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Rapid Assessment of Chlorophyll Content in Sugarcane using a SPAD Chlorophyll Meter across Different Water Stress Conditions

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Abstract: *In vitro* assessments of chlorophyll content are expensive, laborious and time consuming. The alternative methods which are more rapid and straightforward could be very useful. The aim of this study was to investigate the effects of water stress on chlorophyll content and SPAD Chlorophyll Meter Reading (SCMR) and the relationship between chlorophyll content and SCMR under well-watered and drought conditions. Ten sugarcane genotypes (Uthong 6, Khon Kaen 80, K86-161, Khon Kaen 3, 03-4-425, KU60-1, Phill.66-07, B34-164, Uthong 2 and LF82-2122) and two water regimes (well-watered control and water stress at early growth stage) were laid out in factorial experiment in a randomized complete block design with two replications. Drought was imposed to the crop for 10 days during 90 days after transplanting (DAT) to 100 DAT. Data were recorded for total chlorophyll content by N, N-dimethylformamide extraction and SCMR on the second fully expanded leaf from the top at 90 DAT, 100 DAT and 110 DAT. Drought significantly reduced chlorophyll content and SCMR. The reduction in chlorophyll content was more severe than SCMR. Similar responses of sugarcane genotypes to drought and well-watered conditions were observed for chlorophyll content and SCMR. The breeding line 03-4-425 and the cultivars KU60-1 were identified as the best genotypes for chlorophyll content and SCMR. The correlation coefficients between chlorophyll content and SCMR were significant under well-watered conditions and the relationships were more strong and consistent under drought and drought relief. The SCMR can be used for evaluation of chlorophyll content under different water regimes.

Key words: Chlorophyll content, drought, recovery, *Saccharum officinarum* L., SCMR

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is known as an effective crop for biomass production and it is used mainly for sugar production and bio-fuel. In order to have a good biomass production, sugarcane crop must produce high yield of cane and high quality of juice. These biomass cane products, however, seem to be greatly reduced by drought stress (Wiedenfeld, 2000).

The effect of drought on sugarcane is that it reduces gas exchange between leaf and outside air primarily by leaf stomatal closure, leading to restriction of CO_2 diffusion (Zlatev and Yordanov, 2004). Despite harsh condition like drought, plant grown in semi-dryland has photosynthetic apparatus that are remarkably resistant to leaf dehydration. This allows sugarcane to be quite resistant to drought and can maintain the photosynthetic capacity under drought condition (Zlatev and Yordanov, 2004).

Photosynthetically active radiation is absorbed by chlorophyll and accessory pigments of chlorophyll-protein complexes and it migrates to the reaction centers of PS I and II, where the conversion of the quantum photosynthetic process takes place (Rong-hua *et al*., 2006). Analysis of chlorophyll content is important for evaluating the health or integrity of the internal apparatus during photosynthetic process within a leaf (Rong-hua *et al*., 2006; Clark *et al*., 2000) and provides a rapid and accurate technique of detecting and quantifying plants tolerant to drought stress (Rong-hua *et al*., 2006; Percival and Sheriffs, 2002).

Leaf chlorophyll concentration is determined directly by using organic extracting solvents such as acetone (Efeoglu *et al*., 2009; Ting *et al*., 2009; Liu *et al*., 2008), methanol (Cenkci *et al*., 2010), dimethylsulphoxide (DMSO) (Netto *et al*., 2005) and N, N-dimethylformamide (DMF) (Cubas *et al*., 2008) and Chlorophyll content is subsequently measured in a spectrophotometer. Such

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in vitro assessments are expensive, laborious and time consuming. Therefore, the alternative methods which are more rapid and straightforward could be very useful for estimating leaf chlorophyll concentration.

A Chlorophyll Meter SPAD-502 is used for measuring the absorbance of the leaf in two regions, a red 650 nm and an infrared 940 nm (Minolta, 1989). The SPAD Chlorophyll Meter Reading (SCMR) can be recorded on intact leaves at any time throughout the growing process of the leaves (Minolta, 1989). The SCMR was suggested for a rapid assessment of chlorophyll content in many crops, such as corn (Rostami *et al*., 2008) and papaya (Netto *et al*., 2002). The SCMR has been positively correlated with chlorophyll content in *Amaranthus vlitus* L., a common weed (Kapotis *et al*., 2003), soybean (Monje and Bugbee, 1992), cutleaf coneflower (Neufeld *et al*., 2006), rice (Turner and Jund, 1991), coffee (Netto *et al*., 2005), *Lindera melissifolia* (Hawkins *et al*., 2009) birch and wheat (Uddling *et al*., 2007) across a range of plant ages, growing conditions and genotypes. Good association between SCMR and chlorophyll content under a range of water regimes has also been reported in peanut (Arunyanark *et al*., 2009), winter wheat (Barraclough and Kyte, 2001) and sorghum (Xu *et al*., 2000).

In sugarcane, Silva *et al*. (2007) reported that long term drought of 90 days reduced SCMR and the reduction was more severe in leaves of susceptible genotypes. They also found that SCMR could identify drought tolerant genotypes correctly. However, they studied in few sugarcane genotypes and under long term drought conditions.

Sugarcane under rainfed conditions can impose to short period of drought and then recover from drought. The question underlying the investigation is that there would be differential responses for SCMR and chlorophyll content among a wide range of sugarcane genotypes to water stress and recovery from the stress and the relationships between SCMR and chlorophyll content under these conditions have not been well researched. The objectives of this study was to investigate the effects of water stress on chlorophyll content and SCMR and the relationship between chlorophyll content and SCMR in a range of sugarcane genotypes across available soil water regimes.

MATERIALS AND METHODS

Plant culture and stress treatment: Ten sugarcane breeding lines and cultivars (Uthong 6, Khon Kaen 80, K86-161, Khon Kaen 3, 03-4-425, KU60-1, Phill.66-07, B34-164, Uthong 2 and LF82-2122) kindly provided by the Khon Kaen Field Crops Research Center, Khon Kaen, Thailand were used in this study during January to May 2009 at Khon Kaen University.

The pot experiment was undertaken under greenhouse conditions. The plastic containers with 27.5 cm in diameter and 35.0 cm in height were filled with 22 kg of dry soil to create uniform bulk density of 1.5 g cm^{-3} from the bottoms of the containers to 10 cm below the top of the pots. The soil consisted of sand (73.03%), silt (22.67%), clay (4.30%) and organic matter (0.25%) and was identified as sandy loam. The soil chemical properties were pH 5.0, total nitrogen 0.040%, available phosphorus 72 ppm and extractable potassium 67 ppm. The soil moisture contents were 11.5% at Field Capacity (FC) and 2.67% at permanent wilting point (PWC).

The seed canes of 10 cultivars were cut into short pieces each of which had one active bud and they were pre-germinated in germinating trays containing moisten absorbent paper. The uniformly-germinated seed canes were then planted in the plastic containers. There was a plant in each pot.

A factorial experiment in a randomized complete block design with two replications was carried out in a greenhouse during January to May 2009 at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province. Two water regimes (field capacity and drought stress followed by recovery) were assigned as factor A and 10 sugarcane cultivars were assigned as factor B.

Water was supplied daily to the experiment at field capacity level from transplanting to 90 days after transplanting (DAT) and the amount of water was calculated as described previously (Songsri *et al*., 2009). After 90 DAT, water level at field capacity was maintained throughout the experiment for well-watered control. For drought treatment, water was withheld at 90 to 100 DAT. Re-watering was applied after 100 DAT and the stressed treatments were maintained at field capacity.

Calculation of total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Crop water requirement was calculated using the equation as described previously (Songsri *et al*., 2009):

$$ET_{crop} = ET_o \times K_c$$

Where:

ET_{crop} = Crop water requirement (mm day^{-1})

ET_o = Evapotranspiration of a reference plant under specified conditions calculated by pan evaporation method

K_c = Crop water requirement coefficient for sugarcane, which varies with genotype and growth stage

Surface evaporation (E_s) was calculated as described previously (Songsri *et al.*, 2009):

$$E_s = \beta \times \left(\frac{E_o}{t}\right)$$

Where:
E_s = Soil evaporation (mm)
β = Light transmission coefficient measured depending on crop cover
E_o = Evaporation from class A pan (mm day^{-1})
t = Days from the last irrigation

Soil moistures content were measured by gravimetric method at 90 DAT, 100 DAT and 110 DAT.

SPAD readings and chlorophyll extraction: Soil moisture content, chlorophyll concentration and SCMR were observed before the imposition of drought (90 DAT), after drought (100 DAT) and drought recovery (110 DAT). Soil moisture content was measured in order to monitor if the water treatments were controlled properly.

The SCMR was measured on the second fully expanded leaf from the top of the main stem of each plant using an SPAD-502 meter (Minolta SPAD-502 meter, Tokyo, Japan). The data points were recorded at six positions along the length of the leaf blade and then the data points were averaged as a single value. Care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and the interference from veins and midribs was avoided.

The chlorophyll content in leaves was measured by the method described by Moran (1982). Briefly, the second fully-expended leaf was taken from the stem and then the leaf blade was cut into two small leaf discs with the area of 1 cm^2 using a cork borer. The leaf discs were placed in a vial containing 5 mL DMF (N, N-dimethylformamide) and then incubated for 24 h in the dark. A 3 mL of chlorophyll extract was spectrophotometrically (Jasco V530 UV/VIS Spectrometer, Jasco, Japan) measured at 647 and 664 nm, respectively. The equations to calculate for total chlorophyll (Chl t), chlorophyll a (Chl a) and chlorophyll b (Chl b) were as follows: Chl a = 12.64 A_{664} – 2.99 A_{647}, Chl b = -5.6 A_{664} + 23.24 A_{647}, Chl t = 7.04 A_{664}+20.27 A_{647}, expressed in µg cm^{-2}.

Statistical analysis: Analysis of variance was performed for chlorophyll content and SCMR according to a factorial design. Where main effects were significant, Duncan's Multiple Range Test (DMRT) was used to compare means (Hoshmand, 2006). Correlation coefficients between chlorophyll content and SCMR were calculated to understand the relationship between chlorophyll content and SCMR.

RESULTS

Soil moisture content: At 90 DAT, soil moisture contents for both non-stressed treatment and stressed treatment were maintained at field capacity and, therefore, the soil moisture contents were rather similar being 12.4% for non-stressed treatment and 12.0% for stressed treatment compared to 11.5% of calculated soil moisture content (Fig. 1). At 100 DAT when drought was imposed to the crop for 10 days, the soil moisture content of stressed treatment was 3.9% which was near permanent wilting point (2.6%), whereas soil moisture content of non-stressed treatment was 11.7%. At 110 DAT when the stressed crop fully recovered, the soil moisture content of stressed treatment was 13.0%, whereas the soil moisture content of non-stressed treatment was 10.5%.

Effected of soil water deficit and recovery on chlorophyll content and SCMR: At the end of drought period of 10 days (100 DAT), drought significantly reduced chlorophyll content and SCMR (Table 1), the reductions were from 6.64 to 3.78 µg cm^{-2} for chlorophyll content and 33.06 and 27.36 for SCMR. The data showed that reduction in chlorophyll content was much greater than SCMR. However, the water regimes were not significantly different in chlorophyll content and SCMR both before the imposition of drought and after recovery.

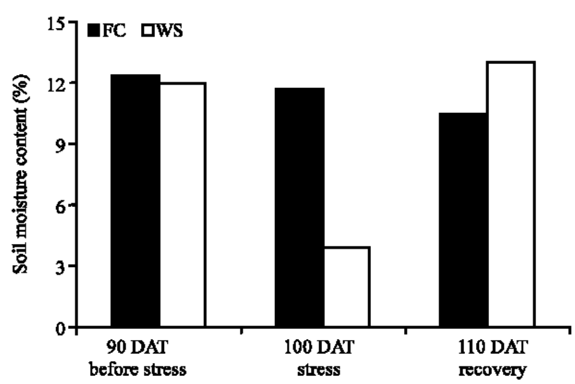

Fig. 1: Soil moisture content (%) in two water regimes (well-watered (FC) ■ and water stress (WS) □) at 90 days after transplanting (DAT), 100 DAT and 110 DAT

Table 1: Chlorophyll content and SPAD chlorophyll meter reading (SCMR) of 10 sugarcane cultivars at 90 DAT, 100 DAT and 110 DAT

	Chlorophyll content ($\mu g\ cm^{-2}$)			SCMR		
Treatment	90 DAT	100 DAT	110 DAT	90 DAT	100 DAT	110 DAT
Soil moisture						
FC	5.43A	6.64A	6.86A	33.23A	33.06A	33.44A
Stress	5.95A	3.78B	6.30A	32.89A	27.36B	31.64A
F-test	NS	**	NS	NS	**	NS
Cultivars						
Uthong 6	6.08abc	5.50ab	7.32b	36.58abc	31.78a	35.92ab
Khon kaen 80	5.22bcd	4.28bc	6.08bcd	33.40bcd	31.90a	33.30bc
K86-161	6.33abc	5.51ab	6.76bc	33.00bcd	31.65a	35.95ab
Khon kaen 3	6.16abc	5.14abc	5.95bcd	34.18abcd	30.38ab	31.63bc
03-4-425	7.65a	6.07a	9.21a	37.30ab	34.05a	39.55a
KU60-1	6.51ab	5.78a	7.47b	38.25a	33.20a	36.73ab
Phill.66-07	3.93d	4.28bc	4.89d	30.38d	26.18b	31.20bc
B34-164	6.18abc	4.07c	5.23cd	23.88e	20.55c	21.58d
Uthong 2	4.07d	5.89a	7.05b	30.98d	30.70ab	29.63c
LF82-2122	4.77cd	5.59ab	5.85bcd	32.68cd	31.68a	29.90c
F-test	**	*	**	**	**	**
A×B	NS	NS	NS	NS	NS	NS
CV (%)	18.96	17.81	17.06	8.80	11.46	11.89

NS, *,** Non significant and significant at $p \leq 0.05$ and 0.01 probability levels, respectively. Means in the same column with the same letters are not different by 95% LSD

Because the interactions between cultivar and water regime were not significant for both chlorophyll content and SCMR, the data were then combined and reported as the averages of two water regimes. Significant differences among sugarcane cultivars were observed for chlorophyll content and SCMR at all evaluation times. The cultivars showed some differential responses for chlorophyll content and SCMR when the ranks of the cultivars were compared. However, combined analysis across different times of evaluation was not performed.

Across the experiment, the range of chlorophyll content was between 3.93 and 9.21 $\mu g\ cm^{-2}$, whereas the range of SCMR was between 20.55 and 39.55. The breeding line 03-4-425 had the highest chlorophyll content (6.07 $\mu g\ cm^{-2}$) and SCMR (34.05) at 100 DAT, the breeding line B34-164 had the lowest chlorophyll content (4.07$\mu g\ cm^{-2}$) and SCMR (20.55). When the crop recovered from drought (110 DAT), the line 03-4-425 was also the highest for chlorophyll content (9.21 $\mu g\ cm^{-2}$) and SCMR (39.55), whereas the cultivar Phill.66-07 was the lowest for chlorophyll content (4.89 $\mu g\ cm^{-2}$) and the line B34-164 was the lowest for SCMR (21.58). The line 03-4-425 also had the highest chlorophyll content (7.65 $\mu g\ cm^{-2}$) under well-water conditions. It should be noted that the cultivars with high chlorophyll content under drought and after recovery also had high potential for these characters under well-watered conditions.

Relationship between chlorophyll content and SCMR: The correlation coefficients between chlorophyll content and SCMR were calculated from the means of 10 sugarcane cultivars at 90 DAT, 100 and 110 DAT. At 90 DAT, when drought was not imposed to the crop, the correlation coefficient between chlorophyll content and SCMR was positive and significant ($r = 0.78$, $p \leq 0.01$) (Fig. 2).

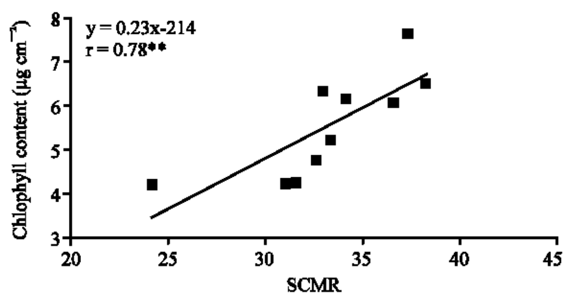

Fig. 2: Relationship between total chlorophyll content ($\mu g\ cm^{-2}$) and SPAD chlorophyll meter reading (SCMR) at 90 days after transplanting (DAT). r = correlation coefficient (n = 10), **significant at $p \leq 0.01$ level

At 100 DAT, when the drought session was ended, the correlation coefficients were calculated separately for well-watered treatment and drought treatment. The correlation coefficients between chlorophyll content and SCMR were positive and significant for both water regimes and the correlation coefficient for drought conditions ($r = 0.90$, $p \leq 0.05$) was much higher than that for well-watered conditions ($r = 0.74$, $p \leq 0.01$) (Fig. 3). Drought group and well-watered group were clearly separated.

At 110 DAT, when the crop recovered from drought, the correlation coefficients between chlorophyll content and SCMR were not significant for well-watered treatment ($r = 0.46$) but significant for drought treatment

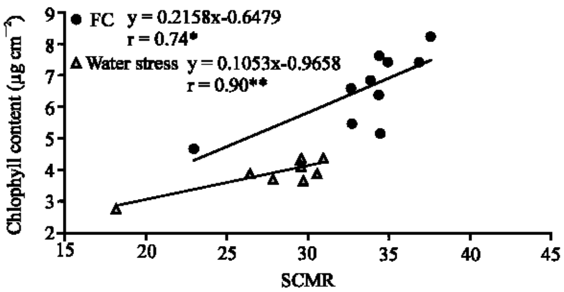

Fig. 3: Relationship between total chlorophyll content ($\mu g\ cm^{-2}$) and SPAD chlorophyll meter reading (SCMR) at 10 days after dehydration (100 DAT) r = correlation coefficient (n = 10) *and **significant at $p \leq 0.05$ and 0.01 level, respectively

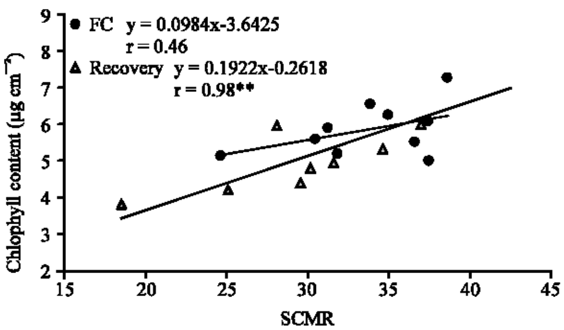

Fig. 4: Relationship between total chlorophyll content ($\mu g\ cm^{-2}$) and SPAD chlorophyll meter reading (SCMR) at 10 days after recovery (110 DAT) r = correlation coefficient (n = 10), **significant at $p \leq 0.01$ level

after re-watering ($r = 0.84$, $p \leq 0.01$) (Fig. 4). This could be due to the differential responses of sugarcane genotypes to drought. Some genotypes had slow recovery and some genotypes had rapid recovery.

DISCUSSION

Plants respond to drought differently for chlorophyll content. In most plant species, chlorophyll is generally sensitive to drought (Rong-hua *et al*., 2006). However, drought can increase chlorophyll content in some cases (Mensah *et al*., 2006) or has no detrimental effect on chlorophyll content (Schlemmer *et al*., 2005). In this study, drought significantly reduced chlorophyll content and SCMR and the reduction in chlorophyll content was more severe than that in SCMR. The findings support previous findings that chlorophyll in sugarcane is sensitive to drought (Silva *et al*., 2007). However, drought increases chlorophyll content in sesame (Mensah *et al*., 2006) and chlorophyll density in peanut (Arunyanark *et al*., 2009), but had no detrimental effect on chlorophyll content in corn (Schlemmer *et al*., 2005). The contrasting results could be possibly due to the differences in degrees of drought, plant species and the timing of drought imposed to the crops.

The interactions between sugarcane cultivar and water regime for chlorophyll content and SCMR were not significant across evaluation times from 90 to 110 DAT. Nigam and Aruna (2008) suggested that SCMR is easy to operate, reliable and fairly stable and low cost. They also found low genotype x time of observations. Therefore, these characters are simpler than cane yield in terms of low genotype by environment interaction and these characters might be used as surrogate traits for drought resistance.

Although, number of sugarcane genotypes used in the experiment was small, differences among sugarcane cultivars for chlorophyll content and SCMR were observed. The results indicated that the variations in SCMR and chlorophyll content existed in these sugarcane genotypes. Silva *et al*. (2007) also found that stalk productivity was highly correlated with SCMR and some sugarcane cultivars showed better growth and productivity with limited soil moisture. Andrew *et al*. (2000) reported that sorghum hybrids possessing the stay-green trait had a significant yield advantage under postanthesis drought compared with hybrids not possessing this trait. Similarly, genetic variations in SCMR were also reported in durum wheat and barley (Giunta *et al*., 2002).

However, 10 sugarcane genotypes showed similar responded to both under fully-irrigated and drought conditions and recovery after drought imposition as they had similar reductions under drought and similar increases after drought relief.

The results indicated that the sugarcane genotypes responded similarly to drought stress and selection of genotypes for high chlorophyll content and SCMR should be straight forwards and should be similarly effective under both drought and full-irrigated conditions.

The cultivar showing consistently high chlorophyll content and SCMR could be readily identified. The genotypes 03-4-425 and KU60-1 performed best for these characters under both well-watered and drought and after drought conditions. The results revealed that drought may be not necessary for evaluation of sugarcane genotypes because either selection under drought or well-irrigated conditions the results were similar. However, screening of a large number of sugarcane genotypes under field conditions is also required to confirm the results.

Prior to the imposition of drought, the correlation coefficient between chlorophyll content and SCMR was positive and significant. After the end of drought session, the relationship of these characters was highest although it was calculated for lower number of data points. After recovery, the relationship between chlorophyll content and SCMR was still high compared to those of well-irrigated sugarcane.

As could be seen in Fig. 2-4, the relationship was rather variable under well-water conditions, depending on the times of assessment and the relationship under drought and after drought relief was rather strong and consistent. Similar to these findings, Nigam and Aruna (2008) found in peanut that SCMR observations can be recorded at any time after 60 days of crop growth, preferably under moisture deficit conditions. In sorghum, SCMR showed significant linear relationships with total leaf chlorophyll and with visual stay green rating under severe post-flowering drought conditions (Xu *et al*., 2000).

The results indicated that assessment of chlorophyll content in sugarcane, using SCMR, is effective and assessment under drought and drought recovery is much better than under well-watered conditions because of the higher relationship of the traits.

In conclusion, drought reduced chlorophyll content and SCMR in sugarcane and the reductions were rather similar among 10 sugarcane cultivars under investigation. Because of low G x E interactions, the cultivars with high chlorophyll content and SCMR were readily identified. The breeding line 03-4-425 and the cultivar KU60-1 were the best genotypes for chlorophyll content and SCMR. The relationships between chlorophyll content and SCMR under drought and drought recovery conditions were strong and more consistent than those under well-watered conditions and therefore a better assessment of chlorophyll content using SCMR under drought and drought recovery was suggested.

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REFERENCES

Andrew, K.B., G.L. Hammer and R.G. Henzell, 2000. Does maintaining green leaf area in sorghum improve yield under drought II. Dry mater production and yield. Crop Sci., 40: 1037-1048.

Arunyanark, A., S. Jogloy, N. Vorasoot, C. Akkasaeng, T. Kesmala and A. Patanothai, 2009. Stability of relationship between chlorophyll density and soil plant analysis development chlorophyll meter readings in peanut across different drought stress conditions. Asian J. Plant Sci., 8: 102-110.

Barraclough, P.B. and J. Kyte, 2001. Effect of Water Stress on Chlorophyll Meter Readings in Winter Wheat. In: Plant Nutrition - Food Security and Sustainability of Agro-Ecosystems, Horst, W.J., M.K. Schenk, A. Burkert, N. Claassen and H. Flessa *et al*. (Eds.). Kluwer Academic Publishers, Netherlands, ISBN: 978-0-7923-7105-2, pp: 722-723.

Cenkci, S., I.H. Cigerci, M. Yýldýz, C. Ozay, A. Bozdag and H. Terzi, 2010. Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. Environ. Exp. Bot., 67: 467-473.

Clark, A.J., W. Landolt, J.B. Bucher and R.J. Strasser, 2000. Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll fluorescence performance index. Environ. Pollut., 109: 501-507.

Cubas, C., M.G. Lobo and M. Gonzalez, 2008. Optimization of the extraction of chlorophylls in green beans (*Phaseolus vulgaris* L.) by N,N-dimethylformamide using response surface methodology. J. Food Compos. Anal., 21: 125-133.

Efeoglu, B., Y. Ekmekci and N. Cicek, 2009. Physiological responses of three maize cultivars to drought stress and recovery. S. Afr. J. Bot., 75: 34-42.

Giunta, F., R. Motzo and M. Deidda, 2002. SPAD readings and associated leaf traits in durum wheat, barley and triticale cultivars. Euphytica, 125: 197-205.

Hawkins, T.S., E.S. Gardiner and G.S. Comer, 2009. Modeling the relationship between extractable chlorophyll and SPAD-502 readings for endangered plant species research. J. Nat. Conserv., 17: 123-127.

Hoshmand, A.R., 2006. Design of Experiments for Agriculture and the Natural Sciences. 2nd Edn., Chapman and Hall, New York.

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. Physl., 50: 395-397.

Liu, Y., T. Zhang and J. Wang, 2008. Photosynthesis and metabolite levels in dehydrating leaves of *Reaumuria soongorica*. Acta Biol. Cracov. Bot., 50: 19-26.

Mensah, J.K., B.O. Obadoni, P.G. Eroutor and F. Onome-Irieguna, 2006. Simulated flooding and drought effects on germination, growth and yield parameters of Sesame (*Sesamum indicum* L.). Afr. J. Biotechnol., 5: 1249-1253.

Minolta, 1989. Chlorophyll Meter SPAD-502. Instruction Manual. Minolta Co., Ltd., Radiometric Instruments Operations, Osaka, Japan.

Monje, O.A. and B. Bugbee, 1992. Inherent limitations of nondestructive chlorophyll meters: A comparison of two types of meters. Hortic. Sci., 27: 69-71.

Moran, R., 1982. Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. Plant Physiol., 69: 1376-1381.

Netto, A.T., E. Campostrini, J.G. de Oliveira and O.K. Yamanishi, 2002. Portable chlorophyll meter for the quantification of photosynthetic pigments, nitrogen and the possible use for assessment of the photochemical process in *Carica papaya* L. Braz. J. Plant. Physiol., 14: 203-210.

Netto, A.T., E. Campostrini, J. Goncalves do oliveria and R.E. Bressan-Smith, 2005. Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. Scientia Hortic., 104: 199-209.

Neufeld, H.S., A.H. Chappelka, G.L. Somers, K.O. Burkey, A.W. Davison and P.L. Finkelstein, 2006. Visible foliar injury caused by ozone alters the relationship between SPAD meter readings and chlorophyll concentrations in cutleaf coneflower. Photosynth. Res., 87: 281-286.

Nigam, S.N. and R. Aruna, 2008. Stability of soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) and their association across varying soil moisture stress conditions in groundnut (*Arachis hypogaea* L.). Euphytica, 160: 111-117.

Percival, G.C. and C.N. Sheriffs, 2002. Identification of drought tolerance woody perennials using chlorophyll fluorescence. J. Arboric., 28: 215-223.

Rong-hua, L.I., G.U.O. Pei-pol, M. Baumz, S. Grando and S. Ceccarelli, 2006. Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. Agric. Sci. China., 10: 551-557.

Rostami, M., A.R. Koocheki, M.N. Mahallati and M. Kafi, 2008. Evaluation of chlorophyll meter (SPAD) data for predictin of nitrogen status in corn (*Zea mays* L.). American-Eurasian. J. Agric. Environ. Sci., 3: 79-85.

Schlemmer, M.R., D.D. Francis, J.F. Shanahan and J.S. Schepers, 2005. Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. Agron. J., 97: 106-112.

Silva, M.A., J.L. Jifon, J.A.G. Silva and V. Sharma, 2007. Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. Braz. J. Plant. Physiol., 9: 193-201.

Songsri, P., S. Jogloy, C.C. Holbrook, T. Kesmala, N. Vorasoot, C. Akkasaeng and A. Patanothai, 2009. Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agric. Water Manage., 96: 790-798.

Ting, A.S.Y., L.M. Tan and A.P.K. Ling, 2009. *In vitro* assessment of tolerance of *Orthosiphon stamineus* to induced water and salinity stress. Asian J. Plant Sci., 8: 206-211.

Turner, F.T. and M.F. Jund, 1991. Chlorophyll meter to predict nitrogen topdress requirement for semidwarf rice. Agron. J., 83: 926-928.

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

Wiedenfeld, R.P., 2000. Water stress during different sugarcane growth periods on yield and response to N fertilization. J. Agric. Water Manage., 43: 173-182.

Xu, W., D.T. Rosenow and H.T. Nguyen, 2000. Stay green trait in grain sorghum: Relationship between visual rating and leaf chlorophyll concentration. Plant Breeding, 119: 365-367.

Zlatev, Z.S. and I.T. Yordanov, 2004. Effects of soil drought on photosynthesis and chlorophyll fluorescence in bean plants. Bulg. J. Plant. Physiol., 30: 3-18.