

Nondestructive and Rapid Estimation of Leaf Chlorophyll Content of Sugarcane using a SPAD Meter

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Abstract: This study evaluated the feasibility of using a SPAD-502 (Minolta, Japan) chlorophyll meter for estimating leaf chlorophyll and active iron content of sugarcane with respect to combined application of amendments. The SPAD chlorophyll meter readings were used to measure the leaf greenness. Leaf chlorophyll contents were also determined using the acetone extraction method. The metabolically active iron content of leaf was analysed by 1,10-orthophenanthroline extract. SPAD readings were significantly correlated with leaf chlorophyll content, cane yield, sugar yield and active iron contents were 0.900, 0.723, 0.711 and 0.860 respectively. These results suggest that the readings from a SPAD meter can be used for rapidly and nondestructively estimating leaf chlorophyll and it could facilitate in-situ decision making for application of amendments to iron deficiency for the perfection of sugarcane yield.

Keywords: Sugarcane, SPAD meter, chlorophyll content, chlorosis, active iron.

1. Introduction

Sugarcane (*Saccharum officinarum*) is one of the most important commercial crops in India and plays a key role in the Indian economy. It is grown in an area of 4.83 million hectares during 2006-07 with a production of 345 million tonnes of cane. Sugar industry is the second largest agro-based industry and there are 501 sugar mills in the country producing 28.33 million tonnes of white sugar. In India, the deficiencies of some micronutrients in sugarcane have been observed in light textured soils, calcareous soils and highly alkaline or acid leached soils. Serious deficiency of micronutrients, particularly, iron and zinc have sharply focused the attention of soil scientists in recent years. Iron, an important micronutrient is present in abundant quantity in soils; but its availability to crops and its utilization are limited by several factors. Iron is essential for chlorophyll synthesis, protein formation, photosynthesis, electron transfer, oxidation and reduction of nitrates and sulphates and enzymatic activities. Iron exists in soil as oxides, carbonates, hydroxides and organic compounds. Among the various forms, ferrous iron (reduced form) is available to crops whereas ferric (oxidized) form is not available. Presence of adequate amount of biologically active iron (Fe^{2+}) is very important for optimum photosynthesis. Iron deficiency causes interveinal chlorosis in newly emerged leaves due to reduced chlorophyll synthesis resulting in reduced photosynthesis, poor growth, yield and quality. Iron chlorosis is more frequently noticed in sugarcane crop than in others due to higher removal of iron. Singh (1972) observed cane yield loss as high as 74 % and reduction in sucrose content to the tune of 42 % due to iron deficiency. Hence this study was taken up to alleviate iron deficiency and to identify tolerant varieties to this malady using SPAD meter reading as an indicator for chlorophyll content and active iron content of foliage and sugarcane yield.

2. Materials and Methods

An investigation was carried out at Research Farm of Sugarcane Breeding Institute, Coimbatore to study the effect of amelioration for iron deficiency and the response of sugarcane varieties to iron nutrition in a sandy loam iron deficient soil (Typic Haplustert) by planting 24 sugarcane clones/varieties with two treatments viz., control and combined application of amendments (organic manure – 10 t/ha + iron fortified organic manure – 2.5 t/ha + gypsum – 7.5 t/ha + sulphur – 0.5 t/ha). Initial soil sample was collected and analysed for various physico-chemical properties using standard procedures (Table 1). The soil of the experimental field was alkaline in reaction (pH 8.51) with the EC of 0.26 dS m⁻¹. The soil was low in available nitrogen (264 kg ha⁻¹) with high available phosphorus (40 kg ha⁻¹) and potassium (816 kg ha⁻¹). The soil had organic carbon content of 0.59 per cent and the soil was deficient in available iron (3.20 ppm) while available zinc (2.10 ppm) was above critical level.

Table 1: Basic properties and nutrient status of the experimental field

S. No.	Soil Character	
1	Textural class	Sandy clay loam
2	pH	8.51
3	EC (dS m ⁻¹)	0.26
4	Organic carbon (%)	0.59
5	Available N (kg ha ⁻¹)	264
6	Available P (kg hsa ⁻¹)	40
7	Available K (kg ha ⁻¹)	816
8	Available Fe (ppm)	3.20
9	Available Zn (ppm)	2.10

The chlorophyll meter reading was taken in the first fully expanded leaf from the top at 120th day after planting by using Minalto SPAD 502 chlorophyll meter. Ten SPAD readings were taken around the midpoint of each leaf and

averaged its values. The same randomly selected leaves were collected from individual plots and the midribs were removed. The mid portions were cut into small pieces with stainless steel scissors and the leaf samples were taken for chlorophyll analysis (Arnon, 1949) and active iron (Katyal and Sharma, 1980). The soil samples were collected at tillering stage (120th day), and analysed pH, EC, OC and for available nutrients (N, P, K, Fe and Zn). The crop was harvested at maturity (12th month) and cane yield was recorded for each plot and sugar yield was computed using commercial cane sugar percent and cane yield.

3. Results and Discussion

The chlorophyll meter reading, chlorophyll content, metabolically active iron content, cane yield and sugar yield was improved due to ameliorative treatment for iron deficiency regardless of varieties (Table 2). Among the twenty four sugarcane clones/varieties investigated, five varieties namely, Co 8021, Co 86032, Co 88025, Co 88028 and Co 94005 did not produce chlorosis and the varieties Co 419, Co 7219, Co 85019, Co 86010, Co 87025 and Co 94003 showed severe chlorosis. Remaining varieties showed moderate effect in control plot. While in ameliorative plot none of varieties showed chlorosis (Plate 1).

SPAD meter reading

About hundred per cent increase of SPAD reading was noticed due to ameliorative treatment (32.5) over control (15.9). Varieties, Co 8021, Co 86032, Co 88025, Co 88028 and Co 92020 recorded higher chlorophyll meter reading (> 18.4 in control and > 34.0 in ameliorated plot) and Co 740, Co 7219, Co 86010, Co 87025, Co 94003 and 971862 registered lower chlorophyll meter reading (< 13.6 in control and > 31.0 in ameliorated plot) in both control and ameliorative treatment. In ameliorative plot, the chlorophyll meter reading varied from 25.4 to 39.3 with the mean of 32.5 (plate. 1a) and in control plot, it varied from 6.2 to 28.6 with the mean of 15.9 (plate. 1b).

Chlorophyll meter reading is an indirect measure of chlorophyll content in leaf blade. Since the chlorosis is mainly due to iron deficiency, the chlorophyll meter reading reflects the effect of amendments on iron availability and concentration of active iron in leaf blade as well as chlorophyll content. Different plant characteristics were used to assess the plant iron nutritional status to observe the effectiveness of the ameliorants. The plant characteristics most affected by iron chlorosis and the leaf SPAD index that markedly increased in ameliorated plot over control. Significant correlations were found between SPAD reading and chlorophyll content ($r = 0.900^{**}$), active iron content of leaf index ($r = 0.860^{**}$). The use of SPAD with other few plants has been tried by Westerveld *et al.*, 2004 and Yan-Ju Liu *et al.*, 2006 and results showed that using SPAD to monitor tissue nutrient is easy and cost advantageous. However, in general, the use of SPAD with other crops has rarely been reported.

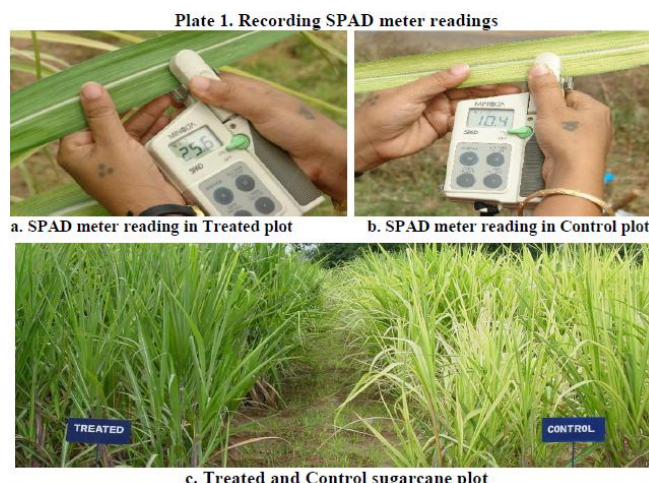


Plate 1: Recording SPAD meter readings

Total chlorophyll content

Total chlorophyll content of leaves followed the same trend as that of SPAD readings. In the control plot, 70% of the varieties showed varying degree of chlorosis indicating differential varietal response to iron deficiency except Co 8021, Co 86032, Co 88025, Co 92020 and Co 94005. None of the varieties expressed chlorosis in the ameliorated plot (Plate 1c). About three-fold increase in mean chlorophyll content (0.376 to 1.206 mg g^{-1}) was observed due to amelioration treatment over control. In the control plot, it varied from 0.139 to 0.993 mg g^{-1} with the mean of 0.376 mg g^{-1} while in ameliorative plot it ranged from 0.818 to 2.305 mg g^{-1} with the grand mean of 1.206 mg g^{-1} of fresh weight. With respect to varieties, Co 8021, Co 86032 and Co 92020 recorded higher chlorophyll content (> 0.467 mg g^{-1} in control and > 1.331 mg/g in ameliorated plot) while lower content was registered in Co 419, Co 740, Co 87025, Co 91010 and Co 93009 (< 0.285 mg g^{-1} in control and < 1.080 mg g^{-1} in ameliorated plot). A phenomenal increase in chlorophyll content was observed in these clones. This clearly indicates that Co 419, Co 740, Co 87025, Co 91010 and Co 93009 are more susceptible to iron deficiency and inefficient utilizer of iron. The varieties Co 8021, Co 86032 and Co 92020 recorded higher chlorophyll content in both control and ameliorated plot revealed that resistance to iron deficiency being an efficient utilizer of soil iron.

The results of the study revealed an increase in the total chlorophyll content as a result of combined application of amendments. Iron is essential for the synthesis of chlorophyll. When iron becomes limiting, the chlorophyll synthesis slows down and the chlorophyll gets diluted due to continuous leaf expansion (Miller *et al.*, 1982). Hence increased availability of Fe with the application of various amendments improved the chlorophyll content of leaves. The positive correlation was observed between active iron and total chlorophyll content of leaves ($r = 0.933^{**}$). Marsh *et al.* (1963) and Terry and Low (1982) also reported close correlation between chlorophyll content of leaves and iron content. The soil of the experimental field was deficient in iron with substantial amount of CaCO_3 . This could have interfered with iron nutrition and hence combined application showed better response. Marsh *et al.*, (1963) pointed out the importance of iron in the formation of

chlorophyll due to its role in the formation of α -aminolevulinic acid. Deficiency of iron may therefore restrict chlorophyll synthesis and consequently lead to chlorosis. This explanation holds true for the increased chlorophyll content observed in the leaf tissues of clones grown in iron deficient condition under combined application treatment and was chlorotic in the control treatment. The variability in the chlorophyll content among varieties was also seen. It might be due to genetic variability on impairing iron availability to the crop.

Metabolically active iron content

The importance of Fe^{2+} content of leaves in imparting chlorotic symptoms was well evidenced by the low Fe^{2+} content in susceptible varieties under iron deficient conditions while in the tolerant varieties high active iron content and the plants were free from chlorosis. The metabolically active iron content between the treatments, the control recorded lower active iron content of 209 ppm than the ameliorative treatment (385 ppm). The improvement due to ameliorative treatment was about 84 %. Among the varieties, Co 8021, Co 86032 and Co 88028 recorded higher active iron content in both control and ameliorative treatment (> 231 ppm in control and > 412 ppm in ameliorated plot). The lower active iron content was noticed in Co 740, Co 86027, Co 87025, Co 91010, Co 93009, Co 94008, Co 97001 and Co 97008 (< 188 ppm) in the control plot while in ameliorative plot Co 7219, Co 87025, Co 88025, Co 89010, Co 91010, Co 93009 and Co 97001 recorded lower active iron content of < 359 ppm.

Chlorotic plants should be evaluated by the quantification of active iron (Katyal and Sharma, 1980 and Zohlen, 2000). Active iron (Fe^{2+}) is fundamental in the synthesis of protoporphyrin IX, the precursor of chlorophylls, the close relationship of Fe^{2+} to chlorophylls and chlorosis makes the determination of Fe^{2+} a good indicator of the nutrient status of crops. Orthophenanthroline extractable iron which is the physiologically active fraction of iron correctly reflects the iron status of the plant. It is worth to mention here that the susceptible varieties exhibited well defined iron deficiency symptoms in iron deficient conditions. However, the tolerant varieties the symptom was absent. The metabolically active iron (Fe^{2+}) decreased with the increasing intensity of iron chlorosis (Gupta *et al.*, 2004). In the present study the active iron increased with increasing chlorophyll and SPAD readings as evidenced from the positive association of active iron with chlorophyll content ($r = 0.933^{**}$) and SPAD reading ($r = 0.860^{**}$). This is in accordance with the suggestions of Oserkowsky (1933) that significant variation occurred between active Fe content and chlorophyll content.

Cane yield

Iron chlorosis can limit crop yield, especially on calcareous soil. Typical management for iron chlorosis includes the use of iron fertilizers, various amendments or chlorosis tolerant cultivars. Yield improvement was observed in all the varieties in varying levels due to ameliorative treatment over

control. The ameliorative treatment recorded higher cane yield of $84.5 t ha^{-1}$ than the control ($56.2 t ha^{-1}$). In the control plot the cane yield ranged from 15 to $99 t ha^{-1}$ with the mean of $56.2 t ha^{-1}$, while in the ameliorated plot it varied from 55 to $126 t ha^{-1}$ with the mean of $84.5 t ha^{-1}$. The improvement due to ameliorative treatment was 52 % over control in cane yield.

Varieties/clones Co 8021, Co 85019, Co 86032, Co 86249, Co 88028, Co 94005 and Co 94012 were found relatively tolerant to iron deficiency and recorded fairly good yield in control also ($> 68 t ha^{-1}$ in control and $> 94 t ha^{-1}$ in ameliorated plot). These varieties could be recommended for cultivation in iron deficient soils. While Co 419, Co 740, Co 7219, Co 86010, Co 86027, Co 87025, Co 91010, Co 93009 and Co 94003 were highly susceptible to this malady and recorded less than $44 t ha^{-1}$ in control and in ameliorated plot also these varieties recorded low yield ($< 75 t ha^{-1}$). These varieties could be used as an indicator variety to detect iron deficiency in soil (Rakkiyappan *et al.*, 2002), which exhibited higher intensity of chlorosis as revealed by chlorophyll content and SPAD meter reading, yielded much lower than others, indicating thereby, an adverse effect of chlorosis on crop yield.

The cane yield seemed to be associated with the active Fe content of the plants as supported by a significant correlation ($r = 0.718^{**}$). The SPAD reading and chlorophyll content also showed significant relationship ($r = 0.723^{**}$ and $r = 0.753^{**}$) with the cane yield. Varietal differences were noticed markedly in respect of their yield, occurrence of chlorosis, leaf active iron and chlorophyll content (Chhibba *et al.*, 2004 and Radhamani *et al.*, 2015). Lingenfelser *et al.* (2005) proved that using resistant genotypes to be the most effective treatment in reducing chlorosis scores and yield loss. This agrees with the results of Naeve and Rehm (2006). Cane yield was improved due to application of ferrous sulphate, sulphur and zinc sulphate (Tomer and Malik, 2004). Sharma *et al.* (2006) reported that application of gypsum gave maximum cane yield with the proper tune of quality. It is due to improved soil condition which may result into optimum uptake of plant nutrients from the soil and thereby ultimately resulted into per hectare higher cane yield. Varietal difference in cane yield was reported by Osman *et al.* (2006) and Radhamani *et al.* (2015). The cane yield of sugarcane varieties Co 86249 and CoC 99061 were significantly higher due to application of sulphur (Saravanan *et al.*, 2006). Singh *et al.* (2002) reported that application of amendments viz., gypsum, pyrites and FYM singly and in combinations to sodic soils reduced the pH of soils and increased the yield.

Sugar yield

The ameliorative treatment recorded higher sugar yield of $11.45 t ha^{-1}$ than the control ($7.16 t ha^{-1}$) with 60% improvement. The varieties Co 8021, Co 85019, Co 86032, Co 86249, Co 88028, Co 94005 and Co 94012 recorded relatively higher sugar yield in ameliorated plot as well as in the control plot. The lowest sugar yield was

Table 2: Effect of ameliorative treatment on SPAD reading, chlorophyll content, active Fe, cane yield and sugar yield of 24 sugarcane varieties

S. No.	Clones / Varieties	SPAD reading		Chlorophyll Content (mg g ⁻¹)		Active Fe (ppm)		Cane yield (t ha ⁻¹)		Sugar yield (t ha ⁻¹)	
		C	T	C	T	C	T	C	T	C	T
1	Co 419	9.1	33.2	0.146	0.947	188	363	42	61	5.08	7.84
2	Co 740	11.4	30.9	0.224	0.925	169	363	42	67	4.64	7.49
3	Co 7219	13.2	28.4	0.414	1.118	206	356	19	69	1.98	8.11
4	Co 8021	26.0	39.3	0.859	2.305	306	506	97	125	13.84	18.60
5	Co 85019	10.7	34.9	0.312	1.111	256	375	81	123	10.06	16.19
6	Co 86010	9.5	29.4	0.182	1.152	188	431	42	64	4.83	8.42
7	Co 86027	15.9	38.4	0.282	1.153	181	400	26	56	2.13	7.77
8	Co 86032	28.4	36.6	0.758	1.652	281	500	99	126	13.53	15.42
9	Co 86249	14.6	31.4	0.276	1.245	189	363	96	101	13.83	19.06
10	Co 87025	10.7	25.4	0.220	1.008	143	308	15	55	1.55	6.46
11	Co 88025	22.6	36.1	0.637	1.209	244	340	67	91	8.28	12.17
12	Co 88028	22.7	36.5	0.374	1.633	269	494	82	104	11.03	14.36
13	Co 89010	15.8	30.4	0.304	1.048	188	350	64	78	8.57	11.08
14	Co 91010	16.1	31.7	0.248	0.838	150	300	20	64	2.51	8.65
15	Co 92020	18.8	25.7	0.620	1.355	313	406	22	89	2.53	11.51
16	Co 93009	15.6	34.0	0.279	0.818	181	319	40	62	5.89	7.86
17	Co 94003	6.2	29.6	0.139	1.089	188	406	34	62	4.20	8.85
18	Co 94005	28.6	31.8	0.993	1.223	313	366	97	105	12.25	14.68
19	Co 94008	13.7	33.4	0.207	1.167	170	469	81	92	9.19	11.90
20	Co 94012	13.6	31.3	0.325	0.927	193	360	98	114	13.75	17.86
21	Co 97001	13.7	32.6	0.291	1.125	175	201	21	82	2.34	9.73
22	Co 97008	18.4	37.4	0.333	1.330	131	438	48	79	5.51	9.65
23	Co 97009	16.3	32.6	0.305	1.229	219	413	54	72	6.45	9.30
24	971862	11.3	29.0	0.303	1.336	188	425	61	88	7.88	11.77
	Mean	15.9	32.5	0.376	1.206	209	385	56.2	84.5	7.16	11.45
	SD	5.99	3.70	0.23	0.314	53	65	30	23	4.22	3.81
	CI	2.40	1.48	0.090	0.126	21	26	12	9	1.69	1.52
	UCI	18.4	34.0	0.467	1.331	231	412	68	94	8.85	12.97
	LCI	13.6	31.0	0.285	1.080	188	359	44	75	5.47	9.92

C – Control, T – Treated, SD - Standard Deviation, CI - Confidence Interval,
 UCI - Upper Confidence Interval, LCI - Lower Confidence Interval.

recorded in the varieties Co 419, Co 740, Co 7219, Co 86010, Co 86027, Co 87025, Co 91010, Co 92020 and Co 94003 even in ameliorated plot. In control plot, the sugar yield ranged from 1.55 (Co 87025) to 13.84 t ha⁻¹ (Co 8021) with the mean of 7.16 t ha⁻¹ while in ameliorated plot 6.46 (Co 87025) to 19.06 t ha⁻¹ (Co 86249) with the mean of 11.45 t ha⁻¹. Kannapan (2006) reported that application of sulphur, gypsum and ammonium phosphate sulphate was essential for getting the maximum sugar yield. The varieties recorded higher cane yield also gave higher sugar yield as could be seen from the positive association ($r = 0.971^{**}$) between cane yield and sugar yield. There was also a significant correlation between SPAD meter reading and sugar yield ($r = 0.711^{**}$).

Significant increase in sugar and cane yield due to sulphur, gypsum application was observed (Jamal *et al.*, 2003; Shukla and Menhilar, 2002). Treatments containing Fe were generally effective in increasing the chlorophyll concentration of iron deficient sugarcane leaves. The combined application of amendments was the more effective for ameliorating the iron deficiency of sugarcane.

The effect of ameliorative treatment on soil characters

Iron chlorosis is a widely occurring nutritional malady of sugarcane, especially in calcareous soils. Iron is essential for

processes such as photosynthesis, respiration, nitrogen fixation and for DNA, chlorophyll and hormone synthesis. Although iron is one of the micronutrients, its chemistry is so influenced by various factors that even when high amount of total iron is present in the soil, visible symptoms of iron deficiency are observed. The ameliorative treatment showed significant effect on plant characteristics as well as yielded superior over control due to improved soil properties (Table 3).

Correlation between SPAD reading and other parameters

SPAD readings were significantly correlated with total chlorophyll content, metabolically active iron content, cane yield and sugar yield of sugarcane (Table 4). This result suggests that the portable chlorophyll meter may be suitable to use for the estimation of leaf chlorophyll content and active iron content of sugarcane plants.

Table 3: Effect of ameliorative treatment on soil characters at tillering stage

S. No.	Soil character	Control	Treated
1	pH	8.36	7.24
2	EC (dS m ⁻¹)	0.60	0.79
3	Organic carbon (%)	0.59	0.72
4	Available N (kg ha ⁻¹)	272	286
5	Available P (kg hsa ⁻¹)	57	69
6	Available K (kg ha ⁻¹)	910	986
7	Available Fe (ppm)	3.46	4.51
8	Available Zn (ppm)	3.65	4.50

Table 4: Interrelationship (r) among SPAD reading and other parameters as influenced by ameliorative treatment on twenty four sugarcane varieties

	SPAD	Total chlorophyll	Active Iron	Cane yield	Sugar Yield
SPAD	1	0.900**	0.860**	0.723**	0.711**
Total chlorophyll		1	0.933**	0.753**	0.747**
Active Iron			1	0.718**	0.674**
Cane yield				1	0.971**
Sugar Yield					1

** - significant at 1 % level

4. Conclusion

The present work demonstrated that foliar chlorophyll and metabolically active iron content could be reliably estimated using the SPAD-502 meter. This method is simple, nondestructive and quickly reports a large number of readings. The portable chlorophyll meter readings (SPAD readings) may provide an efficient means by which to monitor the Fe deficiency of sugarcane and to ameliorate the iron chlorosis. From the present work, it can also be concluded that the combined application of amendments for iron deficiency may be a good way to increase the yield of sugarcane under iron deficient condition. Based on the comparison among plant and yield parameters, the varieties Co 8021, Co 86032, Co 86249, Co 88028, Co 94005 and Co 94012 were recommended for cultivation under iron deficient condition, varieties Co 7219, Co 86010, Co 87025 and Co 91010 could serve as indicator varieties to detect iron deficiency in soils.

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