ESTIMATION OF CHLOROPHYLL CONCENTRATION IN MAIZE USING SPECTRAL REFLECTANCE

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ABSTRACT:

The objective of this research was to identify the relationship between chlorophyll contents and spectral measurements in a maize crop. Spectral measurements were taken and the chlorophyll content was determined in leaf samples in a field experiment with different N rates. Analysis of variance showed differences in the chlorophyll content and spectral indices for the treatments and strong correlations between some reflectance indices and chlorophyll content. Due to a relationship between the N and chlorophyll contents, the results proved to be important for making decisions related to the nitrogen supply for crops, which is crucial in agriculture

1. INTRODUCTION

Maize is an important crop in Colombia, the production is about 1,400,000 tons per year and imports almost reach 2,000,000 tons per year (Venegas, 2010). In Colombia, the application of fertilizers to crops is done, in many cases, without technical criteria; sometimes soil chemical analysis is used and, in only a few cases, a plant tissue analysis is carried out as a basis for nutritional diagnosis. Laboratory analyses require a lot of time to get the results and are expensive; traditional sampling schemes are not suitable for representing the spatial variability of the crop. Currently, it is important to have reliable and fast information on the condition of crops to make input applications in a timely manner and at appropriate amounts. Measurements of spectral responses are an important alternative for identifying plant status to make decisions on input applications and for monitoring the crop status.

Spectral measurements can be extracted from remote sensing images, photographs taken with cameras mounted on unmanned aerial vehicles and proximal sensors, and, therefore, are alternatives with a lot of potential for generating the information required for more efficient crop management.

Chlorophyll is a pigment that has a clear impact on the spectral responses of plants, mainly in the visible spectrum portion. N is a key element in chlorophyll, therefore is usually a high correlation between them (Schlemmer, Francis, Shanahan, & Schepers, 2005). Knowledge on the relationship between spectral reflectance and chlorophyll content is an important alternative for making decisions related to the nitrogen supply for crops, which is crucial in agriculture.

Nitrogen is one of the most important nutrients in agriculture due to the role it plays in growth and crop production and due to the amount that plants require. Nitrogen is a component of chlorophyll and, therefore, is essential for photosynthesis. It is also the basic element of plant and animal proteins, including the genetic material DNA and RNA, and is important in periods of rapid plant growth. In agriculture, nitrogen applications represent an important percentage of production costs; therefore, decisions about the application rate, time and source are key to agricultural competitiveness and sustainability. Currently, N and/or chlorophyll estimations are important to a range of applications, such as precision agriculture and the global carbon cycle.

Several researchers have evaluated the role of spectral measurements in crop diagnosis and monitoring. Recent studies have demonstrated the feasibility of estimate chlorophyll content from hyperspectral vegetation indices composed by the reflectance of specific bands (Schlemmer et al., 2005)(Peñuelas, Garbulsky, & Filella, 2011).The position of the inflexion point in the red edge region (680 to 780 nm) of the spectral reflectance signature, termed the red edge position (REP), is affected by biochemical and biophysical parameters and has been used as a means to estimate foliar chlorophyll and nitrogen content (Cho & Skidmore, 2006)(Curran, Dungan, Macler, & Plummer, 1991).

Remote sensing images have been evaluated in several studies as a source of data for crop mapping and monitoring. In the case of Colombia, elevated cloudiness is an important characteristic that prevents the use of these images in an efficient manner. Therefore, it is necessary to identify and develop new ways to take low-rise, low cost photographs that can be processed quickly. UAV cameras are important alternatives that can be tailored to the needs of the user, which requires knowledge of the spectral responses of different species and crop conditions in order to know the optimal spectral zone for determining the nutritional status of the plants.

To make efficient and reliable use of data from satellite and proximal sensors, it is necessary to know the spectral response of each crop at different growth stages as this depends on the biochemical and physiological characteristics of the plant. Then, indices can be developed that will later be used for identification and characterization of the crops. These indices can serve as indicators of stress, senescence, and disease in higher plants. Research is needed to assess these relationships and to predict leaf nitrogen and chlorophyll contents for a wide variety of plant species.

The objective of this study was to evaluate the relationship between the chlorophyll contents of maize crop with spectral measurements as a basis to improve the nutritional diagnosis of this crop in Colombia. The research included nitrogen measurements, crop yield evaluation, satellite images and local photographs processing, however, in this paper only the results of chlorophyll and spectral measurements are presented.

2. METHODS AND MATERIALS

2.1 Study areas

The study area for the maize (*Zea mays*) experiment was located in the Meta Department, on the eastern plains of Colombia, at the CORPOICA Taluma research Station, 4°22'24.39"N and 72°13'5.30"W. Soils are fine and medium-textured, nutrient-poor, and acid Typic Hapludox, with a high content of aluminum, which is toxic for several crops (Instituto Geográfico Agustin Codazzi, 1998) .The climate is an Am type according to Köppen's classification, with a short dry season in January and February. The average annual rainfall varies between 2500 and 2800mm throughout the area. The mean annual temperature is about 26°C. Cattle grazing is the traditional, principle land use, but now agriculture with rubber plantations, maize, rice and soybean is being promoted.

2.2 Data collection and analysis

An experiment, was carried out with randomized blocks with four repetitions. There were 5 N treatments, including 0 as the control and 50, 100, 150, and 200 kg of N ha $^{-1}$. Spectral measurements were taken at 36 days, 56 days and 108 days after sowing, with a leaf clip using a Fieldspec 4 spectroradiometer (ASD Inc.) and one measurement from the canopy. This radiometer measures in the range of 350-2500nm, with a sampling interval of 1.4 nm at the spectral range of 350 - 1050 nm, while it is 2 nm at the spectral range of 1050 - 2500 nm. The chlorophyll (a and b) was estimated in a laboratory, measuring the absorbance at 647 nm and 665nm for pooled extracts using Lichtenthaler equations (Lichtenthaler, 1987)

Several spectral vegetation indices were calculated (table 1) to identify the better indices that are related to the chlorophyll content. Analysis of variance (ANOVA procedure) was used to determine the effect of the treatments on the spectral measurements. Simple and multiple correlation and regression analyses were employed to identify the relationships between the studied variables. The relationships that were explored mainly concerned the chlorophyll content in the leaves in relation to spectral reflectance and vegetation indices.

Table 1: Spectral vegetation indices used in the analysis

Index	Name	Formula	Source
REP-LE	Red edge position: linear extrapolation method	Linear extrapolation of straight lines on the far-red (680 to 700 nm) and NIR (725 to 760 nm) flanks of the first derivative reflectance spectrum	Cho and Skidmore (2006)
REP-LI	Red edge position: linear interpolation method	700+40(Rre−R700)/(R740−R700) Rre: (R670+R780)/2	Guyot et al. (1988)
Ch-RE	Chlorophyll index red edge	(R750 - R700)/R700	(Gitelson, Merzlyak, & Lichtenthaler, 1996)
REPIG	Red edge position: Inverted Gaussian fitting	RMAX-(RMAX-RMIN)*EXP(-((780(nm)-660(nm))^2)/((2*30^2)))	(Pu, Gong, Biging, & Larrieu, 2003)
PRI	Photochemical reflectance index	(R531 - R570)/(R531 + R570)	(Peñuelas et al., 2011)
NDVI	normalized difference vegetation index	Measure with GreenSeeker (Trimble)	
REP-P	Polynomial fitting	$R(\lambda) = a_0 + \sum_{i=1}^{5} a_i \lambda^i$	(Pu, Gong, Biging, & Larrieu, 2003)

3. RESULTS

The figure 1 shows the leaf spectral signature at three growing stages for the treatments without fertilization. Although the general trend of the spectral responses were similar, differences were found mainly in the 850 nm to 2350 nm, where water content has more effect in reflectance and in the visible range, where the chlorophyll had more influence. Higher values of reflectance were found at the 108 days after sowing when the crop is near to senescence. In the visible range the maximum reflectance was found at 560nm in the three growing stages (figure 2). According to Liang (2004), in the visible part of the spectrum, absorption is due to pigments such as chlorophyll a+b and carotenoids, while the near-IR and middle-IR absorption by water is more important in the variation of reflectance.

Figures 3 and 4 show the spectral responses for the different rates of N in the maize at 108 and 56 days after sowing in part of the visible spectrum. The higher reflectance values were found in the control without N and dropped to the lower values with the 200 kh $^{-1}$ N rate.

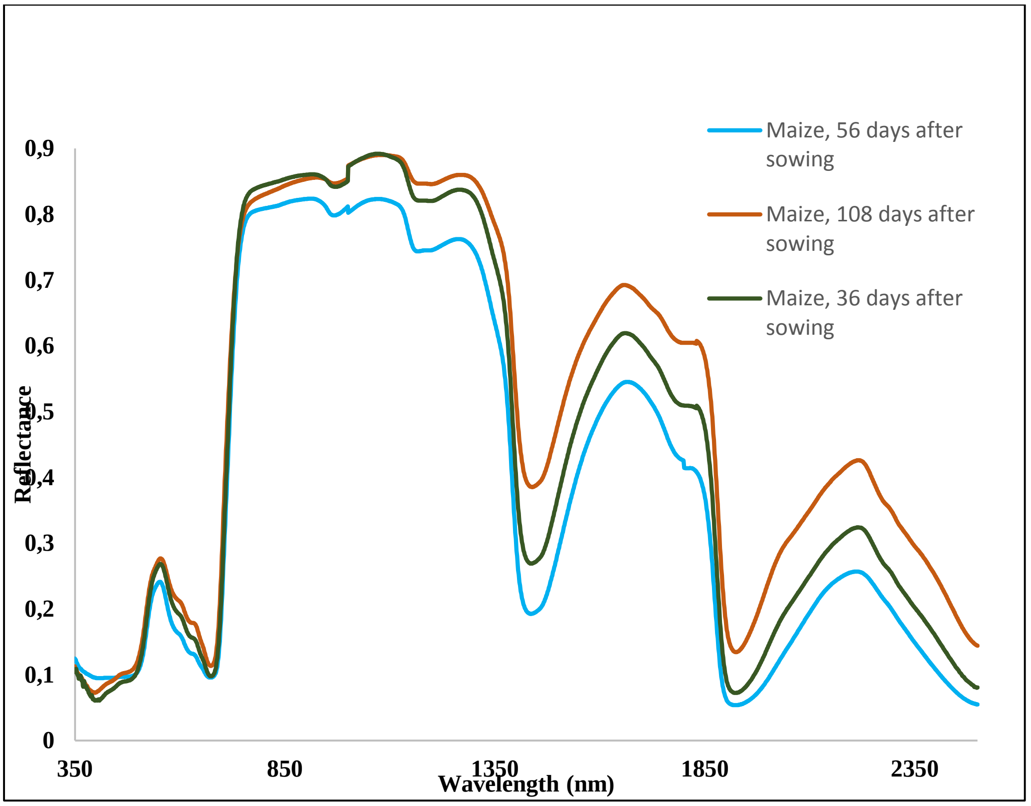

Figure 1: spectral reflectance for leafs in maize at different growing stag

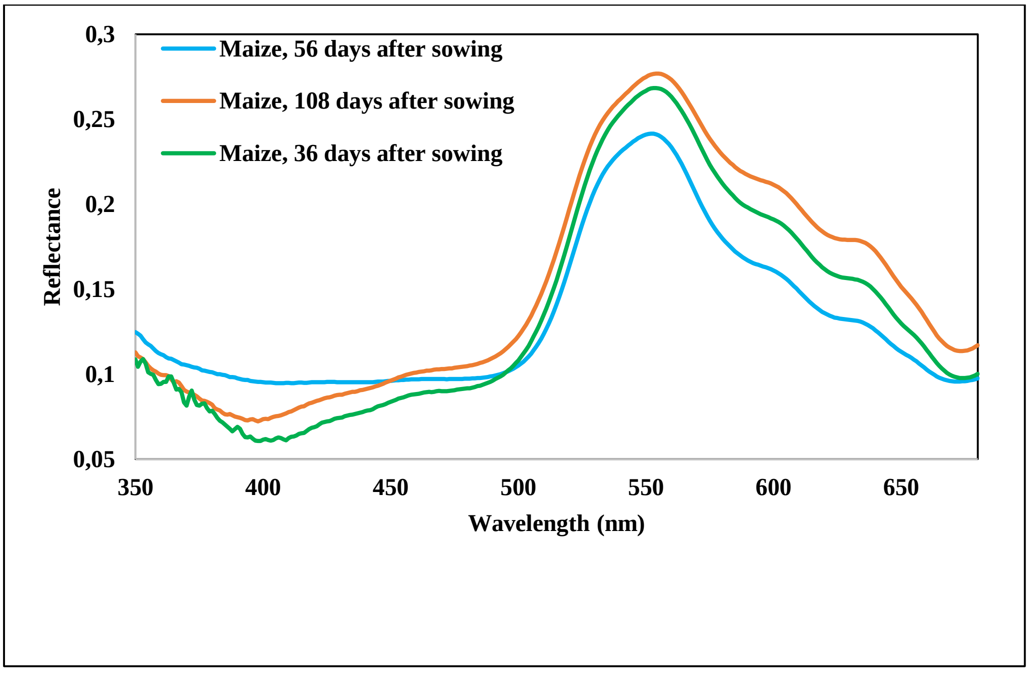

Figure 2: Spectral reflectance in the visible range for leafs in maize at different growing stages

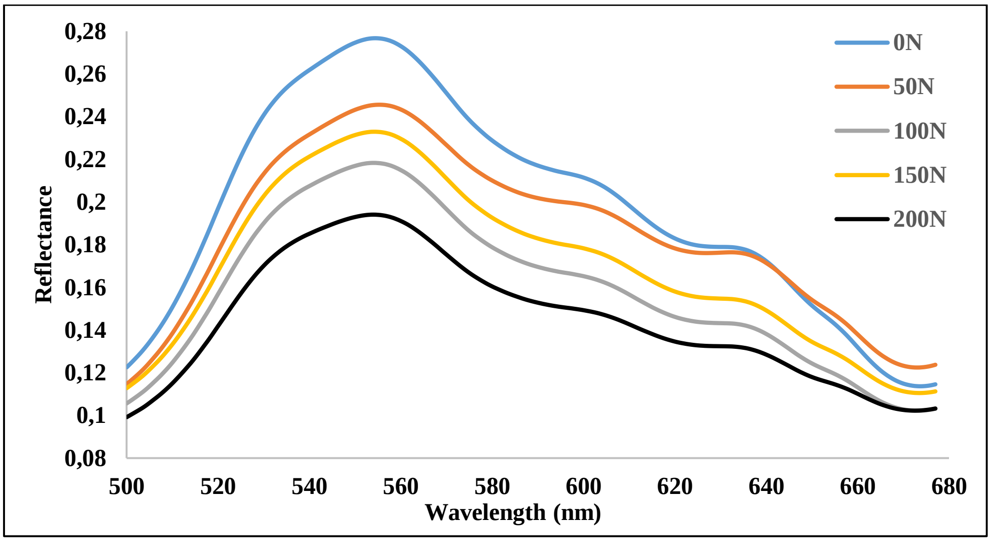

Figure 3**:** Spectral reflectance for maize, at 108 days after sowing, with different N rates.

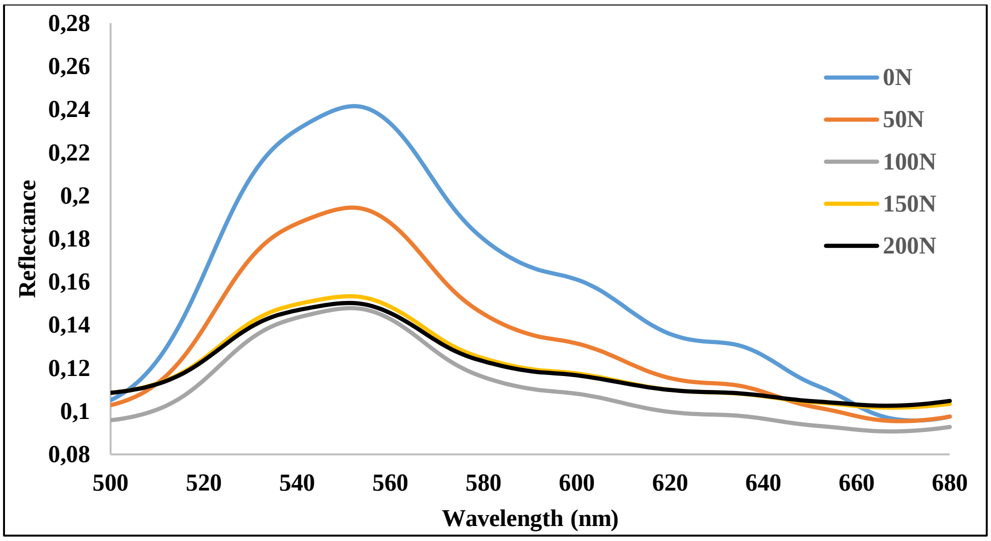

Figure 4: Spectral reflectance for maize, at 56 days after sowing, with different N rates.

Likewise, differences were found between the periods. The higher reflectance was found at 108 days after planting, which may have been related to the incidence of some brown pigments that increase when a plant is in senescence.

Tables 2, 3 and 4 show the Pearson correlation coefficients between the chlorophyll and some spectral vegetation indices for the maize at 36 and 56 days after sowing. The higher correlations in the first stage presented with the red edge position, as determined by the linear interpolation method (REP-LI) (Guyot, G., & Baret, 1988) for chlorophyll b, a and a+b. . The low correlation for NDVI in tables 2 and 3 is because this indice was not calculated but was measured with the Green Seeker sensor (Trimble) and is a canopy measurement. When compared NDVI with canopy reflectance the correlation with chlorophyll increases

The differences in spectral responses due to N rates are important for nutritional diagnosis of the crop as they are the basis for identifying nitrogen deficiencies in plants and making timely decisions for inputs application

In this way can be designed cameras to take images in the portion of the visible and infrared spectrum that most closely correlated to the chlorophyll content and generate information about the status of the crop. Similarly, when cloud conditions permit, remote sensing images can be used to have an approach to the general state of the crops

Table 2: correlation coefficients between chlorophyll and leaf spectral indices for maize, 36 days after sowing (*n=39)*

	NDVI	Ch-RE	PRI	REP - L	REP -P	REPIG	REP-LE
Chlorophyll a	0,40*	0,57**	0,49**	0,97**	0,93**	0,93**	0,54**
Chlorophyll b	0,40*	0,57**	0,49**	0,97**	0,93**	0,93**	0,54**
Chlorophyll a+b	0,44**	0,60**	0,52**	0,97**	0,92**	0,92**	0,55**

* $p < 0.01$, ** $p < 0.001$

Table 3: correlation coefficients between chlorophyll and leaf spectral indices for maize, 56 days after sowing *(n=120)*

	NDVI	Ch -RE	PRI	REP - L	REP- P	REPIG	REP-LE
Chlorophyll a	0,12	0,45**	0,32**	0,91**	0,86**	0,86**	0,93**
Chlorophyll b	0,25**	0,56**	0,47**	0,82**	0,77**	0,77**	0,85**
Chlorophyll a+b	0,22*	0,55**	0,45**	0,87**	0,82**	0,82**	0,89**

* $p < 0.01$, ** $p < 0.001$

Table 4: correlation coefficients between chlorophyll and canopy spectral indices for maize, 56 days after sowing (n=120)

	NDVI	Ch -RE	PRI	REP - L	Rre	REP-LE
Chlorophyll a	0,81**	0,70**	0,47*	0,85**	0,36	0,80**
Chlorophyll b	0,71**	0,57**	0,42	0,76**	0,33	0,74**
Chlorophyll a+b	0,75**	0,61**	0,43	0,80**	0,33	0,77**

* $p < 0.01$, ** $p < 0.001$

For 56 days (table 3), the better correlations were found with the REP calculated by the linear extrapolation method (Cho and Skidmore, 2006), also other indices such as REP-L, REP-P and REPIG showed good correlation indices. For the canopy measures, the better correlations with the chlorophyll were found with REP-LE, NDVI and REP-L.

Figures 5 and 6 represent the relationship between chlorophyll content and REP-LI on 56 days plants, and indicates a good estimation of the chlorophyll content. Other indices showed god results, although results are not presented here. Regression analysis between chlorophyll content and some indices indicates the potential of these indices to estimate the chlorophyll content in the states of crop growth when plants are still young and indirectly estimate the nitrogen content. This information is useful for correcting the deficiencies of this nutrient by applying fertilizer.

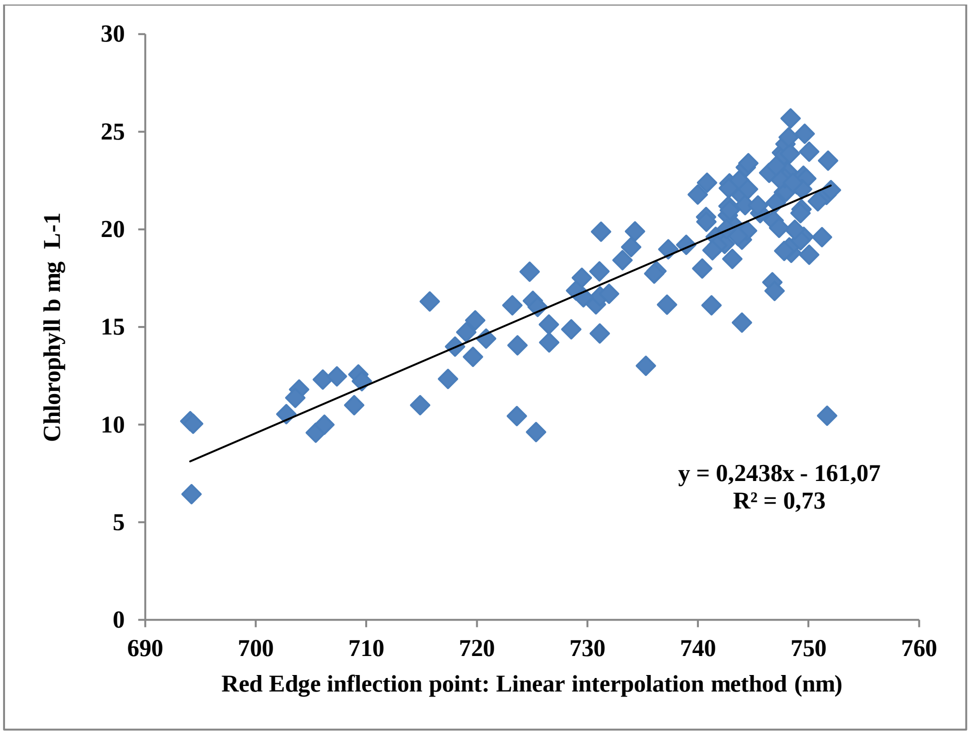

Figure 5: Regression analysis for chlorophyll b content and REP-LI at 56 days after sowing

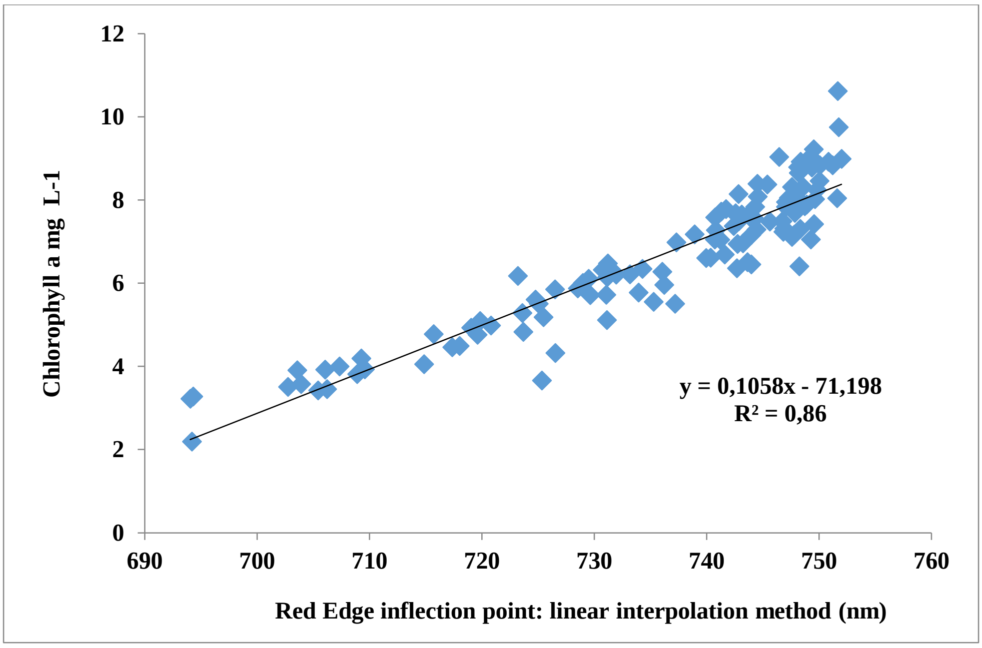

Figure 6: Regression analysis for chlorophyll a content and REP-LI at 56 days after sowing

4. CONCLUSIONS

The nitrogen rates applied had a significant effect on chlorophyll content of maize and on spectral responses. Various vegetation indices had a highly significant correlation with chlorophyll content, which makes them potentially suitable for estimating chlorophyll and indirectly identify N deficiencies in crops to help decisions on fertilizer application.

5. ACKNOWLEDGEMENTS

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