Australian Journal of Crop Science
AJCS
AJCS 6(2):188-193 (2012) ISSN:1835-2707

Application of a spectral sensor for the assessment of nitrogen content in lettuce plants

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Abstract

Nowadays, current heavy reliance on agricultural chemicals has raised many environmental and economic concerns. Therefore, farmers need to find a way for optimal application of agricultural chemicals. Nitrogenous compounds are the most important items for the growing crops. In this research, a nitrogen sensor was developed to assess nitrogen status of lettuce plants. For this purpose, a spectral sensor (TCS230, TAOS, USA) along with an AVR microcontroller (ATmega32, Atmel, USA) (620-700 nm) was used. Moreover, the SPAD test on the leaves of lettuce plants was preformed to compare and confirm the results. The results showed that intensity of solar influence on the sensor and the light source can be fixed by this approach. Spectral sensor were found to be linearly correlated (r^2=86) with chlorophyll content and sensor data. In addition, it was found that the distance between leaf and sensor is very important, where the best results were obtained within 1-3 cm. The increase of distance over 8 cm created a lot of meaningless data. Data analyses showed a correlation between sensor and chlorophyll meter as $r^2=0.22$. Results shows that the sensor used in this research have better performance than leaf color chart. In addition, commercial sensors have better performance compared with the sensor used in detection of nitrogen plant content.

Keywords: Nitrogen content; Lettuce; Spectral sensor; Chlorophyll; SPAD.
Abbreviations: Chl: Chlorophyll; NDVI: Normalized Difference Vegetation Index; N: Nitrogen; SPAD: Signaling Pathway Data; LLC: Leaf Color Chart; GND: Ground; R: Red.

Introduction

Farmers need to find a way for applying optimum usage of agricultural chemicals to achieve maximum yield and minimum environmental risks. The current heavy reliance on agricultural chemicals has raised many environmental and economic concerns in the recent decades. However, among agricultural chemicals, nitrogen (N) based chemicals are the most important materials for crop growing and on the other hand, the most concerning nutrient element for maintaining clean environment. The accurate assessment of nitrogen content in plants and soil is a key point to nutrient management. By application of nitrogen sensors, fertilizers can be applied in an accurate amount when required, by which a lot of expenses would be saved and environmental concerns reduced (Lee and Searcy, 2000). The spectral differences from different canopy colors are not usually affected by plant variety but by nitrogen content itself in the leaves (Lee et al., 1999). A nitrogen sensor was made and tested in a commercial corn field, along with laboratory analysis for nitrogen content in sample leaves (Lee and Searcy, 2000). Values derived from spectral measurements admittedly depend on the amount of chlorophyll. The best relationships between chlorophyll and reflectance measurements have been obtained on the leaf level (Cartelat et al., 2005; Gitelson et al., 2003). Unfortunately, the relationship between chlorophyll and nitrogen content (upon which spectral based measurements of nitrogen nutrition index will change) is disputed. Read et al. (2002) found only a weak correlation (R^2 = 0.32) between chlorophyll and nitrogen content (variables) compared to very strong correlation value of R^2 = 0.97 reported by Cartelat et al. 2005. On the canopy level; however, spectral measurements appear to be correlated well with total aerial nitrogen (Lukina et al., 2001; Schmidhalter et al., 2003; and Mistele and Schmidhalter, 2008). Numerous studies have been conducted to assess moisture and nutrient status by measuring of spectral responses (Blacmer et al., 1996; Evain et al., 2004; Botha et al., 2007; and Mistele and Schmidhalter, 2008). These studies reported that the reflectance within 550-700 nm showed good separation of nitrogen content. The chlorophyll meters have also been used as an alternative to assess nitrogen stress in plants (hardwood foliar, sugar maple and paddy) (Chang and Robison, 2003; Van den Berg and Pekins, 2004; Li Jinwen et al., 2009). These studies found reasonable relationships among yield, nitrogen and chlorophyll meter reading; however it seems that chlorophyll meter requires many measurements to accurately assess plant status. Utility of aerial photographs or satellite imagery are another ways to assess the plant nitrogen status and yield (Junior et al., 2007; Peng et al., 2009). Another way of nitrogen stress assessment is to implement a real-time nitrogen sensor system. Heege and Thiessen, (2008) developed a sensor system for nitrogen

Table 1. The value of chlorophyll content and N-sensor at two different distances.

Chlorophyll content (SPAD)	N-sensor reading within 1-3 cm (%)	N-sensor reading at distances over 8 cm (%)
51.5	72	01
42.6	66	02
27.7	58	32
16.7	44	22
10.3	38	25
6.9	20	19
5.5	16	21
4.1	13	69
3.1	11	68
1.1	05	54

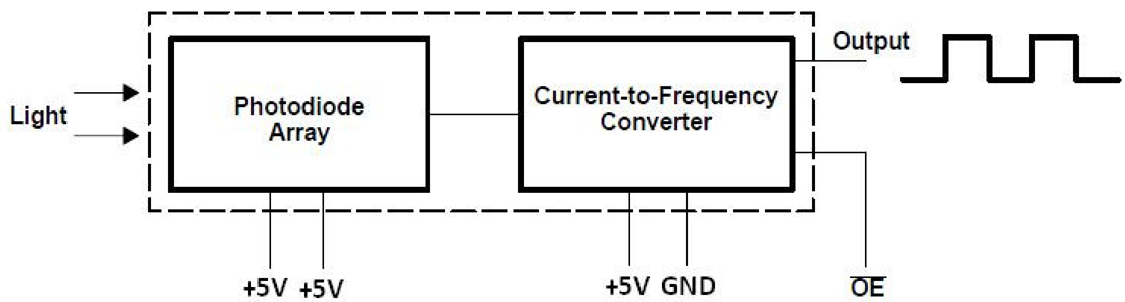

Fig 1. Functional block diagram of light sensor, light detection and conversion to frequency.

and red edge inflection point. They tested their sensors on corn plant and used a NDVI (Normalized Difference Vegetation Index) system. Nitrogen is one of the most important components of protein structure and chlorophyll in plant. One nitrogen and four carbon atoms are included in the rings inside chlorophyll, in such a way, nitrogen has a common bind with carbon and magnesium atoms. Therefore, lack of nitrogen causes yellow color in leaves. The nitrogen content of leaves can be predicted by calculation of determination coefficient (R^2) more than 90 % (Seilsepor and Momayezi, 2005). Measurement of nitrogen content by spectral sensor has not been reported in lettuce yet. The main objective of this research is to develop an intelligent system with capability of detection of required nitrogen to the lettuce plant, by which the consumption of nitrogen fertilizers will be reduced, consequently.

Results and discussion

Towards the young leaves of lettuce, the chlorophyll content decreases; therefore the chlorophyll meter showed lower number but the sensor revealed a higher value. This shows that the light reflection from the leaf surfaces with low chlorophyll content is higher than that of outer leaves. This phenomenon is due to lighter colors of young leaves compared to outer ones. Thus, the sensor receives less light. Precise assessment of data obtained from sensor, can imply that this sensor provides much broader range of values than chlorophyll meter. This fact is a promising prospective of the sensor used in the current study. Data analyses showed a correlation between sensor and chlorophyll meter $r^2=0.86$ (Fig 4).

$$N\text{-}sensor = (-10.977.\ spad) + 659.65 \quad (2)$$

Error was reduced significantly when misleading effects of sunlight intensity decreased. Fig 4. shows the changes resulting from increase of chlorophyll content and the ratio between SPAD and sensor data. It shows that SPAD unit has lesser steep in the range of 20-50, than those in the range of 1.1-20. As a consequence, the sensor provides more convenient data range. A separate chart for each obtained SPAD and sensor are shown in Figs 6 and 7, respectively. The change in the distance between the plant and the sensor, caused alterations in obtained data. The best result was observed for the distance within 1-3 cm. It can be concluded that the more distance will cause more errors as well as sunlight effectiveness. However, sensor detection range was found by 8 cm gap. Nonetheless, an increase in the distance over 8 cm created a lot of meaningless data in the information collected. Data analyses showed a correlation between sensor and chlorophyll meter as $r^2=0.22$ (Fig 8 and Fig 9):

$$N\text{-}sensor= (629.01.\ spad)-6.302 \quad (3)$$

A considerable research works on application of sensors are found in the literature (Ahmad et al., 2010; Gamea et al., 2011). Fernandez et al. (1994) found that nitrogen content of wheat can be estimated independently from plant fertilization treatment through a linear combination of green and red canopy reflectance. However, they could not estimate

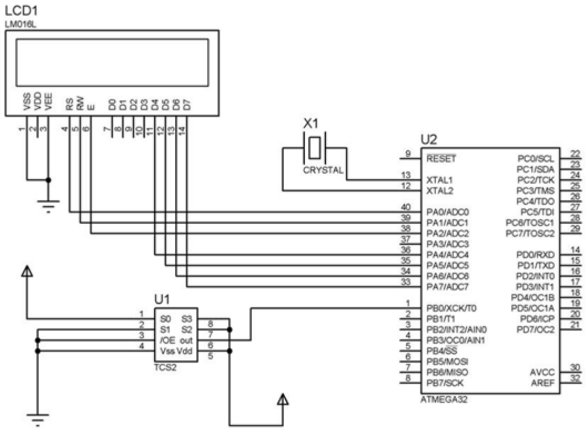

Fig 2. The circuit of N-sensor; connection of LCD, crystal and TCS to microcontroller port.

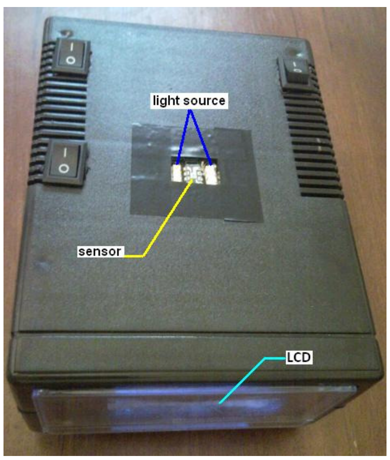

Fig 3. N-sensor used in the current study; put the leaf at the top of the sensor and is read nitrogen content from LCD.

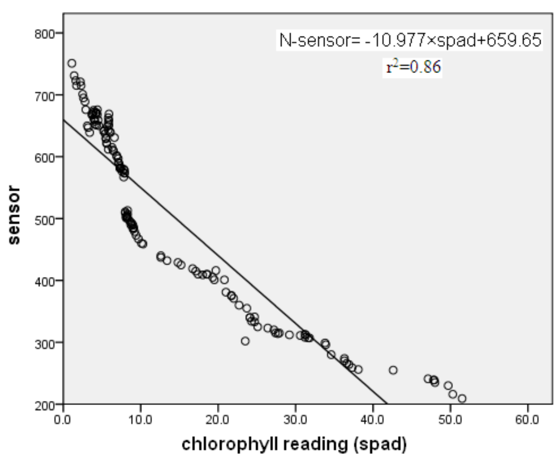

Fig 4. Relationship between chlorophyll content in SPAD and chlorophyll content in sensor.

chlorophyll content through NDVI independent from plant treatment, despite the high linear correlation between the content of nitrogen and chlorophyll. Combinations of reflectance at 620-700 nm and all the red edge parameters provided better chlorophyll estimations than NDVI index. Recently Fitzgerald et al., (2010) used spectral index. The canopy chlorophyll content index and Canopy nitrogen index were r^2=0.83 and r^2=0.60 for nitrogen content of wheat, respectively. Canopy chlorophyll content index resulted closer to the results of N-sensor used in the current study.

This is noteworthy to be mentioned that spectral measurements were useful to describe the chlorophyll meter reading in lettuce, whether the canopy was deprived or supplied adequately with nitrogen or not. The results showed high R^2-values between canopy reflectance and chlorophyll meter reading, which were slightly better than those between canopy reflectance and total aerial nitrogen. Results indicated that SPAD and LCC can be used to estimate total Chl content in leaf 1 of cassava, in which the variables were influenced by leaf position, growth stage, cv. and N fertilizer rate (Anand et al., 2008). Leaf 1 had a significantly lower SPAD, LCC, and Chl a+b than leaves 2 and 3 in two cassava cultivars, differed in their response to each of the variables, up to 60 days after planting. The values of each of the variables increased with increment of N application rate at all growth stages. Relationships between tuber yield and SPAD (r^2 = 0.83), LCC (r^2 = 0.85), and Chl a+b (r^2 = 0.80) were significant ($p<0.05$) and positive at 30 and 60 DAP. A single regression equation was used to describe the relationship between LCC and Chl a+b and between LCC and SPAD, but not between SPAD and Chl a+b (Anand et al., 2008).

$$Chl=0.05spad+0.977 \quad (4)$$
$$Chl=0.507spad+0.948 \quad (5)$$
$$Spad=10.981LCC-3.51 \quad (6)$$

The LCC is one of the methods for assessment of nitrogen content of plants, but the results showed that the sensor used in our experiment has a better performance than LCC. Study of Tremblay et al. (2009) compared the performance of two commercial sensors, the Yara N-Sensor/FieldScan (Yara International ASA, Germany) and the GreenSeeker (NTech Industries Inc., Ukiah, California, USA), for assessing the status of N content in spring wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.). Both of sensors detected nitrogen content accurately more than r^2=0.90 (Tremblay et al., 2009).

Materials and methods

Nitrogen sensor design

This device includes a spectral sensor (TCS230, TAOS, USA). The light reflected from the leaf surface is sent to a microcontroller as a value using photodiode array and a current to frequency converter (Fig 1). An AVR microcontroller (ATmega32, Atmel, USA) program language code vision AVR (C language) was written and frequency meter mode for sending data from sensor was employed and the sensor data with the wavelength values within 620-700 nm were collected. Ciganda et al. (2009) used the Red Edge Chlorophyll Indices as follows: $CI_{red\ edge} = (R_{NIR}/R_{red\ edge})-1$, based on reflectance, R, in the red edge (720–730 nm) and

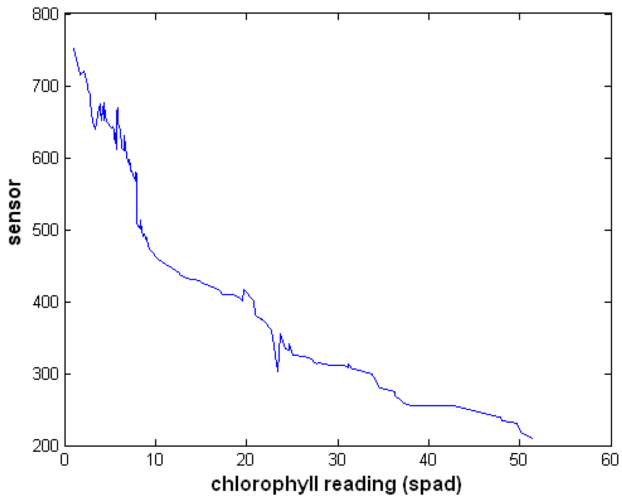

Fig 5. Histogram of typical lettuce leaves at the spectral bands (620-700 nm).

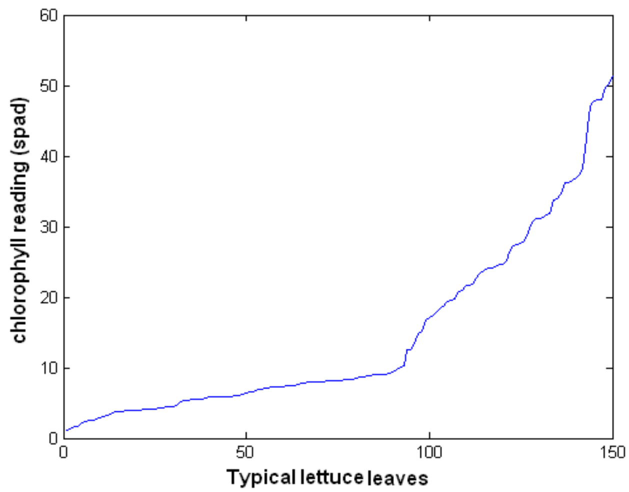

Fig 6. Chlorophyll content of typical lettuce leaves by SPAD.

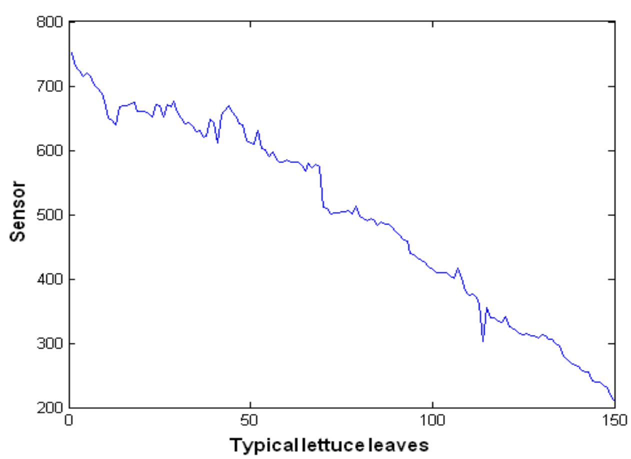

Fig 7. Chlorophyll content of typical lettuce leaves by N-sensor.

near infrared (770–800 nm) was found to be an accurate measure of maize leaf chlorophyll. The TCS230 programmable color light-to-frequency converter combines configurable silicon photodiodes and a current-to-frequency converter on single monolithic CMOS integrated circuit. The output is a square wave (50% duty cycle) with frequency directly proportional to light intensity (irradiance). The full-scale output frequency can be scaled by one of three preset values via two control input pins. Digital inputs and digital output allow direct interface to a microcontroller or other logic circuitry. Output enable (OE) places the output in the high-impedance state for multiple-unit sharing of a microcontroller input line. ATmega32 is a low-power CMOS 8-bit microcontroller based on the AVR enhanced RISC architecture. By executing powerful instructions in a single clock cycle, the ATmega32 achieves throughputs approaching 1 MIPS per MHz allowing the system designer to optimize power consumption versus processing speed. Constant light source was considered for reducing the sun light effects to prevent the error frequency of internal micro controller. Moreover, a crystal 16MHZ was used (Fig 2 and Fig 3). Microcontroller program was written in such a manner that the data outside the range of the received 620 to 700 nm do not register. Active sensors create the signals on a reflector objects and measure reflected signal attributes accordingly. Currently, the images created by passive sensors are more valuable in remote applications used in agricultural products. Passive sensors conveniently receive the signals reflected from the sensed objects.

Those signals which are developed by natural sun radiation could provide some important information on sensed objects; so the latter approach was employed in the current research.

Testing of the nitrogen sensor

To obtain the amount of the chlorophyll in the leaf, a SPAD (model 502, Konica Minolta Co. Japan) was used. For each section of the leaf, 10 data were recorded and then the averages for any receiver as one sensor data were recoded (Table 1). Due to the need to compare a wide range of chlorophyll data obtained from the spectroscopy, device of the lettuce plant chlorophyll had a different range, in which by approaching to the center, the chlorophyll content decreases. Tests in the middle of the day and evening were performed to observe the sunlight effects on the sensor. For establishing a relationship between *N-sensor* and chlorophyll content, the data collected in March, 2010 was considered. A linear relationship between *N-sensor* and chlorophyll found using SPSS 17 software in the following form:

$$N\text{-}sensor = (a \cdot spad) + b \quad (1)$$

Conclusion

This study discusses the development of a sensor for assessment of nitrogen content in plant leaves. Light source can reduce sunlight effect on the sensor. However, the limits in the spectrum range (620-700 nm) can lead to a better data set. Distance between the sensor and the leaf is an important factor. Decrease of distance will result more accurate data. Further research is under consideration to enhance the measurement capability of the system under the controlled displacement.

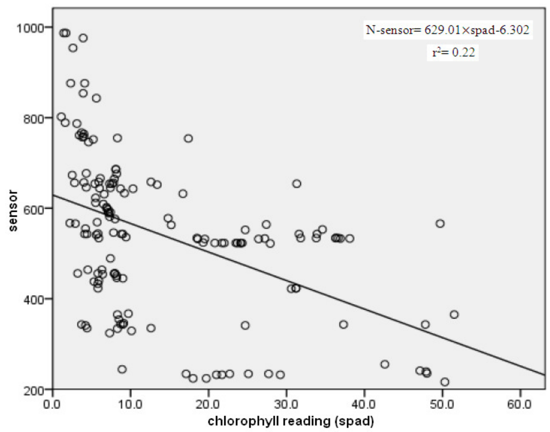

Fig 8. Relationship between chlorophyll content in SPAD and chlorophyll content in sensor at increased distance over 8 cm.

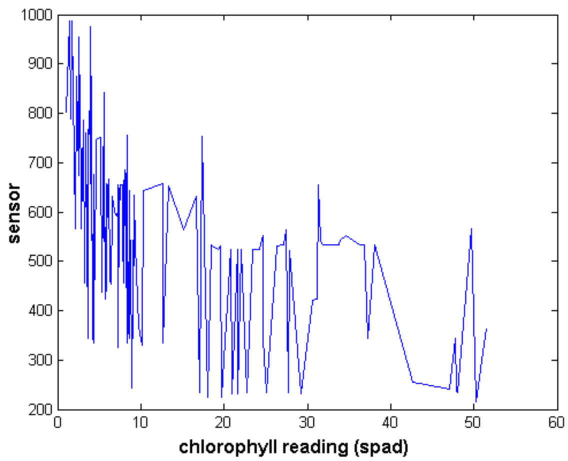

Fig 9. Histogram of typical lettuce leaves at the spectral bands (620-700 nm) at increased distance over 8 cm.

Results shows that the sensor used in this research have better performance than LCC. In addition, commercial sensors have better performance than our sensor in detection of plant nitrogen content.

Acknowledgements

The authors gratefully thank Mahdi Ghasemi-Varnamkhasti for his collaboration regarding this paper.

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