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Chlorophyll meter and leaf colour chart to estimate chlorophyll content, leaf colour, and yield of cassava

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Abstract

A field experiment was conducted with two cassava cultivars and eight levels of nitrogen to examine the relationship between extractable chlorophyll (Chl) content of cassava leaves and both the Chl meter value (SPAD) and leaf colour chart (LCC) score. The SPAD, LCC, and Chl *a*+*b* content were influenced by leaf position, growth stage, cultivar (cv.), and N fertilization. The cvs. and N fertilization had significant effect on SPAD, LCC, and Chl *a*+*b* content of youngest fully expanded leaf (leaf 1) blade in most cases. An F-test indicated that common equations pooled across cvs., N fertilization, and growth stages could be used to describe the relationships between Chl *a*+*b* content and LCC and between SPAD and LCC, but not between SPAD and Chl *a*+*b* content. Relationships between tuber yield and SPAD, LCC, and Chl *a*+*b* content were significant ($p<0.05$) and positive at 30 and 60 d after planting. Thus LCC and SPAD can be used to estimate leaf Chl content which is an indicator of leaf N status.

Additional key words: cultivar; *Manihot esculenta*; N fertilization; SPAD.

Introduction

Cassava (*Manihot esculenta* Crantz), a native of South America, is the most important root crop and fourth most important source of food calories in the tropics (Cock 1982). In plants, green leaf colour is determined primarily by the spectral properties of chlorophyll (Chl). Leaf colour can be estimated by visual assessments, which are subjective and cannot be accurately quantified (cf. Šesták 1971), and a significant positive correlation exists between leaf colour and leaf Chl content (Madeira *et al.* 2000). *In vitro* determinations are destructive, expensive, and time consuming, and may therefore not be applicable for all purposes (Uddling *et al*. 2007). There are more rapid methods and tools for estimating the leaf Chl and leaf colour non-destructively *in vivo*.

The Chl meter and leaf colour chart (LCC) (Balasubramanian *et al*. 1999) are two simple and non-destructive, farmer friendly tools which can be used to monitor leaf colour and leaf Chl content. A portable Chl meter uses spectral transmittance properties of leaves. High correlations of Chl meter readings and LCC values with extractable Chl content have been reported for several plant species (Dwyer *et al*. 1991, Madeira *et al*. 2000, 2003, Uddling *et al*. 2007).

The relationship between the leaf Chl content and leaf colour determined by Chl meter (SPAD value) and LCC in cassava was determined (*1*) to find the empirical relationships of leaf Chl contents with SPAD and LCC of three leaf positions of two cassava cultivars (cvs.) and at three time intervals, (*2*) to relate the LCC with SPAD, and (*3*) to determine the relationship of tuber yield of cassava with SPAD, LCC, and leaf Chl content.

Materials and methods

Field experiment was conducted in the experimental farm of Central Tuber Crops Research Institute, Kerala, India (latitude 8º32'N; longitude 76º55'E; altitude 50 m a.s.l.) during two seasons, 2005−2006 and 2006−2007. We used a split plot layout, with main plot treatments in a randomized complete block design (RCBD) and three replicates per treatment. Main plots were two popular cassava cvs. Sree Vijaya and M-4. Sree Vijaya was

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released from CTCRI, India in 1998, contains 26 % starch and matures in 6 months. M-4 was introduced into India from Malaysia, contains 29 % starch and matures in 10 months. Sub-plots were the eight N fertilizer application rates, 0, 12.5, 25, 50, 75, 100, 150, and 200 kg ha^{-1} N applied as urea (solid granules, 46 % N); half as basal at the time of planting and the remaining applied 60 d later. Phosphorus and potassium fertilizers and all other agronomic practices were standard (Mohankumar 2000). Each individual square plot of 5.4×5.4 m consisted of 36 cassava plants, each planted on mounds taken at a spacing of 0.9×0.9 m. The different rates of N were in order to develop different shades of green colour to the leaves, different leaf Chl contents, and to decide the critical leaf colour shades of N top dressing for optimum tuber yield. The soil of the experimental site is a clayey, skeletal, isohyperthermic, typic plinthustults. Over the 10 month growing season, total rainfall was 1 546 (2005−2006) and 1 820 (2006−2007) mm. The maximum and minimum temperatures over the growth period were 33.86 and 21.83 ºC and 35.20 and 21.27 ºC during the two seasons, respectively.

Leaf colour chart (LCC) developed from a Japanese prototype (Furuya 1987) was standardized along with the Chl meter. The LCC is made of high impact plastic and consists of six colour shades from yellowish green (No. 1) to dark green (No. 6) and the holder is gray in colour. The leaf colour of youngest fully expanded leaf blade (leaf 1) and two nearest leaves (2 and 3) was measured under the same environmental conditions. The colour of a single leaf was measured by placing the middle part of the middle lobe of the leaf in front of the colour strip for comparison. Since the leaf colour reading is affected by the sun's angle and sunlight irradiance, LCC measurements were made by shading the measured leaf with the body of the experimenter at the same time of the day. Readings of five leaves at random for each plot were taken and average LCC was computed. If the colour of a particular leaf was between two colour shades, the mean of the two values was taken as the reading. All observations were recorded on 30, 60, and 90 d after planting (DAP).

Chl meter *SPAD-502* (*Minolta Camera Co*., Japan) uses a silicon photodiode to detect the transmittance of radiation emitted by two diodes, peaking at 650 nm (high absorbance by Chl) and at 940 nm (negligible absorbance by Chl). The meter values, therefore, represent transmittance ratio through the leaf tissue at these wavelengths. Four Chl meter readings were taken around the midpoint of the middle lobe of each leaf, two from each side of the midrib of the middle lobe 30 mm apart. Readings of the same five leaves used for LCC measurements were used for SPAD values also and the means of 20 readings were taken as the mean value of each plot. All observations were recorded on 30, 60, and 90 DAP. After taking the reading, the leaves were pooled for estimation of Chl *a*, *b*, and *a*+*b* contents.

Chl was extracted in 80 % acetone. The contents of Chl *a* and *b* [g kg^{-1}(fresh tissue)] were measured using a spectrophotometer at A_{645} and A_{663} and calculated using the equations given in AOAC (1984).

The cassava plants were harvested manually by pulling out the tubers after a growing period of 6 months for Sree Vijaya and 10 months for M-4. Excluding the outer border row of 20 plants from each square plot, 16 inner plants were harvested to assess the effect of the cv. and N fertilizer rates on tuber yield of cassava.

Statistical analysis of variance was performed on SPAD, LCC, leaf Chl contents, and tuber yield to determine the effects of cvs., N fertilization, and their interaction and different sampling dates using *SYSTAT* (1992). Least significant difference (LSD) test was used at 0.05 level of probability to test differences between treatment means. The empirical relationships of leaf Chl contents with tuber yield, LCC, and SPAD were analyzed by regression models and were compared for different leaf positions as well as for three different growth stages using F-test and were deemed significant at $p<0.05$.

Results and discussion

Effects of cvs. and N fertilizer rates: The SPAD, LCC, and leaf Chl content of leaf 1 were significantly lower than those of leaves 2 and 3, which were similar. Piekielek and Fox (1992) also reported that SPAD values were influenced by the leaf position in maize (*Zea mays*). The effects of cv. and N fertilization on SPAD, LCC, and leaf Chl content of leaves 2 and 3 were mostly non-significant (data not shown). Howeler (1996) reported that the youngest fully expanded leaf reflects the most recent history of N availability to cassava and an immediate reflection will be noticed in the green leaf colour of leaf 1 and not in lower leaves. However, in most cases cv. and N rate had a significant influence on the values of leaf 1 (Tables 1 and 2). Interactions between cv. and N fertilization were not significant (data not shown). The interaction between cv. and DAP was significant, the values were at par between 30 and 60 DAP while it was significantly different at 90 DAP in most cases. Thus one value could be used up to 60 DAP to predict Chl content and tuber yield of cassava.

With leaf 1 at 30 and 60 DAP there was a significant difference in SPAD, LCC, Chl *a*, and Chl *a*+*b* contents of leaf 1 between the two cvs. (Table 1). At 90 DAP, these values were also lower for Sree Vijaya compared to

Table 1. SPAD value, LCC score, and leaf chlorophyll (Chl) contents [g kg^{-1}] of youngest fully expanded leaf blade at 30, 60, and 90 d after planting (DAP) in two cassava cultivars across eight N fertilizer rate treatments (mean of 2 years).

DAP	Cultivar	SPAD	LCC	Chl *a*	Chl *b*	Chl *a*+*b*
30	Sree Vijaya	25.11	2.58	1.802	0.614	2.408
	M-4	26.42	2.74	1.963	0.670	2.633
	LSD (0.05)	1.10	0.12	0.143	NS	0.162
60	Sree Vijaya	25.61	2.56	1.869	0.451	2.320
	M-4	26.94	2.69	1.974	0.571	2.545
	LSD (0.05)	1.00	0.11	0.102	NS	0.165
90	Sree Vijaya	25.63	2.52	1.821	0.663	2.564
	M-4	26.91	2.65	1.865	0.697	2.648
	LSD (0.05)	1.00	0.11	NS	NS	NS

Table 2. SPAD value, LCC score, and leaf chlorophyll (Chl) contents [g kg^{-1}] of youngest fully expanded leaf blade at 30, 60 and 90 d after planting (DAP) at different N doses [kg ha^{-1}] across two cultivars of cassava (mean of 2 years).

DAP	N	SPAD	LCC	Chl *a*	Chl *b*	Chl *a*+*b*
30	0	19.80	1.83	1.67	0.569	2.249
	12.5	22.22	2.14	1.711	0.577	2.299
	25.0	25.74	2.44	1.763	0.582	2.352
	50.0	26.13	2.57	1.845	0.631	2.384
	75.0	26.47	2.65	1.871	0.645	2.443
	100.0	27.39	2.78	1.894	0.660	2.559
	150.0	27.89	2.85	1.895	0.664	2.569
	200.0	28.52	2.89	1.897	0.665	2.571
	LSD (0.05)	0.51	0.10	NS	NS	0.231
60	0	19.32	1.87	1.724	0.436	2.162
	12.5	22.73	2.18	1.757	0.457	2.215
	25.0	24.94	2.47	1.844	0.482	2.329
	50.0	27.78	2.66	1.932	0.506	2.441
	75.0	28.25	2.85	1.991	0.543	2.533
	100.0	29.48	2.91	2.064	0.589	2.656
	150.0	30.08	2.92	2.069	0.592	2.663
	200.0	30.78	3.03	2.069	0.593	2.666
	LSD (0.05)	1.21	0.15	0.211	0.035	0.130
90	0	18.84	1.91	1.741	0.598	2.345
	12.5	20.07	2.18	1.813	0.623	2.447
	25.0	21.14	2.31	1.847	0.649	2.506
	50.0	24.66	2.54	1.869	0.698	2.578
	75.0	27.79	2.63	1.943	0.731	2.691
	100.0	28.49	2.80	1.967	0.768	2.743
	150.0	29.74	2.82	1.971	0.771	2.753
	200.0	30.38	2.87	1.973	0.775	2.756
	LSD (0.05)	1.04	0.12	0.110	NS	0.206

M-4 but there was no significant difference in Chl content between the cvs. Yang *et al*. (2003) reported that N rates and cvs. had a significant effect on LCC score and SPAD value in rice (*Oryza sativa*). Significantly better relationships between SPAD and extractable Chl were observed by Azia and Stewart (2001) in muskmelon (*Cucumis melo* L.) leaves and by Madeira *et al*. (2003) in sweet pepper (*Capsicum annuum*).

The SPAD, LCC, and leaf Chl contents of leaf 1 increased as N fertilizer rate increased in all the three growth stages (Table 2). The N fertilization had larger effect on SPAD than on LCC and leaf Chl contents especially at 60 DAP. At 0 kg(N) ha^{-1}, both LCC and SPAD were very low and there was a significant increase in both the values at 12.5 kg(N) ha^{-1}. At 30 DAP, the LCC for 0 kg(N) ha^{-1} was 1.83 which increased significantly to 2.78 at 100 kg(N) ha^{-1} and to 2.89 at 200 kg(N) ha^{-1}. A similar trend was observed for SPAD and Chl *a*+*b* content.

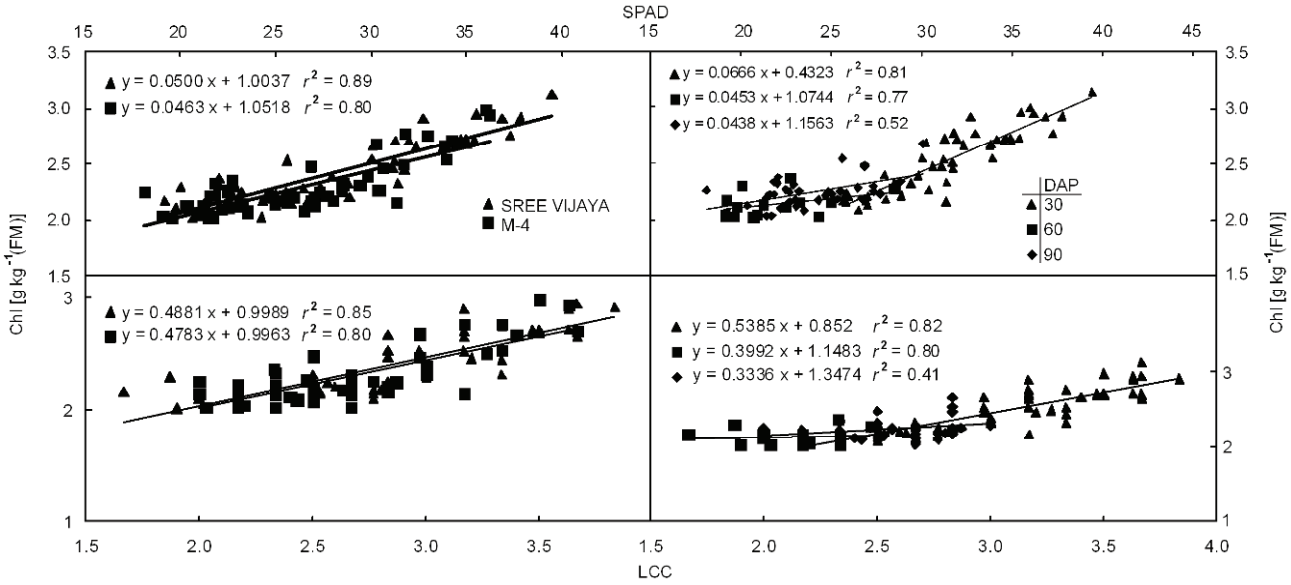

Fig. 1. Relationship between chlorophyll (Chl) *a*+*b* contents and Chl meter (SPAD) values (*top*) or leaf colour chart (LCC) values (*bottom*) of leaf 1 of two cassava cultivars and at 30, 60, and 90 d after planting (DAP).

Relationship between leaf Chl content and SPAD of leaf 1 was positive and significant (p<0.05) for both cassava cvs. (Fig. 1). Relationships between Chl *a* and *b* and SPAD were not significant (p>0.05) (data not shown). The relationship between SPAD and leaf Chl content was significantly better with leaf 1 (Fig. 1) than with leaves 2 and 3 (data not shown). The relationship between SPAD and leaf Chl content of leaf 1 varied significantly with growth stage. The relationship was significant (p<0.05) and positive at 30 and 60 DAP (Fig. 1), but not at 90 DAP. When leaf 1 data were combined for cvs. and N fertilizer rates at 30 and 60 DAP, the relationship between Chl *a*+*b* content and SPAD was described by the equation: Chl *a*+*b* [g kg^{-1}] = 0.050 SPAD + 0.977 (r^2 = 0.90). Thus SPAD provides a good estimate of the Chl *a*+*b* content in cassava up to 60 DAP at which time N fertilizer application needs to be completed (Mohankumar 2000). Although very good relationships between SPAD and both Chl *a*+*b* and Chl *a* have been reported in many plant species (Dwyer *et al*. 1991, Vos and Bom 1993, Madeira 2003), this was not evident in our study with cassava. However, we found that SPAD can be used to accurately predict the Chl *a*+*b* content in leaf 1 of cassava which is the index plant part for determining critical nutrient concentration (Howeler 1996).

Relationship between leaf Chl content and LCC: With leaf 1, the linear relationship between LCC and Chl *a*+*b* content (but not Chl *a* and *b*) of both cvs. was significant (p<0.05) and positive (Fig. 1). The relationship was stronger in leaf 1 (Fig. 1) than in leaves 2 and 3 (data not shown).

With leaf 1, the relationship between LCC and Chl *a*+*b* content was positive and significant (p<0.05) at both 30 and 60 DAP (Fig. 1), and the strength of the relationship was greater than at 90 DAP. When leaf 1 data were combined across cvs., N fertilizer rates, and at 30 and 60 DAP, the relationship between LCC and Chl *a*+*b* content was described by the equation: Chl *a*+*b* [g kg^{-1}] = 0.507 LCC + 0.948 (r^2 = 0.85).

Relationship between SPAD and LCC: With leaf 1, the linear relationship between SPAD and LCC was significant (p<0.05) and positive at 30 and 60 DAP (Fig. 2). In addition, there was a generally better relationship between LCC and SPAD than with LCC and Chl *a*+*b* content (cf. Figs. 2 and 1). The relationships were not significant (p>0.05) for leaves 2 and 3 at all three growth stages (data not shown).

For leaf 1, the LCC had a close relationship with SPAD across growth stages (r^2 = 0.80). Thus the LCC could substitute for Chl meter in estimating Chl *a*+*b* content and leaf colour in cassava. When the data of leaf 1 were combined across cvs., N fertilization, and for 30 and 60 DAP, the regression between LCC and SPAD was SPAD = 10.981 LCC – 3.510 (r^2 = 0.82).

The F-test applied to the common regression equations indicated that a single regression equation was valid for LCC and total Chl (p=0.13) and for LCC and SPAD (p=0.11) while a common regression equation should not be used for the relation of SPAD and total Chl content (p<0.05).

Relationship between tuber yield and SPAD, LCC, and leaf Chl: The SPAD of leaf 1 at 30 DAP accounted for 83 % and at 60 DAP for 81 % of the variation in tuber yield of cassava. The LCC scores of leaf 1 at 30 DAP accounted for 85 % and at 60 DAP for 81 % of the

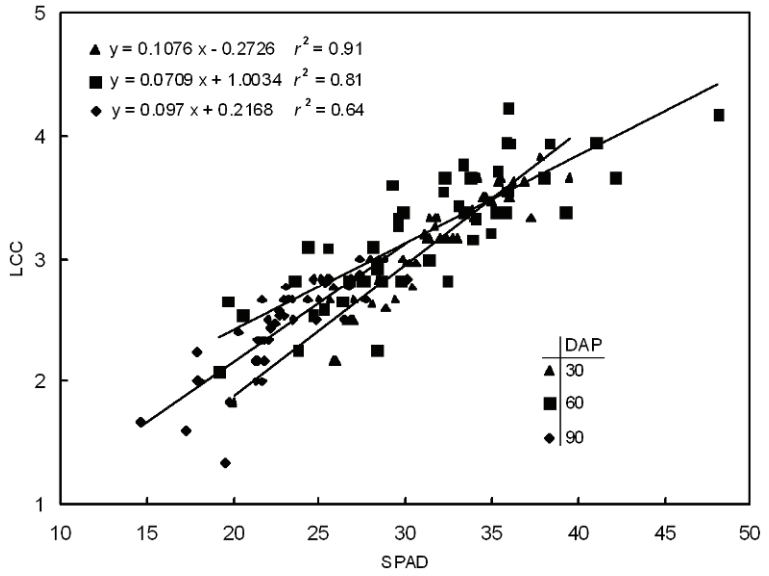

Fig. 2. Relationship between LCC score and SPAD value of leaf 1 of cassava at 30, 60, and 90 d after planting (DAP).

Table 3. Linear coefficients relating tuber yield of cassava to SPAD value, LCC score, and chlorophyll (Chl) *a*+*b* of leaf 1 at each growth stage (DAP – days after planting) in the form: Yield = a + b (SPAD), Yield = a + b (LCC), or Yield = a + b (Chl *a*+*b*).

DAP	LCC			SPAD			Chl *a*+*b*		
	a	b	r^2	a	b	r^2	a	b	r^2
30	−15.49	14.811	0.85	−20.91	1.713	0.83	24.610	1.111	0.80
60	−13.65	13.582	0.81	−14.93	1.378	0.81	25.050	−1.697	0.65
90	−57.06	32.244	0.48	−45.07	2.907	0.39	−1.307	10.690	0.46

variation in cassava tuber yield (Table 3). The relationship of tuber yield with SPAD or LCC was significant at 30 and 60 DAP ($p<0.05$) and not significant ($p>0.05$) at 90 DAP. The correlation between SPAD or LCC and tuber yield was significantly higher than with Chl *a*+*b* content (r^2 = 0.80 and 0.65, respectively, at 30 and 60 DAP). If used between 30 and 60 DAP, both SPAD and LCC seem to accurately predict tuber yield of cassava. Both leaves 2 and 3 had insignificant correlation of tuber yield with SPAD, LCC, and Chl *a*+*b* content (data not shown). Thus there exists significant and positive correlation between tuber yield and SPAD or LCC of leaf 1. Since the correlation was significantly higher with SPAD or LCC than with Chl contents, these two tools can very effectively be used in predicting tuber yield of cassava and for N management.

In conclusion, our results indicate that SPAD and LCC can be used to estimate total Chl content in leaf 1 of cassava. These variables were influenced by leaf position, growth stage, cv., and N fertilizer rate. Leaf 1 had a significantly lower SPAD, LCC, and Chl *a*+*b* than leaves 2 and 3 and the two cvs. differed in their response to each of the variables up to 60 DAP. The values of each of the variables increased with increasing N application rate at all growth stages. Relationships between tuber yield and SPAD ($r^2 = 0.83$), LCC ($r^2 = 0.85$), and Chl *a*+*b* ($r^2 = 0.80$) were significant ($p<0.05$) and positive at 30 and 60 DAP. A single regression equation can be used to describe the relationship between LCC and Chl *a*+*b* and between LCC and SPAD, but not between SPAD and Chl *a*+*b*.

References

AOAC: Official Methods of the Association of Official Analytical Chemists. − Pp. 231-232. Arlington, 1984.

Azia, F., Stewart, K.A.: Relationships between extractable chlorophyll and SPAD values in muskmelon leaves. − J. Plant Nutr. **24**: 961-966, 2001.

Balasubramanian, V., Morales, A.C., Cruz, R.T., Abdulrachman, S.: On-farm adaptation of knowledge-intensive nitrogen management technologies for rice systems. − Nutr. Cycling Agroecosyst. **53**: 59-69, 1999.

Cock, J.H.: Cassava: A basic energy source in the tropics. − Science **218**: 755-762, 1982.

Dwyer, L.M., Tollenaar, M., Houwing, L.: A nondestructive method to monitor leaf greenness in corn. − Can. J. Plant Sci. **71**: 505-509, 1991.

Furuya, S.: Growth diagnosis of rice plants by means of leaf color. − Jap. agr. Res. quart. **20**: 147-153, 1987.

Howeler, R.H.: Mineral nutrition of cassava. – In: Craswell, E.T., Asher, C.J., O'Sullivan, J.N. (ed.): Mineral Nutrient

Disorders of Root Crops in the South Pacific. Pp. 110-116. ACIAR, Canberra 1996.

Madeira, A.C., Ferreira, A., Varennes, A., Vieira, M.I.: SPAD meter *versus* tristimulus colorimeter to estimate chlorophyll content and leaf color in sweet pepper. − Commun. Soil Sci. Plant Anal. **34**: 2461-2470, 2003.

Madeira, A.C., Mendonca, A., Ferriera, M.E., Taborda, M. de L.: Relationship between spectroradiometric and chlorophyll measurements in green beans. − Commun. Soil Sci. Plant Anal. **31**: 631-643, 2000.

Mohankumar, C.R.: Cultural and manurial requirement of cassava. – In: Mohankumar, C.R. (ed.): Production Technology of Tuber Crops. Pp. 7-41. CTCRI, Thiruvananthapuram 2000.

Piekielek, W.P., Fox, R.H.: Use of a chlorophyll meter to predict sidedress nitrogen requirements for maize. − Agron. J. **84**: 59-65, 1992.

Šesták, Z.: Determination of chlorophylls *a* and *b*. – In: Šesták, Z., Čatský, J., Jarvis, P.G. (ed.): Plant Photosynthetic Production. Manual of Methods. Pp. 672-701. Dr W. Junk Publ., The Hague 1971.

Systat: Systat software. − Systat Inc., Evaston 1992.

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

Vos, J., Bom, M.: Hand-held chlorophyll meter: a promising tool to assess the nitrogen status of potato foliage. − Potato Res. **36**: 301-308, 1993.

Yang, W.H., Peng, S.B., Huang, J.L., Sanico, A.L., Buresh, R.J., Witt, C.: Using leaf color charts to estimate leaf nitrogen status of rice. − Agron. J. **95**: 212-217, 2003.