Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Estimation of lettuce shoot nitrogen via a portable spectrophotometer

Fernando Ferrari Putti
fernando.putti@unesp.br

São Paulo State University (UNESP)
Pedro Henrique Lupo Guerrero
São Paulo State University (UNESP)
Jéssica Pigatto Queiroz Barcellos
São Paulo State University (UNESP)
Bruna Arruda
São Paulo State University (UNESP)
Angela Vacaro de Souza
São Paulo State University (UNESP)

Research Article

Keywords: Chlorophyll. Photosynthesis. Lactuta sativa L. Macronutrient. Sensor

Posted Date: May 30th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4389878/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Lettuce are vegetables with a high commercial value and a short cycle production, which requires precise managements to guarantee a profitable production. For this, the nutritional providing is an important factor, highlining the nitrogen, a macronutrient considered essential for the proper development of plants due to its participation in the composition of the main plant molecules, such as chlorophyll. In this sense, non-destructive strategies to monitor the balance nutrition is fundamental to avoid deficiency or excess of nutrients during the lettuce cycling, and the agriculture 4.0, brings to light new technological means to achieve this goal. This work aimed to verify the potential to use a portable spectrophotometer to estimate N shoot in lettuce, as a non-destructive, precise, quickly, cheap, waste free option to be operated by the lettuce farmers, on farm, for the N monitoring, based on the destructive laboratory analysis for N shoot and chlorophyll, and the widely used sensor chlorophyll meter (SPAD). For this, a greenhouse experiment was conducted with lettuce cropped under N levels input, corresponding to 0, 25, 50, 75, 100 and 125% of the lettuce recommendation. The estimation for N in the lettuce shoot was performed by destructive analysis: *i)* shoot N; *ii)* chlorophyll and non-destructive analysis: *iii)* chlorophyll meter SPAD and *iv)* a portable spectrophotometer. Non-destructive measurements were performed 3 times at: 20, 23 and 26 days after transplanting (DAT). The lettuce harvest occurred 28 DAT, to determine N shoot uptake and chlorophyll, using a destructive way, and lettuce production. The estimation of N shoot in lettuce using the portable spectrophotometer showed a high correlation to the standard destructive laboratory analysis and the chlorophyll meter (SPAD), showing high explanation of the data and so a high potential to estimate N shoot in lettuce using the proposed portable spectrophotometer as an optimum non-destructive, precise, quickly, cheap, waste free option to be operated by the lettuce farmers, on farm, for the N monitoring.

1 Introduction

Lettuce (*Lactuta sativa* L.) is a culture native from Asia, but nowadays is cultivated in all over the world, figuring among the most widespread leafy crops, with great production and consumption. According to data from the United Nations Food and Agriculture Organization (FAOSTAT, 2021), in 2020, approximately 27.3 million Mg of lettuce were cropped, where China was responsible for 52% of world production, followed by the United States and India, and Brazil figured with 576 thousand Mg. The introduction of lettuce in Brazil took place in the 16th century by the Portuguese settlers, and nowadays is the most consumed vegetable in this country. The lettuce production in Brazil is concentrated in small areas (average of five hectares per farm), cultivated in all Brazilian territory to attend the demand due to its high perishability and short time for transport and storage.

Due to their morphological leaf characteristics, most of the lettuce cultivars are responsive to elements that promote vegetative growth, such as the macronutrient Nitrogen (N). Plants metabolize this macronutrient through biochemical reactions involving oxidation and reduction, thus creating organic compounds such as nucleic acids, cell walls, and amino acids (proteins) (Marschner, 2011; Taiz et al., 2017). The availability of N to the plant influences the chlorophyll content, number, and composition of chloroplasts (Marchiori et al., 2014), which is directly related to its photosynthetic capacity and indirectly to the alterations in the synthesis of proteins related to the cell division in the photosynthetic area, and so plant growth (Lawlor, 2002). Chlorophyll is among the pigments responsible for absorbing sunlight and carrying out a wide range of plant processes, such as phototropism, photomorphogenesis, stomata opening and leaf photosynthetic functioning. This pigment has the spectrum of absorption of light energy in the blue regions (430 nm wavelength) and in the red regions (660 nm wavelength), being a narrow light spectrum close to the maximum absorbance of chlorophyll (Muneer, et al., 2014), whereas in the green region the absorption of light energy is low, with higher reflectance values (Taiz et al., 2017).

The decrease in the amount of chlorophyll in the plants reduces the light energy absorbed by the plant, thus increases the reflectance in this waveband responsible for providing the necessary energy to start the photosynthetic chain (Taiz et al., 2017). Thus, the N deficiency may lead to a reduction in the plant capacity to carry out the photosynthesis (Mu et al., 2018), and hence may reduce the plant biomass accumulation (Taiz et al., 2004) by the decreasing in the number of leaves and plant growth (de Almeida et al., 2011). Therefore, N becomes a limiting factor for the satisfactory development of lettuce if provided in deficient amount (Santos et al., 2011).

However, the excess of N also has a negative impact on lettuce productivity, as this macronutrient increases leaf senescence and impairing head formation (Sala et al., 2008). Additionally, the excess of N in lettuce may lead to high concentrations of nitrate in the leaf, which may represent a risk to human health if ingested in excess (Miyazawa et al., 2001).

The above raises the importance of the adequate management of this macronutrient for a sustainable production of this type of leafy crop, which highlighting the importance of monitoring the proper handling of this element to obtain a final product with satisfactory nutritional and commercial quality (Milhomens et al., 2015). As lettuce is a crop with short production cycle the fertilization must be precise, and the monitoring become important. Under adequate production conditions, the lettuce cultivation can be conducted throughout the year, generating a quick financial return to the producer and high availability to the consumer (Candido et al., 2018). However, the standard nutrition analysis requires destructive sampling of the plants.

To proceed the analysis for monitoring purpose, the farmers must send the plant material to a specialized laboratory, which may not be close to the farm. In general, the nutritional analyses are based on tissue extractions and digestions, representing a waste generation. Therefore, the traditional nutrient monitoring represents costs, spending of time until the farmers get to the results and requires an adequate management of the waste generation to avoid environmental impacts.

In this sense the use of technologies related to precision agriculture has become popular among the farmers from the most diverse crops to monitor variables that may affect the productivity, which is associated with the popularization of Agriculture 4.0. New technologies are being studied to allow non-destructive analysis, that does not generate contaminating waste, with quickly measuring, high precision, using equipment that are easily operated by no specialized labor, and that require minimal maintenance.

In this context a wide range of equipment are being used, such as sensors. A sensor can be defined as a device responsive to provoke a stimulus, which can be physical or chemical, generating a signal that can be translated into magnitudes to measure and/or monitor variables. There are sensors designed for the most varied magnitudes, with different ranges, frequencies, and precision.

The chlorophyll meter (SPAD, Soil Plant Analysis Development) is widely used to perform indirect, quick, and non-destructive measurements on the chlorophyll content present in plants (Primo et al., 2014). The analysis consists in the measurement of the difference between the absorbed and not absorbed light by the chlorophyll molecule, generating values proportional to the amount of chlorophyll present in the plant leaves (Martins et al., 2010). The variations in the chlorophyll amount present in the leaves may conduct to some inferences regarding the of N content in the plant (Bassi et al., 2018), where low amount of chlorophyll may be related to N deficiency. However, this sensor presents a high acquisition cost and requires specialized labor for handling.

Thus, other sensors, cheaper and easy handle, have been developed and present potential to be used to determine the photosynthetic activity and N content in the shoot, depending on the calibration for it. One of these sensors is the portable spectrophotometer, a sensor that has a potential to be used for biological and physical-chemical measurements. This sensor can measure, after contact with the sample, the number of absorbed photons, and so could be applied to measure the photosynthetic capacity of plants, considering that the greater the light absorption, the higher the photosynthetic rate (Gil et al., 2002). Through a spectrophotometer, part of the light is absorbed by the chlorophyll present in the leaves, and the rest is reflected by encountering the detector cell of the equipment, being converted into an electrical signal, and subsequently interpreted by sensors. So, this sensor is capable to measure the amount of light that is reflected by the leaves, where the amount of light that is absorbed by the sensor is inversely proportional to the amount of light absorbed by the chlorophyll molecules (Maria et al., 2007). The advantage of the spectrophotometer is more precise, quicker, and low cost, suitable for small farmers, in comparison to the chlorophyll meter (SPAD).

The hypothesis tested here is that there is a correlation between the photosynthetic activity estimation obtained with destructive analysis of chlorophyll and non-destructive analysis for chlorophyll using SPAD, the standard equipment used nowadays, and destructive N content determination with the results obtained by a portable spectrophotometer, as an innovative equipment, in lettuce. This work aimed to verify the potential to use a portable spectrophotometer to estimate N shoot in lettuce, as a non-destructive, precise, quickly, cheap, waste free option to be operated by the lettuce farmers, on farm, for the N monitoring, based on the destructive laboratory analysis for N shoot and chlorophyll, and the widely used sensor chlorophyll meter (SPAD).

2 Material and methods

2.1 Experimental area

The experiment was carried out in a greenhouse (22 ± 4 °C), in the Experimental Area of the Faculty of Sciences and Engineering, Tupã *campus*, São Paulo, with geographic location defined by the coordinates 22° 51' South Latitude (S) and 48° 26' Longitude West (W) and average altitude of 786 meters above sea level.

The greenhouse was a tunnel type, having the following dimensions: 25 m long; 6 m wide, in arch shape, with 5 m heigh in the center and 3 m heigh on the sides covered in the top and sides with an additive transparent polyethylene film (150 μm thick).

2.2 Experimental design and treatments

The experiment adopted a randomized block design and consisted of six N concentrations availability, applied as urea (45% N), corresponding to 0, 25, 50, 75, 100 and 125% of the recommendation (0, 10, 20, 30, 40, 50 kg ha^{-1}) by Boletim 200 (da Eira Aguiar et al., 2014), with 4 replications, totaling 24 pots.

2.3 Planting seedlings and crop management

A curly lettuce (Verônica cultivar) was used, and the seedlings were obtained at the company Proteto Agrícola, specialized in horticulture, in the municipality of Tupã - São Paulo. The transplant occurred 25 days after of sowing into polyethylene pots (2 L capacity) filled with soil classified as Eutrophic Red Yellow Argisol (Soil Survey Staff, 1999), with a sandy/medium texture. Soil liming and fertilization were carried out according to the recommendations of Boletim 200 for lettuce (da Eira Aguiar et al., 2014), except for N, which followed the treatments. Liming occurred 15 days before transplanting and the fertilization and treatment application was performed during the transplanting. The irrigation was carried out manually twice a day during the entire experimental period, at 08:00 and at 18:00, to maintain the field capacity.

2.4 Non-destructive analysis for N estimation of lettuce

The estimation for N in the lettuce shoot was performed by non-destructive analysis, using two equipment: *i)* a CFL-1030 chlorophyll meter (SoilControl – Instrumentos de Medição & Pesquisa) (Falker, 2008) and *ii)* a portable Nix Spectro 2 spectrophotometer (NIX SENSOR LTDA, 2021). Measurements were performed 3 times (72 h interval) at: 20, 23 and 26 days after transplanting (DAT). For this, the youngest and fully expanded leaves, more externally to the main vein of the leaf, were sampled. The analyses were conducted in the morning, at 9:00 am.

The chlorophyll meter analyzed three bands of wavelength in the light spectrum, showing the content of chlorophyll A (μmol m^2), chlorophyll B (μmol m^2) and total chlorophyll (A + B) (μmol m^2). The portable spectrophotometer generated absorbance values, in the intervals of 400–700 nm wavelengths (λ) (10 nm interval). The amount of light absorbed by the chlorophyll molecules A(λ) was obtained by the Eq. (1):

$$A(\lambda) = 1 - R(\lambda) \quad (1)$$

Where: A(λ) is the light absorbed by the chlorophyll, R(λ) is the light reflected by the leave (reading of the spectrophotometer).

The absorbance (A) values were considered for the wavelengths λ = 430 and λ = 660 nm (standard for chlorophyll).

2.5 Destructive analysis for N and chlorophyll determination and production of lettuce

The lettuce harvest occurred 28 DAT. In this occasion, plants were removed from the soil, the leaves were detached from the stem, quantified through manual counting and fresh biomass weight was obtained. A leaf per plant was sampled and kept in -80 °C until photosynthetic pigment analysis in the laboratory. Chlorophylls a and b were determined according to the methodology proposed by Lichtenthaler (1987). The left shoot material was dried in the oven (72 h; at 65 °C) until constant weight to obtained by shoot dry matter. The N content in the shoot was obtained by crushing the shoot dried material using a Wiley mill (Tecnal Equipamentos, Piracicaba-SP, Brazil) and sieved through 1 mm mesh. The N content in the shoot were determined according to the method proposed by Malavolta et al. (1997). Lettuce shoot N uptake (N_{uptake}) was calculated using the followed Eq. (2):

$$N_{uptake} = DM \times N_{content} \quad (2)$$

Where: N_{uptake} is the N up taken by the lettuce (mg plant^{-1}); DM is the shoot dry matter per plant (g plant^{-1}); and $N_{content}$ is the lettuce shoot N content (mg g^{-1}).

2.6 Statistical analysis

All sets of data obtained were submitted to a regression adjustment. The graphs were elaborated using SigmaPlot 12.5 software developed by Systad Software Inc. (2022). A Principal Component Analysis was performed, with biplot, using Past 4.10 software.

3 Results

3.1 Effect of N availability levels on lettuce N shoot uptake and production

The N doses used in experiment did not affect the number of leaves per plant (Fig. 1A). However, the N uptake increased as the N level incremented, following the regression (2nd order) (Fig. 1B), and hence increased the fresh shoot mass per plant (2nd order) (Fig. 1C).

Source: the authors. Scale

3.2 Lettuce shoot chlorophyll measurement by chlorophyll meter SPAD under N availability levels

Using a chlorophyll meter SPAD, the standard method, the Chlorophyll A and B were measured, and the measurements increased as N input treatments increased, allowing a regression adjustment (2nd order). The results showed the same trend among the days evaluated (20, 23 and 26 DAE) (Fig. 2).

3.3 Lettuce shoot absorbance obtained with portable spectrophotometer under N availability levels

The multivariate analysis showed a separation among the results from the N dose treatments across the axis x (76.832% data explanation), where, on the left side were located the lower input of N treatments, below the recommended dosage for lettuce. On the other hand, on the right side, the higher N input, the recommended dose (40 kg ha^{-1}) and the overdose (50 kg ha^{-1}), were located (Fig. 3). Using a biplot, the data of reflectance (1 - abs), obtained in the wavelength range 400 to 700 nm (10 nm interval) were correlated to the recommended dose, but opposite to the low dosages, where high correlation were obtained in the 430 and 660 wavelengths, highly related to the chlorophyll range.

In this way, a linear adjustment (1st order) between N application (kg ha^{-1}) and reflectance, for both 430 and 660 nm was observed, following the same trend for 20, 23 and 26 DAE (Fig. 4). For all the linear adjustments, high explanation was observed with $R^2 \geq 0.95$, in average (Fig. 4).

3.4 Validation of lettuce N shoot estimation using destructive method

Using the destructive method for chlorophyll and N determination a high correlation was observed, with $R^2 = 0.8828$ and $R^2 = 0.8898$, for chlorophyll A and B, respectively (Fig. 5).

3.5 Validation of lettuce N shoot estimation using non-destructive methods

The relationship between lettuce shoot N uptake and chlorophyll A and B showed high explanation. Chlorophyll A showed the highest explanation at 26 DAT ($R^2 = 0.9467$) and chlorophyll B at 20 DAT ($R^2 = 0.9554$) (Fig. 6).

In the same way, the relationship between the absorbance (A) and the lettuce shoot N uptake, showed higher explanation, where the results obtained at 26 DAT, for both 430 and 660 nm, showing a value of $R^2 = 0.9349$ and $R^2 = 0.9276$, respectively (Fig. 7).

The relationship between the absorbance A (430nm) and A (660 nm), obtained with the portable spectrophotometer, and the chlorophyll a and b, obtained with chlorophyll meter SPAD, showed in average a $R^2 = 0.5$ (Fig. 8).

4 Discussion

The N application under the recommended dosage guaranteed the lettuce production. Applications below the recommended N dose harmed the lettuce development, showing effect of N deficiency, reflecting in low leaves development. Broadley et al. (2000) investigated the effects of nitrogen deficiency on growth components of lettuce and observed that there were effects of treatment on morphological and physiological components of growth, where the assimilation of carbon and the leaf weight ratio decreased in N- limited plants.

On the other hand, higher N dosages showed a trend to reduce the N shoot lettuce uptake, indicating a waste of N input, as the plant is not able to uptake more that its need. The application of N under higher doses also showed a trend to reduce the fresh shoot mass per plant. Bottoms et al. (2012) examined the Nitrogen requirements in California lettuce production areas under pressure to improve nitrogen (N) and concluded that high soil NO_3-N (greater than 20 $mg{\cdot}kg^{-1}$) was a reliable indicator that N application could be reduced or delayed. Therefore, the present study focused on the monitoring for the recommended N dosage application.

To monitor the N level, some destructive methods may be used by the farmers as strategies to monitor the plants nutritional levels. The determination on laboratory for the chlorophyll and N content are great indicator for the N nutritional levels. The results showed high correlation between those destructive analysis for lettuce, indicating that the determination of one of them are enough to monitor the nutritional state of the lettuce. The choose for the analysis may be relay on the cost, availability of the analysis in the closest laboratory and the amount of residual material generated by the analysis. The results indicated that high N in the plant reduced the chlorophyll A and B determined in the laboratory, indicating that the optimum levels are 40 and 13 for chlorophyll A and B, respectively.

Using non-destructive methods to estimate the nutritional state of the lettuce, the chlorophyll meter SPAD, as standard equipment, was used and showed a positive relationship for the N dosages input. When the N recommended dose for lettuce was applied the chlorophyll a was about 20 and for chlorophyll b showed values about 3.0. Considering the lettuce response to the N input levels, regarding the N up taken by the lettuce shoot, the chlorophyll SPAD data showed high relationship with N uptake. As N is an important constituent of the chlorophyll molecule, the measure of chlorophyll is a recommended parameter to estimate N in the plants. Mendoza-Tafolla et al. (2019) also estimating Nitrogen and Chlorophyll Status of Romaine Lettuce using chlorophyll meter, SPAD and atLEAF, and found high, positive, and significant linear correlations with N (R^2 = 0.90) and Chlorophyll (R^2 = 0.97).

However, nowadays, the improvement of the agricultures system is leading to the searching for advances equipment and studies with new sensors are become fundamental. In this sense, a wide range of portable spectrophotometer has risen the attention for the evaluation of parameters that involve color. Barros et al. (2021) studied a sensor to evaluate the quality control of extra virgin olive oil and adulterated compound oils. Moreover, a pocket-sized sensor was studied by Borges et al. (2022) to control color acquisition of plant leaves and observed that, in both RGB and CIELab color systems, the

Nix™ Pro color sensor was able to successfully predict differentiate crops, mainly for mango, quinoa, peach, pear, and rice.

In the same way, here, the portable Nix Spectro 2 spectrophotometer (NIX SENSOR LTDA, 2021) was tested to estimate the N, highly related to the chlorophyll pigmentation, reflected by green color. The results for the proposed portable spectrophotometer showed a high correlation with the calculated absorbance. The data indicated a high association with the recommended dose and the absorbance on the wavelength 430 and 660 nm. This may be related to the wavelength related to the chlorophyll range. For the portable spectrophotometer, the recommended N doses indicated absorbance values around the 0.94 for the A (430 nm) and 0.92 for the A (660 nm). Values below may represent a N deficiency. On the other hand, higher values may represent an excess of N in the shoot, related to luxury uptake and high production costs.

Studying the correlation between the chlorophyll and the data obtained with the portable spectrophotometer, values of $R^2 = 0.5$ were observed. Malek et al. (2012) studied the performance of a spectral sensor (TCS230, TAOS, USA) along with an AVR microcontroller (ATmega32, Atmel, USA) (620–700 nm) for the assessment of nitrogen content in lettuce plants and obtained correlation between sensor and chlorophyll meter of $R^2 = 0.22$. Therefore, the portable spectrophotometer tested here indicated a higher correlation with the standard, which may be related to higher technology applied and wider range of wavelength of the sensor. Likewise, the results showed a high relationship between the absorbance and N shoot uptake, with a strong stability for the absorbance A (660 nm), with high explanation of the data (R^2), for all the data set (20, 23, and 26 DAT). Therefore, for the greenhouse measurements, the recommendation for the N shoot monitoring, is to get the readings with the portable spectrophotometer at 20 to 26 DAT, obtaining reflectance around 0.08, corresponding the absorbance of 0.92, for the wavelength 660 nm. The results obtained in this greenhouse experiment are important to provide an initial background for larger scale areas.

5 Conclusions

The estimation of N shoot in lettuce using the portable spectrophotometer showed a high correlation to the standard destructive laboratory analysis of N, and non-destructive analysis, with the chlorophyll meter (SPAD), showing high explanation of the data and so a high potential to estimate N shoot in lettuce using the proposed portable spectrophotometer as an optimum non-destructive, precise, quickly, cheap, waste free option to be operated by the lettuce farmers, on farm, for the N monitoring.

Declarations

Authorship contribution statement

Fernando Ferrari Putti: Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Bruna Arruda; Pedro Lupo Gueireiro: Writing – original draft, Writing – review & editing, Visualization. Jessica Pigatto Queiroz Barcelos: Conceptualization, Writing – review & editing, Angela Vacaro de Souza: Resources, Funding acquisition, Investigation, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Funding Declaration

Fernando Ferrari Putti would like to thank the São Paulo Research Foundation (FAPESP) for the research fellowship (Grant number 2016/20365-1) and Angela Vacaro de Souza the research fellowship (Grant number 2020/ 14166-1)

References

1. Barros, I. H., Paixão, L. S., Nascimento, M. H., Lacerda Jr, V., Filgueiras, P. R., & Romão, W. (2021). Use of portable Raman spectroscopy in the quality control of extra virgin olive oil and adulterated compound oils. Vibrational Spectroscopy, 116, 103299. https://doi.org/10.1016/j.vibspec.2021.103299
2. Bassi, D., Menossi, M., & Mattiello, L. (2018). Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. Scientific reports, 8(1), 1–13. https://doi.org/10.1038/s41598-018-20653-1
3. Borges, C. S., Chakraborty, S., Weindorf, D. C., Lopes, G., Guilherme, L. R. G., Curi, N., … Ribeiro, B. T. (2022). Pocket-sized sensor for controlled, quantitative and instantaneous color acquisition of plant leaves. Journal of Plant Physiology, 272, 153686. https://doi.org/10.1016/j.jplph.2022.153686
4. Bottoms, T. G., Smith, R. F., Cahn, M. D., & Hartz, T. K. (2012). Nitrogen requirements and N status determination of lettuce. HortScience, 47(12), 1768–1774. https://doi.org/10.21273/HORTSCI.47.12.1768
5. Broadley, M. R., Escobar-Gutierrez, A. J., Burns, A., & Burns, I. G. (2000). What are the effects of nitrogen deficiency on growth components of lettuce?. The New Phytologist, 147(3), 519–526. http://doi.org/10.1046/j.1469-8137.2000.00715.x
6. Candido, W. d. S., Soares, R. S., Franco, C. A., Diniz, G. M. M., Silva, E. H. C., Marin, M. V., & Braz, L. T. (2018). Stability and adaptability of curled green-leaf lettuce lines using the REML/Blup mixed model. Pesquisa Agropecuária Brasileira, 53, 298–306. http://dx.doi.org/10.1590/S0100-204X2018000300004

7. da Eira Aguiar, A. T., Gonçalves, C., Paterniani, M. E. A. G. Z., Tucci, M. L. S. A., & de Castro, C. E. F. (2014). Instruções Agrícolas para as Principais Culturas Econômicas: IAC, Campinas. 7 ed. 452 p.
8. de Almeida, T. B. F., de Mello Prado, R., Correia, M. A. R., Puga, A. P., & Barbosa, J. C. (2011). Avaliação nutricional da alface cultivada em soluções nutritivas suprimidas de macronutrientes. Biotemas, 24(2), 27–36. https://doi.org/10.5007/2175-7925.2011v24n2p27
9. Falker (2008). Manual do medidor eletrônico de teor clorofila (ClorofiLOG/CFL 1030). In: Falker Automação Agrícola Porto Alegre.
10. FAOSTAT. Food and Agriculture Organization of the United Nations, 2021. https://www.fao.org/faostat/en/#data/QCL/visualize
11. Gil, P. T. d., Fontes, P. C. R., Cecon, P. R., & Ferreira, F. A. (2002). Índice SPAD para o diagnóstico do estado de nitrogênio e para o prognóstico da produtividade da batata. Horticultura Brasileira, 20, 611–615. https://doi.org/10.1590/S0102-05362002000400020
12. Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. Journal of experimental Botany, 53(370), 773–787. https://doi.org/10.1093/jexbot/53.370.773
13. Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol, 148, 350–382. https://doi.org/10.1016/0076-6879(87)48036-1
14. Malavolta, E., Vitti, G. C., & Oliveira, S. A. D. (1997). Avaliação do estado nutricional das plantas: princípios e aplicações.
15. Malek, M., Massah, J., & Dehghan, M. (2012). Application of a spectral sensor for the assessment of nitrogen content in lettuce plants. Australian Journal of Crop Science, 6(2), 188–193.
16. Marchiori, P. E., Machado, E. C., & Ribeiro, R. V. (2014). Photosynthetic limitations imposed by self-shading in field-grown sugarcane varieties. Field Crops Research, 155, 30–37. https://doi.org/10.1016/j.fcr.2013.09.025
17. Maria, J., Ongarelli, G., Aguila, S., Alfredo, R., Saavedra, J., Sasaki, F. F., Kluge, R. A. (2007). Cebolinha Minimamente Processadas. Revista Iberoamericana de Tecnología Postcosecha, [s. l.], p. 53–59.
18. Marschner, H. (2011). Marschner's mineral nutrition of higher plants. Academic press. 3 ed. 672 p.
19. Martins, A. D., Coelho, F. S., Leao, A. B., Silva, M. C. C., Braun, H., Fontes, P. C. R. (2010). Relação entre índice SPAD, teores de clorofila extraível e nitrogênio na folha de batata. Horticultura Brasileira 28: S835-S841
20. Mendoza-Tafolla, R. O., Juarez-Lopez, P., Ontiveros-Capurata, R. E., Sandoval-Villa, M., Iran, A. T., & Alejo-Santiago, G. (2019). Estimating nitrogen and chlorophyll status of romaine lettuce using SPAD and at LEAF readings. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47(3), 751–756. https://doi.org/10.15835/nbha47311525
21. Milhomens, K. K. B., Nascimento, I. R., de Castro Tavares, R., Ferreira, T. A., & Souza, M. E. (2015). Avaliação de características agronômicas de cultivares de alface sob diferentes doses de nitrogênio. Revista Verde de Agroecologia e Desenvolvimento Sustentável, 10(1), 22.

22. Miyazawa, M., Khatounian, C. A., & Odenath-Penha, L. (2001). Teor de nitrato nas folhas de alface produzida em cultivo convencional, orgânico e hidropônico. Agroecologia Hoje, 2, 23.
23. Mu, X., Chen, Q., Chen, F., Yuan, L., & Mi, G. (2018). Dynamic remobilization of leaf nitrogen components in relation to photosynthetic rate during grain filling in maize. Plant Physiology and biochemistry, 129, 27–34. https://doi.org/10.1016/j.plaphy.2018.05.020
24. Muneer, S., Kim, E. J., Park, J. S., & Lee, J. H. (2014). Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (Lactuca sativa L.). International journal of molecular sciences, 15(3), 4657–4670. https://doi.org/10.3390/ijms15034657
25. NIX SENSOR LTDA. Nix Spectro 2, 2021. https://www.nixsensor.com/wp-content/uploads/2021/10/Nix_Spectro_Spec-Sheet_v2-2_03.pdf
26. Primo, A. A., Melo, M. D., Vieira, L. V., Feitosa, T. S., de Souza, I. M., PEREIRA, G.,.. . de Souza, H. A. (2014). Teores de clorofila e índice SPAD em folhas de mudas de gliricídia em função da aplicação de diferentes doses de composto orgânico oriundo de resíduos de pequenos ruminantes.
27. RDC TEAM. (2013). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
28. Sala, F. C., da Costa, C. P., Teixeira, L. d. D., Fabri, E. G., & Blat, S. F. (2008). Reação de cultivares de alface a Thielaviopsis basicola. Horticultura Brasileira, 26, 398–400. https://doi.org/10.1590/S0102-05362008000300021
29. Santos, R. F., Borsoi, A., Tomazzoni, J. L., & Viana, O. H. (2011). Aplicação de nitrogênio na cultura da alface (Lactuca sativa L.). Varia Scientia Agrárias, 2(2), 69–77. https://e-revista.unioeste.br/index.php/variascientiaagraria/article/view/5441
30. Soil Survey Staff. 1999. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys, 2nd ed. Natural Resources Conservation Service, U.S. Department of Agriculture Handbook, 436p.
31. Taiz, L., Zeiger, E., Moller, I., & Murphy, A. (2004). Fisiologia vegetal. 3 ed. Porto alegre–Artmed, 613–641.
32. Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2017). Fisiologia e desenvolvimento vegetal. Artmed Editora

Figures

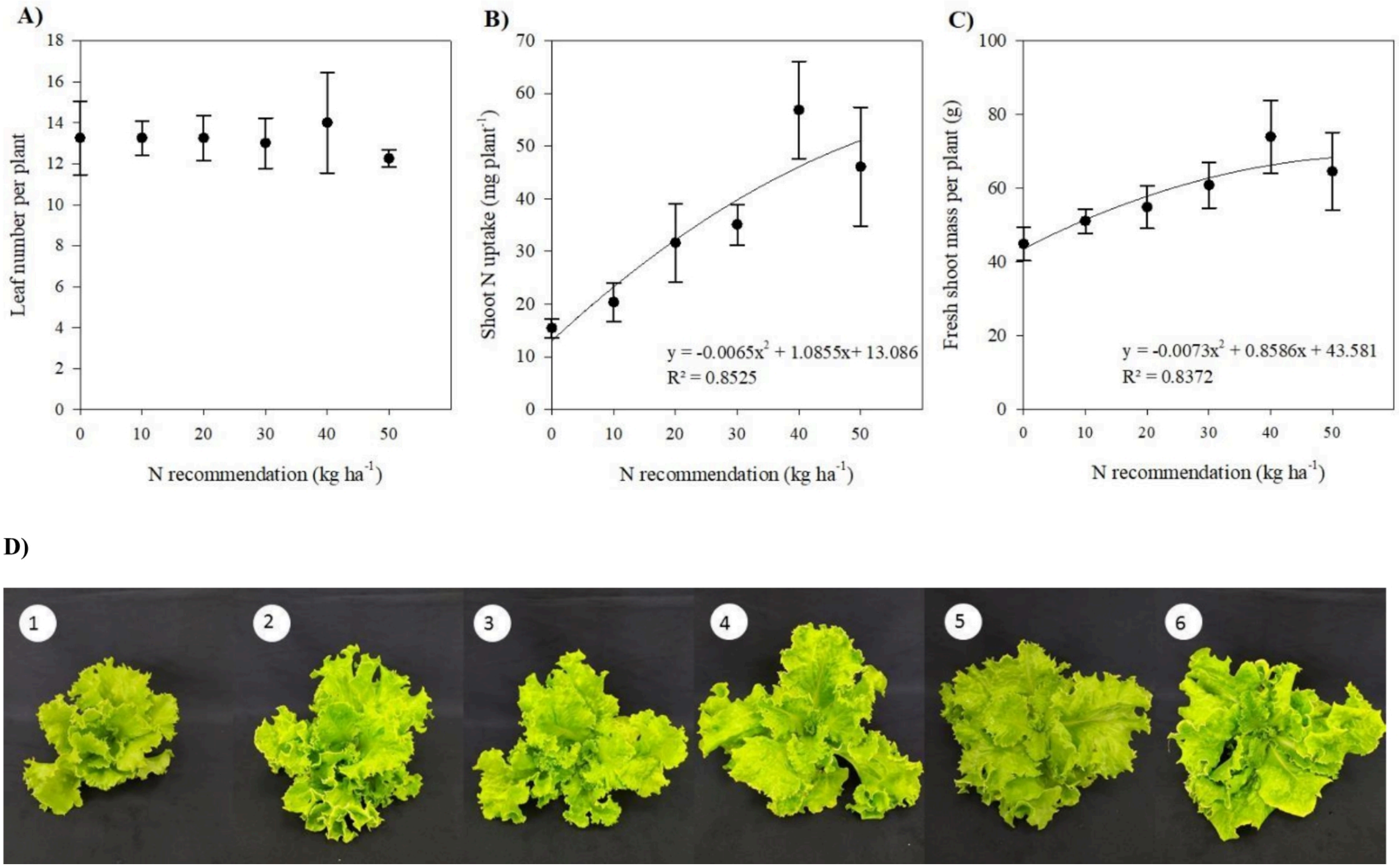

Figure 1

Production parameters: **A)** Leaf number per plant; **B)** Fresh shoot mass per plant **C)** Shoot N uptake per plant and **D)** plants register obtained in a greenhouse experiment, with lettuce, under six levels of N application according to the N recommendation to the culture by Boletim 200 (da Eira Aguiar et al., 2014) in percentage: 0, 25, 50, 75, 100 and 125 %, where 100 % correspond to the recommended dosage to the crop. The harvest was performed 28 days after transplanting (DAT). Error bars mean the standard deviation (n=4).

Source: the authors. Scale

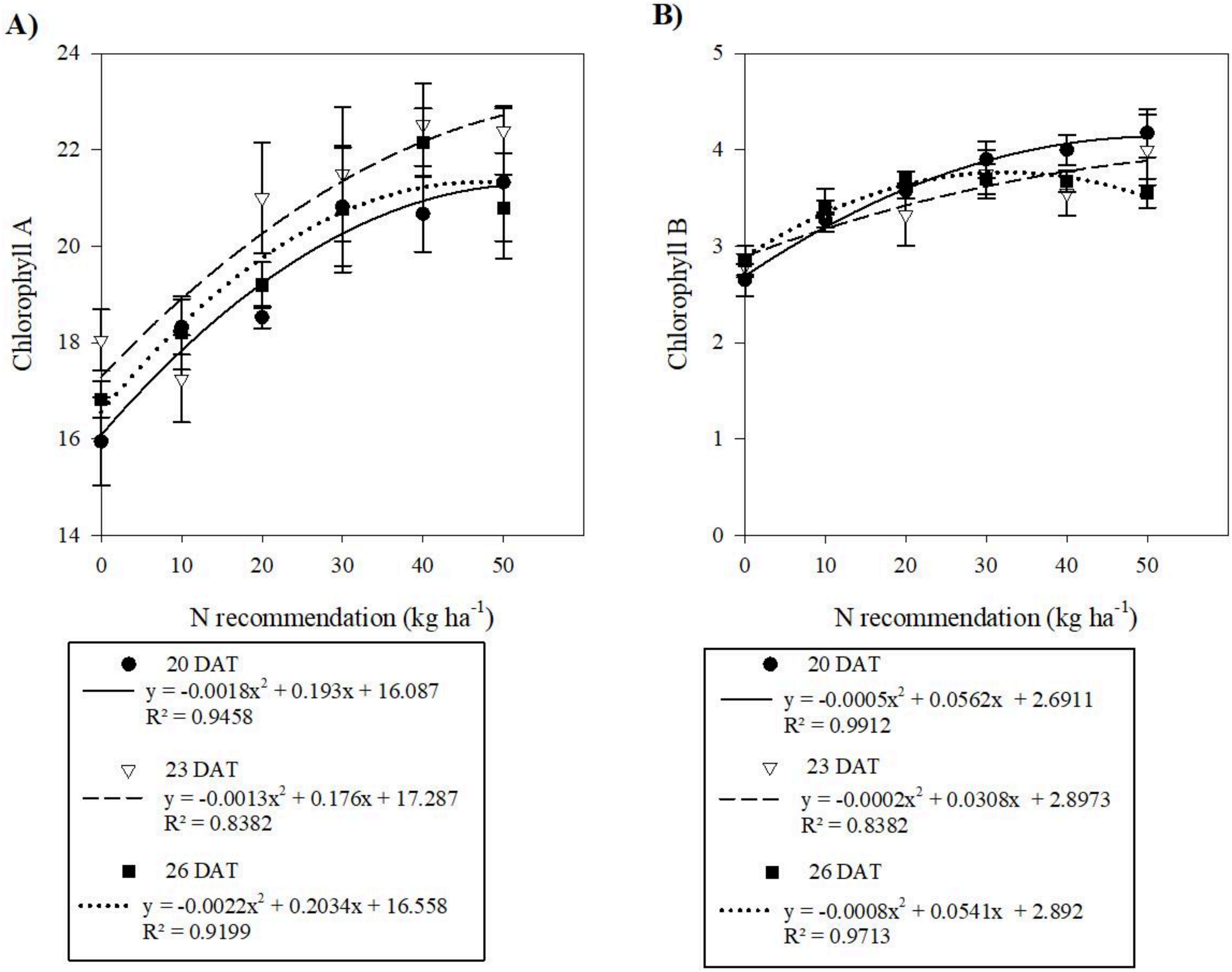

Figure 2

A) Chlorophyll A; **B)** Chlorophyll B obtained with chlorophyll meter SPAD in a greenhouse experiment, with lettuce, under six levels of N application according to the N recommendation to the culture by Boletim 200 (da Eira Aguiar et al., 2014) in percentage: 0, 25, 50, 75, 100 and 125%, where 100% correspond to the recommended dosage to the crop. The measurements were taken in three times: 20, 23 and 26 days after transplanting (DAT). Error bars mean the standard deviation (n=4).

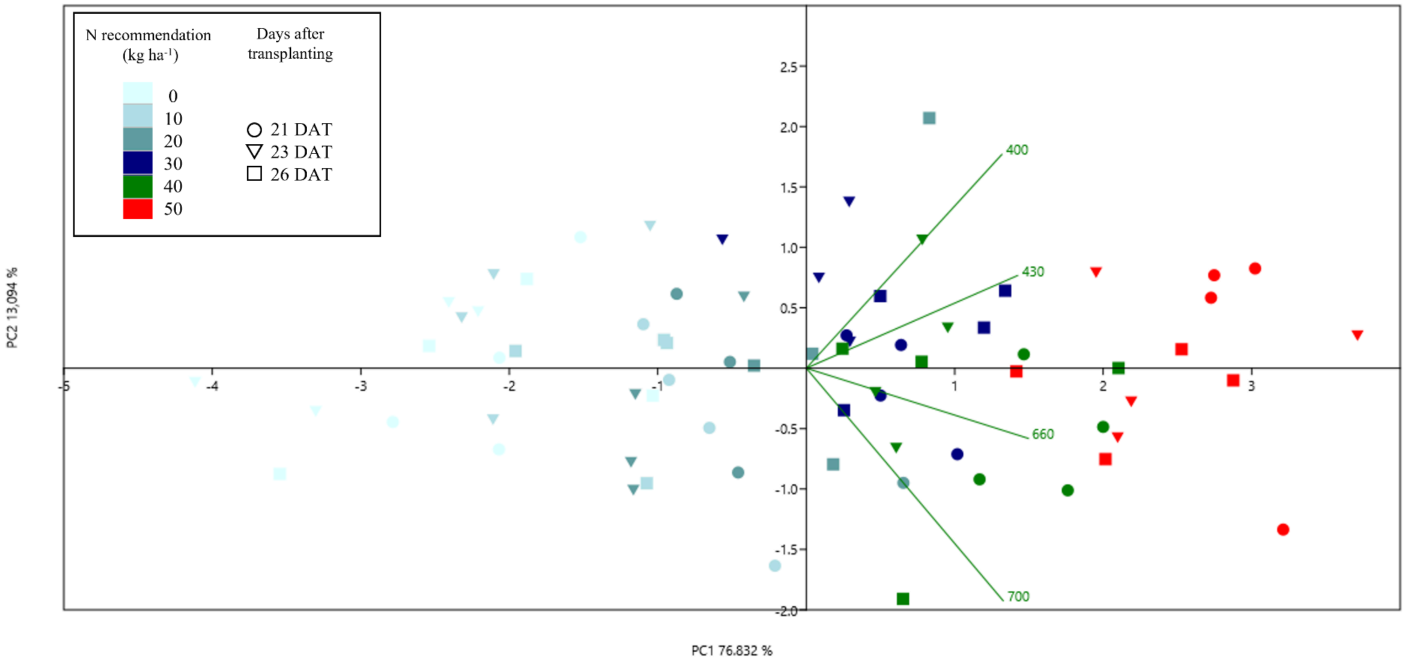

Figure 3

Principal component analysis (PCA) obtained in a greenhouse experiment, with lettuce, under six levels of N application according to the N recommendation to the culture by Boletim 200 (da Eira Aguiar et al., 2014) in percentage: 0, 25, 50, 75, 100 and 125%, where 100% correspond to the recommended dosage to the crop. The measurements were taken in three times: 20, 23 and 26 days after transplanting (DAT). Biplot represent the light absorbed by the chlorophyll (A(λ)): A (400 nm); A (430 nm); A (660 nm) and A (700 nm).

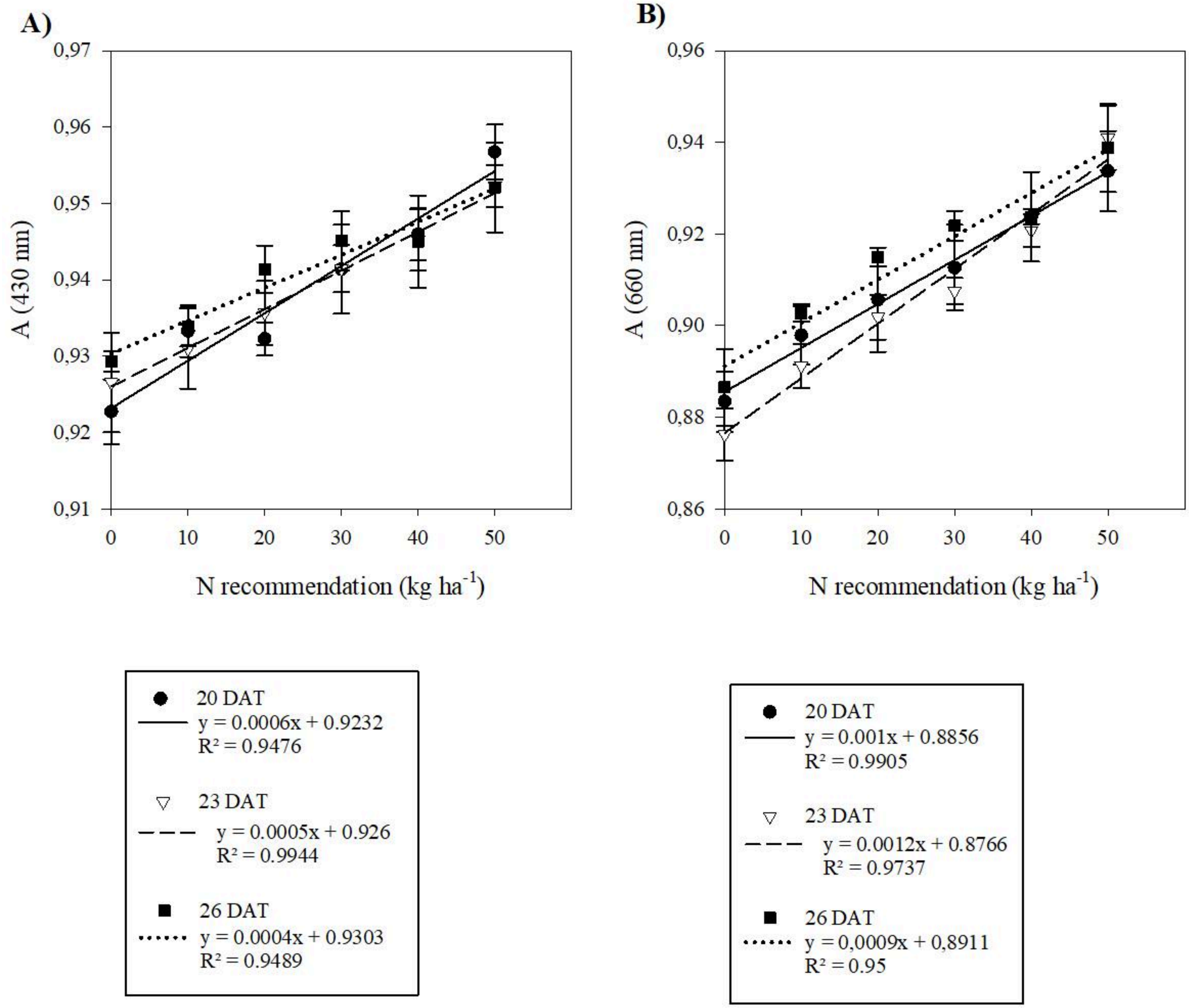

Figure 4

Light absorbed by the chlorophyll (A(λ)): **A)** A (430 nm); **B)** A (660 nm) obtained in a greenhouse experiment, with lettuce, under six levels of N application according to the N recommendation to the culture by Boletim 200 (da Eira Aguiar et al., 2014) in percentage: 0, 25, 50, 75, 100 and 125%, where 100% correspond to the recommended dosage to the crop. The measurements were taken in three times: 20, 23 and 26 days after transplanting (DAT). Error bars mean the standard deviation (n=4).

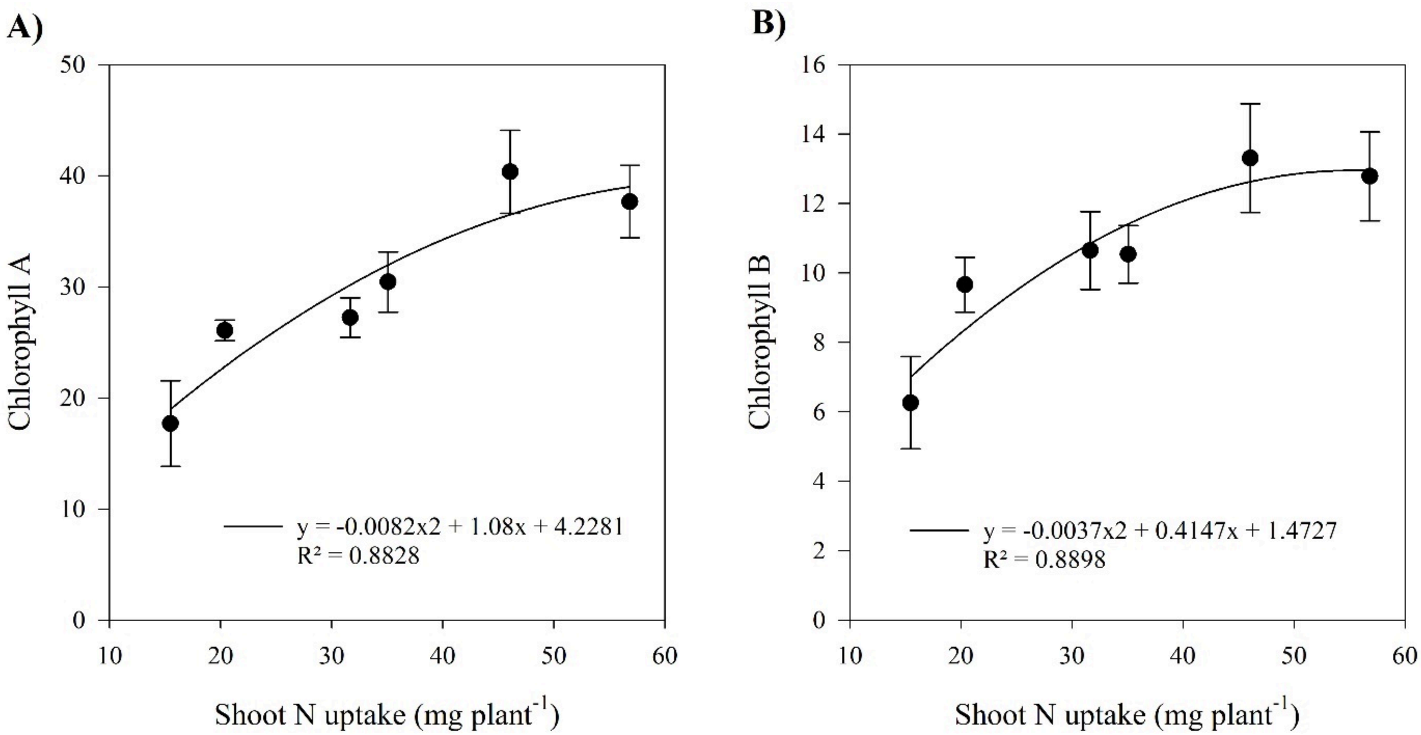

Figure 5

Relationship between N uptake and **A)** chlorophyll A and **B)** chlorophyll B, obtained using a non-destructive method (chlorophyll meter SPAD), in a greenhouse experiment, with lettuce, under six levels of N application according to the N recommendation to the culture by Boletim 200 (da Eira Aguiar et al., 2014) in percentage: 0, 25, 50, 75, 100 and 125%, where 100% correspond to the recommended dosage to the crop The harvest was performed 28 days after transplanting (DAT). Error bars mean the standard deviation (n=4).

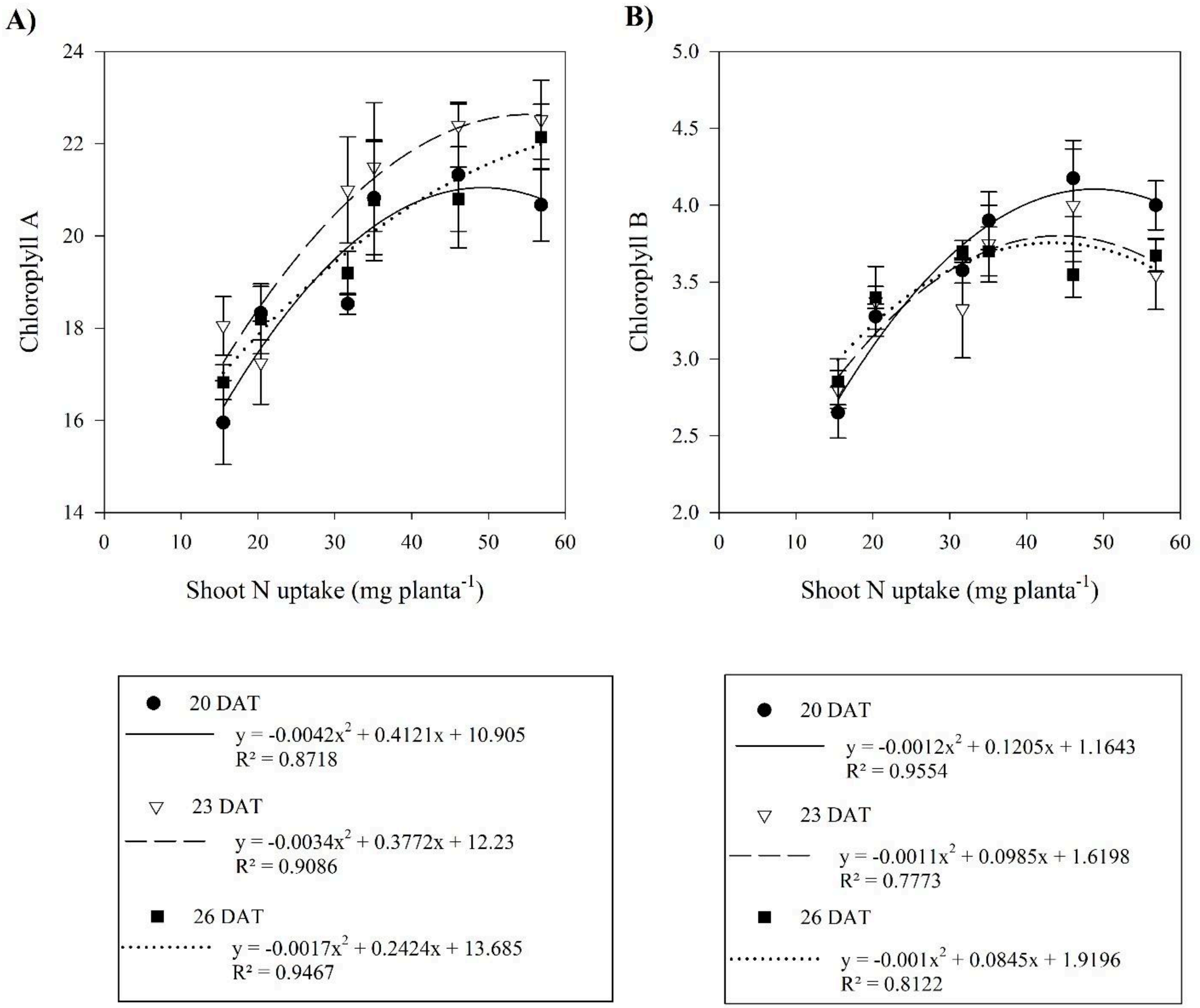

Figure 6

Relationship between N uptake and **A)** chlorophyll A and **B)** chlorophyll B, obtained using a non-destructive method (chlorophyll meter SPAD), in a greenhouse experiment, with lettuce, under six levels of N application according to the N recommendation to the culture by Boletim 200 (da Eira Aguiar et al., 2014) in percentage: 0, 25, 50, 75, 100 and 125%, where 100% correspond to the recommended dosage to the crop. The measurements were taken in three times: 20, 23 and 26 days after transplanting (DAT). Error bars mean the standard deviation (n=4).

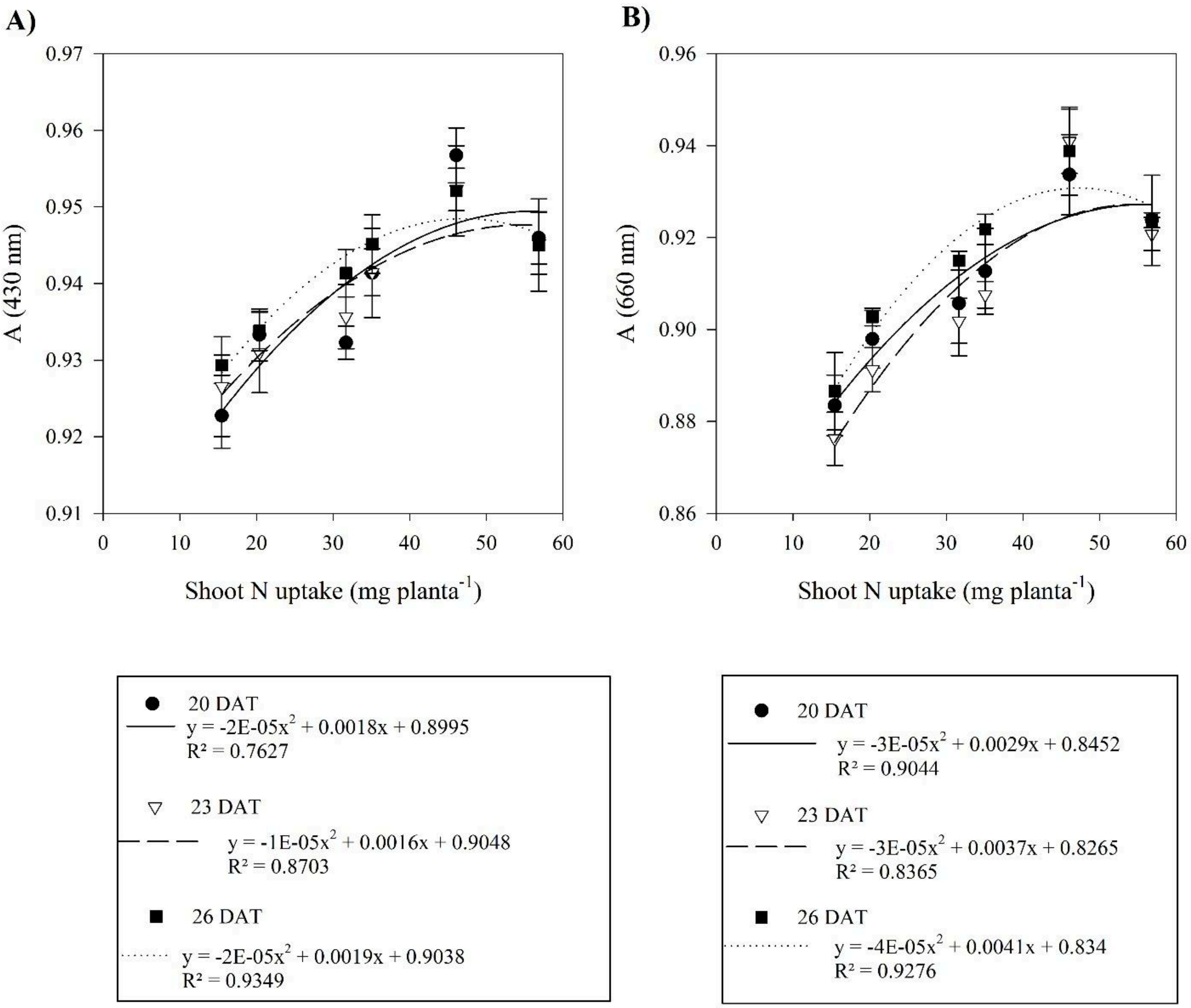

Figure 7

Relationship between light absorbed by the chlorophyll (A(λ)): **A)** A (430 nm); **B)** A (660 nm) and N uptake, obtained in a greenhouse experiment, with lettuce, under six levels of N application according to the N recommendation to the culture by Boletim 200 (da Eira Aguiar et al., 2014) in percentage: 0, 25, 50, 75, 100 and 125%, where 100% correspond to the recommended dosage to the crop. The measurements were taken in three times: 20, 23 and 26 days after transplanting (DAT). Error bars mean the standard deviation (n=4).

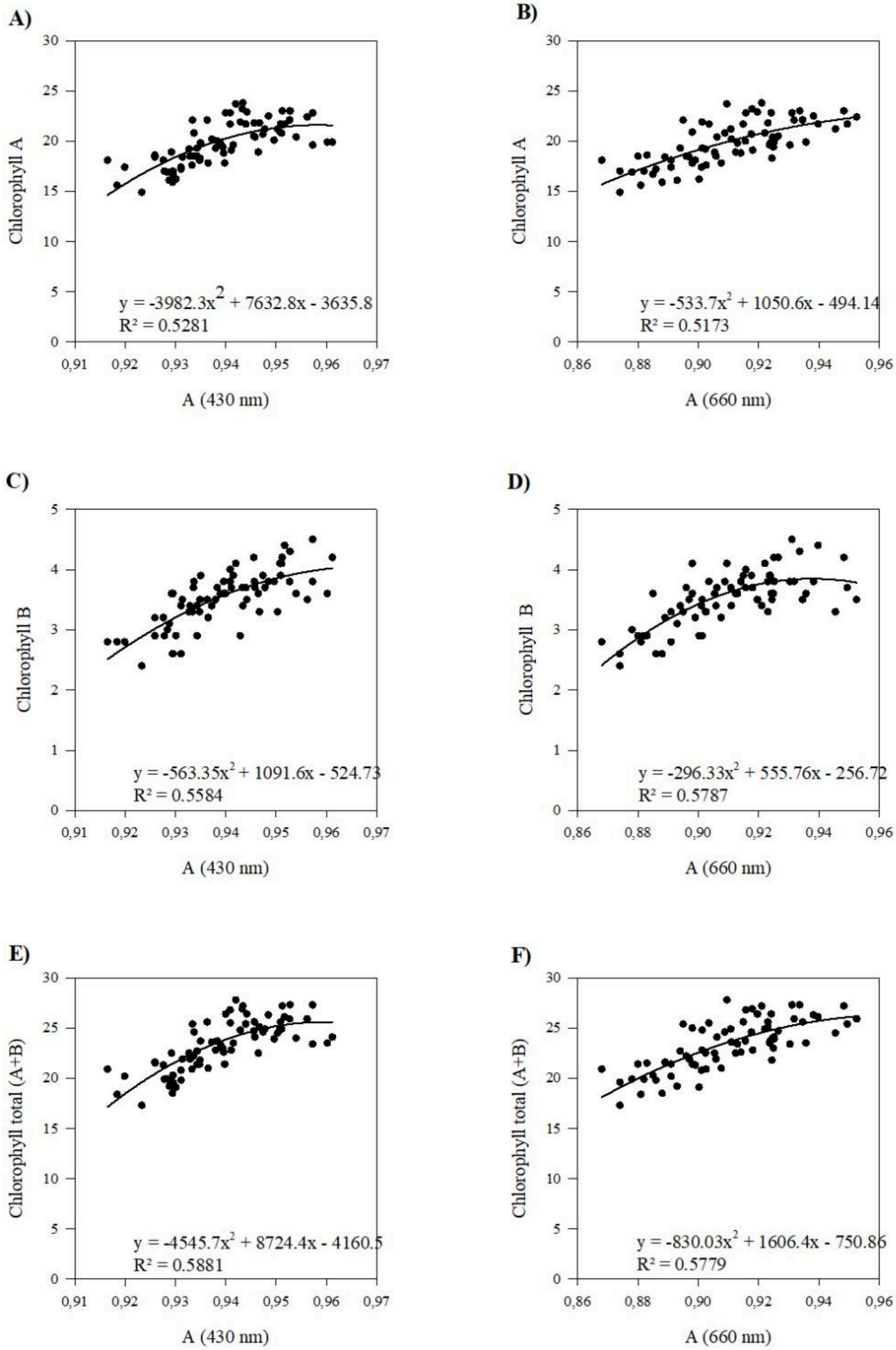

Figure 8

Relationship between chlorophyll and light absorbed by the chlorophyll (A(λ)) obtained in a greenhouse experiment, with lettuce, under six levels of N application according to the N recommendation to the culture by Boletim 200 (da Eira Aguiar et al., 2014) in percentage: 0, 25, 50, 75, 100 and 125%, where 100% correspond to the recommended dosage to the crop. The measurements were taken in three times: 20, 23 and 26 days after transplanting (DAT) and the relationship is presented with the whole set of data according to: **A)** Chlorophyll A; A (430 nm); **B)** Chlorophyll A; A (660 nm); **C)** Chlorophyll B; A (430 nm); **D)** Chlorophyll B; A (660 nm).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Graph.pdf