Journal of Plant Nutrition

ISSN: 0190-4167 (Print) 1532-4087 (Online) Journal homepage: http://www.tandfonline.com/loi/lpla20

A comparison between different chlorophyll content meters under nutrient deficiency conditions

Hazem M. Kalaji, Piotr Dąbrowski, Magdalena D. Cetner, Izabela A. Samborska, Izabela Łukasik, Marian Brestic, Marek Zivcak, Horaczek Tomasz, Jacek Mojski, Henryk Kociel & Balaji M. Panchal

To cite this article: Hazem M. Kalaji, Piotr Dąbrowski, Magdalena D. Cetner, Izabela A. Samborska, Izabela Łukasik, Marian Brestic, Marek Zivcak, Horaczek Tomasz, Jacek Mojski, Henryk Kociel & Balaji M. Panchal (2017) A comparison between different chlorophyll content meters under nutrient deficiency conditions, Journal of Plant Nutrition, 40:7, 1024-1034, DOI: 10.1080/01904167.2016.1263323

To link to this article: http://dx.doi.org/10.1080/01904167.2016.1263323

JOURNAL OF PLANT NUTRITION
2017, VOL. 40, NO. 7, 1024–1034
http://dx.doi.org/10.1080/01904167.2016.1263323

Taylor & Francis
Taylor & Francis Group

A comparison between different chlorophyll content meters under nutrient deficiency conditions

Hazem M. Kalaji[a,b], Piotr Dąbrowski[c], Magdalena D. Cetner[a], Izabela A. Samborska[a], Izabela Łukasik[a], Marian Brestic[d], Marek Zivcak[d], Horaczek Tomasz[e], Jacek Mojski[f], Henryk Kociel[f], and Balaji M. Panchal[g]

[a]Department of Plant Physiology, Warsaw University of Life Sciences, Warsaw, Poland; [b]SI Technology, Warsaw, Poland; [c]Department of Environmental Improvement, Warsaw University of Life Sciences, Warsaw, Poland; [d]Department of Plant Physiology, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic; [e]Department of Plant Production Engineering, Mazowian Reseach Center in Kludzienko, Institute Technology and Life Sciences – ITP, Grodzisk Mazowiecki, Poland; [f]Institute Technology and Life Sciences – ITP, Raszyn, Poland; [g]Nurture Earth Research and Development Pvt. Ltd., Aurangabad, India

ABSTRACT

Chlorophyll content meters have been used successfully to estimate foliar chlorophyll content in various plant species in non-destructive way, especially to study stress physiology and abiotic stresses, such as nutrient deficiency. The main aim of this work was to compare the records of different chlorophyll content meters with the results obtained by the destructive method under the deficiency of main macronutrients in plants growth medium. Four devices (CL-01, SPAD-502, Dualex, and CCM-200) were used to estimate chlorophyll content in maize and tomato plants. In maize plants, all devices validated high accuracy for potassium and nitrogen deficiency and low accuracy for phosphorous and magnesium. In tomato, they showed a high degree of accuracy for calcium, potassium, and iron deficiencies, and low accuracy for phosphorus deficiency. All devices proved to be suitable to provide a reasonably estimation of chlorophyll content under optimal nutrient conditions. However, under nutrient deficiency conditions, tested devices showed different values for the same plant under the same nutrient deficiency. This suggest that, these devices should be validated by a sampling destructive method under such conditions.

ARTICLE HISTORY
Received 5 April 2015
Accepted 16 September 2015

KEYWORDS
maize; tomato; chlorophyll; plant stress

Introduction

Improvement of nutrient availability is one of the reasons of increasing agricultural production in the last century (Ludwig et al. 2010). Many macronutrients and micronutrients, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), and iron (Fe), have been recognized as essential for plants, which cannot complete their life cycles and accomplish normal physiological functions in the absence of any of these nutrients. Usually, growth and yield of crops are reduced due to their deficiencies. Plant growth in relation to the concentration of an essential nutrient element is described by the "generalized dose response curve" (Berry and Wallace 1981).

During photosynthesis, antenna pigments in leaf chloroplasts absorb the solar radiation, and through resonance transfer, the resulting excitation is directed to the reaction center pigments, which release electrons and initiate the photochemical process (Richardson et al. 2002). The chlorophyll

CONTACT Piotr Dąbrowski piotr.andrzej.dabrowski@gmail.com Department of Environmental Improvement, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776, Warsaw, Poland.

(Chl) molecules (mostly Chl *a* and Chl *b*) are thus needed for the transformation of light energy to the chemical bonds. In practical perspective, leaf chlorophyll content is the important parameter for scientists (*e.g.*, physiologists, ecophysiologists, biologists), land managers, and farmers. The amount of the solar radiation absorbed by a leaf is mainly a function of the foliar concentrations of photosynthetic pigments. The reduction of chlorophyll concentrations may limit photosynthetic process and causes lowering in primary production (Curran et al. 1990). Moreover, it is already known that a lot of leaf nitrogen is merged in chlorophyll; thus, measuring the latter content gives an indirect amount of nitrogen status (Moran et al. 2000). Chlorophyll content is also related to stress physiology and abiotic factors, like light and water status (Pinkard et al. 2006). Quantifying chlorophyll content may provide important information about the relationships between plants and their environment (Coste et al. 2010).

There are several methods for determining chlorophyll (Chl) content in leaves. Generally, they can be divided into destructive and non-destructive methods. The destructive methods require an extraction by a solvent, followed by the spectrophotometric determination of absorbance by the Chl solution, and conversion from absorbance to concentration using standard formulas. Other methods, such as high-performance liquid chromatography, can be also used. These methods of determination are accurate, but labor-intensive and expensive to perform (Pinkard et al. 2006). Chlorophyll content meters have been used successfully in many species to estimate foliar chlorophyll (Loh et al. 2002; Abdelhamid et al. 2003) and to allow the measuring of chlorophyll relative content on the same leaf over time (Yamamoto et al. 2002). The readings from chlorophyll content meters can be also used to predict the nitrogen status in leaves (Cabangon et al. 2011). The meters/devices that calculate a chlorophyll content index (CCI) are based on measuring the reflectance or absorbance at particular wavelengths, although the approaches based on chlorophyll fluorescence records are also used to assess the crop nutrient status (Kalaji et al. 2014; Zivcak et al. 2014). Handheld chlorophyll absorbance meters, of which several are commercially available, measure absorbance by the leaf of two different wavelengths of light: red and near-infrared. The red light is strongly absorbed by chlorophyll. The second is a "reference wavelength" necessary to adjust for differences in tissue structure (Richardson et al. 2002).

The main aim of this study was to compare the records of four portable chlorophyll content meters with results obtained by the destructive, spectrophotometric method. In our work, we focused on the devices that measure the parameters based on absorbance of transmitted light. The study analyzed the possibility to emply these devices to uncover the responses of two plant species (maize and tomato) to the deficiency of main macronutrients.

Table 1. The composition of the various culture media in mM. The concentration of minerals was achieved by addition of X cm^3 of concentrated stock solution (1 mol per 1 dm^3) of corresponding component per 1 dm^3 of medium.

	Type of growth medium (ml/dm^3)							
1 M solution	Full	-Ca	-S	-Mg	-K	-N	-P	-Fe
pH	5.05	4.89	4.80	4.88	4.82	4.87	4.94	5.12
$Ca(NO_3)_2{\cdot}4H_2O$	4	—	4	4	4	—	4	4
KNO_3	6	6	6	6	—	—	6	6
$MgSO_4{\cdot}7H_2O$	2	—	—	—	2	2	2	2
$NH_4H_2PO_4$	2	2	2	2	2	—	—	2
$Mg(NO_3)_2{\cdot}6H_2O$	—	4	—	—	—	—	—	—
$MgCl_2{\cdot}6H_2O$	—	—	1	—	—	—	—	—
Na_2SO_4	—	2	—	1	—	—	—	—
$NaNO_3$	—	—	—	—	6	—	—	—
$CaCl_2$	—	—	—	—	—	4	—	—
KCl	—	—	—	—	—	2	—	—
NaH_2PO_4	—	—	—	—	—	2	—	—
NH_4NO_3	—	—	—	—	—	—	1	—
1% Iron citrate	1	1	1	1	1	1	1	—

Table 2. Technical characteristics of used chlorophyll content meters.

Technical specification	CL-01	SPAD-502	Dualex	CCM-200
Producer	Hansatech, United Kingdom	Minolta, Japan	Force-A, France	Optiscience, United States
Measured parameters	Chlorophyll index	Chlorophyll index, nitrogen status	Chlorophyll index, flavonol index, nitrogen status, Chl/Flav ratio, anthocyanin index	Chlorophyll index
Light sources	2 LED, 620 red and 940 nm IR	2 LED, 650 and 940 nm	5 LED, 1 UV-A (528 nm), 1 red, 2 near NIR	2 LED, 650 and 950 nm
Units	0–2000	0–75	—	0–100
Measurement time [s]	2–3	Less than 2	0.5	3–4
Measurement area	1 cm diameter circle	2 mm × 3 mm	5 mm diameter circle	1 cm diameter circle
Number of measurements	60	30	10,000	4090
Weight [g]	250	225	220	168

Materials and methods

Maize (*Zea mays* L.), cultivar "Marignan," and tomato (*Solanum lycopersicum* L.), cultivar "Maeva F1," were grown in a controlled greenhouse in 1 dm^3 dark glass pots filled with a modified Hoagland nutrient solution (see Table 1). The solutions were supplied by air continuously and replaced every 3 days. The medium pH was about 6 for all solution types. The average temperature for day/night was 26/18°C, and the photoperiod for the day/night cycle was 16/8 h. The maximum photosynthetically active radiation was about 1400 mmol m^2 s^{-1}. After 7 days of growth, the seedlings were subjected to nutrient deficiency stress. Later, 14 days after the stress initiation (21 days after germination), chlorophyll content measurements (non-destructive and destructive) were done on 12 leaves (to represent the whole plant) for each treatment. At this stage (21 days after germination), only slight visual symptoms of nutrient deficiencies were observed.

At first, six measurements were conducted on the same spots (6 repetitions along each of 12 leaves) by the use of four chlorophyll content meter: CL-01 (Hansatech, UK), SPAD-502 (Minolta, Japan), Dualex (FORCE-A, Orsay, France), and CCM-200 (Optiscience, UK). The comparison of their technical specification is shown in Table 2.

Just after, the same leaves measured by 4 devices were detached, and the amount of the total chlorophyll was estimated by the spectrophotometric method. Chlorophyll a+b content was measured in the laboratory in fresh leaf samples (0.5 g). Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Leaf samples were homogenized with acetone (80% v/v), filtered, and made up to a final volume of 5 ml. At that point, the solution was centrifuged for 10 min at 3000*g*. Pigment concentrations were calculated from the absorbance of the extract at 663 and 645 nm using the formula (Asharaf et al. 1994) given below:

$$\text{Chlorophyll a} + \text{b (mg / g FW)} = [20.2\times (\text{A645}) \;\; 8.02\times (\text{A663})]\times 0.5$$

Analysis of variance (ANOVA) was used to test the effects of the nutrient deficiency on chlorophyll content in the leaves. The Dunnett test was used to compare significant differences between control and nutrient-deficient samples' means at the 5% level of significance. The slopes and intercepts of the regression lines expressing the relationships between chlorophyll content measured by destruction and non-destruction methods were analyzed using the test of homogeneity of regression coefficients and were compared at the 5% level of significance.

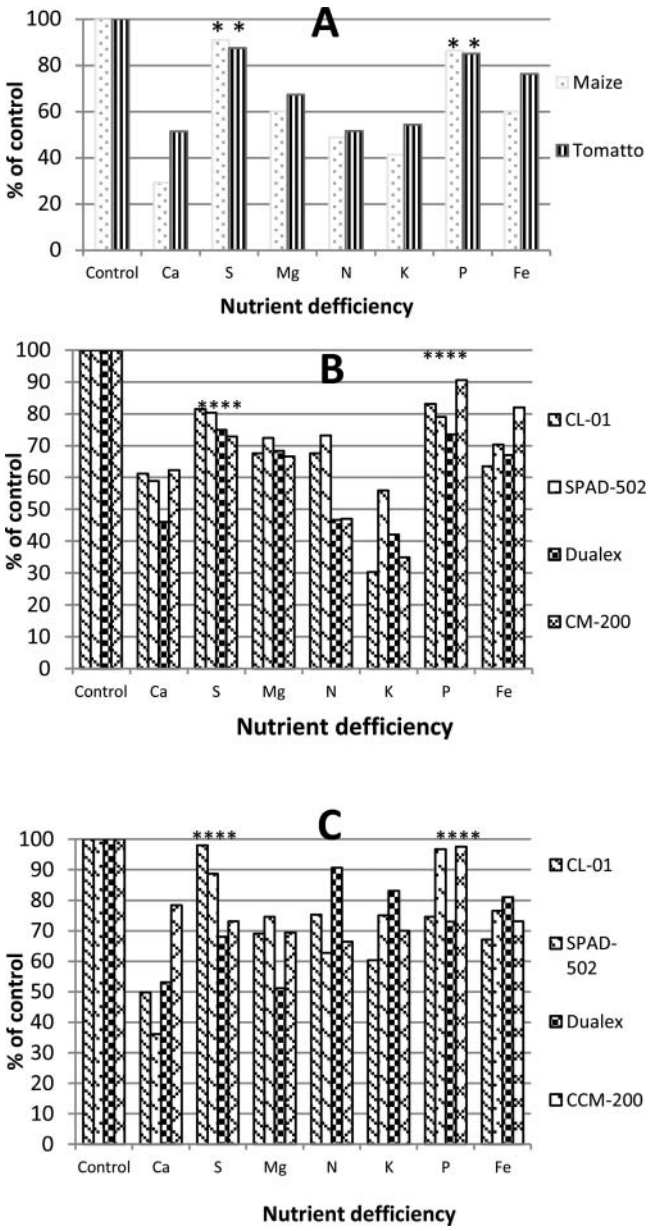

Figure 1. Changes in chlorophyll content and chlorophyll index. Bars marked by asterix symbols indicates values that are not differentiated significantly from control.

Results

On the basis of the ANOVA, it was found that deficiencies of all elements except P and S caused a significant decrease in total chlorophyll content measured by the destructive method in both plant species (Figure 1). Ca deficiency in maize caused a decrease in the total chlorophyll content by 70.1%, Mg deficiency caused a decrease by 40.1%, N deficiency by 51%, K deficiency by 58.7%, and Fe deficiency by 39.9%. In tomato, Ca deficiency caused a decrease in the total chlorophyll content by 48.6%, Mg deficiency caused a decrease by 32.7%, N deficiency by 48.2%, K deficiency by 45.7%, and Fe deficiency by 23.7% (Figure 1). CL-01, SPAD-502, and Dualex demonstrated correctly a significant reduction of the CCI caused by N, K, Mg, and Ca deficiency and lack of

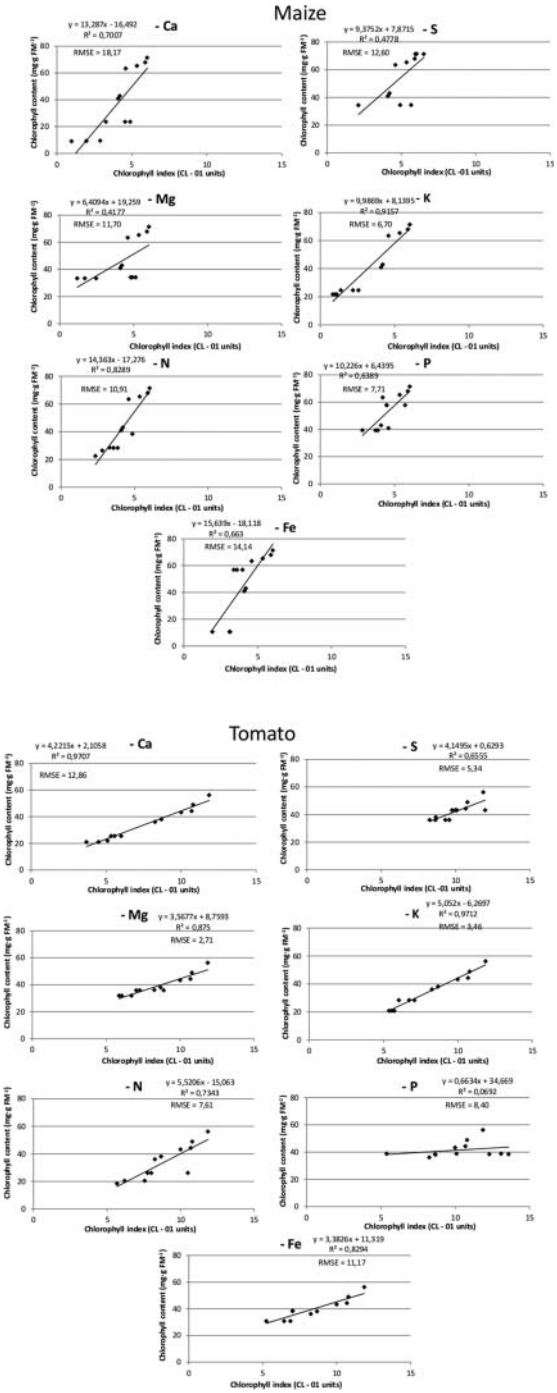

Figure 2. Relation between chlorophyll content and chlorophyll index measured by CL-01.

significant reduction in plants for P and S deficiency. CCM-200 showed a lack of significant reduction of CCI in plants affected by Fe deficiency.

Regression analysis was conducted for all nutrient deficiencies and for all chlorophyll content meters. For maize, all tested devices demonstrated a high degree of accuracy for K and N deficiency ($r^2 \geq 0.70$). Measurements made by CL-01 explains 91.6% of changes of chlorophyll content in plant under K and 82.9% under N deficiency. All tested devices were characterized by a

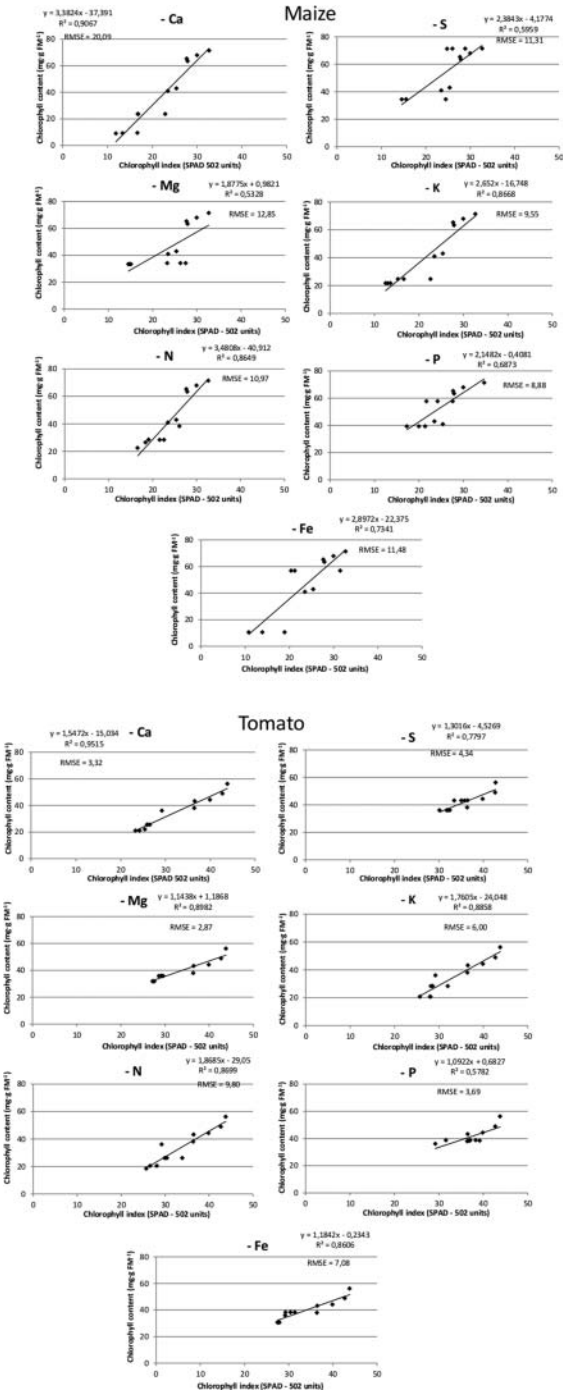

Figure 3. Relation between chlorophyll content and chlorophyll index measured by SPAD-502.

low degree of accuracy for P and Mg. Measurements made by CL-01 explains only 39.6% of chlorophyll content changes under Mg deficiency and 45.1% under P (Figure 2).

Successively, for SPAD-502, it was 53.3% for Mg deficiency and 68.7% for P (Figure 3). For Dualex, it was 39.6% for Mg and 45.1% for P, and for CCM-200, it was, respectively, 52.4% and 42%. For tomato, all tested devices showed a high degree of accuracy for Ca, K, and Fe deficiency ($r^2 \geq 0.70$).

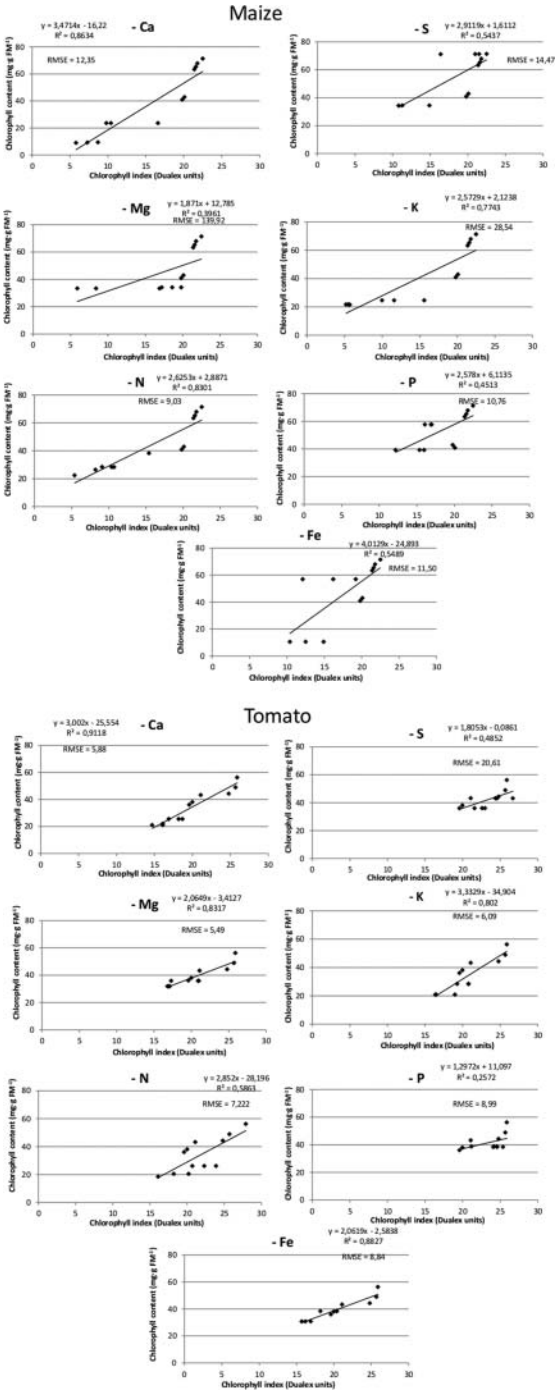

Figure 4. Relation between chlorophyll content and chlorophyll index measured by Dualex.

Mg deficiencies were identified with high accuracy by CL-01, SPAD-502, and Dualex, while the measurements made using CCM-200 for this deficiency can explain only 46.4% of the variation in chlorophyll content. N deficiencies were also measured with high accuracy by 3 devices: CL-01, SPAD-502 and CCM-200. The changes in chlorophyll content caused by P deficiency were measured with a low accuracy of all devices (Figures 3, 4, and 5).

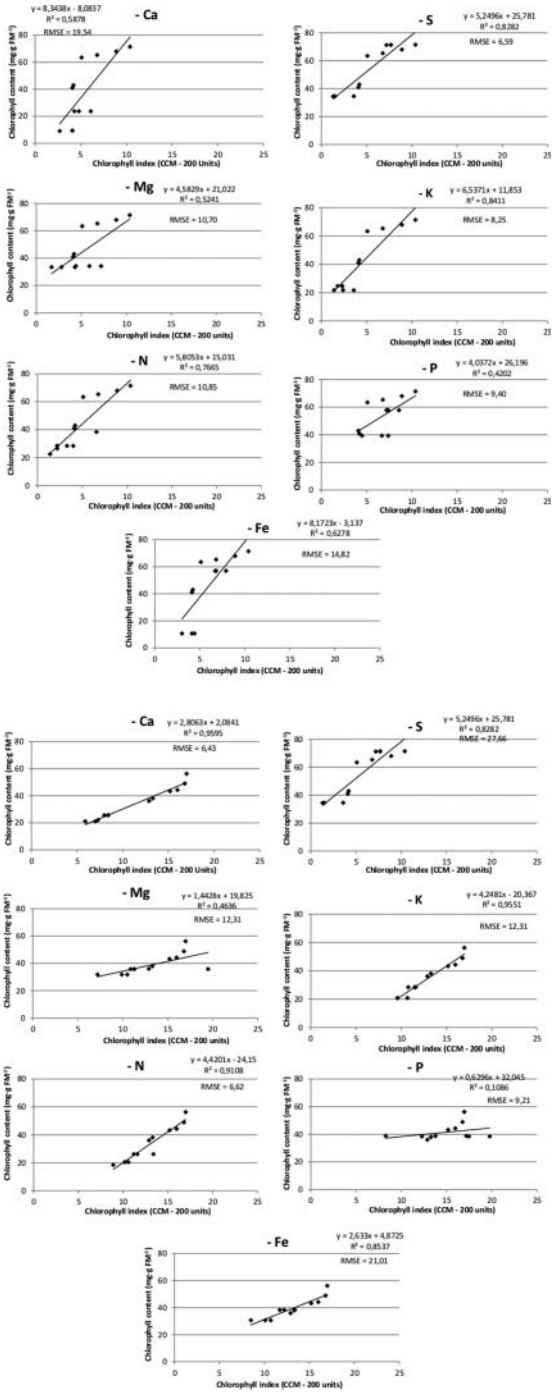

Figure 5. Relation between chlorophyll content and chlorophyll index measured by CCM-200.

Discussion

A lot of the researches have been carried out to investigate the impact of deficits of individual nutrient such as Ca deficiency (Brand and Becker 1984), Mg (Laing et al. 2000), P (Corbridge 1995; Hill et al. 2015), Fe (Bertamini et al. 2002). The effect of N on plants was widely tested, for example by Ciompi et al. (1996); Correia et al. (2005); Riedell (2014) or Singht and Reddy (2014). However, in the

literature, we were not able to find a report that considers all aforementioned nutrients together in terms of their impact on the content of chlorophyll. Our results also revealed that each of the tested plants was characterized by a different response (based on chlorophyll content) to the nutrient deficiencies.

To determine the chlorophyll content in a sample, calibration curves between meter/device readings and the chlorophyll concentration in the tissue sample are needed (Markwell et al. 1995; Djumaeva et al. 2012). Most of realized studies show that the relationship between chlorophyll content and chlorophyll index (relative values) represents a linear regression (Cate and Perkins 2003; Madeira et al. 2003; Wang et al. 2004), but some studies report a non-linear relationship (Markwell et al. 1995; Richardson et al. 2002; Uddling et al. 2007). Hunt and Daughtry (2014) observed physical interaction between chlorophyll content and optical leaf structure affecting leaf transmittances, which is one of the reasons of non-linearity of the relationship between chlorophyll meter estimations and actual chlorophyll content. When a few species are compared, the angular coefficients of the linear equations may be different for each of them. This may be explained by anatomical characteristics of the leaves of different species, or even patterns of the heterogeneous distribution of Chl in leaf, which can interfere in properties of absorption and reflection of radiation used in the determination of the Chl values. Non-uniform distribution among species causes a variable, non-linear response between chlorophyll content and non-invasive measurements (Parry et al. 2014). The effect of non-uniformly distributed Chl is likely to be more important in explaining the non-linearity in the empirical relationships (Cassol et al. 2008). Moreover, the results may be biased because measurements are made along veins, changing the curvilinear shape of the calibration curve. Some authors suggested that differences in leaf mass per area (LMA), among or within species, may lead to different calibration curves through different degrees of mutual shading among chloroplasts. Assuming that higher LMA is related to a greater leaf thickness, mutual chloroplast shading may be avoided in thicker leaves (Hikosaka 2004; Coste et al. 2010).

We consider that our work delivered pioneer results, since there is no comparison of all four chlorophyll content meters in the literature yet. Moreover, there is no comparison of chlorophyll content meters under different nutrient deficiencies, particularly in terms of detecting changes in chlorophyll content. CL-01 has been successfully verified by Cassol et al. (2008) for an estimation of total Chl content in the leaves of some crops (*Zea mays, Cucumis sativus, Raphanus sativus*, and *Ceiba speciosa*). Chl index was linearly and positively correlated to Chl content in all the species. In Pinkard et al. (2006) opinion, the Minolta SPAD-502 is suitable for determining the foliar chlorophyll content of E. globules and E. nitensas. The authors confirmed a good correlation between chlorophyll concentration and chlorophyll index. Similar conclusions were presented by Coste et al. (2010), and Djumaeva et al. (2012). The ability to estimate chlorophyll in the leaves of sugar maple by a CCM-200 was analyzed by van den Berg and Perkins (2004). The authors confirmed the high usefulness of this device. Meyer et al. (2006) suggested that the optical measurements of Chl by SPAD-502 and Dualex are accurate after transformation into mass contents by dividing the obtained values by LMA. Moreover, the data provided by these devices were proportional to N content in tissues. Parry et al. (2014) found that the relationship between values measured by two different types of chlorophylmeters (Minolta–SPAD and CCM-200) was non-linear; anyway, it was possible to develop equations for converting between units from these two meter types. However, our results suggest that in specific conditions caused by nutrient deficiency, the changes in leaf properties limit the ability of some chlorophyll meters to estimate chlorophyll content correctly.

A Chl meter developed by Minolta Corporation for determining the nitrogen status of crops, and the primary application for the Chl meter has been to determine the potential efficacy of additional nitrogen treatments to crop plants. The relationship between the output of the results from these devices and leaf Chl concentration is nonlinear and must be recalculated (Markwell et al. 1995). Moreover, Coste et al. (2010) argued that the values obtained by these devices depend not only on chlorophyll content but also on other aspects of leaf optics, which may be influenced by various environmental and biological factors. On the contrary, Parry et al. (2014) observed no significant effect of environment on the optical/absolute chlorophyll relationship. Anyway, both the results of our experiments and

researches published by other authors clearly indicate that changes in leaf physical and optical properties due to alterations of external factors (such a mineral nutrition level) may result in deviations from the expected linear relationship between chlorophyll meter records and real chlorophyll content, which are, moreover, specific for each type of chlorophyll meter.

Conclusions

Our results clearly showed that, the response of each meter/device varied under nutrient deficiency conditions. Various tested devices gave different results under each specific nutrient deficiency for each plant species. We recommend that, at the beginning of an experiment, to calibrate the device by data validation with a classical destructive method (e.g. spectrophotometric or chemical analysis) in order to determine the exponential equation which can directly convert its output to a leaf chlorophyll concentration. We conclude that all four tested chlorophyll meters are able to provide a rapid and reasonably accurate estimate of leaf chlorophyll content. However, a special attention should be given when the occurrence of nutrient deficiency in plants is expected.

References

Ashraf, M. Y., A. R. Azmi, A. H. Khan, and S. A. Ala. 1994. Effect of water stress on total phenols, peroxides activity and chlorophyll content in wheat. *Acta Physioligiae Plantarum* 16(3):1–18.

Abdelhamid, M., T. Horiuchi, and S. Oba. 2003. Evaluation of the SPAD value in Faba Bean (*Vicia faba* L.) leaves in relation to different fertilizer applications. *Plant Production Science* 6: 185–189.

Berry, W. L., and A. Wallace. 1981. Toxicy: The concept and relationship to the dose response curve. *Journal of Plant Nutrition* 3: 13–19.

Bertamini, M., K. Muthuchelian, and N. Nedumchezhian. 2002. Iron deficiency induced changes on the donor side of PS II in field growth grapevine (*Vitis vinifera* L. cv. Pinot noir) leaves. *Plant Science* 162: 599–605.

Brand, J. J., and D. W. Becker. 1984. Evidence for direct roles of calcium in photosynthesis. *Journal of Bioenergetics and Biomembranes* 16: 239–249.

Cabangon, R. J., E. G. Castillo, and T. P. Tuong. 2011. Chlorophyll meter-based nitrogen management of rice grown under alternate wetting and drying irrigation. *Field Crops Research* 121: 136–146.

Cassol, D., F. S. P. De Silva, A. R. Falqueto, and M. A. Bacarin. 2008. An evaluation of non-destructive methods to estimate total chlorophyll content. *Photosynthetica* 46: 634–636.

Cate, T. M., and T. D. Perkins. 2003. Chlorophyll content monitoring in sugar maple (*Acer saccharum*). *Tree Physiology* 23: 1077–1079.

Ciompi, S., E. Gentili, L. Guidi, and G. F. Soldatini. 1996. The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. *Plant Science* 188: 177–184.

Corbridge, D. E. C. 1995. *Phosphorus: An outline of its chemistry, biochemistry and technology*. Amsterdam: Elsevier.

Correia, C. M., J. M. Pereira, J. F. Coutinho, L. O. Bjorn, and J. M. G. Torres-Pereira. 2005. Ultraviolet-B radiation and nitrogen affect the photosynthesis of maize: A Mediterranean field study. *European Journal of Agronomy* 22: 337–347.

Coste, S., C. Baraloto, C. Leroy, É. Marcon, A. Renaud, A. Richardson, J. C. Roggy, H. Schimann, J. Uddling, and B. Hérault. 2010. Assessing foliar chlorophyll contents with the SPAD-502 chlorophyll meter: A calibration test with thirteen tree species of tropical rainforest in French Guiana. *Annals of Forest Science* 67: 607–617.

Curran, P. J., J. L. Dungan, and H. L. Gholz. 1990. Exploring the relationship between reflectance red edge and chlorophyll content in slash pine. *Tree Physiology* 7: 33–48.

Djumaeva, D., J. P. A. Lamers, C. Martius, and P. L. G. Vlek. 2012. Chlorophyll meters for monitoring foliar nitrogen in three tree species from arid Central Asia. *Journal of Arid Environment* 85: 41–45.

Hikosaka, K. 2004. Interspecific difference in the photosynthesis–nitrogen relationship: Patterns, physiological causes, and ecological importance. *Journal of Plant Research* 117: 481–494.

Hill, M. W., B. G. Hopkins, and V. D. Jolley. 2015. Maize in-season growth response to organic acid-bonded phosphorus fertilizer (Carbond P ®). *Journal of Plant Nutrition* 38: 1398–1415.

Hunt, E. R., and C. S. T. Daughtry. 2014. Chlorophyll meter calibrations for chlorophyll content using measured and simulated leaf transmittances. *Agronomy Journal* 106: 1–9.

Kalaji, H. M., A. Oukarroum, V. Alexandrov, M. Kouzmanova, M. Brestic, M. Zivcak, I. A. Samborska, M. D. Cetner, S. I. Allakhverdiev, and V. Goltsev. 2014. Identification of nutrient deficiency in maize and tomato plants by in vivo chlorophyll a fluorescence measurements. *Plant Physiology and Biochemistry* 81: 16–25.

Laing, W., D. Greer, O. Sun, P. Beets, A. Lowe, and T. Payn. 2000. Physiological impacts of Mg deficiency in *Pinus radiata*: Growth and photosynthesis. *New Phytologist* 146: 47–57.

Loh, F. C. W., J. C. Grabosky, and N. L. Bassuk. 2002. Using the SPAD-502 meter to assess chlorophyll and nitrogen content of benjamin fig and cottonwood leaves. *Horticultural Technology* 12: 682–686.

Ludwig, B., D. Geisseler, K. Michel, R. G. Joergensen, E. Schulz, I. Merbach, J. Raupp, R. Rauber, K. Hu, L. Niu, and X. Liu. 2010. Effects of fertilization and soil management on crop yields and carbon stabilization in soils. A review. *Agronomy Sustainable Development* 31: 361–372.

Madeira, A. C., A. Ferreira, A. deVarennes and M. I. Vieira. 2003. SPAD meter versus tristimulus colorimeter to estimate chlorophyll content and leaf color in sweet pepper. *Communications in Soil Science and Plant Analysis* 34: 2461–2470.

Markwell, J., J. C. Osterman, and J. L. Mitchell. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research* 46: 467–472.

Meyer, S., Z. G. Cerovic, Y. Goulas, P. P. Montpied, S. Demotes-Mainard, L. P. R. Bidel, I. Moya, and E. Dreyer. 2006. Relationships between optically assessed polyphenols and chlorophyll contents, and leaf mass per area ratio in woody plants: A signature of the carbon–nitrogen balance within leaves?. *Plant, Cell and Environment* 29: 1338–1348.

Moran, J. A., A. K. Mitchell, G. Goodmanson, and K. A. Stockburger. 2000. Differentiation among effects of nitrogen fertilization treatments on conifer seedlings by foliar reflectance: A comparison of methods. *Tree Physiology* 20: 1113–1120.

Parry, C., J. Blonquist, and B. Bugbee. 2014. In situ measurement of leaf chlorophyll concentration: Analysis of the optical/absolute relationship. *Plant, Cell and Environment* 37: 2508–2520.

Pinkard, E. A., V. Patel, and C. Mohammed. 2006. Chlorophyll and nitrogen determination for plantation-grown Eucalyptus nitens and E. globulus using a non-destructive meter. *Forest Ecology Management* 223: 211–217.

Richardson, A. D., S. P. Duigan, and G. P. Berlyn. 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytologist* 153: 185–194.

Riedell, W. E. 2014. Nitrogen fertilizer applications to maize after alfalfa: Grain yield, kernel composition and plant mineral nutrients. *Journal of Plant Nutrition* 37: 2026–2035.

Singh, S. K., and V. R. Reddy. 2014. Combined effects of phosphorus nutrition and elevated carbon dioxide concentration on chlorophyll fluorescence, photosynthesis, and nutrient efficiency of cotton. *Journal of Plant Nutrition and Soil Science* 177: 892–902.

Uddling, J., J. Gelang-Alfredsson, K. Piikki, and H. Pleijel. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosyntesis Research* 91: 37–46.

van den Berg, A. K., and T. D. Perkins. 2004. Evaluation of a portable chlorophyll meter to estimate chlorophyll and nitrogen contents in sugar maple (*Acer saccharum* Marsh.) leaves. *Forest Ecology and Management* 200: 113–117.

Wang, Q. B., M. J. Chen, and Y. C. Li. 2004. Nondestructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. *Journal of Plant Nutrition* 27: 557–569.

Yamamoto, A., T. Nakamura, J. J. Adu-Gyamfi, and M. Saigusa. 2002. Relationship between chlorophyll content in leaves of sorghum and pigeon pea determined by extraction method and by chlorophyll meter (SPAD-502). *Journal of Plant Nutrition* 25: 2295–2301.

Zivcak, M., K. Olsovska, P. Slamka, J. Galambosova, V. Rataj, H. B. Shao, M. H. Kalaji, and M. Brestic. 2014. Measurements of chlorophyll fluorescence in different leaf positions may detect nitrogen deficiency in wheat. *Zemdirbyste-Agriculture* 101: 437–444.