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(Research Article)

Regression equation for correlation between Spad index and photosynthetic pigments under saline stress in African mahogany culture

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Abstract

The quantification of the levels of photosynthetic pigments is of paramount importance, especially in the evaluation of the photochemical capacity of plants under saline stress. However, laboratory procedures for the titration of these pigments, such as chlorophyll, have expressive laboratory apparatus and specific reagents. This study aimed to evaluate a regression equation for correlation between SPAD index and photosynthetic pigments in African mahogany plants, *Khaya senegalensis*, under saline stress. The plants were cultivated in vases containing washed sand, in a greenhouse. Salinity was established by adding NaCl, with levels of electrical conductivity: 1.0 • 3.38 • 15.14 • 29.90 • 42.61 • 53.60 dS m $^{-1}$, in a completely randomized design (DIC) . The total chlorophyll contents were determined in the laboratory using a spectrophotometer and, in the plants, the SPAD index was quantified directly under the leaves. By regression analysis, through data evaluation with the STATISTICA program, the significant correlation between the SPAD index and total chlorophyll was expressed by the equation: Total chlorophylls = -41.0166 + 1.0338 EC, allowing this result to be direct quantification of the levels of photosynthetic pigments using the SPAD index.

Keywords: Salt stress; Coefficient of determination**;** Total chlorophylls; Woody plants

1. Introduction

Among the African mahogany species, *Khaya senegalensis* has greater hardiness and relative advantage for cultivation in areas under adverse conditions, especially in regions with high concentration of salts in the soil solution and low rainfall (RIBEIRO; FILHO; SCOLFORO, 2017) .

Salinity is a term related to the excessive accumulation of salts, especially NaCl, in soil or water, which negatively affects the growth and development of many living organisms (PEDROTTI et al., 2015) . It has been one of the environmental factors that can limit plant growth and productivity, especially in arid and semi-arid regions, due to the great environmental contrasts (AKÇA et al., 2020) .

Factors that affect plant biomass also affect photosynthetic activity (RADY et al., 2018) , which under saline conditions, chlorophyll and total carotenoid contents of leaves decrease (ACOSTA-MOTOS et al., 2015) . Chlorophyll contents are significantly affected by salinity with a 50-60% reduction in total chlorophyll content (SEMIDA et al., 2016) .

Leaf chlorophyll concentration is measured in mg/gm (PAL; SENGUPTA, 2016) ; SINGHAL et al., 2019a) , being an important biochemical parameter and primarily responsible for the photosynthesis reaction to energize the plant and represent crop health information (SINGHAL et al., 2019b) .

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One of the standard methods for measuring chlorophyll is chemical extraction and calculation in the laboratory based on the spectrometer reading using the Wettstein equation (VON WETTSTEIN, 1957). The pigments are extracted by macerating the leaf discs, using 80% (v/v) acetone as an extractor. After filtering the extracts, the levels of chlorophyll *a* and *b* are determined through spectrophotometric readings at 645 and 470 nm, respectively, according to Arnon, (1949) .

As an alternative to the laboratory procedure of photosynthetic pigment titration is the SPAD device (soil and plant analysis development meter), being calibrated for several field crops (HASSANIJALILIAN et al., 2020) .

The SPAD-502plus Chlorophyll Meter (Minolta Camera Co., Osaka, Japan) measures transmittance at 650 nm and 940 nm to provide a relative measure of chlorophyll content in leaves (WANG et al., 2019) . The SPAD-502plus is designed as a lightweight portable chlorophyll meter; was employed as a low-cost, fast, simple and non-destructive device to diagnose the nitrogen nutritional status of various crops (YANG et al., 2014) .

Under these conditions, monitoring the total chlorophyll content is very useful for evaluating the adaptability of plants to saline stress conditions. However, the method of titration of total chlorophylls by spectrometer is time-consuming, laborious and difficult to perform on a large number of samples. (ECARNOT; COMPAN; ROUMET, 2013) .

In this context, the present study aimed to define the use of a regression equation model in the correlation between the SPAD index, total chlorophyll and electrical conductivity, allowing the portable chlorophyll meter instrument SPAD-512 to be a reliable alternative to estimate the contents of total chlorophyll in leaves of young *Khaya senegalensis* plants, replacing the method of extracting and quantifying the levels of these pigments , under saline stress conditions.

2. Material and methods

2.1. Characterization of the experimental area

The experiment was conducted in a greenhouse, at the State University of Southwest Bahia (UESB), *campus* of Vitória da Conquista (Figure 01), whose geographic coordinates are 14º 53' 08'' south latitude and 40º 48' 02" west longitude of Greenwich, with an altitude of 881 m. The treatments were administered during the first four months of plant growth (June 2018), time accounted for at planting.

The rainy season in the region comprises the months of November to March. The total annual rainfall is around 700 mm, and the thermal averages show maximums of 26.4 °C and minimums of 16.1 °C, with an annual average of 20.2 °C (Figure 02).

Figure 1 Location of the study area. Vitória da Conquista – BA, northeast Brazil

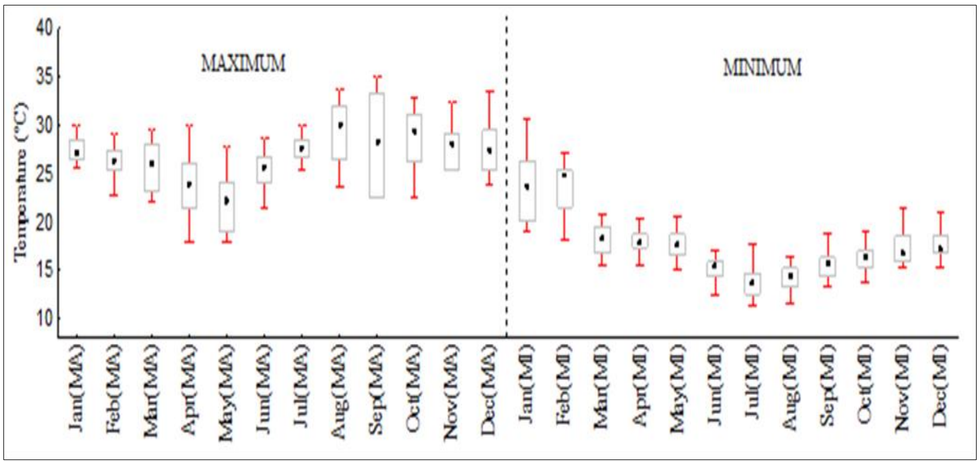

Figure 2 Maximum and minimum temperature for the year 2018 in the municipality of Vitória da Conquista - Bahia (INEMET)

2.2. Experimental design, transplanting and growing conditions

In a completely randomized experiment, young African mahogany plants were cultivated in pots containing washed sand and nutrient solutions with NaCl in varying concentrations – 0, 20, 145, 270, 395 and 520 mM of NaCl, whose concentrations were equivalent to the following levels of electrical conductivity: 1.0 • 3.38 • 15.14 • 29.90 • 42.61 • 53.60 dS m $^{-1}$ with four replications, totaling 24 plots (Figure 03).

Figure 3 Young plants of *Khaya senegalensis* in the experimental units, representing the distribution of treatments in pots in the greenhouse

The seedlings were produced by the Instituto Brasileiro de Florestas, in tubes with a conical model, being acquired at 180 days of age. The seedlings were transplanted into vases with a volume of 15 L, previously filled with sand passed through a 0.005 mm sieve. Small perforations at the base of the pots allowed the collection of drainage water, for later analysis of its chemical characteristics.

The amount of water applied was determined by the pot capacity method, in which the water content of the sand was kept close to the field capacity. Soil water content monitoring was based on daily verification of pot weight.

The addition of NaCl to the nutrient solution, in varying concentrations, was performed every 15 days. Electrical conductivity (dS m $^{-1}$) was monitored using a portable conductivity meter. In the treatment without addition of NaCl, the electrical conductivity was 1 dS m $^{-1}$. In the other treatments, there was an increase in electrical conductivity, due to the addition of NaCl to the nutrient solution, in varying concentrations.

2.3. Estimation of the content of photosynthetic pigments - (SPAD index)

During the experimental period, the SPAD index was verified, as an estimate of the chlorophyll content, using a SPAD-502plus chlorophyll meter (Minolta Corporation, Ltd., Osaka, Japan), whose readings were taken at three points on each side from the midrib of the leaf, on the adaxial face, between 8 am and 10 am, every five days.

2.4. Extraction and quantification of total chlorophylls

At the end of the experimental period, five discs were removed from the blade of the second fully expanded leaf. The pigments were extracted by macerating the leaf discs, using 80% (v/v) acetone as an extractor. After filtering the extracts, the chlorophyll *a* and *b contents* were determined through spectrophotometric readings at 645 and 470 nm, respectively, according to Arnon, (1949) . The total chlorophyll content was considered as the sum of the chlorophyll *a* and *b* contents .

2.5. Statistical analysis

The results were submitted to analysis of variance (ANOVA), Table 1, through the F test to compare means, and regression analysis for the quantitative study of the evaluated characteristics, using the statistical program SISVAR 5.6 and STATISTICA with subsequent regression analysis in the study between each treatment without including the control, and the control treatment will be compared with the others by the Dunnet test ($p < 0.05$).

Table 1 Description of the components of the analysis of variance used in the comparison of SPAD index means and total chlorophylls

Causes of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F calculated
Treatments	I-1	SQTrat	QMTrat	QMTrat/ QMRes
Residue	I (J-1)	SQRes	QMRes	
Total	IJ-1	SQTotal		

On what:

$$SQTotal = \sum_{i=1}^{I}\sum_{j=1}^{J} y_{ij}^2 - C, \text{ on what} C = \frac{\sum_{i=1}^{I}\sum_{j=1}^{J} y_{ij}^{\ 2}}{IJ}$$

Measures the overall variation of all observations.

$$SQTrat = \frac{\sum_{i=1}^{I} y_i^2}{J} - C$$

Sum of squares of groups (treatments), associated exclusively with a group effect (salinity levels).

The sum of squares of the residuals was obtained by difference:

$$SQRes = SQTotal - SQTrat$$

Sum of squares of residuals, due exclusively to random error, measured within groups (salinity levels).

$$QMtrat = \frac{SQtrat}{I - 1}$$

Being the mean square of the groups (treatment).

$$QMRes = \frac{SQRes}{I(J - 1)}$$

Being the mean square of the residues (parameter attributed to unscaled effects in the treatments).

To verify a significant difference between the treatments (SPAD index and total chlorophyll for the different salinity levels), the F test was used, considering that if calculated F > *tabulated F, the null hypothesis* H_0 is rejected , that is, there is evidence of a significant difference between at least one pair of treatment means, at the α *level* of significance chosen, with a 5% probability in the case under study. Otherwise, the null hypothesis H_0 is not rejected , that is, there is no evidence of a significant difference between the treatments, at the chosen significance level α .

To generate the regression equations, the method of least squares was used, being an optimization technique mathematics that seeks to find the best fit for a data set, trying to minimize the sum of squares of the differences between the estimated value (regression equation) and the observed data of SPAD index and total chlorophylls of the African mahogany crop, such differences being called residuals and expressed mathematically by:

$$\sum_{i-1}^{n} e_i^2$$

On what:

n = represents the number of observations, being the number of data sampled from the SPAD index and total chlorophylls of the culture;

e = difference between the actual value of the SPAD index data and total chlorophylls observed during the time period of the experiment (one year) and those estimated by the equation.

To measure the quality of the model in relation to its ability to correctly estimate the values of the SPAD index response variables and total chlorophylls (dependent variables) as a function of the tested salinity levels (SPAD x CE) and intensity of the green color - index SPAD (total chlorophylls x SPAD) (independent variables) the correlation coefficient was generated R^2, determined by:

$$R^2 = 1 - \left(\frac{SQRes}{SQTot}\right)$$

On what:

SQRes = sum of squared residue;

SQTot = sum of squared total.

The value of R^2, can assume values from 0 to 1, and the higher the value of the correlation coefficient, the closer to the real data are the data estimated by the generated equation regression model.

3. Results and discussion

It can be verified by the F test at 5% probability ($p < 0.05$), in the variance analysis chart, ANOVA (Table 2), evidence that the evaluated characteristics, SPAD index and total chlorophyll, were significantly affected by the concentrations NaCl variables.

Table 2 Analysis of variance (ANOVA) for Total Chlorophylls and SPAD Index

Variation sources	**GL**	**SQ**	**QM (Total Chlor.)**	**F**
Treatment	5	879.008265	175.801653	10,637*
Error	18	297.481249	16.526736	
Total	23	1176.489514		
CV (%)	28.26			
Variation sources	GL	SQ	QM (In.SPAD)	F
Treatment	5	700.147083	140.029417	13,160*
Error	18	159.607917	10.640528	
Total	23	901.229583		
CV (%)	6.13			

*significant ($p < 0.05$); ns = not significant; CV = Coefficient of variation.

The SPAD index became different from the control plants from salinity 14.5 dS m $^{-1}$, since lower salinity levels, such as 2 dSm $^{-1}$ did not compromise the ratio of photosynthetic pigments present in the leaves (Figure 4.A), possibly thanks to the plants having minimized sodium entry and concentration in the cytoplasm (FREIRE et al., 2010) , thus ensuring cellular turgor (SOUZA et al., 2017) . Comparing only the plants submitted to the addition of NaCl to the control treatment, the increasing levels of salinity caused a decreasing effect on the SPAD index (Figure 4.A).

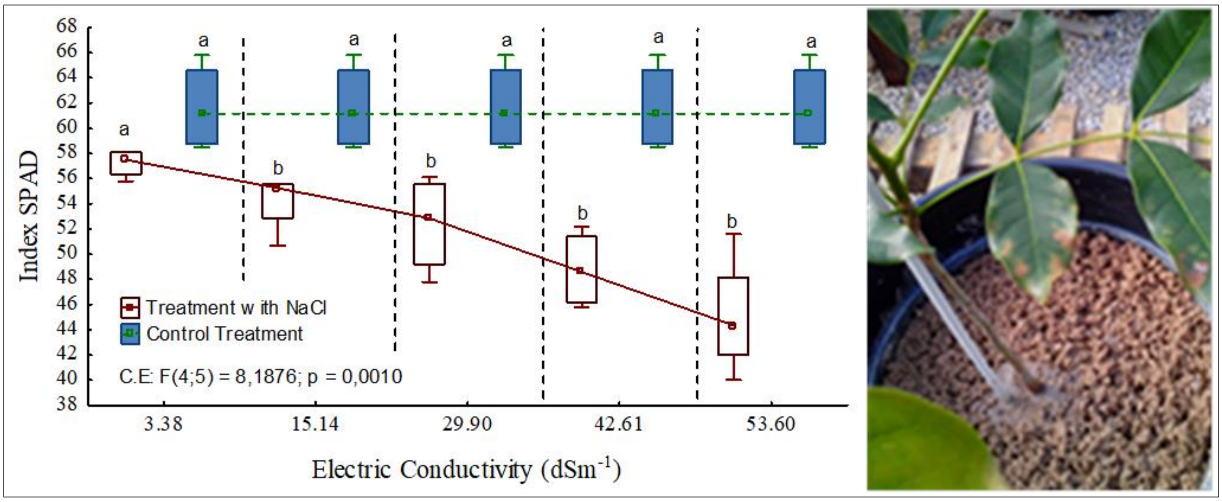

Figure 4 [A] SPAD index in leaves of young *Khaya senegalensis plants* subjected to varying concentrations of NaCl: comparative evaluation of the effects between control plants and treatments with addition of NaCl. [B] Symptoms of toxicity caused by high concentrations of Na^+ in adult leaves of young plants of *Khaya senegalensis* . At each level of EC (electrical conductivity), equal letters in the bars indicate that the data do not differ from each other, by Dunnett's test. * Significant ($p < 0.05$).

The values of total chlorophyll (y axis) and SPAD index (Z axis, represented by the alternation of colors) are directly related to the variation in electrical conductivity (y axis), in which the increase in EC produced a reducing effect on the pigments photosynthetics and SPAD index (Figure 5.A). The EC of 2 dSm $^{-1}$ induced a quantification of 25.8 mg of total chlorophyll and SPAD index greater than 61 (represented by the red color) and less than 64 (brown color), whereas with the increase in EC, for example from the value of 50 dSm $^{-1}$, the titrated total chlorophyll content was 3 mg and the SPAD index, for the same EC value, quantified in an index lower than 53 (yellow color) (Figure 5.A).

In this study, the lowest values found in all indicators of chlorophyll content, in treatments with addition of NaCl, can be attributed to the generalized formation of reactive oxygen species (ROS), which cause damage to cellular components (KAPOOR et al. , 2019) . ROS cause degradation of chlorophylls and peroxidation of membrane lipids, reducing their fluidity and selectivity (HUIHUI et al., 2020) .

The SPAD index values represent the variation in the intensity of the green color in African mahogany leaves, resulting from the content of photosynthetic pigments present (CAO et al., 2017) . The SPAD index titration for electrical conductivities of 2.0; 14.5; 27.0; 39.5 and 52.0 dS m $^{-1}$, are represented in each of the five points of the correlation between the SPAD index and the total chlorophyll content, on the abscissa axis of the linear regression (Figure 6.B). The reduction of photosynthetic pigments is accompanied by a decrease in the intensity of the green color (Figure 4.B) (WANG et al., 2019) , which is perceived by the lowest recorded values of the SPAD index, causing a positive interdependence between these two variables (Figure 5.B), correlated by the mathematical model of regression equation (1), capable of representing the real data in 99% (coefficient of determination 0.99):

$$Clorofilas\ totais\ \left(\frac{mg}{mg}\right) = -41{,}0166 + 1{,}0338\ C.E\ (\text{dSm}^{-1}) \ldots\ldots\ldots\ldots(1)$$

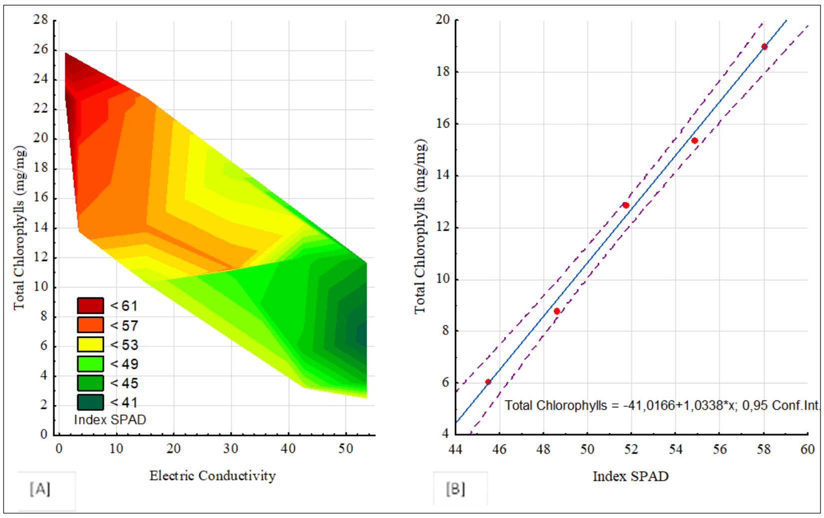

Figure 5 [A] Three-dimensional relationship between total chlorophylls (mg/mg), electrical conductivity (dSm $^{-1}$) and SPAD index in leaves of *K. senegalensis plants.* [B] Direct linear correlation between total chlorophylls and SPAD index for *K. senegalensis* plants under salt stress conditions. * Significant ($p < 0.05$)

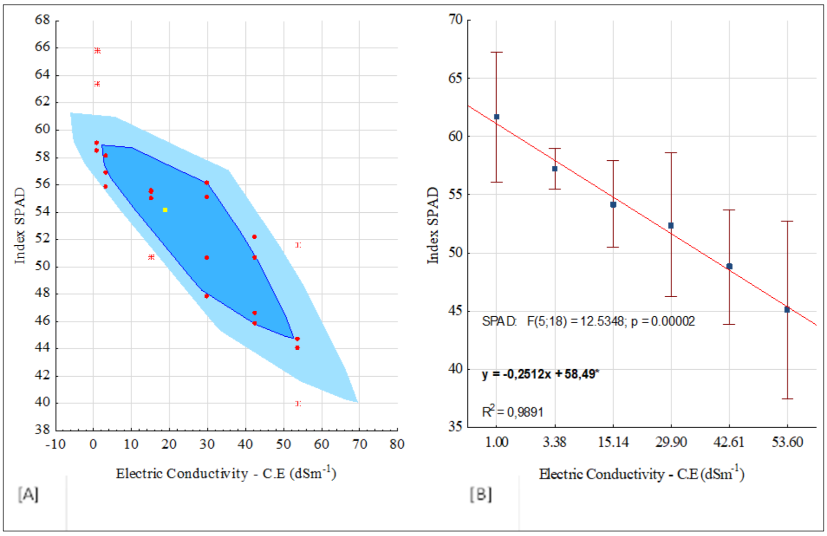

Figure 6 [A] Dispersion of SPAD index values as a function of the variation in electrical conductivity levels. [B] Regression analysis equation for SPAD index as a function of EC * Significant ($p < 0.05$)

The use of the regression equation model in the correlation between the SPAD index and total chlorophyll, allows the SPAD-512 portable chlorophyll meter instrument to be a reliable alternative to estimate the total chlorophyll contents in leaves of young Khaya senegalensis plants, *in* substitution to the method of extraction and quantification of the contents of these pigments.

Positive linear correlations between chlorophyll contents and SPAD indices have been observed in studies with other species, such as *Aniba rosaeodora* and *Swietenia macrophylla* , which are tropical trees from the Amazon (SALLA; CINTRA; ANTONIO, 2007) , and pineapple (LEONARDO et al., 2013) . In practical terms, the estimation of total chlorophyll contents through a method that produces instantaneous and low-cost results, such as the SPAD index, represents an advantage in relation to the chlorophyll extraction and quantification method, which, in addition to being a destructive procedure of plant material, demands more time and cost.

It is observed that the mean value 54, representative of the SPAD index dispersion, is a function of the electrical conductivity of 20 dSm $^{-1}$ (Figure 6.A). In the tested conductivity range, the value of 54 for the intensity of the green color, resulting from the amount of photosynthetic pigments, for all plants in the experimental units, indicates that the African mahogany species Khaya senegalensis *presents* a significant level of tolerance to saline stress conditions , tolerance is also observed in studies with Brazilian mahogany, *Swietenia macrophylla* (SOUZA et al., 2017) .

The value of F (5;18) = 12.5348 shows that the SPAD index variation is inherent to the applied electrical conductivity treatments at 12.5 times greater than the difference between the SPAD index titers attributed to factors external to the treatments residue) not assigned to EC (Figure 6.B).

In the direct linear relationship between electrical conductivity and the SPAD index (Figure 6.B), the increase in EC generated a reduction in the intensity of the green color of African mahogany leaves (SPAD index), behavior represented by the regression equation (2), with a representativeness of 98% of ability to estimate the observed data (R^2 0.9891), indicating a strong correlation between these two variables (SPAD index; CE):

$$SPAD = -0{,}2512\, C.E + 58{,}492 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

4. Conclusion

- The F test at 5% probability ($p < 0.05$) found evidence that the evaluated characteristics, SPAD index and total chlorophyll, were significantly affected by the varying concentrations of NaCl;
- The positive interdependence between the SPAD index and photosynthetic pigments was correlated by the mathematical model of the regression equation: Total chlorophylls = -41.0166 + 1.0338 EC , with capacity to represent the real data in 99% (coefficient of determination 0, 99);
- The regression equation: SPAD = -0.2512 CE + 58.492 represented the behavior in the direct linear relationship between the electrical conductivity and the SPAD index, with a representativeness of 98% of ability to estimate the observed data (R^2 0.9891);
- The use of a regression equation model in the correlation between the SPAD index, total chlorophyll and electrical conductivity, allows the SPAD-512 portable chlorophyll meter instrument to be a reliable alternative to estimate the total chlorophyll contents in leaves of *Khaya senegalensis* plants , under saline stress conditions

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflict of interest.

References

[1] Acosta-Motos J, Diaz-Vivancos P, Álvarez S, Fernández-García N, Sanchez-Blanco MJ, Hernández JÁ (2015) Physiological and biochemical mechanisms of the ornamental Eugenia myrtifolia L. plants for coping with NaCl stress and recovery. Plan 242(4): 829–46.

[2] Akça E, Aydin M, Kapur S, Kume T, Nagano T, Watanabe T, Çilek A, Zorlu K (2020) Long-term monitoring of soil salinity in a semi-arid environment of Turkey. Catena 193(4): 104614. DOI: https://doi.org/10.1016/j.catena.v193n4p104614/2020.

[3] Arnon DI (1949) Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. Plant Physiology 24(1): 1–15.

[4] Cao Q, Miao Y, Li F, Gao X, Liu B, Lu D, Chen X (2017) Developing a new Crop Circle active canopy sensor-based precision nitrogen management strategy for winter wheat in North China Plain. Precision Agriculture 18(1): 2–18.

[5] Ecarnot M, Compan F, Roumet P (2013) Assessing leaf nitrogen content and leaf mass per unit area of wheat in the field throughout plant cycle with a portable spectrometer. Field Crops Research 140: 44–50. DOI: http://dx.doi.org/10.1016/j.fcr.v140p44-50/2013.

[6] Hassanijalilian O, Igathinathane C, Doetkott C, Bajwa S, Nowatzki J, Ali S, Esmaeili H (2020) Chlorophyll estimation in soybean leaves infield with smartphone digital imaging and machine learning. Computers and Electronics in Agriculture 174(4): 105433. DOI: https://doi.org/10.1016/j.compag.n174v4p105433/2020.

[7] Huihui Z, Xin L, Zisong X, Yue W, Zhiyuan T, Meijun A, Yuehu Z, Wenxu Z, Nan X, Guangyu S (2020) Toxic effects of heavy metals Pb and Cd on mulberry (Morus alba L.) seedling leaves : Photosynthetic function and reactive oxygen species (ROS) metabolism responses. Ecotoxicology and Environmental Safety 195(1): 110469. DOI: https://doi.org/10.1016/j.ecoenv.v195n1p110469/2020 .

[8] INMET - National Institute of Meteorology. Meteorological data (2018). Available at: http://www.inmet.gov.br/portal/estacoes/estacoesAutomaticas .

[9] Kapoor D, Singh S, Kumar V, Romero R, Prasad R, Singh J (2019) Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene 19(4): 100182. DOI: https://doi.org/10.1016/j.plgene.v19n4p100182/2019.

[10] Leonardo FDAP, Pereira WE, Silva SDEM, Pereira J, Costa D (2013) Chlorophyll content and SPAD index in pineapple. Revista Brasileira de Fruticultura 35(2): 377–83.

[11] De Oliveira Freire AL, De Sousa Filho GM, De Miranda JRP, Souto PC, De Araújo LV, Coutinho (2010) Growth and mineral nutrition of neem (Azadirachta indica A. Juss.) and cinnamon (Melia azedarach linn.) submitted to salinity . Forest Science 20(2): 207–15.

[12] Pal AK, Sengupta C (2016) Effect of plant growth promoting rhizobacteria on early growth of Rice plant (Oryza sativa L .) under Cadmium (Cd) and Lead (Pb) stress conditio. Ijbst 9(12): 74–81.

[13] Pedrotti A, Chagas RM, Ramos VC, Prata APN, Lucas AAT, Santos PB (2015) Causes and consequences of the soil salinization process. Electronic Magazine on Management, Education and Environmental Technology 19(2): 1308–24. DOI: https://doi.org/10.5902/223611701654 -Rev.Ele.Ges.Ed.Tec.v19n2p1308-24/2015.

[14] Rady MOA, Semida WM, Abd El-Mageed TA, Hemida KA, Rady MM (2018) Up-regulation of antioxidative defense systems by glycine betaine foliar application in onion plants confer tolerance to salinity stress. Scientia Horticulturae 240(2): 614–22. DOI: https://doi.org/10.1016/06-069/j.scienta.v240n2p614-22/2018.

[15] Ribeiro A, Filho ACF, Scolforo JRS (2017) African Mahogany (Khaya spp.) cultivation and the increase of the activity in Brazil. Forest and Environment 24: 504–8. DOI: http://dx.doi.org/10.1590/2179-8087/Flor.Amb.v24e076814p504-8/2017.

[16] Salla L, Cintra J, Antonio R (2007) Chlorophyll contents in tropical trees determined with the SPAD-502. Brazilian Journal of Biosciences 5(2): 159–61.

[17] Semida WM, Abd El-Mageed TA, Howladar SM, Rady MM (2016) Foliar-applied α-tocopherol enhances salt-tolerance in onion plants by improving antioxidant defense system. Australian Journal of Crop Science 10(7): 1030–39. DOI: https://doi.org/10.21475/ajcs.v10n7p1030-39/2016.

[18] Singhal G, Bansod B, Mathew L, Goswami J, Choudhury BU, Raju PLN (2019a) Chlorophyll estimation using multi-spectral unmanned aerial system based on machine learning techniques. Remote Sensing Applications: Society and Environment 15(3): 100235. DOI: https://doi.org/10.1016/J.rsase.v15n3p100235/2019 .

[19] Singhal G, Bansod B, Mathew L, Goswami J, Choudhury BU, Raju PLN (2019b) Estimation of Leaf Chlorophyll Concentration in Turmeric (Curcuma longa) Using High-Resolution Unmanned Aerial Vehicle Imagery Based on Kernel Ridge Regression. Journal of the Indian Society of Remote Sensing 47(7): 1111–22. DOI: https://doi.org/10.1007/12524-019-00969-9/J.Ind.S.Rem.Sen.v47n7p111-22/2019.

[20] Souza RS, Weber OLS, Scaramuzza JF, Ribeiro, ES (2017) Concentration of macronutrients and sodium in mahogany seedlings subjected to salt stress. Native 5(2): 127–32. DOI: https://:doi.org/10.5935/2318-7670-Nat.v05n02p127-32/2017.

[21] Wang JJ, Li Z, Jin X, Liang G, Struik PC, Gu J, Zhou Y (2019) Phenotyping flag leaf nitrogen content in rice using a three-band spectral index. Computers and Electronics in Agriculture 162(5): 475–81. DOI: https://doi.org/10.1016/04-042 - J.compag.v16n5p475-81/2019.

[22] Von Wettstein D (1957) Chlorophyll-letale und der submikroskopische Formwechsel der Plastiden. Experimental Cell Research 12(3): 427–506. DOI: https://doi.org/ 10.1016/0014-4827 - Exp.Cel.Res.v12n3p427-506/1957.

[23] Yang H, Yang J, Lv Y, He J (2014) SPAD values and nitrogen nutrition index for the evaluation of rice nitrogen status. Plant Production Science 17(1): 81–92. DOI: http://dx.doi.org/10.1626/17-18 - Pps.v17n1p81-92/2014.