Effect of the Vertical Profile of Chlorophyll on Maize Canopy Reflectance: Remote Determination under Field Conditions

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Abstract. The chlorophyll (Chl) content of a crop canopy is a biophysical variable that quantitatively expresses the photosynthetic capacity of a vegetation stand and it is related to many important plant functions and parameters. Therefore, it is not surprising that many remote sensing studies have focused on the estimation of Chl content of vegetation canopies to asses the vitality of plants and to detect vegetation stress. However, there is little information regarding how the distribution of Chl within vegetation canopies defines the reflectance signatures measured remotely and the derived spectral indexes. The goal of this study was to determine how deep into a maize canopy, a spectral vegetation index, based on the red edge and NIR spectral bands, senses the Chl content of individual leaves. Reflectance was measured using a hand-held radiometer at both the leaf and canopy level in order to retrieve foliar and total canopy Chl content, respectively. A hierarchical regression analysis was used to find (i) how many maize leaves contribute significantly to total canopy Chl content, and (ii) how many leaves, from top to bottom, are sensed by a field radiometer and by the red edge chlorophyll index, $CI_{red\ edge}$. Results showed that $CI_{red\ edge}$ senses the chlorophyll content of the top 7 to 8 leaves in the maize canopy and, thus, is able to accurately estimate total chlorophyll content in canopy.

1.Introduction

The chlorophyll (Chl) content of a crop canopy is a biophysical variable that quantitatively expresses the photosynthetic capacity of vegetation stand. It is related to canopy biophysical parameters such as nitrogen content, above-ground biomass, green and total leaf area index, net ecosystem CO_2 exchange, and absorbed photosynthetic active radiation (e.g., Evans, 1989; Gitelson et al., 2006). Therefore, it is not surprising that remote sensing have focused on the estimation of Chl content in the canopy to determine the vitality of vegetation and to detect vegetation stress (e.g., Barton, 2000; Gitelson et al., 2005; Le Maire et al., 2008; Ustin et al., 2009).

The variability of photosynthetic apparatus inside the canopy ranges from very dark green photosynthetically active leaves to pale green or senescent leaves (yellow to brown). In addition, the vertical distribution of Chl content in canopies changes during the growing season (Ciganda et al, 2008). Such vertical variability of Chl, largely affected by the vertical distribution of leaf area, defines the total incoming light that is reflected back from canopy.

In many remote sensing studies, the vegetation reflectance data used to compute vegetation indices and to estimate Chl in the canopy (or any other canopy biophysical variable) is considered as it contains information from the entire canopy. For example, Gitelson et al. (2005), among many others, developed a two-spectral band model, so called the red edge chlorophyll index ($CI_{red\ edge}$), to remotely estimate total Chl content in soybean and maize canopies. The authors found that such model related linearly and closely with total Chl content in canopy.The basis for this approach is the assumption that the entire canopy (from the bottom leaves to the uppermost) contributes to the reflected light gathered by the sensor in the three spectral bands used to compute the model. However, a question remains: is the computed $CI_{red\ edge}$ retrieving information from the entire canopy or from just a section of it that is a very good proxy of total Chl in the canopy? If only a section of the canopy is involved, which leaves are contributing most?

The objective of this study is to determine how deep into the maize canopy the $CI_{red\ edge}$ senses and how accurately it estimates the chlorophyll content in canopy. We determined the number of leaves in a maize canopy, from top to bottom, which Chl content is sensed by a sensor and affects the remote estimates of Chl in the canopy as well as identified the relative contribution of Chl in each leaf to the $CI_{red\ edge}$.

2. Materials and Methods

This study took advantage of an established research facility, which was part of the Carbon Sequestration Program at the University of Nebraska-Lincoln. The study took place in the 2005 growing season on three different sites planted with maize hybrids Dekalb 6375 (D-6375), Pioneer brand 33B51 (P-33B51), and Pioneer brand 31G68 (P-31G68), respectively on each site.

2.1 Canopy Reflectance Measurement and Remote Estimation of Canopy Chl Content

Canopy spectral measurements were taken two times a week during the entire growing season of 2005 on each of the three sites. A dual-fiber system, with two inter-calibrated Ocean Optics USB2000 radiometers, mounted on ‘‘Goliath’’, an all-terrain sensor platform (Rundquist et al., 2004), was used to collect data in the range 400-900 nm with a spectral resolution of about 1.5 nm. One of the radiometers, equipped with a 25° field-of-view optical fiber, was pointing downward to measure the upwelling radiance from the crop canopy (L_λ^{canopy}). The other radiometer, was pointing upward to simultaneously measure incident irradiance (E_λ^{inc}).

Reflectance measurements were made within an area of *ca.* 0.8 ha for each of the three sites. During the growing season, the sensor was positioned at the same high *ca.* 4.8m above the top of canopy determining an instantaneous field of view diameter of 2m. A total of 36 spots within these areas were sampled per measurement date and site.

From each reflectance scan, the $CI_{red\ edge}$ was calculated as (Gitelson et al, 2005):

$$CI_{red\ edge} = (\rho_{NIR}/\rho_{red\ edge})-1 \qquad (2)$$

where ρ_{NIR} is reflectance in the near infrared range from 770 through 800 nm and $\rho_{red\ edge}$ is the reflectance in the red edge range from 720 to 730 nm. The mean value of the $CI_{red\ edge}$ of the 36 scans was computed to estimate Chl content in canopy.

2.2 Plant Sampling and Labeling Procedures

Three plants were sampled on 15 dates (DOY 153 through DOY 263) from sites 1 and 3 and on 13 dates (DOY 166 through DOY 263) from site 2. The sampling period covered the period from early vegetative growing stages of three leaves to last reproductive stages when all kernels on the ear have attained their maximum dry weight or maximum dry matter accumulation. A total of 128 plants were sampled resulting in over 2,000 leaves collected for further chlorophyll and leaf area measurements. On each sampling date, plants considered representative of the growing stage of the entire site were selected randomly from an area where remote canopy reflectance measurements were taken. Once the plants were selected, leaves were numerically labeled from top (leaf 1) to bottom positioned leaves using consecutive numbers. After labeling, the leaves were cut from the stem, placed in a sealed plastic bag, and brought to the laboratory inside a cooler.

2.3 Leaf Reflectance and Chlorophyll Content

Reflectance of leaves from sampled plants was measured in the spectral range from 400 to 900 nm using a leaf clip, with a 2.3-mm diam. bifurcated fiber-optic cable attached to both an Ocean Optics USB2000 spectroradiometer and to an Ocean Optics LS-1 tungsten halogen light source. A Spectralon reflectance standard (99% reflectance) was scanned before each leaf measurement. The software CDAP (CALMIT, University of Nebraska-Lincoln Data Management Program) was used to acquire and process the data from the sensor.

Each leaf was visually examined to identify and separate sections that were different in color. Leaf sections were marked, labeled and cut for further measurements. In the case of a leaf that was considered homogeneous in color, ten randomly distributed scans were made along the leaf margin (both sides of midrib). In the case of a leaf with a heterogeneous distribution of color, sections that appeared homogeneous in color were measured independently and ten randomly distributed scans were taken on each such leaf section.

The Chl content (in mg m^{-2}) of each leaf (Chl_{leaf}) or/and leaf section (Chl_{sect}) was estimated using equation developed by Ciganda et al (2009):

$$Chl_{leaf}\,(mg\ m^{-2}) = 37.904 + 1353.7 \times CI_{red\ edge} \qquad (3)$$

2.4 Measurement of Canopy Chlorophyll Content

The total amount of Chl in each leaf (Chl_{leaf}^{total}), in grams of Chl, was calculated following a methodology developed by Ciganda et al. (2008). The area of each leaf, S_{leaf}, or the area of each leaf section (in the case of leaves with sections of different greenness), $S_{section}$, was measured with a leaf area meter (Model LI-3100A, Li-Cor, Inc., Lincoln, NE). Total amount of Chl in each leaf was calculated as the product of leaf area, S_{leaf}, (in m^2) and its Chl content, Chl_{leaf} (in mg Chl m^{-2}) as following:

$$Chl_{leaf}^{total} = Chl_{leaf} \times S_{leaf} \qquad (4)$$

In the case of leaves with two or more sections of different greenness (i.e., “m” sections), total amount of Chl of the entire individual leaf was calculated as the sum of the products for each section using the following equation:

$$Chl_{leaf}^{total} = \sum_{i=1}^{m} Chl_{i\ section} \times S_{i\ section} \qquad (5)$$

Total amount of Chl in the canopy (Chl_{canopy}) expressed as the amount of Chl per unit of ground area (i.e., gChl m^{-2}), was calculated as the sum of the total amount of Chl in leaves of each plant normalized by the ground area beneath one plant (S_g):

$$Chl_{canopy} = \sum_{i=1}^{n} Chl_{leaf}^{total} / S_g \qquad (6)$$

Where n is number of leaves in each plant.

2.5 Statistical analysis

The relationship between $CI_{red\ edge}$ and leaf Chl content Chl_{leaf}^{total} along the vertical profile of the canopy was analyzed using a hierarchical linear multiple regression. In this approach, similarly to other multiple regression analysis, the hierarchical regression consists in establishing a set of independent variables that explain a proportion of the variance of the dependent variable. However, this analysis has the major advantage over the other multiple regression methodologies (e.g., stepwise regression) that the researcher determines the order of entry of the independent variables. Such characteristic fits for this study, in which the order of entering the Chl content of the leaves, *from top to bottom*, is determinant to understand the effect of each leaf Chl content on the $CI_{red\ edge}$ calculated from reflectance measured above the canopy.

In this study, the dependent response variable (Y) computed across sites from the canopy reflectance data was the $CI_{red\ edge}$ calculated using Eq. 3. The predictors or explanatory variables were the leaf Chl content (Chl_i) for each leaf positioned along the vertical profile of the maize canopy from top to bottom. Thus, Chl_i was entered in the model in a precise order: beginning from the uppermost leaf, followed by the leaf positioned immediately below the top one, and going down through the canopy profile up to the 14th leaf (almost at the bottom of the canopy). Hence, the multiple regression took the form:

$$Y = b_{0i} + \sum_{i=1}^{n} b_i * Chl_i \quad (7)$$

Where the b_i are the regression coefficients, representing the amount the dependent variable Y changes with changes in the corresponding independent, Chl_i; b_{0i} is the intercept point where the regression line intercepts the y-axis, and it is different for each model; and n is total number of leaves included in the model. The analysis ended up with a model with 14 parameters (each parameter corresponds to the Chl content in one leaf) not counting the intercept.

The models were evaluated using the adjusted determination coefficient, R^2-adj. The R^2-adj is the R^2 adjusted for the degrees of freedom and does not necessarily increase as the number of variables in the model increases since it penalizes for the number of parameters included in the model. The formula for the R^2-adj is:

$$R^2\text{-adj} = 1 - ((1-R^2)(n-1/n - k - 1)) \quad (8)$$

Where n is the leaf number and k is the number of parameters in the model not counting the intercept (i.e., the number of Chl_i). The R^2-adj and the coefficients, b_{oi} and b_i, of each linear model were obtained using the *lm* function of the R statistical software (Hornik, 2006).

3. Results and Discussion

3.1 Cumulative chlorophyll content

The vertical distribution of Chl content in maize is bell-shaped regardless of crop growth stage (Ciganda et al., 2008). Thus, the cumulative Chl calculated adding the leaf Chl content from top to bottom (Figure 1) shows minimum values at the top of the canopy and progressively increases reaching maximum values and then remains invariant.

The magnitude of cumulative Chl in the vertical profile of maize canopies varied during the growing season; however the shape of Chl distribution was similar in all sites with different hybrids. During the very early vegetative stages (June 2 – 15, day of year, DOY = 153-166), no differences were found among sites regarding both the shape of Chl distribution and cumulative Chl values. After June 15 (DOY = 173, stage V8), the maize in sites 1 and 2 showed higher values of cumulative Chl in the vertical profile. The earlier senescence of the bottom leaves in plants in site 3 (since August 10, DOY = 222, stage R3) made the difference between Chl content among sites even larger. At very late reproductive stages (after September 7, DOY = 250), when senescence became conspicuous in all sites, the differences among cumulative Chl became negligible.

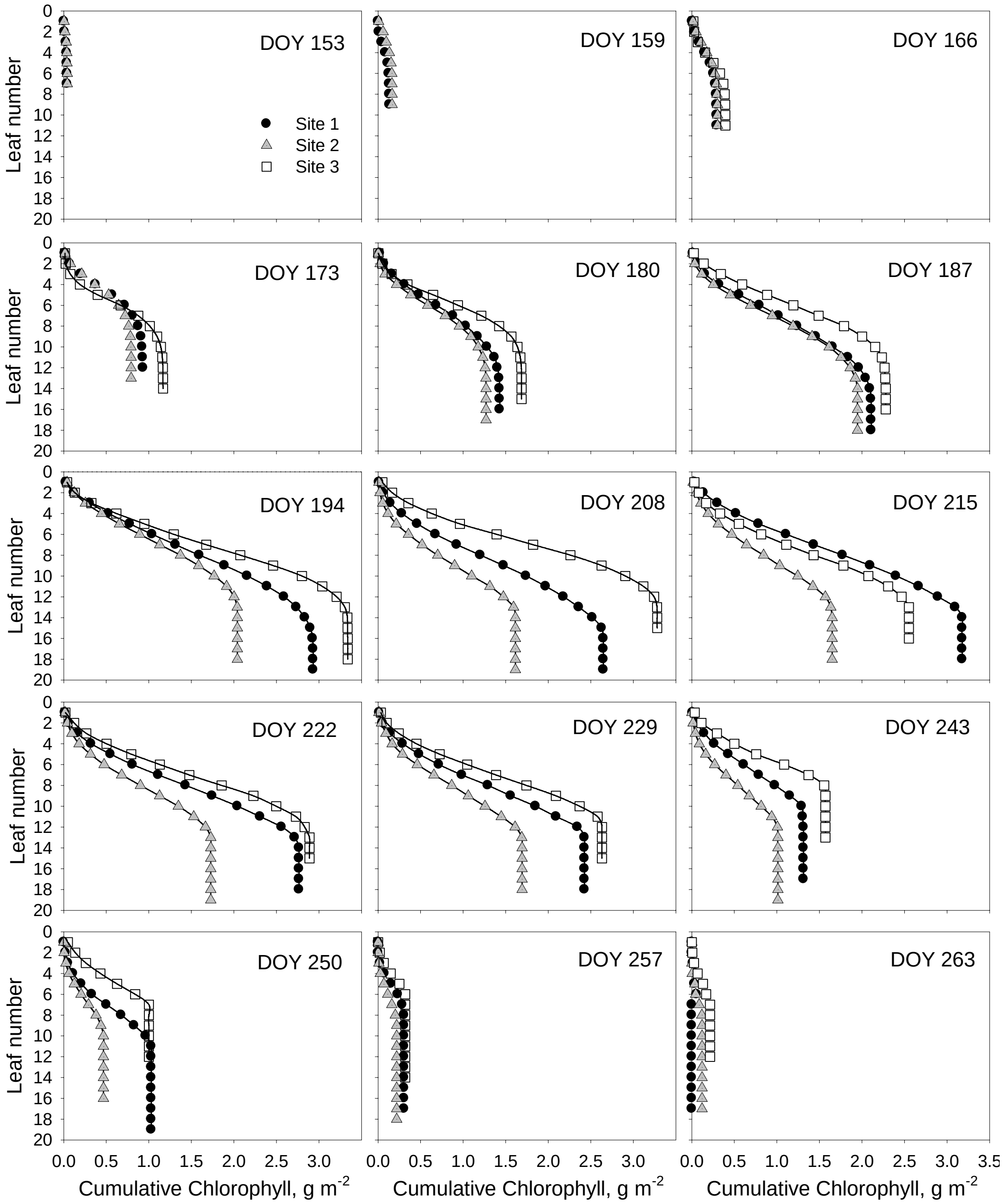

Figure 1. Cumulative chlorophyll in the vertical profile of three maize sites for 15 sampling dates from June 2nd (DOY 153) through September 20th (DOY 263) of 2005. Cumulative Chl at each layer of the canopy was calculated as: $Chl_i^{cumulative} = \sum_{i=1}^{n} Chl_i^{leaf}$ where C_i^{leaf} is chlorophyll content in i-leaf.

In accordance with previous studies (Gitelson et al., 2005, Ciganda et al., 2008, 2009) the $CI_{red\ edge}$ was found to be an accurate proxy of maize canopy Chl content (Figure 2). The data for the three hybrids were pooled together and the overall r^2 for the linear relationship between the CIred edge and Chl content in the canopy was 0.924 with an associated error in the estimation of 0.43.

However, it is still unclear whether this close relationship $CI_{red\ edge}$ vs. Chl is due to the fact that the sensor senses light reflected by leaves located far from top of the canopy or it is due to fact that Chl content in few top leaves well represented total canopy Chl. To answer this question the hierarchical regression analysis was used.

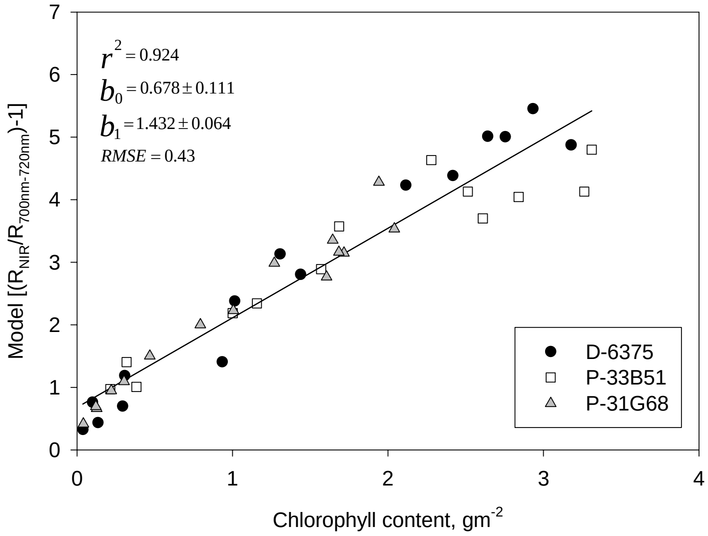

Figure 2. Linear relationship between the model [($R_{NIR}/R_{red\ edge}$)-1] and total chlorophyll content in canopy for three maize hybrids. The intercept, b_0, the slope, b_1, (± their standard errors) and the coefficient of determination, r^2, of the linear fit Chl vs. model are shown in the figure The model was computed from canopy reflectance measured remotely. Chlorophyll content was measured non-destructively at leaf level.

3.2 Hierarchical regression

Using hierarchical regression we tried to understand how canopy chlorophyll, Chl_{canopy}, and chlorophyll index $CI_{red\ edge}$ relate to leaf Chl content. To find hierarchical regression between Chl_{canopy} and leaf chlorophyll, we began calculating the relationship between Chl_{canopy} and $b_{oi} + \sum b_i \times Chl_i$ (where i is the number of leaves that varied from 1 to 14 and Chl_i is chlorophyll content in i leaf) with one the uppermost leaf, and then adding Chl in second leaf and so on until the 14th leaf of the canopy. To find hierarchical regression between $CI_{red\ edge}$ and leaf chlorophyll, as in previous case, we calculated the relationship $CI_{red\ edge}$ vs. $n_{oi} + \sum m_i \times Chl_i$.

The relationships $CI_{red\ edge}$ vs. $n_{oi} + \sum m_i \times Chl_i$ for top four leaves were weak; adjusted determination coefficient R^2-adj was below 0.6 (Figure 4). As the Chl content of successive leaves was added, R^2-adj increased to a point (leaves 7 and 8) that the addition of more parameters to the model did not increase the R^2-adj (Figure 5). Relationship $CI_{red\ edge}$ vs. $n_{oi} + \sum m_i \times Chl_i$ has slightly different maximal R^2-adj values in different sites: in site 1 maximal R^2-adj was for leaves 7 and 8, in site 2 for leaves 9 and 10, and in site 3 for leaves 8 to 10. In Figure 5, average values of R^2-adj are presented with bars showing standard deviation of R^2-adj. R^2-adj decreases with the addition of leaves beyond 8th. Thus, R^2-adj has peak around leaves 7th and 8th, indicating that $CI_{red\ edge}$ relates closely with Chl in top seven to eight leaves.

Adjusted determination coefficient of the relationship Chl_{canopy} vs. b_{oi} $\sum b_i \times Chl_i$, presented in Figure 5, increases sharply as the Chl content of eight-nine successive leaves was added and then the relationship becomes almost flat by leaves 11 through 14. Thus, the analysis using hierarchical regression shows that while R^2-adj of relationship Chl_{canopy} vs. $b_{oi} + \sum b_i \times Chl_i$ increases monotonically with successful addition of leaves from top to bottom, R^2-adj of the relationship $CI_{red\ edge}$ vs. $n_{oi} + \sum m_i \times Chl_i$ reaches its maximum value at leaves 7-8. Such maximum value may be interpreted as the point of maximal sensitivity of the $CI_{red\ edge}$ to chlorophyll content.

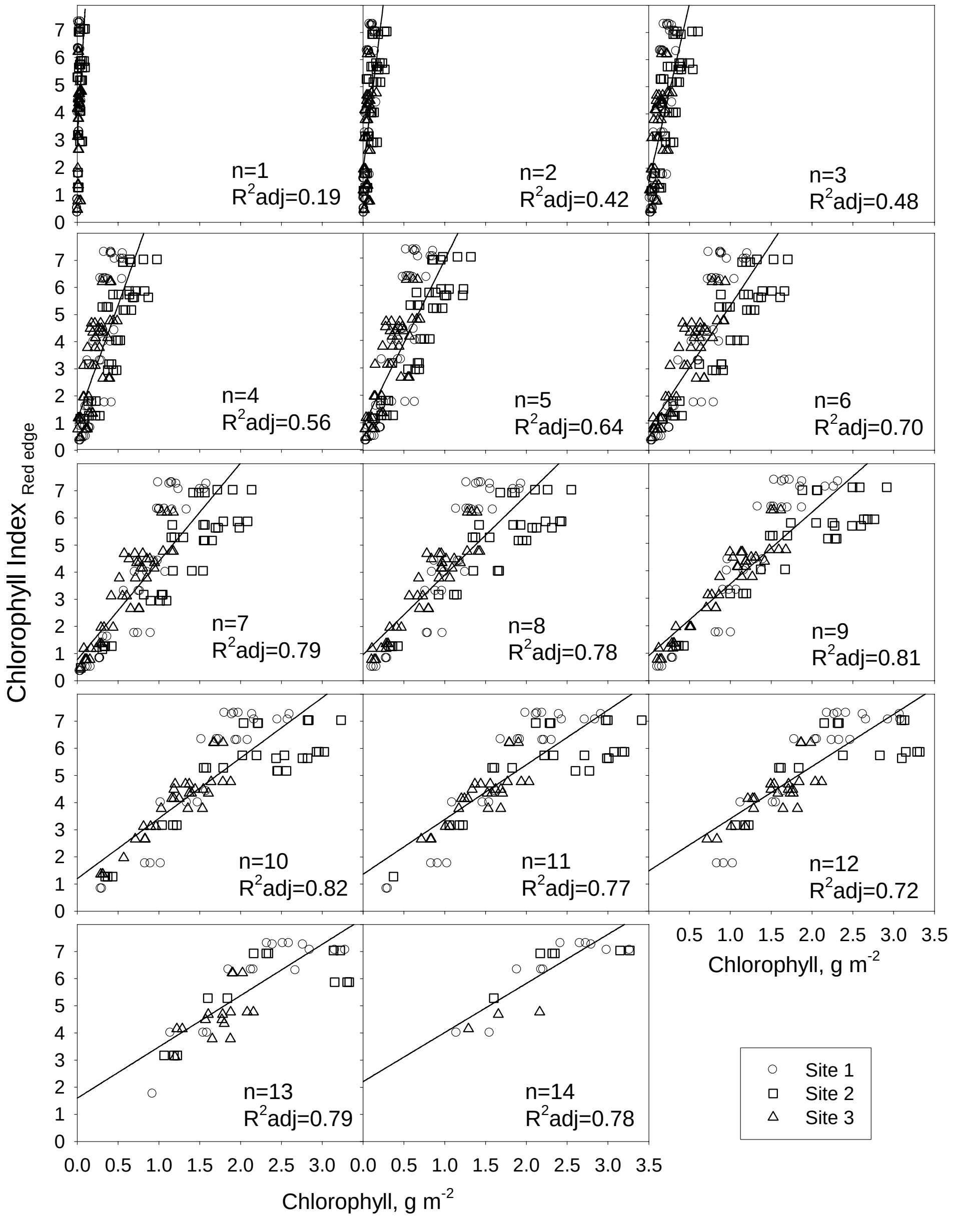

Figure 4. Red Edge chlorophyll index for three maize sites plotted versus chlorophyll cumulated from top to bottom leaf. Chlorophyll in each leaf, from top to bottom, was included successively in the linear model (Eq. 7) as independent predictor variable. The adjusted determination coefficient, R^2_{adj}, and the number of leaves, n, for each step are shown in the figure.

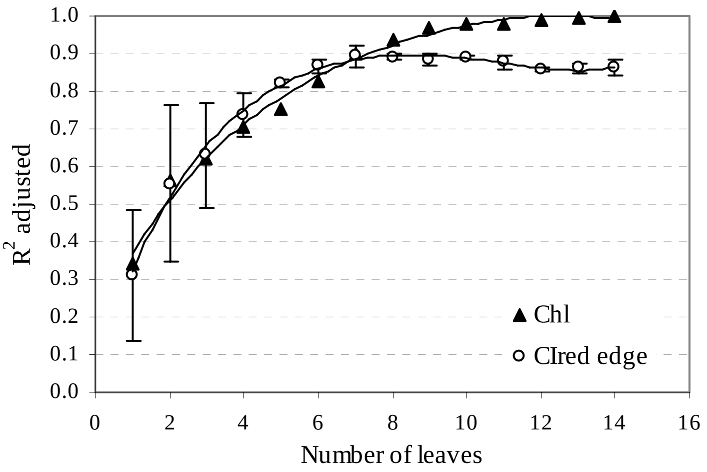

Figure 5. Adjusted determination coefficients of two hierarchical regressions: total canopy chlorophyll, $Chl = b_{0i} + \sum_{i=1}^{n} b_i * Chl_i$, and red edge chlorophyll index, $CI_{rededge} = m_{0i} + \sum_{i=1}^{n} n_i * Chl_i$, plotted versus number of leaves (i.e. parameters). R^2-adjusted for the relationship $CI_{rededge} = m_{0i} + \sum_{i=1}^{n} n_i * Chl_i$ were averaged for three sites and maximal and minimal values are presented by bars.

These results also suggest that as the canopy is composed of plants with six-seven or less leaves (early phenological stages), the $CI_{red\ edge}$ brings the information about Chl content of the entire canopy. Such deep sensing by the $CI_{red\ edge}$ is due to the use of the NIR band where absorption of light by leaves is very low and due to the long wave part of the red edge band (720-730 nm) with quite high reflectance and much lower absorption (Gitelson and Merzlyak, 1996) than in the red band usually employed in vegetation indices. At later growing stages, when plants have more than 7-8 leaves, the reflectance of leaves positioned below the top seven leaves apparently contributes very little to the reflected light gathered by the sensor. Importantly to note that the leaves 7th and 8th in maize have maximal Chl content which closely relates to total canopy Chl (Ciganda et al., 2008; 2009). Knopff and Goudrian (1994) showed that the middle leaves of a canopy (with a leaf area profile similar to a maize canopy) are responsible for the highest absorption of the incident PAR but do not contribute significantly to light reflected by canopy and, therefore, to R^2-adj of the relation $CI_{red\ edge}$ vs. $n_{oi} + \sum m_i \times Chl_i$. On the contrary, top leaves, having less Chl content, absorb less light but contribute significantly to canopy reflectance in visible spectrum and thus to R^2-adj.

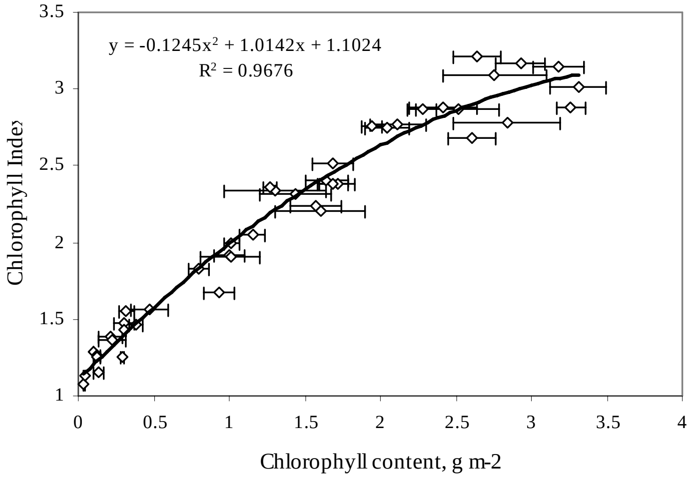

Figure 6. Averaged for three plants total chlorophyll content in maize canopy for three sites estimated using Red Edge chlorophyll index $CI_{red\ edge}$. Bars are minimal and maximal Chl content among three plants.

4.Conclusions

The results showed that red edge chlorophyll index that employs the NIR and the red edge (720-730 nm) spectral bands senses the chlorophyll content of 7 to 8 top leaves in the maize canopy allowing very accurate estimation of maize canopy chlorophyll content. A hierarchical regression procedure made it possible to assess the importance of the Chl content of each leaf in defining total Chl content in maize canopy.

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