American-Eurasian J. Agric. & Environ. Sci., 3 (1): 79-85, 2008
ISSN 1818-6769

Evaluation of Chlorophyll Meter (SPAD) Data for Prediction of Nitrogen Status in Corn (*Zea mays* L.)

Majid Rostami, Ali Reza Koocheki, Mahdi Nassiri Mahallati and Mohammad Kafi

Department of Agronomy, Ferdowsi University of Mashhad, Iran

Abstract: Improving N management would eventually depend on the accuracy with which the N status of plant can be assessed. The usefulness of SPAD chlorophyll meter readings for plant N assessment is based on the direct proportionality between leaf chlorophyll and leaf N concentration. Field experiments were conducted during 2005 and 2006 to determine the response of grain yield, leaf and grain nitrogen concentration and chlorophyll content of leaf to nitrogen fertilizer application rate. Dynamics of chlorophyll was measured by using a SPAD chlorophyll meter on the youngest fully developed leaves of a full season hybrid of maize. The average of SPAD values were increased with the increasing of nitrogen fertilizer application, irrespective of the growth stage. SPAD readings at R1 growth stage accurately predicted yield and nitrogen concentration in leaf. Increasing nitrogen fertilizer up to 400 kg ha^{-1} significantly increased maize biological yield and SPAD readings, but higher N application did not significantly increase these parameters further. In general results showed that using SPAD meter is not a good technique for early prediction of N status in corn. Based on our results photosynthetic maturity is the best time for prediction of N status in corn, because at this time leaf chlorophyll reaches to its maximum.

Key words: Chlorophyll meter · Corn · Grain yield · Nitrogen fertilization

INTRODUCTION

Maize is an economically important crop because of its widespread commercial production and utilization. It is well know that nutrient deficiency in most cultivated crops during the growth season causes imbalances, leading to reduce yield. Among the essential macronutrients, nitrogen is described as the most important element for crop growth [1]. Nitrogen plays a pivotal role in several physiological processes in the plant. It is fundamental to establishment of the plant's photosynthetic capacity, it prolongs the effective leaf area duration, delaying senescences, and it is important for ear and kernel initiation, contributing to define maize sink capacity [2]. About half of the 110 kg ha^{-1} annual increase in maize yields over the last half century can be attributed to improve cultural practices, especially N fertilizer use [3]. To maximize grain yield, farmers often apply a higher amount of N fertilizer than the minimum required for maximum crop growth [4]. When N application is not synchronized with crop demand, N losses from the soil-plant system are large, leading to low fertilizer efficiency [5]. The amount of N applied to maize must be carefully managed to ensure that N will be available throughout the growing season. However, the application of N at rates exceeding plant utilization, represent an unnecessary input cost to maize producer and may harm aquatic and terrestrial environments [6].

Adjusting the N input to an economically and ecologically compatible level would require reliable information on the N status of maize. Information on the N status can be obtained either from the crop side or from the soil side of the system. Crop-related indicators can be classified mainly in to three groups, namely those where the N status is monitored by (i) nitrate concentration, (ii) optical methods, or (iii) total N concentration [7]. Standard methods for N determination involve tissue collection which is a destructive and time-consuming procedure. Leaf N concentration of normal plants varies from as low as 2 - 3% up to 4 - 5% depending primarily on plant species [8].

Because of the direct relationship between N and chlorophyll the portable chlorophyll meter has become a popular non-destructive means for measuring leaf N status in some crops [9,10]. The SPAD_502 chlorophyll meter utilize two light-emitting diodes (650 nm & 990 nm) and a photodiode detector to sequentially measure

Corresponding Author: Dr. Majid Rostami, Department of Agronomy, Ferdowsi University of Mashhad, Iran

transmission of red and infrared light through leaves. The obtained SPAD values are proportional to the chlorophyll content of leaves [11, 12]. Earl and Tollenaar [2] found a close correlation (R2=0.98) between SPAD reading and maize leaf absorptance. Recent research indicates a link between chlorophyll content, leaf N status and crop yield [13,14]. Chlorophyll meter readings enable users to quickly and easily measure leaf greenness, which is determined by leaf chlorophyll content. However factors other than N can influence growth, chlorophyll and N relationships and thus the interpretation of SPAD meter reading [5]. The main objective of this study was to test whether a SPAD chlorophyll meter can be effectively used to predict grain yield, grain nitrogen concentration and leaf nitrogen concentration of maize in field, based on simple measurements before harvest.

MATERIALS AND METHODS

Field experiments were conducted in 2005 and 2006 growing seasons on a loamy clay soil at the experimental field of Lorestan University (48° 17' N., 33° 36' E., 1148 latitude). Annual rainfall at the region is typically 512 mm and maximum July temperature is 47°C. The climate during two years was close to 30 year averages. Soil preparation included disking, chisel plowing and harrowing. Chemical properties of the soils are shown in Table 1.

Full season hybrid of maize (S. C. 704) was sown on May18, 21, 2005 and 2006, respectively at a density of 72000 plants ha^{-1}. Various levels of nitrogen fertilizer were applied in two experiments. Dates of application and amounts of fertilizer are given in Table 2.

The experimental treatments were arranged in complete block design with four replicates. Each plot was 6 m long and 4.5 m wide and consisted of six rows with a 0.75 m inter-row spacing. Plots were irrigated whenever soil water potential reached to F.C, to ensure that no water deficit occurred during the crop growth cycle. Crops were fully protected against weeds and pests in the two experiments. Previous crops were barley (*Hordeum voulgaris* L.) and wheat (*Tirticum aestivum* L.) in 2005 and 2006, respectively. Weekly chlorophyll meter (SPAD-502, Minolta, Ramsy) readings were taken in all plots starting at V6.

SPAD readings were taken on the mid-point of the youngest fully expanded leaf (before silking) and on the ear leaf (after silking). Because insufficient sample size does not allow for the detection of small, but real differences between treatment means according to

Table 1: Soil characteristic in two years of experiment

Soil year	type	pH	Ec	%N	P (mg/kg)	K (mg/kg)	%OC	Manure application
1	Clay loam	7.6	1.4	0.039	27	235	0.59	no
2	Clay loam	7.8	1.9	0.035	18	270	0.48	no

Table 2: Fertilizer treatments with rates and timing of application in two years of experiment

year	N treatment kgh^{-1}	First application at V1	Second application at V6
2005	0	-	-
	100	50	50
	200	100	100
	300	150	150
	400	200	200
	500	250	250
2006	0	-	-
	100	40	60
	200	80	120
	300	120	180
	400	160	240
	500	200	300

recommendation of Costa *et al.* [15], twenty leaves were measured at random in the plot and a main SPAD value was calculated for each plot.

Herbage samples were dried at 80°C for 48 h to assess their mass and then ground through a 1 mm screen. Total nitrogen content in the dried sample was determined using the Kjeldahl digestion technique. Grain yield was calculated per hectare and adjusted to a standard moisture of 155g kg^{-1}.

For prediction purposes, relationships between variables were analyzed by fitting simple linear or quadratic regressions. More complicated function such as the logistic which may have greater biological validity than the quadratic were used only where benefits were worthwhile. The aims of this study was to determine relationship between nitrogen fertilization and leaf chlorophyll content as measured using a SPAD chlorophyll meter and study the effect of different levels of nitrogen fertilization on the grain yield and grain nitrogen concentration of maize.

RESULTS AND DISCUSSION

Grain yield (reported as 15.5 percent moisture) was increased with increasing N rates. (Fig. 1a). A highly significant quadratic regression equation (Eq. (1)) was obtained between grain yield and applied N. Grain yield ranged from 5.93 to 12.85 t/ha depending on N rate.

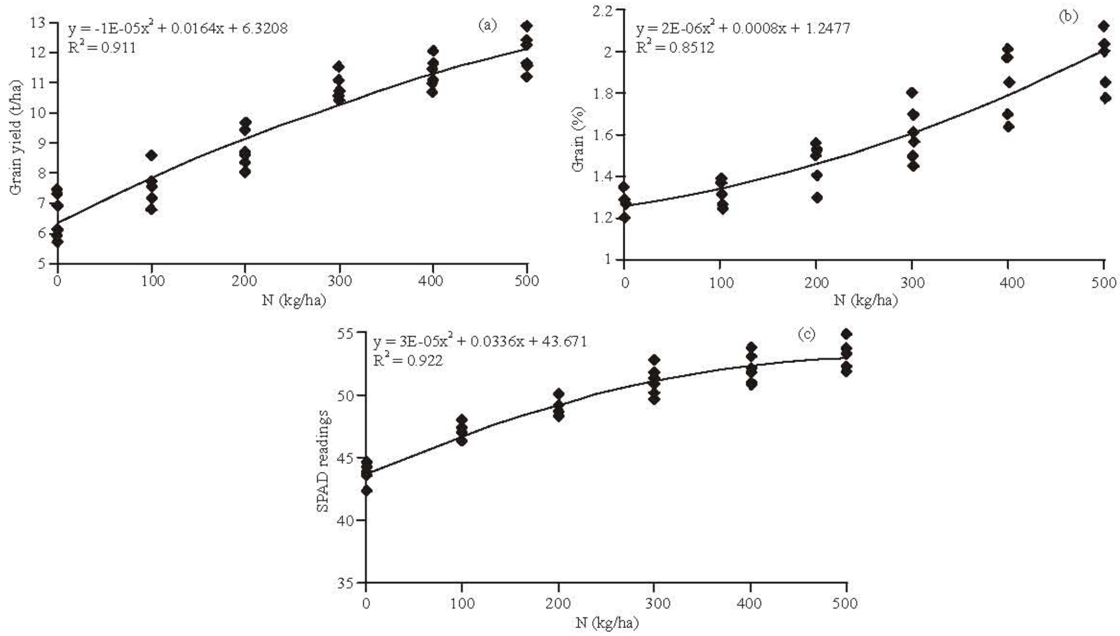

Fig. 1: Effect of nitrogen fertilizer rate on (a) grain yield, (b) %N in grain and (c) SPAD readings at R1 growth stage.

Varvel *et al.* [16] demonstrated N fertilizer significantly increased both corn grain yield and SPAD readings. Results showed that with increasing N fertilizer application response of yield decreased; this indicated that high N rates increased N loss, so nitrogen use efficiency decreased with increasing N rates. Results of Arreguie *et al.* [5] showed that the response of the wheat yield in relation to N fertilizer application followed two models of behavior. In most of the their trials there was a response to the first N applications, but once the optimal dose was reached, a maximum yield was obtained and did not improve with additional increase of N fertilizer.

$$\text{Grain Yield} = -1E - 05\ N^2 + 0.0164\ N + 6.32\quad R^2 = 0.91 \qquad (1)$$

By increasing rates of N fertilizer, %N in grain increased (Fig. 1b). The SPAD readings were significantly increased by N application up to 400 kg ha^{-1} (Fig. 1c). Higher correlation coefficients were observed for the relationship between SPAD readings and N fertilization level than for that between SPAD readings and grain yield. Regression analyses indicated a significant quadratic plateau equation for SPAD values versus different level of N fertilizer.

It was observed that SPAD readings rose in the period V7 onwards to the R1 growth stage and after that SPAD values were decreased (Fig. 2). The increase in SPAD reading and subsequent decrease has also reported where SPAD readings were low at V7, maximized at R1 and

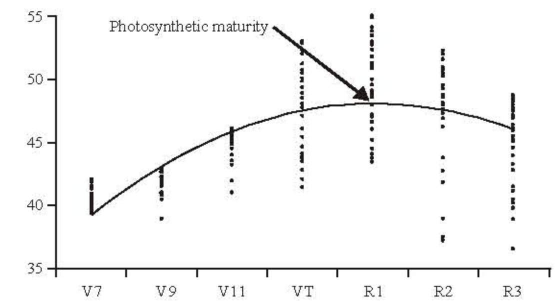

Fig. 2: SPAD Chlorophyll meter readings over the course of the growth

low but higher than those found for V7, at R4 growth stage. [17]. The point at which leaf chlorophyll reaches its maximum, has been termed "photosynthetic maturity" and dose not necessarily correspond to maximum leaf size [18]. The increase of SPAD readings with plant growth has already been documented by Schepers *et al.* [19]; Smeal and Zhang [20]; Costa *et al.* [10]. The lowest leaf chlorophyll content was found during the early stage of corn development and the quadratic increase in SPAD readings after these early stages until R1 indicate that high part of absorbed N was used to produce other plant structure rather than chlorophyll.

The SPAD value reflecting crop N status is correlated to corn grain yield (Fig. 3a). The correlation matrix between corn grain yield and SPAD values in different growth stages is presented in Table 3. The SPAD

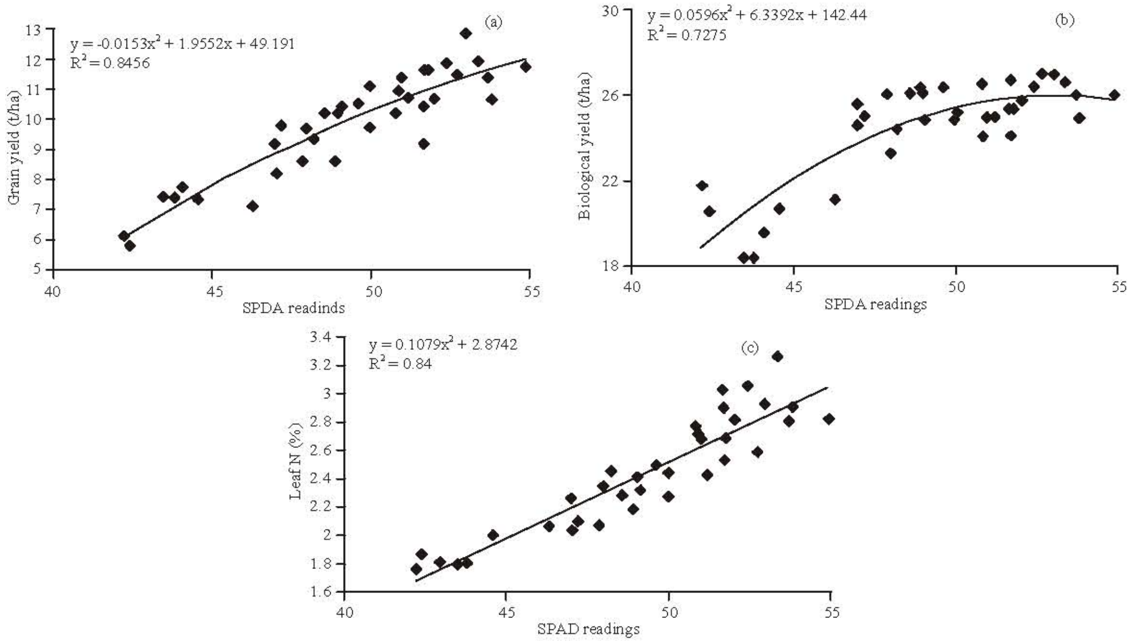

Fig. 3: Relationships between SPAD readings at R1 growth stage and (a) grain yield, (b) biological yield and (d) %N in ear leaf.

Table 3: Correlation matrix between corn grain yield and SPAD readings

Parameter	Y	X1	X2	X3	X4	X5	X6	X7
Y	1							
X1	0.173	1						
X2	0.289^{*}	0.05	1					
X3	0.744^{**}	0.245	0.281^{*}	1				
X4	0.835^{**}	0.054	0.327^{*}	0.808^{**}	1			
X5	0.929^{**}	0.131	0.172	0.713^{**}	0.773^{**}	1		
X6	0.865^{**}	0.082	0.184	0.715^{**}	0.777^{**}	0.896^{**}	1	
X7	0.816^{**}	0.077	0.231	0.742^{**}	0.793^{**}	0.879^{**}	0.934^{**}	1

Y= Corn grain yield and X1, X2, X3, X4, X5, X6 and X7 are the SPAD readings at V7, V9, V11,VT, R1, R2 and R3 respectively.

* and ** indicate significance at 5% and 1% respectively.

readings at all growth stages after V7 were positively correlated with corn grain yield. At the early growth stage low correlation observed, but correlation between SPAD readings and grain yield was higher during later stages of corn development. Many studies [21, 22, 16] using the SPAD to assess maize nitrogen status have shown reliable indication of N stress and relationship to relative yield, especially with later season sensing.

To study the relationship between biological yield and SPAD readings at R1 growth stage, we determined the correlation coefficients for several continuous models, the quadratic model being the one which best fitted the data (Fig. 3b). Increasing N fertilizer up to 400 kg ha^{-1} significantly increased biomass of corn. However, higher N application did not increase this parameter further.

Regression analysis of the data showed that nitrogen concentration in the leaves of corn is linearly correlated with the SPAD readings (R^2= 0.84). Leaf N concentration greatly influences both the development of maize canopies and their photosynthesis. The linear regression of leaf N concentration and SPAD values at VT, R1 and R2 was highly significant at each growth stage (results not presented) and the same was true when data for all stage were pooled (Fig. 3c). The signs of nitrogen deficiency always appear first as yellow discoloration and withering of the older parts of plant, whilst the younger parts remain green longer. As a rule however, the younger parts are also paler than usual because the remobilized nitrogen obtained from intrinsic sources is far from adequate for normal growth or optimal chlorophyll synthesis [23]. Schepers *et al.* [19] found that at silking stage in maize, readings from a SPAD was correlated well with leaf N concentration for a given hybrid and location, but that calibration of the meter was not practical due to unique greenness characteristics of hybrids. Peng *et al.* [24] showed that the linear relationship between leaf N concentration and SPAD readings was differed depending on plant developmental stage, position of the measurement on the leaf and genotype. According to Dwyer *et al.*, [25] the narrow range of chlorophyll meter readings measured at growth stage V6 makes it difficult to separate N-deficient from N-sufficient field areas.

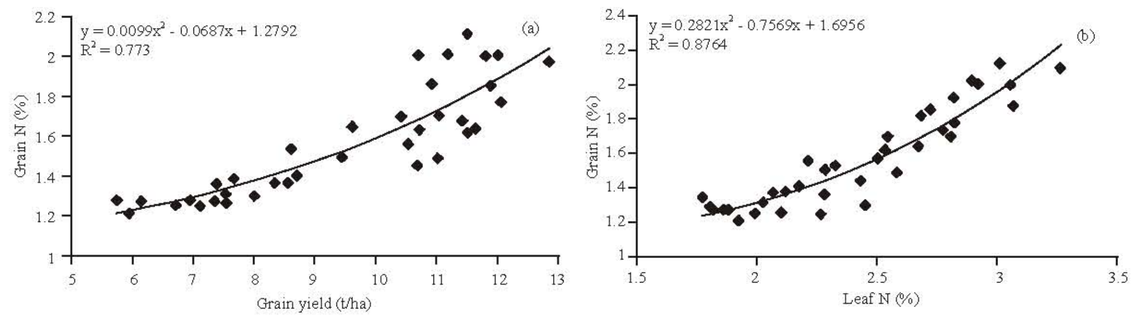

Fig. 4: Relationships between %N in grain and (a) grain yield and (b) %N in ear leaf.

Our results showed that SPAD readings at growth stages after V7 can fairly describe the N status of corn in the field and practically determine whether N fertilizer application is needed. Varvel *et al.*, [16] found that only large N deficiencies could be detected using the chlorophyll meter at the V8 stage.

Schepers *et al.* [19] found a direct relationship between SPAD readings and ear leaf N concentration taken from the exact sampling sites, using leaf disks and reported that these finding cannot be related directly to the traditional practice, where N concentration has been recorded on a whole-leaf basis. SPAD meter values may level off since the nitrogen that is taken up by the crop in excess of the amount that enables optimal growth may be converted into chlorophyll in a progressively lower fraction [26]. Consequently, in the lower range SPAD meter readings are a reliable indication of nitrogen deficiency, but in the higher range SPAD meter readings do not distinguish between adequate and excessive nitrogen levels [16, 27-29].

In the lowest grain yield, grain N concentration was 1.2% and it was gradually increased till 7 t/ha. Beyond 7 t/ha, grain N% was increased with yield with greater variation and in the highest grain yield grain N concentration was 1.97% (Fig. 4a).

Increases in grain %N with yield are commonly observed in individual response trials, but when comparisons are made across locations or seasons a more common observation for a given N supply is that grain %N was decreased with increasing yield [14].

There was a good relationship between %N in grain and %N in leaves (Fig. 4b). By increasing SPAD values and N concentration in leaf, %N in grain increased, because a large proportion of the N in grain is remobilized from leaves and stems after anthesis, rather than being taken up from the soil. SPAD reading at the R1 growth stage accurately predicted yield and nitrogen concentration in grain because the relationship between grain N concentration and SPAD reading at R1 was much stronger than those for other stages. Lopez-Bellido *et al.* [14] concluded that the chlorophyll meter could be used to predict the grain N concentration of wheat in England. Results of Arregui *et al.* [5] showed that it is possible to use chlorophyll meter to distinguish between the extreme values (those of very low grain N concentration and those very high N concentration), but intermediate readings did not present a good relationship with grain N concentration.

CONCLUSIONS

Since SPAD readings are closely related to leaf nitrogen concentration, the SPAD meter can be used to monitor the N status of maize and thereby to adjust the rates of N fertilization in order to increase nitrogen use efficiency [30-32]. Analyses of data collected at different growth stages were used to determine how early in the season SPAD data could be used to predict future crop N need. The proposed method allows the N fertilization in corn to be managed dynamically, adjusting the N recommendations to the crop N requirements during the growing season. SPAD readings taken at later stages offered better relationship with grain yield and leaf N concentration than those taken at V7 growth stage, therefore assessment of the crop N status should be done between V11 and R1, if we are willing to take corrective fertilizer actions for grain yield. A delayed measurement from V7 to VT generally improves the prediction of yield. At VT it is still possible to affect the grain yield through application of nitrogen fertilizer. Only less than 20% of the total N uptake by corn occurs before V8 [33]. To avoid substantial yield loss, in-season nitrogen fertilizer should be applied before VT to R1 growth stages [34]. In general our results showed that using SPAD meter is not a good technique for early prediction of N status in corn. Based on our results photosynthetic maturity (R1 stage in our experiment) is the best time for prediction of N status in corn, because at this time leaf chlorophyll reaches to its maximum.

SPAD-based N fertilizer management resulted in a higher N use efficiency and agronomic N use efficiency than the fixed-rate treatment. However, from a practical point of view, there are three limitations to the use of SPAD. First, SPAD values do not indicate how much N should be applied and only indicate the need for additional N. Secondl this method is quite sensitive to sampling details and errors made at this stage could easily influence the measurement values. Any thing causing plant stress may affect amount of chlorophyll in plants, thus affect SPAD readings [35]. Third since plant chlorophyll is affected by many factors, it is impossible to identify a universal meter reading that indicates sufficient N for all varieties of a specific crop, thus the reading must be calibrated for different varieties and environments [36]. Moreover this method is not capable of detecting luxury N uptake since maize plants achieve maximum chlorophyll content irrespective of the level of over-fertilization [37].

REFERENCES

1. Shaahan, M.M., A.A. El-Sayad and E.A.A. Abou El-Nour, 1999. Predicting nitrogen, magnesium and iron nutritional status in some perennial crops using a portable chlorophyll meter. Scientia Horticulturae., 82: 339-348.
2. Earl, H.J. and M. Tollenaar, 1997. Maize leaf absorptance of photosynthetically active radiation and its estimation using a chlorophyll meter. Crop Sci., 37: 436-440.
3. Sinclair, T.R., 1995. Effect of nitrogen supply on maize yield. I. Modeling physiological responses. Agron. J., 87: 632–641.
4. Lemaire, G. and F. Gastal, 1997. Nitrogen uptake and distribution in plant canopies. In: Lemaire, G. (Ed.), Diagnosis of the Nitrogen Status in Crops. Springer–Verlag, Berlin, pp: 3-43.
5. Arregui, L.M., B. lasa, A. Lafarga, I. Iraneta and E. Baroja and M. Quemada, 2006. Evaluation of chlorophyll meters as tools for N fertilization in winter wheat under humid Mediterranean conditions. Europ. J. Agronomy., 24: 140-148.
6. Vidal, I., L. Longeri and J.M. Hetier, 1999. Nitrogen uptake and chlorophyll meter measurement in spring wheat. Nutr. Cycl. Agroecosyst., 55: 1-6.
7. Herrmann, A. and F. Taube, 2004. The range of the critical nitrogen dilution curve for maize (*Zea mays* L.) can be extended until silage maturity. Agron J., 96: 1131-1138.
8. Parvizi, Y., A. Ronaghi, M. Maftoun and A. Karimian, 2004. Growth, nutrient status and chlorophyll meter readings in wheat as affected by nitrogen and manganese. Commun. Soil Sci. Plant Anal., 35: 1387-1399.
9. Chapman, S.C. and H.J. Barreto, 1997. Using a chlorophyll meter to estimate specific leaf-nitrogen of tropical maize during vegetative growth. Agron. J., 89: 557–562.
10. Costa, C., L.M. Dwyer, P. Dutilleul, D.W. Stewart, B.L. Ma and D.L. Smith, 2001. Inter-relationships of applied nitrogen, SPAD and yield of leafy and non-leafy maize genotypes. J. Plant Nutr., 24: 1173-1194.
11. Kapotis, G., G. Zervoudakis, T. Veltsistas and G. Salahas, 2003. Comparison of chlorophyll meter readings with leaf chlorophyll concentration in *Amaranthus vlitus*: correlation with physiological processes. Russ. J. Plant Physiol., 50: 442-444.
12. Yamamoto, A., T. Nakamura, J.J. Adu-Gyamfi and M. Saigusa, 2002. Relationship between chlorophyll content in leaves of sorghum and pigeonpea determined by extraction method and by chlorophyll meter (SPAD-502). J. Plant Nutr., 25: 2295-2301.
13. Cartelat, A., Z.G. Cerovic, Y. Goulas, S. Meyer, C. Lelarge, J.L. Prioul, A. Barbottin, M.H. Jeuffroy, P.G. Gata and I. Agati, 2005. Optically assessed contents of leaf polyphenolics and chlorophyll as indicator of nitrogen deficiency in wheat (*Triticum aestivum* L.). Field Crops Res., 91: 35-49.
14. Lopez-Bellido, R.J., C.E. Shepherd and P.B. Barraclough, 2004. Predicting post anthesis N requirements of bread wheat with a Minolta SPAD meter. Eurp. J. Agronomy., 20: 313-320.
15. Costa, C., D. Frigon, P. Dutilleul, L.M. Dwyer, V.D. Pillar, D.W. Stewart and D.L. Smith, 2003. Sample size determination for chlorophyll meter readings on maize hybrids with a broad range of canopy types. J. Plant Nutr., 26: 1117-1130.
16. Varvel, G.E., J.S. Schepers and D.D. Francis, 1997. Ability for in season correction of nitrogen deficiency in corn using chlorophyll meters. Soil Sci. Soc. Am. J., 61: 1233-1239.
17. Bullock, D.G. and D.S. anderson, 1998. Evaluation of the Minolta SPAD-502 chlorophyll meter for nitrogen management in maize. J. Plant Nutr., 21: 741-755.

18. Sestak, Z. and J. Catsky, 1962. Intensity of photosynthesis and chlorophyll content as related to leaf age in *Nicotiana senderae*. Hort. Biol. Plant., 4: 131-140.
19. Schepers, J.S., D.D. Francis, M. Vigil and F.E. Below, 1992. Comparison of maize leaf-nitrogen concentration and chlorophyll meter readings. Commun. Soil Sci. Plant Anal., 23: 2173-2187.
20. Smeal, D. and H. Zhang, 1994. Chlorophyll meter evaluation for nitrogen management in maize. Commun. Soil Sci. Plant Anal., 25: 1495-1503.
21. Vetsch, J.A. and G.W. Randall, 2004. Corn production as affected by nitrogen application timing and tillage. Agron J., 96: 502-509.
22. Fox, R.H., W.P. Piekielk and K.M. Macneal, 2001. Comparison of late-season diagnostic test for predicting nitrogen status of corn. Agron J., 93: 590-597.
23. Bergmann, W., (ed). 1992. Nutritional Disorders of Plants: development, visual and analytical diagnosis. G. Fisher Verlag; Jena; Stuttgart; New York, pp: 741.
24. Peng, S., F.V. Garcia, R.C. Laza and K.G. Cassman, 1993. Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. Agron. J., 85: 987-990.
25. Dwyer, L.M., M. Tollenaar and L. Houwing, 1991. A nondestructive method to monitor leaf greenness in corn. Can. J. Plant Sci., 71: 505-509.
26. Schroder, J.J., J.J. Neeteson, O. Oenema, P.C. Struik, 2000. Dose the crop or soil indicate how to save nitrogen in maize production. Reviewing the state of the art. Field Crops Res., 66: 151-164.
27. Dwyer, L.M., A.M. anderson, B.L. Ma, D.W. Stewart, M. Tollenaar and G. Gregorich, 1995. Quantifying the nonlinearity in chlorophyll meter response to maize leaf-nitrogen concentration. Can. J. Plant Sci., 75: 179-182.
28. Waskom, R.M., D.G. Westfall, D.E. Spellman and P.N. Soltanpour, 1996. Monitoring nitrogen status of maize with a portable chlorophyll meter. Commun. Soil Sci. Plant Anal., 27: 545-560.
29. Wood, C.W., D.W. Reeves, R.R. Duffield and K.L. Edmisten, 1992. Field chlorophyll measurements for evaluation of maize nitrogen status. J. Plant Nutr., 15: 487-500.
30. Hussain, F., K.F. Bronson, Y. Singh, B. Singh and S. Peng, 2000. Use of chlorophyll meter sufficiency indices for nitrogen management of irrigated rice in Asia. Agron. J., 92: 875-879.
31. Martinez, D.E. and J.J. Guiamet, 2004. Distortion of the SPAD-502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie., 24: 41-46.
32. Varvel, G.E., W.W. Wilhelm, J.F. Shanahan and J.S. Schepers, 2007. An algorithm for corn nitrogen recommendations using a chlorophyll meter based sufficiency index. Agron. J., 99: 701-706.
33. Schepers, J.S., D.D. Francis and J.F. Power, 1995. Tissue analysis to improve nitrogen management practices. p. 195–198. In Proc. Clean Water-Clean Environment-21st Century. Vol. II: Nutrients. Kansas City, MO. 5-8 Mar. 1995. Am. Soc. Agric. Eng., St. Joseph, MI.
34. Binder, D.L., D.H. Sander and D.T. Walters, 2000. Maize response to time of nitrogen application as affected by level of nitrogen deficiency. Agron. J., 92: 1228-1236.
35. Sandoval-Villa, M., C.W. Wood and E.A. Guertal, 2002. Tomato leaf chlorophyll meter readings as affected by variety, nitrogen form and nighttime nutrient solution strength. Journal of Plant Nutr., 25: 2129-2142.
36. Murdock, L., S. Jones, C. Bowley, P. Needham, J. James and P. Howe, 1997. Using a Chlorophyll Meter to Make Nitrogen Recommendations on Wheat; University of Kentucky, Cooperative Extension Service: Lexington, KY.
37. Hawkins, J.A., J.E. Sawyer, D.W. Barker and J.P. Lundvall, 2007. Using relative chlorophyll meter values to determine nitrogen application rates for corn. Agron. J., 99: 1034-1040.