

Estimating Nitrogen and Chlorophyll Status of Romaine Lettuce Using SPAD and at LEAF Readings

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Abstract

Nitrogen (N) is an essential nutrient for plant growth and development and is especially important in the production of high quality leafy green crops. In this experiment, leaf N concentration, chlorophyll concentration (Chl) and weight above fresh matter (AFM) of romaine lettuce (*Lactuca sativa* L. var. *longifolia*) were estimated by correlations between *in situ* SPAD and atLEAF readings. Lettuce was grown in high tunnels during 42 days and was irrigated at five nitrogen levels: 0, 4, 8, 12 and 16 mEq·L⁻¹ of NO₃⁻, with a modified Steiner nutrient solution. The N concentration, Chl concentration and AFM were determined in the laboratory, while SPAD and atLEAF readings were measured *in situ* weekly. SPAD readings had high, positive and significant linear correlations with N (R² = 0.90), Chl (R² = 0.97) and AFM (R² = 0.98); The atLEAF readings had a similar linear correlation with N (R² = 0.91), Chl (R² = 0.92) and AFM (R² = 0.97). Both, SPAD and atLEAF readings had high, positive, and significant linear correlation (R² = 0.96). Suggesting that, SPAD and atLEAF meters can be used to non-destructively and accurately estimate the N status of lettuce, in a reliable and quick manner during the crop production cycle.

Keywords: above fresh matter; crop nutrition; *Lactuca sativa*; non-destructive sampling; soilless culture

Introduction

Nitrogen (N) is an essential nutrient for plant growth and development. It is found in amino acids, nucleic acids, proteins and in chlorophyll (Chl). The Chl concentration of the leaf is closely related to N concentration in the plant (Zebarth *et al.*, 2002), therefore, monitoring plant Chl and N concentrations during production could be used as a management strategy to enhance plant growth, yield and marketability (Gitelson *et al.*, 2003; Peng and Yuan, 2017).

Current destructive methods for the determination of N and Chl are precise, but time-consuming and relatively expensive (Kalaji *et al.*, 2017). In contrast, portable non-destructive equipment has been used successfully with some

plant species to quickly estimate the leaf Chl or N concentrations *in situ* (Loh *et al.*, 2002; Abdelhamidg *et al.*, 2003; Padilla *et al.*, 2018a) and enable repeat measurements from the same leaf during a production cycle (Kalaji *et al.*, 2017; Yamamoto *et al.*, 2002).

The SPAD 502 Plus Chlorophyll Meter (Konica Minolta®, Japan) is used for rapid and non-destructive determination of relative leaf Chl concentration (Gianquinto *et al.*, 2004). Transmission of light through the leaf is measured at 650 and 940 nm; the wavelength of 650 nm coincides with the spectral region with maximum chlorophyll activity, while transmittance at 940 nm is used to compensate for factors, including leaf moisture content and thickness (Zhu *et al.*, 2012). It has been reported that the SPAD readings are related to the concentration of foliar

chlorophyll (Uddling *et al.*, 2007; Basyouni *et al.*, 2015; Basyouni and Dunn, 2017).

The atLEAF CHL chlorophyll meter (FT Green LLC, USA) works in a similar way to the SPAD meter, but uses a wavelength of 660 nm instead of 650 nm (Zhu *et al.*, 2012). Readings obtained with an atLEAF CHL PLUS chlorophyll meter are similar to those obtained with the SPAD meter (Basyouni *et al.*, 2015), but the atLEAF meter is currently more affordable when compared to the SPAD meter (Zhu *et al.*, 2012; Basyouni and Dunn, 2017).

It has been reported that the Chl and N concentrations determined in the laboratory have high correlations with the SPAD readings for a variety of plant species, including: corn (Hurtado *et al.*, 2010), tomato (Ferreira *et al.*, 2006), cabbage (Westerveld *et al.*, 2003), rice (Huang *et al.*, 2016) and mangrove (Dou *et al.*, 2018). High correlations have also been reported with atLEAF meter in corn, wheat (Zhu *et al.*, 2012) and sage (Dunn *et al.*, 2018a).

Padilla *et al.* (2018b) have reported relatively lower sensitivity of the atLEAF meter in sweet pepper, but it was suggested that there was limited literature available for the atLEAF and that in general, there is limited research focused on vegetable crops when compared to cereal crops like wheat or maize (Padilla *et al.*, 2018a).

Lettuce is the most important crop in the group of leafy vegetables around the world, due its high nutritive value and because it is a source of many vitamins and contains minerals (Noumedem *et al.*, 2017). In 2017, worldwide production of lettuce was 26.9 million tons (Food and Agriculture Organization of the United Nations, 1998) with China leading production with 15.2 million tons. Mexico ranks tenth globally for lettuce production with 480,808 tons produced (SIAP, 2019) and fourth globally for lettuce exports, with an estimated value of 268 million dollars (OEC, 2019).

The objectives of this study were to estimate leaf N concentration, chlorophyll concentration (Chl) and weight above-ground fresh matter (AFM) of romaine lettuce using SPAD and atLEAF readings.

Materials and Methods

Cultural conditions

This study was conducted in high tunnels, at the Faculty of Agricultural Sciences Autonomous University of the State of Morelos, Chamilpa, Cuernavaca, Morelos, Mexico (18° 58' 51" N, 99° 13' 55" W, altitude of 1,866 m.a.s.l.), from April to May of 2018. Temperature, relative humidity, and light intensity were recorded hourly from Hobo® datalogger U12-012 (Onset Computer Co., USA) for the duration of the experiment. The maximum and minimum temperatures were 40 °C and 13 °C, respectively, with an average of 26 °C. The maximum and minimum relative humidity was 83% and 15%, respectively, with an average of 45%. Average light intensity for the duration of the experiment was 28,000 lux.

Seeds of romaine lettuce 'Green Star' (Johnny's Seeds, USA) were sown (March 13, 2018) into 200-well polystyrene trays (30 mL), previously filled with BM2 Berger® commercial substrate and were transplanted (April 13, 2018) into 10 L polyethylene bags, which had been filled with volcanic rock (locally called tezontle) with a granulometry of 1 to 7 mm.

Lettuce seedlings were irrigated daily with approximately 0.5 L of Steiner's nutrient solution, modified to provide nitrate at 0, 4, 8, 12 and 16 mEq·L⁻¹ (Mercado-Luna *et al.*, 2010) for the first three weeks, and then with 1.5 L of nutrient solution for the remaining period of the study (42 Days After Transplanting). Plants were irrigated to achieve approximately 20% leaching fraction by volume. Micronutrients were added as a commercial mixture of chelates (Microsol Rexene® Mix SQM) in doses of 80 g m⁻³, to achieve a supply of 3 mg·L⁻¹ of iron. The pH of the nutrient solutions was acidified with sulfuric acid to values between 5.6 and 6.0.

Measurements and observations

In situ sampling using the SPAD-502® Plus Minolta and atLEAF® CHL Plus portable meters (Fig. 1) was conducted weekly at approximately solar noon as described by León *et al.* (2017). Briefly, SPAD and atLEAF value were obtained from six plants per treatment, with ten readings per leaf (five on each side of the leaf midrib), from three fully expanded, recently matured leaves per plant. Immediately after finishing the measurements *in situ*, the fresh aerial biomass of the plants was harvested to determine the AFM with an OHAUS lab balance.

The same leaves used in SPAD and atLEAF measurements were then immediately sampled to determine Chl concentration (mg·g⁻¹) as described by Mackinney (1941) and von Wettstein (1957). Briefly, a portion of fresh biomass (1 g) was harvested from the plant, homogenized with 20 mL of concentrated acetone, filtered and adjusted to 50 mL of 80% acetone. Leaf samples and suspensions were kept in the dark between the processing steps. Absorbance (A) readings were taken at 645 nm and 663 nm on a UV-Vis Spectrophotometer DR 5000 (HACH, Canada). The calculations were made with the following equation:

$$\text{Total Chl} = (20.2 \times A_{645\text{nm}}) + (8.02 \times A_{663\text{nm}})$$

Samples were then dried in a forced air oven at 70 °C for 72 hours and then ground. Total N was quantified by the micro Kjeldahl method as described by Kalra (1997).

Design and statistical analysis

A completely randomized experimental design was used, at five nitrogen levels (0, 4, 8, 12 and 16 mEq·L⁻¹ of NO₃⁻), with six plants per treatment. There was one plant per container, with each container as a single replication (Fig. 2). Correlations were made of the SPAD and atLEAF readings against N concentration, Chl concentration and AFM using SigmaPlot® Ver. 12.5 (SYSTAT Inc., USA).

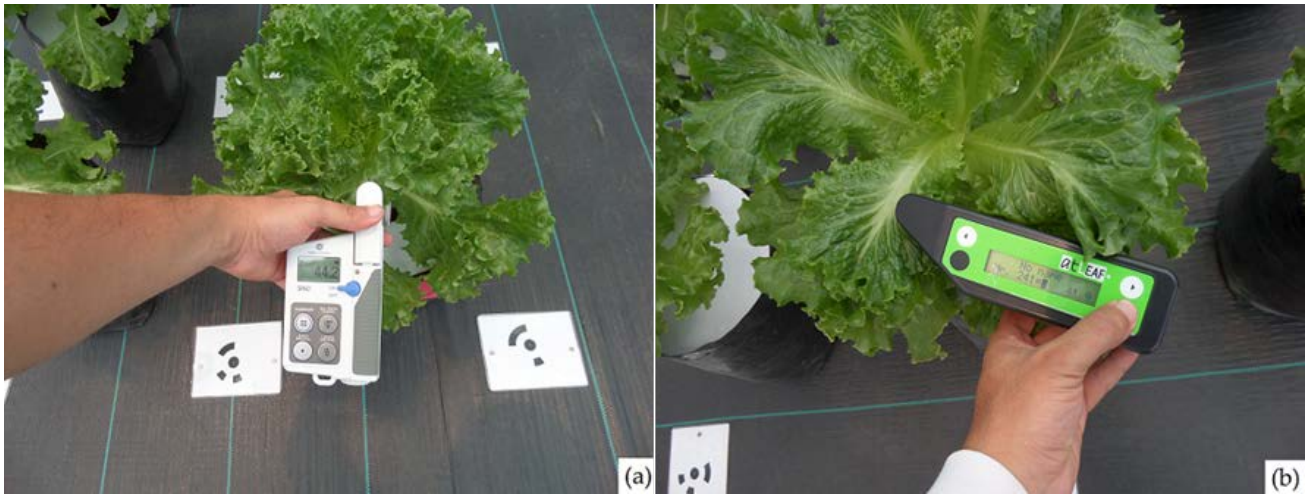


Fig. 1. Use of SPAD (a) and atLEAF (b) to measure relative chlorophyll concentration in romaine lettuce

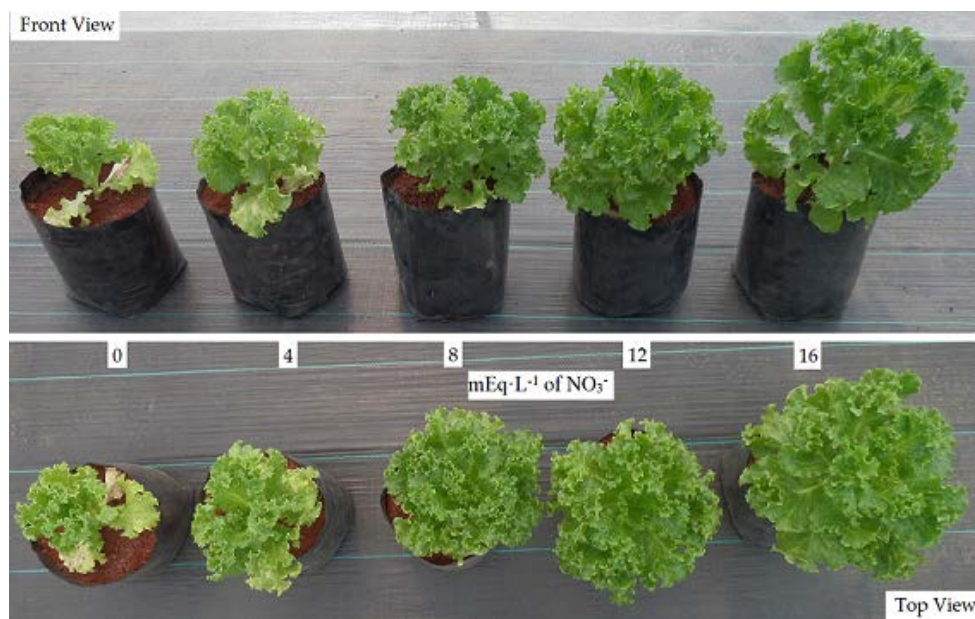


Fig. 2. Front view and top view of romaine lettuce growth as a function of N-NO₃ level at harvest

Results and Discussion

Relationship between N concentration, SPAD and atLEAF readings

SPAD readings had a high correlation ($\alpha = 0.01$) with leaf N concentration in romaine lettuce. The simple linear regression (Fig. 3) has a positive correlation coefficient ($r = 0.95$), a coefficient of determination (R^2) of 0.90 and a RMSE of 0.02. These values were higher than those reported in other plant species, for example oregano ($r = 0.80$, $R^2 = 0.64$) (Calderón-Medellín *et al.*, 2011) for correlation of SPAD and leaf N concentration. The results of the present study suggest that SPAD is a reliable instrument for estimating the concentration of N in lettuce plants, as reported for basil (Ruiz-Espinoza *et al.*, 2010), spinach (Liu *et al.*, 2006) and poinsettia (Dunn *et al.*, 2018b).

N concentration and atLEAF readings show a high linear correlation coefficient ($r = 0.96$, $R^2 = 0.91$, RMSE = 0.02, $\alpha = 0.01$) (Fig. 3), which indicates that the atLEAF

meter can be used for reliable and rapid estimation of N concentration in romaine lettuce plants. These results are similar to the studies carried out by Zhu *et al.* (2012) who reported that the atLEAF meter could be an affordable and reliable alternative ($R^2 = 0.78-0.92$) for the estimation of N concentration in corn, wheat, barley, potato and canola. Similarly, Basyouni *et al.* (2015) reported high correlation values ($R^2 = 0.75-0.83$) between N concentration and atLEAF readings when evaluating induced N deficiencies in two varieties of poinsettia.

Relationship between Chl concentration, SPAD and atLEAF readings

The correlation between SPAD readings and the Chl concentration (Fig. 4) was highly significant and positive ($r = 0.99$, $R^2 = 0.97$, RMSE = 0.03, $\alpha = 0.01$), which are similar to those of Fenech-Larios *et al.* (2009) who found high correlation of SPAD readings and Chl concentration ($r = 0.99$) in basil seedlings. The correlations of our present study are stronger than those reported by Martín *et al.*

(2007) who in viburnum, pittosporum and arbutus, and Ruiz-Espinoza *et al.* (2010) in basil found correlation coefficients between 0.36 and 0.52, and 0.57, and 0.67, respectively.

Chl concentration and atLEAF readings had a high linear correlation ($r = 0.96$, $R^2 = 0.92$, $RMSE = 0.09$, $\alpha = 0.01$). In that, our results had a higher adjustment when compared to those described by Zhu *et al.* (2012) who reported coefficients of determination between 0.72-0.88 for Chl concentration and atLEAF readings in corn, wheat, barley, potato and canola. While, Hebbar *et al.* (2016) reported coefficients of correlation between 0.68-0.95 in coconut palm leaves.

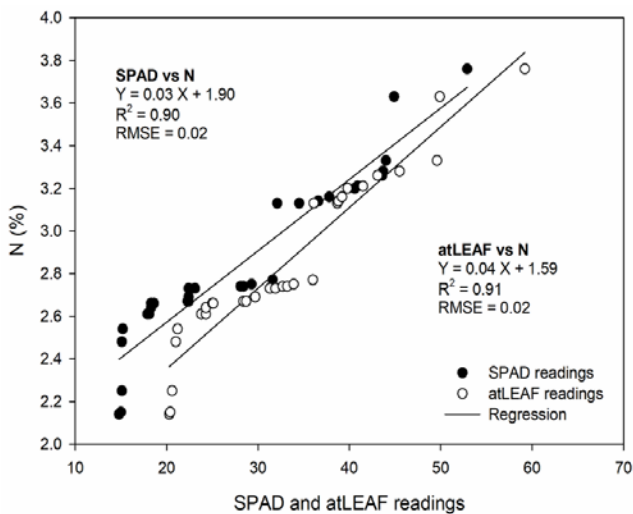


Fig. 3. Relationship between N concentration, SPAD and atLEAF readings in romaine lettuce

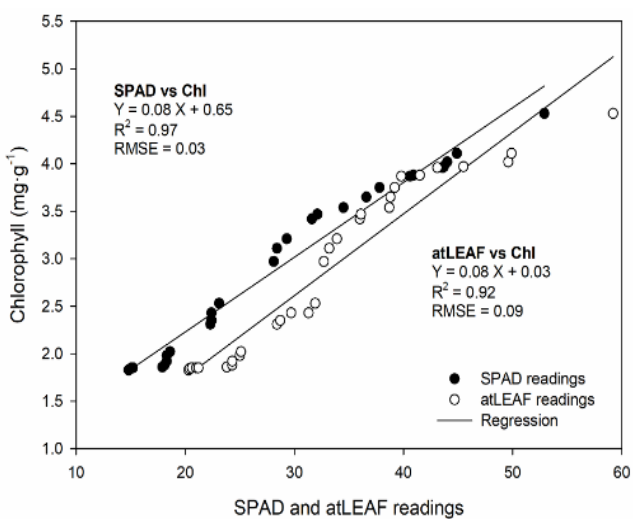


Fig. 4. Relationship between Chl concentration, SPAD and atLEAF readings in romaine lettuce

Relationship between AFM, SPAD and atLEAF readings

SPAD readings had a high correlation ($\alpha = 0.01$) with AFM in romaine lettuce. The simple linear regression (Fig. 5) shows a positive correlation coefficient (r) of 0.99 and a coefficient of determination (R^2) of 0.98. These results are similar to those reported by Cunha *et al.* (2015) with correlation coefficients between 0.92-0.98 in arugula. Also, Cho *et al.* (2007) suggested that the SPAD readings could be used in the field to estimate and project growth rates and predict yields in the cucumber crop. Therefore, SPAD can be considered as a tool to quickly and reliably estimate the AFM in lettuce.

Correlation between the atLEAF readings and AFM (Fig. 5) was highly significant ($\alpha = 0.01$) and presented a 'good fit' to the linear regression ($r = 0.98$, $R^2 = 0.97$, $RMSE = 0.04$). These results are similar to those reported by Dey *et al.* (2016) who obtained values of $r = 0.97$ and $R^2 = 0.95$ in betel vine.

AFM in lettuce is important because it is closely related to the yield of this crop. According to our results, the use of both SPAD and atLEAF meters could be used as a tool to predict potential yield.

Correlation between SPAD and atLEAF readings

SPAD and atLEAF readings show a high linear correlation coefficient ($r = 0.98$, $R^2 = 0.96$, $RMSE = 0.06$, $\alpha = 0.01$) (Fig. 6). These results are similar to those reported by Dunn *et al.* (2018b) who obtained a correlation coefficient of 0.80 to 0.95 between the SPAD and atLEAF readings in poinsettia leaves. Zhu *et al.* (2012) found significant correlations between the SPAD and atLEAF readings for corn, barley and potato ($R^2 = 0.90-0.92$). Both of these studies concluded that the atLEAF meter represented a cheaper alternative to the SPAD meter.

AtLEAF meter may be preferable as it is more affordable and is 80% cheaper than SPAD meter. However, it is important to mention that the readings of SPAD and atLEAF in leaves may not be representative of the entire plant canopy and should therefore be viewed with a certain amount of caution.

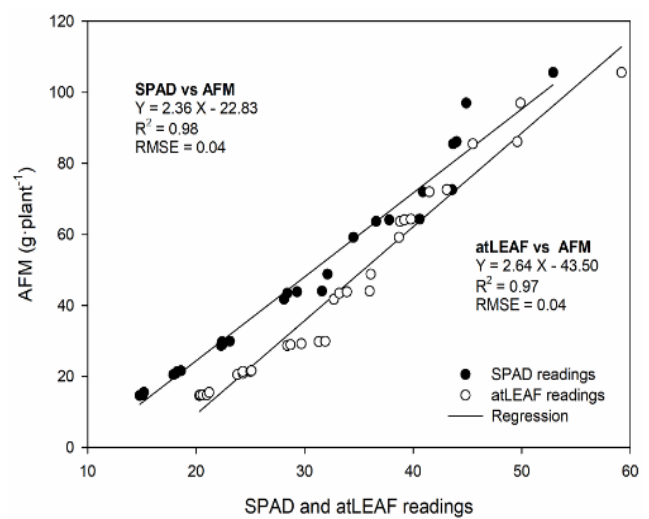


Fig. 5. Relationship between AFM, SPAD and atLEAF readings in romaine lettuce

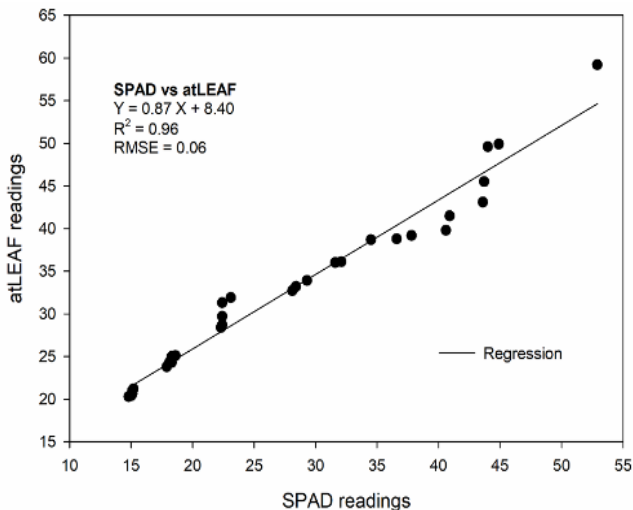


Fig. 6. Correlation between SPAD and atLEAF readings in romaine lettuce

Conclusions

This study shows that both meters (SPAD and atLEAF) accurately predict leaf N and Chl concentrations and AFM in romaine lettuce from linear regression equations. SPAD readings had high, positive and significant linear correlations with N ($R^2 = 0.90$), Chl concentration ($R^2 = 0.97$) and AFM ($R^2 = 0.98$), AtLEAF readings had a similar linear correlation with N ($R^2 = 0.91$), Chl concentration ($R^2 = 0.92$) and AFM ($R^2 = 0.97$). Both the SPAD and atLEAF readings allow for early estimates of plant nitrogen and chlorophyll status that can be used to schedule fertilizer application more efficiently during the growing season without affecting the development of the plants. The relationship between SPAD and atLEAF readings was also high, positive, and had a significant linear correlation ($R^2 = 0.96$). Both SPAD and atLEAF meters can be used to non-destructively and accurately estimate the N status of lettuce, in a reliable and quick manner during the crop production cycle. In addition, atLEAF meter is currently more affordable than SPAD meter.

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