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APPLICATION OF SCMR AND FLUORESCENCE FOR CHLOROPHYLL MEASUREMENT IN SUGARCANE

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ABSTRACT: Chlorophyll is necessary for photosynthesis in plant and affecting for crop yield. Chlorophyll content can be directly measured, however, that method is destructive leaves. In sugarcane, indirect methods were used for drought or stress condition however literature is lacking for normal condition. The objective was to study the relationship between direct and indirect chlorophyll measurement. The experiment was conducted under 2 locations in Mahasarakham province, Thailand. RCBD with 4 replications and 16 varieties were used. The chlorophyll content (CC), SPAD chlorophyll meter reading (SCMR) and chlorophyll fluorescence (CF) were measured five times and a month interval between 8-12 months after planting. The results revealed that CC and SCMR of 16 varieties in both locations were significantly different, however, CF was not significantly different. The interaction between location and varieties were found. The relationships between methods of measurement were also found. At Kut Rung district, CC and SCMR were positively correlated in 8 to 12 months except for 11 months (0.71**, 0.55*, 0.77**and 0.78** respectively). Another location, CC and SCMR were correlated in 8 and 9 months (0.51*and 0.73** respectively) and CF was not correlated with CC and SCMR. Combined analysis, CC was positively correlated with SCMR at 8-12 months except for 11 months (0.61*, 0.55*, 0.63**and 0.50* respectively). SCMR is a useful strategy for indirectly chlorophyll measurement in sugarcane. CF could not apply for measurement in this case. For further research, we are looking for a relationship between chlorophyll content and sugarcane yield or other desirable characteristics in sugarcane.

Keywords: Correlation, Chlorophyll content, SPAD chlorophyll, SCMR

1. INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is known as an effective crop for sugar and biomass production according to bio-fuel production. It can produce high cane yield and high quality of juice, however, seem to be greatly reduced by drought stress and others [1]. Sugarcane is one of the most important cash crops in Thailand which occupies around 1.76 million ha [2], and most of the sugarcane production in Thailand is under rain-fed condition and drought is usually appearance during the season and it affects to reduce growth and yield of sugarcane. Several previously researches studied on effect of drought to physiological characteristics such as chlorophyll content, SCMR [3]-[5], chlorophyll fluorescence [5], cane yield [6]-[7], root length, root dry weight, root/shoot ratio, stalk diameter and biomass [8]. Chlorophyll is a pigment that is important for photosynthesis in plant and also related to crop yield. Photosynthetically active radiation (PAR) is absorbed by chlorophyll and accessory pigments of chlorophyll-protein complexes and related to PSI and II [9]. Analysis of chlorophyll content is important for evaluating health or detecting and quantifying plants tolerance to drought stress [10]. Chlorophyll measurement can be measured both direct and indirect methods however direct method must be destructive leaves, expensive, laborious and time-consuming. Indirect methods are alternative methods which are more rapid and straightforward. Generally, chlorophyll measurements are usually using for drought condition for detecting drought tolerance in the plant including sugarcane, however, literature is lacking for normal condition and the relationship between direct and indirect methods require more study in normal condition. Therefore, the objective of this research was to study the relationship between directly and indirectly chlorophyll measurement to determine the rapid and accurate methods for chlorophyll measurement in sugarcane.

2. MATERIAL AND METHODS

2.1 Plant Material

Sixteen varieties of sugarcane namely KK06-501, KK07-478, NSUT08-22-3-13, RT2004-085, CSB06-2-15, CSB06-2-21, CSB06-4-162, CSB06-5-20, TBy27-1385, TBy28-0348, MPT02-458, MPT03-166, 91-2-527, KK3, LK92-11 and KPS01-12 were used. All of varieties were improved from government agency and private company in Thailand.

2.2 Experimental Design

The experiment was conducted in 2016-2017 under a rain-fed condition at two locations in Maha Sarakham province where is located in Northeast of Thailand. The first location was Kut Rang district and 16 varieties were planted on November 9, 2016. Another location was Wapi Prathum district, and sugarcane was planted on December 18, 2016. The experiment was laid out in a randomized complete block design (RCBD) with 4 replications, 4 rows a plot, 50 cm between plant and 130 cm between row, plot size was 5.2 × 5 m^2 (Wapi Prathum district) and 5.2 × 6 m^2 (Kut Rang district). Chemical fertilizer formula 15-15-15 at rate 50 kg/rai at 4 and 6 months after planting.

2.3 Data Collection

Chlorophyll content (CC), SPAD chlorophyll meter reading (SCMR) and chlorophyll fluorescence (CF) were observed 5 times from 8 to 12 months after planting and a month interval, three parameters were measured on 2^{nd} or 3^{rd} expanded leaf from the top. The SCMR was measured using by SPAD-502 meter (Minolta SPAD-502 meter, Tokyo, Japan). The data points were recorded at three positions along the length of the leaf blade (avoid veins and midribs) and then data points were averaged as a single value. The CC in leaves was measured by the method described by Moran [11]. Briefly, the leaf was cut one small leaf disc with the area 1 cm^2 using cork border, the leaf disc was placed in a vial containing 5 ml DMF (N, N-dimethyl formamide) and incubated in 4 °C for 24 h in dark. The chlorophyll extract was measured at 647 and 664 nm by a spectrophotometer**.** The equations to calculate for total chlorophyll, chlorophyll a (Chl a) and 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}, expressed in µg cm^{-2}. The CF was measured two positions in the middle of leaf and midrib was avoided, Fv/Fm was used and averaged as a single value, the CF was measured using chlorophyll fluorescence meter (PAM-2000, Heinz Walz GmbH, Germany). The measured leaf was dark- adapted for 30 min using leaf clips (FL-DC, Opti-Science) before fluorescence measurements. The chlorophyll fluorescence was determined following the procedures of Maxwell and Johnson [12].

2.4 Statistical Data Analysis

Analysis of variance (ANOVA) was conducted on the collected data using STATISTICS 9 and treatment means were separated using DMRT at 5 % probability level. Location × treatment was analyzed and correlation among CC SCMR and CF were done.

3. RESULTS AND DISCUSSIONS

The results showed that SCMR and CC were significantly different in both locations, however, CF was not significantly different.

For combine analyzed between location we found the interaction between location and varieties in SCMR and CC and also two parameters were significantly different, however, an interaction between CF was not found. At 8 months after planting, KKU06-501 had the highest SCMR (44.15 SPAD unit) following CSB06-2-21 and NSUT08-22-3-13 (42.30 and 41.86 SPAD unit, respectively), however, at 9 months NSUT08-22-3-13 had the highest SCMR following KK06-501 (42.06 and 40.76 SPAD unit, respectively). Whereas 91-2-527 had the highest SCMR at 10 months following RT2004-085 and CSB06-2-15, however at 11 and 12 months KK06-501 had the highest SCMR (40.73 and 40.13 SPAD unit, respectively) following CSB06-4-162 (40.09 and 39.24 SPAD unit, respectively) (Table 1). In previous studies, SCMR was measured at 90, 100 and 110 days after transplanting (DAT), they reported that SCMR at 90 and 110 DAT compared between drought and FC was not significantly different (average SCMR at 90 and 100 DAT was 33.23 and 33.44 SPAD unit, respectively) but SCMR at 100 DAT had significant between stress and FC (27.36 and 30.06 SPAD unit, respectively) [3]. In normal condition, SCMR was observed at 60, 90, 120 and 150 days after planting (DAP), they found that increasing trend of SCMR up to 120 DAP in all promising clones and decreasing trend was noticed in some clones and SCMR of all clones was under 50 SPAD unit [6] and this value was similar to the present study, however, in the present study SCMR was observed during 8 to 12 months after planting whereas the previous study was done 4 times during 60-150 DAP. Sudhakar [4] reported that SCMR at 60 and 120 DAP of 14 genotypes under irrigated condition was significantly different and the value ranged between 35.0 to 40.5 for 60 DAP and 35.5 to 46.6 for 120 DAP, the SCMR values were slightly lower than the present study. Radhamani, Kannan and Pakkiyappan [13] reported that SCMR at 3 growth stages was investigated in 15 cultivars of sugarcane and they showed significant at different stages, average SCMR value at tillering, grand growth and maturity was 24.34, 24.04 and 22.00 respectively. SCMR value at 120 DAP of 12 cultivars was significant that ranged between 39.63 to 51.15 [14]. Moreover, SCMR was measured in other crops in normal condition i.e. sweet sorghum [15], [16], sorghum and barley. The SCMR of sweet sorghum in 50% days to flowering ranged between 36.03-48.36 that the values were not different with sugarcane and SCMR had related to biomass yield (r=0.39*) [15] but this relationship did not study in our research. It would be studied in further research. Chlorophyll content (CC) at Kut Rung was significantly different all of five times interval a month during 8-12 months. At 8 months, NSUT08-22-3-13 had highest CC whereas KK06-501 had the highest CC at 9 months. However, at 10 months, NSUT08-22-3-13 and CSB06-5-20 had highest CC, moreover, NSUT08-22-3-13 had the highest CC at 11 months as well and CSB06-4-162 had the highest CC at 12 months (data not show). The CC at Wapi Prathum district was significantly different, KK06-501 had the highest CC at 8 months whereas CSB06-5-20 had the highest CC at 9 months. However, at 10 months CSB06-2-15 gave the highest CC and MPT02-458 gave the highest CC at 11 months, 91-2-527 have the highest CC in 12 months (data not show).

Table 1 SCMR of 16 sugarcane varieties during 8-12 months after planting under two locations

variety	SCMR (SPAD unit)				
	8 m	9 m	10 m	11 m	12 m
KK06-501	44.15a	40.76ab	35.04abc	40.73a	40.13a
KK07-478	37.94c-g	33.18e	29.20e	33.30fgh	33.49cde
NSUT08-22-3-13	41.86abc	42.06a	34.86abc	34.66d-h	33.78cde
RT2004-85	37.90d-g	33.31e	35.63ab	36.05c-f	36.37a-d
CSB06-2-15	40.14b-f	39.28abc	35.60ab	36.40cde	39.16ab
CSB06-2-21	42.30ab	37.20bcd	31.81d	36.70cd	35.40bcd
CSB06-4-162	41.45a-d	37.94bc	33.69a-d	40.09ab	39.24ab
CSB06-5-20	37.85d-g	37.54bcd	35.01abc	33.69e-h	32.06de
TBy27-1385	41.13a-d	37.03cd	34.25a-d	32.84ghi	33.63cde
TBy28-0348	35.56g	36.08cde	32.01d	38.28abc	36.66abc
MPT02-458	37.06efg	36.50cde	35.53ab	36.60cd	36.14a-d
MPT03-166	36.50fg	34.18de	31.81d	35.45c-g	33.78cde
91-2-527	40.74a-e	36.43cde	35.86a	37.38bcd	39.09ab
KK3	38.61b-g	38.74abc	34.20a-d	32.08hi	33.80cde
LK92-11	39.04b-g	36.60cde	32.71cd	30.08i	32.41cde
KPS01-12	40.56a-e	37.48bcd	33.21bcd	34.71d-h	29.64e
F-test	**	**	**	**	**
CV (%)	10.07	9.71	7.54	8.02	12.31

** significant at P< 0.01,
Mean in the same column followed by the same letter(s) are not significantly different

For combined analysis, CC was significantly at 8-12 months except 11 months after planting. At 8 months, both variety KK06-501 and CSB06-4-162 gave the highest CC following NSUT08-22-3-13 (8.92, 8.68 and 7.41 µg cm^{-2} respectively). At 9 months we found that CSB06-2-15 had the highest CC following CSB06-5-20, whereas NSUT08-22-3-13 showed the highest CC at 10 months following CSB06-5-20 and CSB06-2-15 and 12 months CSB06-4-162 had the highest CC following 91-2-527 and KK06-501 (8.84. 8.41 and 8.26 µg cm^{-2} respectively) (Table 2).

The previous researches, chlorophyll content under drought and field capacity (FC) was not significantly different at 90 and 110 except 100 DAT, it was different CC between drought and FC (3.78 and 6.64 µg cm^{-2} respectively). The average CC of 10 cultivars ranged between 4.07 to 7.65 (90 DAT), 4.07 to 6.07 (100 DAT) and 4.89 to 9.21 µg cm^{-2} (110 DAT) [3], in the one hand, total chlorophyll under stress was lower than non-stress condition [17], that results were slightly lower than present study especially CC under FC condition because present research studied on non-stress condition and chlorophyll content was observed at different growth stage. Chlorophyll content was measured under a non-stress condition at 3 growth stage; tillering, grand growth and maturity stage, the results showed that chlorophyll content of 15 varieties had significant and total chlorophyll content decreased with the stage of the plant [13].

However, present study chlorophyll content was observed 5 times and a month interval, therefore, the results of both study was different.

The association between SCMR, CC and CF, at Kut Rung, CC and SCMR were positively correlated in 8 to 12 months except for 11 months (0.71**, 0.55*, 0.77** and 0.78** respectively) (data not show). For another location, CC and SCMR were positively correlated only in 8 and 9 months (0.51* and 0.73** respectively) (data not show) and CF was not correlated with CC and SCMR.

Table 2 Chlorophyll content (CC) of 16 sugarcane varieties during 8-12 months after planting under two locations

variety	Chlorophyll content (μg cm^{-1})				
	8 m	9 m	10 m	11 m	12 m
KK06-501	8.92a	9.03abc	13.85a-d	5.89	8.26ab
KK07-478	5.09e	5.70g	12.56efg	4.81	4.97e
NSUT08-22-3-13	7.41b	8.40a-e	14.94a	6.29	7.07bcd
RT2004-85	5.93cde	8.24a-e	12.89d-g	6.56	5.77de
CSB06-2-15	6.73bc	9.39a	14.48ab	6.14	6.11cde
CSB06-2-21	6.14cde	7.88c-f	12.31fg	5.36	5.97cde
CSB06-4-162	8.68a	8.74a-d	14.16abc	6.04	8.84a
CSB06-5-20	6.50bcd	9.37ab	14.63ab	4.87	7.37bc
TBy27-1385	6.53bcd	7.72c-f	12.54efg	4.61	5.97cde
TBy28-0348	6.42bcd	6.69fg	12.21fg	6.54	6.74cd
MPT02-458	5.96cde	7.19ef	13.97a-d	7.24	6.65cd
MPT03-166	5.63cde	8.12a-e	12.04g	5.79	7.27bc
91-2-527	6.11cde	7.53def	13.76a-e	6.21	8.41ab
KK3	6.06cde	7.49def	12.95c-g	4.86	6.30cde
LK92-11	5.84cde	8.04b-e	13.80a-d	6.24	6.71cd
KPS01-12	5.31de	8.79a-d	13.44b-f	4.88	6.16cde
F-test	**	**	**	ns	**
CV (%)	19.55	16.95	9.23	19.94	21.05

** and ns; significant at P< 0.01 and not significant,
Mean in the same column followed by the same letter(s) are not significantly different

For combined analysis, the correlation coefficients between SCMR and CC were calculated from means of 16 sugarcane cultivars of two locations we found that SCMR had positive correlated with CC at 8-12 months except 11 months after planting. At 8 months after planting, the correlation coefficient between SCMR and CC was positive and significant (r=0.61, p≤0.05) (Fig. 1a). At 9 months, SCMR had positive correlated with CC and significantly different (r=0.55, p≤0.05) (Fig. 1b) whereas at 10 months the relationship between SCMR and CC was positive and highly significant (r=0.63, p≤0.01) (Fig. 1c) and 12 months, the correlation coefficient between SCMR and CC was positive and significantly different (r=0.50, p≤0.05) (Fig. 1d). For previous studies, the correlation coefficients between chlorophyll content and SCMR were calculated from means of 10 sugarcane cultivars at 90 and 100 DAT (r=0.78**, 0.74**) [3] whereas SCMR and CC were calculated from 15 cultivars and significant correlation was found (r=0.833**) [13]. The relationship between SCMR and chlorophyll content of 24 cultivars both control and iron deficiency had positively correlated (0.900**) [18], these results were slightly higher than the present study.

In species more closely related to sugarcane such as sweet sorghum, the relationship between SCMR and CC was found during 40 to 100 DAP [16], wheat (r=0.90) [19] and also found in maize [20], moreover, SCMR was positively correlated with biomass yield in sweet sorghum (r=0.39**) [15]. However, SCMR had positive correlated with other traits i.e. stalk number and tiller number (r=0.72* and 0.77** respectively) [7].

4. CONCLUSIONS

Chlorophyll content and SCMR of 16 varieties in both locations were significantly different however, CF was not significantly different. The interaction between location and varieties were found. The relationships between methods of measurement were also found. At Kut Rung, CC and SCMR had correlated in 8 to 12 months except for 11 months (0.71**, 0.55*, 0.77** and 0.78** respectively). For another location, CC and SCMR were correlated in 8 and 9 months (0.51* and 0.73** respectively) and CF was not correlated with CC and SCMR both locations. Combined analysis, CC was significantly correlated with SCMR in 8 to 12 months except for 11 months (0.61*, 0.55*, 0.63** and 0.50* respectively). SCMR is a useful tool for indirectly chlorophyll measurement in sugarcane. However, CF could not apply for measurement in this case.

For the further study, we are looking forward to using the knowledge from present study to estimation yield or desirable traits in sugarcane. It could be alternative methods for selection or investigation good performances of sugarcane.

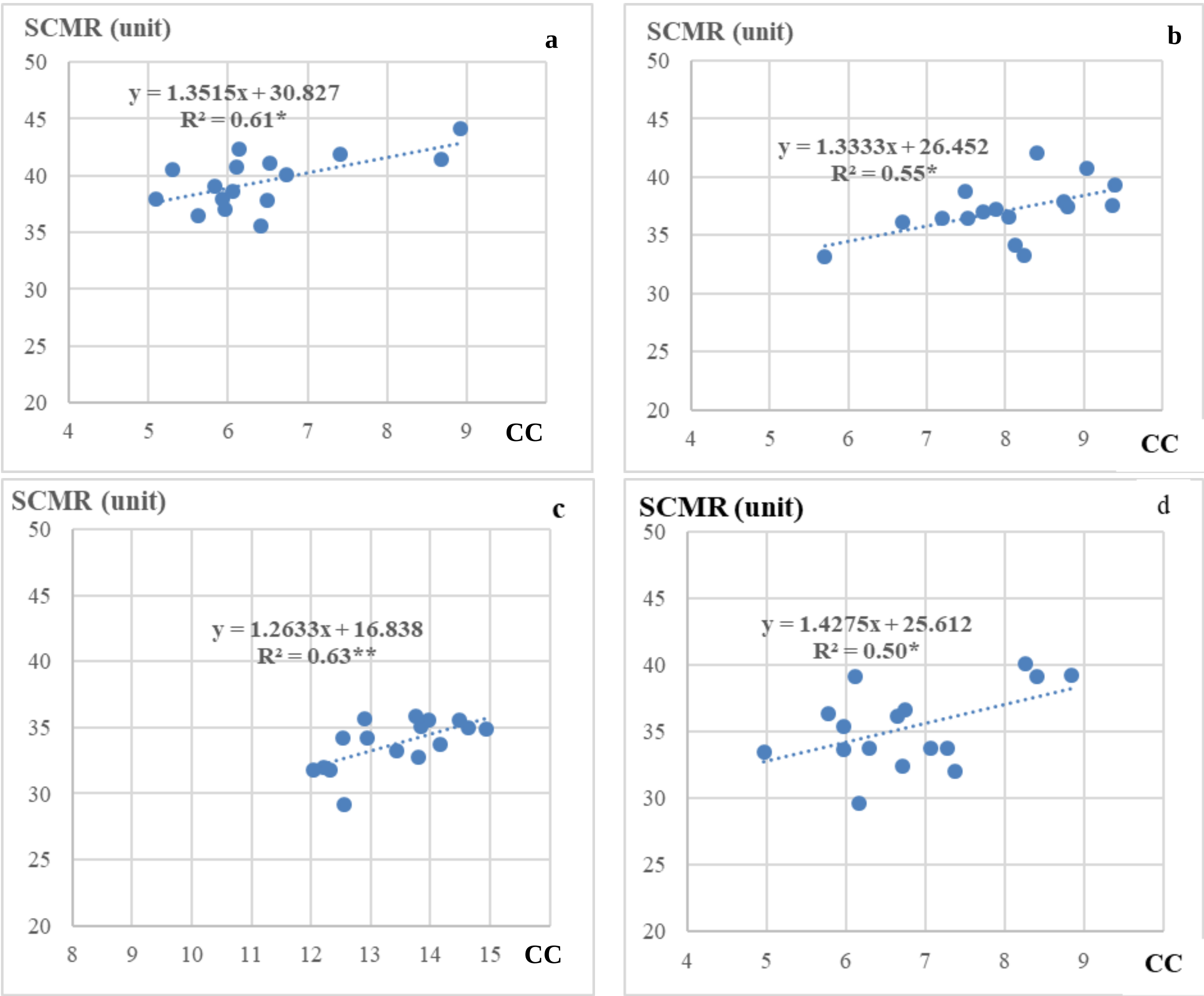

Fig. 1 Relationship between SCMR and CC of 16 sugarcane varieties at 8, 9, 10 and 12 months after planting (1a, 1b, 1c, and 1d, respectively) under two locations in Maha Sarakham province.

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6. REFERENCES

[1] Wiedenfeld R.P., Water Stress During Different Sugarcane Growth Periods on Yield and Response to N Fertilizer. Journal of Agriculture Water Management, Vol.43, 2000, pp. 173-182.

[2] Office of the Cane and Sugar Board (OCSB), Sugarcane production in Thailand report, 2017, Available source: http://www.ocsb.go.th/upload/journal/fileupload/923-9999.pdf.

[3] Jangpromma N., Songsri P., Thammasirirak S. and Jaisil P., Rapid Assessment of Chlorophyll Content in Sugarcane using a SPAD Chlorophyll Meter Across Different Water Stress Conditions, Asian Journal of Plant Sciences Vol.9, No.6, 2010, pp. 368-374.

[4] Sudhakar P., Latha P. and Babu A.M., Evaluation of Sugarcane Genotypes for High Water Use Efficiency and Thermostability

Tolerance Under Imposed Moisture Stress at Formative Stage, Sugar Tech Vol.12, No.1, 2010, pp. 72-75.

[5] Khonghintaisong J., Songsri P., Toomsan B. and Jongrunglang N., Rooting and Physiology Traits Responses to Early Drought Stress of Sugarcane Cultivars, Sugar Tech Vol.20, No.4, 2018, pp. 396-406.

[6] Reddy A.N.G., Madhudi K.V.N., Rajeswari V.R. and Sudhakar P., Evaluation of Different Morpho-Physiological Traits of Pre-release Early Sugarcane Clones, International Journal of Applied Biology and Pharmaceutical Technology Vol.5, Issue3, 2014, pp. 233-238.

[7] Khonghintaisong J., Songsri P., and Jongrunglang N., Growth and Physiological Patterns of Sugarcane Cultivars to Mimic Drought Conditions in Late Rainy Season System, Naresuan University Journal: Science and Technology Vol.25, No.2, 2017, pp. 102-112.

[8] Jangpromma N., Thammasirirak S., Jaisil P. and Songsri P., Effect of Drought Recovery from Drought Stress on Above Ground and Root Growth, and Water Use Efficiency in Sugarcane (*Saccharum officinarum* L.), Australian Journal of Crop Science Vol.6, No.8, 2012, pp. 1298-1304.

[9] Rong-hua L.I., Pei-pol G.U.O., Baumz M., Grando S., and Ceccarelli S., Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley, Agricultural Sciences in China Vol.10, 2006, pp. 551-557.

[10] Clark A.J., Landolt W., Bucher J.B. and Strasser R.J., Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll fluorescence performance index, Environmental Pollution Vol. 109, 2000, pp. 501-507.

[11] Moran R., Formulae for determination of chlorophyllous pigments extracted with N, N dimethylformamide, Plant Physiology Vol.69, 1982, pp.1376-1381.

[12] Maxwell K. and Johnson G.N., Chlorophyll fluorescence-A practical guide, Journal of Experimental Botany Vol.51, No.345, 2000, pp. 659-668.

[13] Radhamani R., Kannan R. and Rakkiyappan P., Leaf chlorophyll meter reading as an indicator for sugarcane yield under Iron deficient Typic Haplustert, Sugar Tech Vol.18, No.1, 2016, pp.61-66.

[14] Bhavana M., Kumar M.V., Bindu G.S.M. and Reddy D.V.V., Study on Morpho-Physiological Characters in Different Clones of Sugarcane (*Saccharum officinarum* L.), International Journal of Current Microbiology and Applied Sciences Vol.6, No.11, 2017, pp. 205-217.

[15] Bunphan D., Jaisil P. and Sanitchon J., Genetic Variation and Correlation of Some Agronomic Traits, Biomass and Ethanol Yield in Diverse Sweet Sorghum (*Sorghum bicolor* L. Moench) Cultivars, Khon Kaen Agriculture Journal Vol.46, No.1, 2018, pp. 155-162.

[16] Bunphan D., Jaisil P., Sanitchon J., Knoll J.E. and Anderson W.F., Association of Chlorophyll Content and Spad Chlorophyll in Diverse Sweet Sorghum Cultivars Under Different Environments, Khon Kaen Agriculture Journal Vol.42, No.4, 2014, pp. 605-616.

[17] Medeiros D.B., da Silva E.C., Nogueira R.J.M.C., Teixeira M.M., Buckeridge M.S., Physiological limitations in Two Sugarcane Varieties Under Water Suppression and After recovering, Theoretical and Experimental Plant Physiology Vol.25, No.3, 2013, pp. 213-222.

[18] Radhamani R. and Kannan R., Nondestructive and Rapid Estimation of Leaf Chlorophyll Content of Sugarcane using a SPAD Meter, International Journal of Science and Research Vol.5 No.4, 2013, pp.2392-2397.

[19] Udding J., Gelang-Alfredsson J., Piikki K. and Pleijel H., Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings, Photosynthesis Research Vol. 91, No.1, 2007, pp. 37-46.

[20] Markwell J., Osterman J.C. and Mitchell J.L, Calibration of the Minolta SPAD-502 leaf chlorophyll meter, Photosynthesis Research Vol. 46, 1995, pp. 467-472.