of the chlorophyll will be removed. As a result, chlorophyll becomes pheophytin (chlorophyll without Mg2C) and inactivates the photochemical reaction furthermore leaves decrease the absorptance of blue and red light while increasing the reflectance at the corresponding wavelength bands. The ability to accurate estimation of plant chlorophyll content and concentration may give growers valuable information to allow estimation of crop yield potential and to make decisions in fertilizer management.

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II. RELATED WORK

C. Lin et. Al. designed three independent experiments to collect data from three leaf sample sets for the construction and validation of Chls estimation models. Firstly, a reflectance experiment was conducted to collect foliar Chls and reflectance of leaves with changing water stress using the ASD FieldSpec spectroradiometer. Second, a chlorophyll meter (SPAD-502) experiment was conducted to collect foliar Chls and meter readings. Finally, an experiment was conducted to collect the third data set for the validation of Chls models by using the root mean squared error (RMSE) and the mean absolute error (MAE). A hardwood species, namely Camphor tree was selected for experiments. Leaf samples of the data sets for chlorophyll reflectance experiment & chlorophyll SPAD experiment is 50 and 45, respectively, and the additional evaluation data set is 70 leaves [1].

Anatoly A. Gitelson et. Al. conducted an experiment in which there objective was to examine the spectral behavior of the relationship among reflectance and chlorophyll content and to develop a technique for non-destructive chlorophyll estimation within leaves with a wide range of pigment content and composition using reflectance in some of broad spectral bands. Spectral reflectances of maple, chestnut, wild vine and beech leaves in a wide range of pigment content and composition were investigated [2].

L. J. Martinez et. Al. has done a research in order to indentify the relationship between chlorophyll content and spectral measurement in maize crop. Spectral measurements were taken and the chlorophyll content was determined in leaf samples in a field experiment. Spectral measurements were taken at 36 days, 56 days and 108 days after sowing, with a leaf clip using Fieldspec 4 spectroradiometer (ASD Inc.) and one measurement from the canopy. Although the general trend of the spectral responses were identical, differences were found mainly in the 850 nm to 2350 nm, where water content has great effect in reflectance and in the visible range, where the chlorophyll had more influence [3].

M. R. Schlemmer et. Al. examined the relationship of corn leaf spectral response to its chlorophyll content and relative water content. The effects of N(Nitrogen) stress and water stress were examined on each of these physiological parameters. leaf spectral reflectance was measured with the ASD Fieldspec FR spectroradiometer, chlorophyll content estimated by the SPAD-502 meter. Results of this study indicate that spectral reflectance appears to have promise for estimating certain physiological parameters at the leaf level [4].

Y. Özyigit performed a study to determine nitrogen, phosphorus, and potassium contents of rangeland plants using spectral reflectance value. The measurements were made in $1m^2$ area of different parts of a rangeland. Stepwise linear regression was used to select wavelengths to study relationships between laboratory analysis results and spectral data. According to the result, significant relationship existed between predicted and measured nutrients, with R^2 values of 0.85 for nitrogen, 0.43 for phosphorus, and 0.84 potassium [5].

Bo Liu et.al. peformed an experiment in which, FISS was used to collect spectral information from soybean leaves. The chlorophyll content was evaluated using Multiple Linear Regression (MLR), Partial Least Squares (PLS) regression and support vector machine (SVM) regression [6].

R. Casa et. al. have evaluated chlorophyll content by using SPAD-502 and Dualex devices and compared results to spectral reflectance indices & full spectral information (400–2500 nm) acquired by a spectroradiometer (ASD FieldSpec) equipped with a contact probe and leaf clip. The calibration models obtained on experimental data for SPAD (on maize) and Dualex (on four crops) which showed intermediate or high estimation accuracy with root-mean-square error (RMSE) values ranging between 7 and 11 $\mu g/cm^2$ banking on the species. These results were slightly better than those achieved using spectral reflectance indices, which were lesser though to those provided by PLSR using full spectral resolution [7].

Sergej Bergsträsser et. Al. performed a study in which they introduce a tailor-made hyperspectral absorption-reflectance-transmittance imaging (HyperART) system, achieving a non-invasive determination of both reflectance and transmittance of the whole leaf. They compared the data obtained from HyperART with ASD FieldSpec. The result of comparision showed good correlation which underlying the accuracy of the HyperART system [8].

Yunseop Kim et. Al. used hyperspectral camera to analyze the spectral signature of plant leaves to identify the plant water stress. The experimental results indicate that intelligent optical sensors could deliver decision support for plant stress detection and management [9].

Chaoyang Wu et. Al. performed a study in which a set of vegetation indices belonged to three categorize (normalized difference vegetation index (NDVI), modified simple ratio (MSR) index and the modified chlorophyll absorption ratio index (MCARI, TCARI) and the combined forms (MCARI/OSAVI and TCARI/OSAVI))were tested using the PROSPECT and SAIL models to examine their potentials in chlorophyll content estimation. The results showed that

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the combined indices TCARI/OSAVI[705,750] and MCARI/OSAVI[705,750] are most accurate for chlorophyll estimation with high correlation coefficients R^2 of 0.8808 and 0.9406 [10].

Shigeto Kawashima et. Al. used colour video camera and a personal computer to estimate chlorophyll content. It is shown that, for a specific purposes, the chlorophyll content of leaves can be estimated with sufficient accuracy using a portable video camera [11].

Carol L. Jones et. Al. used multi-spectral imaging system was to determine spectral reflectance and to estimate top-view surface area. An ultrasonic distance sensor provided vegetation height estimates. Surface area estimates and height data were combined to estimate plant biomass. The product of biomass estimate and normalized difference vegetative index provided the best estimate of chlorophyll content per plant [12].

Lucie Kupkova et. Al. have taken Silver Birch leaves and Scots Pine needles .The goal of their study was to prove the correlation among the spectral reflectance data and the chlorophyll content determined spectrophotometrically using distinct spectral indices (MCARI, TCARI / OSAVI, mNDVI705, $ANMB_{650-725}$) and to find a mathematical description of this relation. The relations between different indices and the chlorophyll content showed similar trends, the $ANMB_{650-725}$ index revealed the finest results regarding the statistical significance [13].

E. Raymond Hunt Jr et. Al. estimated chlorophyll content to detect crop nitrogen requirement. The triangular greenness index (TGI) was developed depend on the area of triangle surrounding the spectral features of chlorophyll with points at (670 nm, R_{670}), (550 nm, R_{550}), and (480 nm, R_{480}). They investigated that TGI may be the best spectral index to detect crop nitrogen requirements with low-price digital cameras mounted on low-altitude airborne platforms [14].

Cameron Proctor et. Al. have studied vegetation indices to estimate foliar pigment concentration from floating macrophytes. In the study they surveyed 39 wetland species (12 floating macrophytes (FM), 8 grasses/sedges/rushes (GSR) and 19 herbs/wildflowers (HWF)). They investigated that vegetation indices that exploit the red-edge region were a reasonable compromise that had great explanatory power for estimation of foliar pigments across the sampled wetland vegetation [15].

Kang Yu et. Al. performed a study in which all possible two-bands combinations for the simple Ratio and the normalized difference vegetation index were evaluated using linear regression analysis against the leaf chlorophyll concentration (LCC). Compared with published indices, freshly selected SRs and NDVIs improved the predictive ability for LCC. The most significant improvement was detected with increasing of R^2 values by 13 % for SR and 6% for NDVI [16].

Yaohuan Huang et. al. studied potentiality of hyperspectral indices to detect Chl-a concentrations in Tangxun Lake. Three kinds of hyperspectral methods, including single-band reflectance, first derivative of reflectance, and reflectance ratio, were evaluated from the spectral profiles of all bands of the hyperspectral sensor. Evaluation results indicated that two methods, that is the first derivative of reflectance and reflectance ratio, were highly correlated (R_2 > 0.8) with the measured Chl-a concentrations [17].

Jan-Chang Chen et. Al. conducted a study to investigate variations of leaf chlorophyll content and surface spectral reflectance of various tree species (Daphniphyllum glaucescens, Michelia formosana, Illicium dunnianum and Machilus kusanoi) over contrasting terrain. The results suggest that the REP could be used to determine the chlorophyll content in tree leaves. The index $mNDVI_{705}$ seemed to be more sensitive to detecting chlorophyll content in a wide range of tree species over a terrain [18].

Wesley J. Moses et. Al. conducted a study to investigate the performance of NIR-red models when applied to multi-temporal airborne reflectance data captured by the hyperspectral sensor, Airborne Imaging Spectrometer for Applications (AISA), with non-uniform atmospheric effects over the dates of data acquisition. Two atmospheric correction procedures, that is, Fast Line-of-sight Atmospheric Adjustment of Spectral Hypercubes (FLAASH) and QUick Atmospheric Correction (QUAC), were applied to AISA data. QUAC produced a robust atmospheric correction, which led to NIR-red algorithms that were able to precisely estimate chlorophyll-a concentration, with a root mean square error of 5.54 mg m^3 for chlorophyll a concentrations in the range 2.27e81.17 mg m^3 [19].

Driss Haboudane et. Al. conducted a study in which Field spectral measurements were collected across corn and wheat canopies. They were used to test and evaluate several combined indices for chlorophyll estimation using hyperspectral imagery. Across all the set of indices tested during study, index integerations like Modified Chlorophyll Absorption Ratio Index/Optimized Soil Adjusted Vegetation Index (OSAVI), Triangular Chlorophyll Index/OSAVI, Moderate Resolution Imaging Spectrometer Terrestrial Chlorophyll Index/Improved Soil-Adjusted Vegetation Index (MSAVI), and Red-Edge Model/MSAVI seem to be relatively consistent and more constant as estimators of crop chlorophyll content [20].

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III. METHODOLOGY

The C. Lin et. Al. designed three experiments to collect data for the construction and validation of chlorophyll estimation model. First, a reflectance experiment was conducted, by ASD FieldSpec Spectroradiometer, Second, a chlorophyll meter (SPAD-502) experiment. Finally experiment was conducted to collect the third data set for the validation of Chls models using the root mean square error (RMSE) and the mean absolute error (MAE). Anatoly A. Gitelson et. Al. conducted a study where reflectance and transmittance spectra of the leaves were taken in a spectral range in between 400 and 800 nm with a spectral resolution of 2 nm by a Hitachi 150–20 spectrophotometer equipped with an integrating sphere. L. J. Martinez et. Al. performed 5 N treatments, including 0 as the control and 50, 100, 150, and 200 kg of N ha^{-1}. Spectral measurements were taken with a leaf clip using a Fieldspec 4 spectroradiometer to calculate chlorophyll variation. Analysis of variance (ANOVA procedure) was used to determine the effect of the treatments on the spectral measurements. Bo Liu et. Al. used FISS(Field Imaging Spectrometer System) to gather spectral information from soybean leaves. The chlorophyll content was evaluated using a multiple linear regression (MLR), partial least squares (PLS) regression & support vector machine (SVM) regression. R. Casa et. Al. conducted a study where full spectral data were manipulated using partial least squares regression (PLSR). Sergej Bergsträsser et. Al. conducted a study where they introduced a tailor-made hyperspectral absorption-reflectance-transmittance imaging (HyperART) system, Turning out a non-invasive determination of both reflectance and transmittance of the entire leaf. Shigeto Kawashima et. Al. performed a study where they use portable colour video camera and a personal computer for estimating chlorophyll content. Kang Yu et. Al. conducted a study in which, throughout the range of 350 to 1800 nm, all possible two-bands combinations with the simple ratio (SR= Rj/Ri) & the normalized difference vegetation index (NDVI= (Rj-Ri)/(Rj+Ri)) were calculated using linear regression analysis across the leaf chlorophyll concentration (LCC) [16]. Yaohuan Huang et. Al. used three types of hyperspectral methods, containing single-band reflectance, first derivative of reflectance, and reflectance ratio were used out of which two methods, the first derivative of reflectance & reflectance ratio, were hugely correlated (R_2 > 0.8) with the measured Chl-a concentrations.

IV. SIMULATION AND RESULTS

C. Lin et. Al. found one of the basic assumption during the study which underpins that a spectral index that effectively integrates the reflectance at the blue and red bands, at which the light particularly absorbed by chlorophyll is only used for plant photosynthesis, is better for foliar Chls estimation than the indices that use other than the blue and red bands. An effective chlorophyll indicator with the form of (P_{645}– P_{455}) = PREP proved to be the most accurate and stable predictor for foliar Chls concentration. This model was calculated with an R^2 of 0.90 (P <0.01) from the training samples and evaluated with RMSE 0.35 & 0.38 mg $g^{-1}$for the validation samples of fresh and water-stressed leaves, respectively.

Anatoly A. Gitelson et. Al. used two independent data sets to validate the developed algorithms. The root mean square error of the chlorophyll prediction did not exceed 50 μmol/m^2 in leaves with total chlorophyll ranged from 1 to 830μmol/m^2.

M. R. Schlemmer et. Al. found that the normalized difference between the first derivatives at 525 and 570 nm, as well as the wavelength location of the red edge, represented a strong association with chlorophyll content (r^2 = 0.81 and 0.80, respectively). Even stronger association to chlorophyll content were observed with the ratios of 600/680 nm (r^2=0.83) and 630/680 nm (r^2=0.83).

Y. Özyigit et. Al. investigated a study and according to the result, significant relationships existed between predicted and computed nutrients, with R^2 values of 0.85, 0.43, and 0.84 for nitrogen, phosphorus and potassium, respectively.

Bo Liu et. Al. conducted a study where the smallest RMSE of the chlorophyll content retrieved using FISS data was 0.201 mg/g, a relative reduction of more than 30% analyzed with the RMSE based on a non-imaging ASD spectrometer, which represents a great estimation accuracy compared with the mean chlorophyll content of the sampled leaves (4.05 mg/g). Their study indicates that FISS could retrieve both spectral and spatial detailed information of high quality.

Sergej Bergsträsser et. Al. performed a study where they investigated that the use of leaf reflectance and transmittance, plus sum (by which the non-absorbed radiation is calculated) obtained by the HyperART system gave considerably enhanced results in classification of Cercospora leaves spot disease and determination of chlorophyll content than high-resolution ASD FieldSpec.

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Yunseop Kim et. Al. investigated that the highest correlation was found with Red Edge NDVI at 705 nm and 750 nm in narrowband indices and NDVI at 680 nm and 800 nm in broadband indices.

Chaoyang Wu et. Al. conducted a study and their results showed that the integrated indices TCARI/OSAVI[705,750] and MCARI/OSAVI[705,750] are most suitable for chlorophyll estimation with high correlation coefficients R^2 of 0.8808 and 0.9406, respectively .

Carol L. Jones et. Al. investigated that the product of biomass estimate and normalized difference vegetative index ($NDVI_{680}$) provided the prime estimate of chlorophyll content per plant (r^2 = 0.91).

Lucie Kupková et. Al. investigated that although the relations between different indices and the chlorophyll content show similar trends, the $ANMB_{650-725}$ index revealed the best results with respect to the statistical significance [13]

Kang Yu et. Al. Compared their result with published indices, newly selected SRs and NDVIs improved the predictive ability for LCC. The most significant improvement was noticed with increasing of R^2 values by 13 % for SR and 6% for NDVI.

Yaohuan Huang et. Al. conduct a study and results indicated that two methods, the first derivative of reflectance and reflectance ratio, were greatly correlated (R^2 > 0.8) with the measured Chl-a concentrations.

Jan-Chang Chen et. Al. investigated that the index $mNDVI_{705}$ seemed more sensitive to detecting chlorophyll content in a wide range of tree species across a terrain.

Driss Haboudane et. Al. tested some indices in their study and among the set of indices tested, index combinations like a Modified Chlorophyll Absorption Ratio Index/Optimized Soil-Adjusted Vegetation Index (OSAVI), Triangular Chlorophyll Index/OSAVI, Moderate Resolution Imaging Spectrometer Terrestrial Chlorophyll Index/Improved Soil-Adjusted Vegetation Index (MSAVI), and Red-Edge Model/MSAVI seems to be relatively consistent and more stable as estimators of crop chlorophyll content.

V. CONCLUSION AND FUTURE WORK

In spite of complex morphological and optical properties and still imprecise understanding of leaf optics, considerable progress has been reached in the development of non-destructive techniques for sensing of plant physiological status via quantitative estimation of chlorophyll content. This paper contains study of non-destructive and remote estimation methods of chlorophyll estimation of plant leaves which gives physiological status of vegetation and detects the early stages of stress. The result presented show that reflectance spectroscopy is useful and efficient tool for quantification of pigment content. Fundamental spectral features of leaf reflectance and its relation with chlorophyll content are reviewed here, which may provide a solid basis for the development of the reliable technologies for monitoring vegetation status. The developed models are closely related to chlorophyll content and are able to give nearly an accurate estimate of foliar pigments in wide range of their content.

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