



# Comparison of Genotypes and Cultural Practice to Control Iron Deficiency Chlorosis in Sugarcane

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## ABSTRACT

Iron deficiency chlorosis is a common problem for sugarcane (*Saccharum officinarum*) grown on iron deficient calcareous soils. Iron is essential for chlorophyll synthesis and any deficiencies may hinder photosynthetic capabilities of the plant that may result in yield loss. Cultivar selection and/or proper management may reduce yield loss. The objective of this investigation was to identify the cultivars and determine the effect of ameliorative treatment for controlling iron deficiency chlorosis in sugarcane in Tamilnadu, India. A field experiment was conducted for fifteen sugarcane varieties with two treatments (control and amelioration). The leaf greenness was evaluated for chlorosis by SPAD chlorophyll meter readings and also using acetone extraction method. The metabolically active iron content of leaf was analysed by 1,10-orthophenanthroline extract. The ameliorated treatment was consistently better at reducing iron chlorosis scores and yield loss. The resistant genotypes had significantly lower chlorosis scores compared to the susceptible varieties. Iron deficiency chlorosis was adequately controlled by amelioration treatment and/or using resistant genotypes may be the more effective treatment for yield improvement.

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## 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 ( $\text{Fe}^{2+}$ ) 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 compare the genotypes for identifying tolerant varieties and cultural practice to this malady using SPAD meter readings as an indicator for chlorophyll, active iron content of leaves and sugarcane yield.

## Materials and Methods

Field evaluation was carried out at Research Farm of Sugarcane Breeding Institute, Coimbatore to compare the genotypes and the response of amelioration treatment to fifteen sugarcane varieties in a sandy clay loam iron deficient (Typic Haplustert) soil in Split Plot Design with two main plot treatments control and amelioration (combined application of organic manure – 10 t/ha + Fe and Zn fortified organic manure – 2.5 t/ha + gypsum – 7.5 t/ha + sulphur – 0.5 t/ha) and 15 sugarcane varieties as sub plot treatments and replicated thrice. 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 8.49) with the EC of 0.54 dS  $\text{m}^{-1}$  and organic carbon content of 0.52 per cent. The soil was low in available nitrogen (211 kg  $\text{ha}^{-1}$ ) with high available phosphorus (39 kg  $\text{ha}^{-1}$ ) and potassium (764 kg  $\text{ha}^{-1}$ ). The soil was deficient in available iron (3.43 ppm) while the other parameters such as available zinc (2.80 ppm), manganese (12.51 ppm) and copper (2.12 ppm) were above critical level.

The chlorophyll meter reading was taken in the first fully expanded leaf from the top at 120<sup>th</sup> day after planting by using SPAD 502 (Minolta, Japan) 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 content estimation (Katyal and Sharma, 1980). The soil samples were collected at tillering stage (120<sup>th</sup> day), and analysed pH, EC, OC and for available nutrients (N, P, K, Fe, Zn, Mn and Cu). The crop was harvested at maturity (12<sup>th</sup> month) and cane yield was recorded for each

plot and sugar yield was computed using commercial cane sugar percent and cane yield.

### Results and Discussion

Ameliorative treatment and varieties showed significant effect on plant characters and yield parameters. Among the fifteen sugarcane clones/varieties investigated, four varieties namely, Co 7219, Co 87025, Co 91010 and 971862 showed severe chlorosis and the varieties Co 8021, Co 86032, Co 86249, Co 94005 and Co 94012 did not produce chlorotic symptoms. Remaining varieties showed moderate effect in control plot. While in ameliorative plot none of varieties showed chlorosis (Plate 1). The SPAD meter reading, chlorophyll content and metabolically active iron content of leaves (Table 2), cane yield and sugar yield of sugarcane clones (Table 3) showed significant effect on amelioration treatment regardless of varieties.

#### Chlorophyll meter readings (SPAD readings)

Between the main plot treatments, the ameliorative treatment registered higher chlorophyll meter reading of 31.78 than the control (24.34). In sub plot, variety Co 86032 recorded significantly higher chlorophyll meter reading of 34.97, which was on par with Co 8021, Co 86249, Co 94005 and Co 94012 were recorded the SPAD meter readings of 33.62, 33.00, 33.97 and 33.82 respectively. The lowest reading was recorded in Co 87025 (19.75), which was on par with Co 91010 (21.12) and 971862 (20.70).

The chlorosis is mainly due to iron deficiency, the chlorophyll meter reading reflects the iron availability and concentration of active iron in leaf blade as well as chlorophyll content. Since SPAD reading is an indirect measure of chlorophyll content in leaf blade. Significant correlations were found between SPAD reading and chlorophyll content ( $r = 0.816^{**}$ ), 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 use of SPAD to monitor tissue nutrient is easy and cost advantageous. However, in general, the use of SPAD with other crops has rarely been reported. SPAD units and the concentration of chlorophylls decreased as severity of Fe chlorosis increased. These results coincide with those reported in the literature (Fan and Faust, 1984, Abadia *et al.*, 1991 and Morales *et al.*, 1991). The ameliorative treatment considerably improved the chlorophyll meter reading by about 7 units over control.

**Plate 1. Recording SPAD meter readings of Treated and Control sugarcane plot**



#### Total chlorophyll content

The total chlorophyll content differed significantly due to ameliorative treatments and varieties. Between the main plot treatments, the ameliorative treatment recorded higher total chlorophyll content of  $1.348 \text{ mg g}^{-1}$  than the control ( $0.649 \text{ mg g}^{-1}$ ). The ameliorative treatment improved the total chlorophyll content to about two fold over control. Among the sub plot treatments, the variety Co 86032 registered the highest total chlorophyll content of  $1.325 \text{ mg g}^{-1}$ , which was significantly higher than other varieties and the lowest was recorded in

971862 ( $0.697 \text{ mg g}^{-1}$ ), which was on par with Co 97001 ( $0.756 \text{ mg g}^{-1}$ ). Such varietal difference was reported by Shrivastava *et al.* (2000); Goos and Johnson (2000) and Radhamani *et al.* (2013). The probable reason might be that these varieties were tolerant/susceptible to iron deficiency malady as indicated by the high/low iron uptake by them.

The data on the total chlorophyll content had clearly shown that amelioration had a marked positive effect on the chlorophyll content of the leaves of different sugarcane varieties. Similar increase in the chlorophyll content due to amelioration was reported by other workers (De Kock *et al.*, 1960, O'Sullivan, 1969 and Del Rio *et al.*, 1978). Better performance of certain varieties compared to the others due to ameliorative treatment may have to be explained based on SPAD meter readings, chlorophyll content and active iron content, which Fe might have played. 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). The positive correlation was observed between active iron and total chlorophyll content of leaves ( $r = 0.853^{**}$ ). 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 and nitrogen with substantial amount of other nutrients. 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  $\alpha$ -aminolevulinic acid. Deficiency of iron may therefore restrict chlorophyll synthesis and consequently lead to chlorosis. This explanation holds true for the variability in the chlorophyll content among varieties were observed in the leaf tissues of clones grown in iron deficient condition. It might be due to genetic variability on impairing iron availability to the sugarcane plant.

#### Metabolically active iron content

The foliage metabolically active iron content differed significantly due to ameliorative treatments and varieties. The ameliorative treatment considerably improved the active iron content of 254 ppm over control (187 ppm). In sub plot, the variety Co 86032 recorded the highest active iron content of 256 ppm, which was on par with Co 8021 and Co 88028 were recorded the active iron contents of 255 and 253 respectively. The lowest active iron content of 189 ppm was registered in Co 87025, which was on par with Co 91010 (191 ppm).

Application of amelioration caused a remarkable increase in the active iron content in the index leaves about 70 ppm over control in all varieties indicating thereby, the role of amelioration in increasing the active Fe content in plants. Chlorotic plants should be evaluated by the quantification of active iron (Katyal and Sharma, 1980; Zohlen, 2000). Katyal and Sharma (1980) have reported that ferrous iron in fresh leaves is a better indicator of iron deficiency in plants unlike total Fe. The metabolically active Fe content of leaf tissues seemed to be a better index of Fe nutrition in different genotypes of sugarcane besides the chlorosis rating (chlorophyll meter reading). The active iron content in the leaves was also more when the plants were raised under ameliorative treatments and may be attributed to the favourable changes in soil properties caused by ameliorative treatment. The  $\text{Fe}^{2+}$  content of the leaves as one could expect, increased with supply of iron through iron fortified organic manure. The positive effect of organics on  $\text{Fe}^{2+}$  content of the leaf tissues and the tune of increase was marked when organics was added along with  $\text{FeSO}_4$ . It is to be

mentioned that the DTPA-Fe content of the soil was also increased to a phenomenal extent by the ameliorative treatment included organic manure and iron and zinc fortified organic manure. Similar observations were made by Yerriswamy *et al.*, (1994). Active iron ( $\text{Fe}^{2+}$ ) is fundamental in the synthesis of protoporphyrin IX, the precursor of chlorophylls, the close relationship of  $\text{Fe}^{2+}$  to chlorophylls and chlorosis makes the determination of  $\text{Fe}^{2+}$  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, in the tolerant varieties the symptom was absent. The metabolically active iron ( $\text{Fe}^{2+}$ ) 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 ( $r = 0.853^{**}$ ) and SPAD reading ( $r = 0.860^{**}$ ).

### Cane yield

The amelioration treatment and varieties had significant influence on cane yield. Ameliorative treatment improved the cane yield of all the varieties studied over control but the increase in yield varies with varieties. The ameliorative treatment improved the cane yield by  $31.5 \text{ t ha}^{-1}$  over control. The control treatment registered the mean cane yield of  $65.9 \text{ t ha}^{-1}$  and ameliorative treatment recorded the cane yield of  $97.4 \text{ t ha}^{-1}$  with a mean of  $81.7 \text{ t ha}^{-1}$ . Among the varieties, the highest cane yield of  $112.0 \text{ t ha}^{-1}$  was recorded in Co 86032, which was on par with Co 86249, Co 94005 and Co 94012. The variety Co 87025 registered significantly lowest cane yield of  $28.7 \text{ t ha}^{-1}$ . Iron chlorosis can limit crop yield, especially on calcareous soil. Typical management for iron chlorosis includes the use of iron fertilizers or chlorosis tolerant cultivars. Obviously, maximum varieties had varying degree of incipient deficiency of Fe and they must be fertilized or ameliorated, when grown in areas prone to Fe deficiency in order to realize optimum yield from them. Yield increases of various crops, including sugarcane, have been reported following addition of organic amendments to soil (Bevacqua and Mellano, 1994 and Hallmark *et al.*, 1995). 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 and sugar yield.

Varities/clones Co 8021, Co 86032, Co 86249, Co 94005 and Co 94012 were found relatively tolerant to iron deficiency and recorded fairly good yield (more than  $90 \text{ t ha}^{-1}$ ) in control plot. These varieties could be recommended for cultivation in iron deficient soils. Variety Co 87025 was highly susceptible to this malady and recorded less than  $15 \text{ t ha}^{-1}$  in control and less than  $50 \text{ t ha}^{-1}$  in ameliorated plot. This variety 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 SPAD meter reading, yielded much lower than others, indicating thereby, an adverse effect of chlorosis on cane yield. The cane yield seemed to be associated with the active Fe content of the plants as supported by a significant positive correlation ( $r = 0.776^{**}$ ). The SPAD reading and chlorophyll content also showed significant relationship ( $r = 0.853^{**}$  and  $r = 0.761^{**}$ ) 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.* (2013). 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). Varietal difference in cane yield was reported by Osman *et al.* (2006) and Radhamani *et al.* (2013). The cane yield of sugarcane varieties Co 86249 and CoC 99061 were significantly higher due to application of sulphur (Saravanan *et al.*, 2006).

### Sugar Yield

The amelioration treatment and varieties showed significant effect on sugar yield. Between the main plot treatments, ameliorated plot recorded significantly higher sugar yield of  $14.51 \text{ t ha}^{-1}$  than the control plot ( $9.34 \text{ t ha}^{-1}$ ). The ameliorated plot had improved the sugar yield of more than  $5 \text{ t ha}^{-1}$  over control. Among the varieties, Co 86032 registered the highest sugar yield of  $18.18 \text{ t ha}^{-1}$ , which was on par with Co 94012 ( $17.33 \text{ t ha}^{-1}$ ). The variety Co 87025 recorded significantly lowest sugar yield of  $3.83 \text{ t ha}^{-1}$ . The sugar yield ranged from  $3.83$  to  $18.18 \text{ t ha}^{-1}$  with the mean of  $11.93 \text{ t ha}^{-1}$  in sub plot treatments. The relatively lower sugar yield was recorded in Co 7219, Co 87025 and Co 91010 in control. The varieties Co 8021, Co 86032, Co 86249, Co 94005 and Co 94012 recorded higher cane yield also gave higher sugar yield as could be seen from the positive association ( $r = 0.984^{**}$ ) between cane yield and sugar yield. There was also a significant correlation between SPAD meter reading and sugar yield ( $r = 0.859^{**}$ ).

### 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 (N) 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 improved the soil properties over control (Table 4), which yielded superior cane yield.

### 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 varieties (Table 5). This result suggests that the portable chlorophyll meter may be suitable to use for the estimation of leaf chlorophyll and active iron contents of sugarcane plants.

### Conclusion

The present work demonstrated that foliage 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 for amelioration of iron chlorosis. The varieties Co 8021, Co 86032, Co 86249, Co 94005 and Co 94012 were recommended for cultivation under iron deficient condition, variety Co 87025 could serve as indicator variety to detect iron deficiency in soils. The leaf chlorophyll concentrations in the sugarcane plants treated with ameliorants was always greater than in the sugarcane leaves that did not receive ameliorants (control) indicates that the effect of combined application of ameliorants for economic yield improvement.

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

S.No.	Soil Character	
1	Textural class	Sandy clay loam
2	pH	8.49
3	EC (dS m <sup>-1</sup> )	0.54
4	Organic carbon (%)	0.52
5	Available N (kg ha <sup>-1</sup> )	211
6	Available P (kg hsa <sup>-1</sup> )	39
7	Available K (kg ha <sup>-1</sup> )	764
8	Available Fe (ppm)	3.43
9	Available Zn (ppm)	2.80
10	Available Mn (ppm)	12.51
11	Available Cu (ppm)	2.12

Table 2. SPAD meter readings, total chlorophyll and active Fe contents of fifteen sugarcane varieties

S. No.	Clones / Varieties	SPAD Reading			Chlorophyll Content (mg g <sup>-1</sup> )			Active Fe (ppm)		
		C	A	Mean	C	A	Mean	C	A	Mean
1	Co 7219	16.07	29.13	22.60	0.295	1.242	0.769	170	228	199
2	Co 8021	32.40	34.83	33.62	0.803	1.713	1.258	234	275	255
3	Co 85019	23.30	30.97	27.14	0.805	1.361	1.083	200	231	216
4	Co 86032	33.87	36.07	34.97	0.938	1.712	1.325	231	281	256
5	Co 86249	32.73	33.27	33.00	0.837	1.417	1.127	205	244	225
6	Co 87025	14.07	25.43	19.75	0.415	1.354	0.885	150	227	189
7	Co 88025	21.50	33.83	27.67	0.720	1.296	1.008	172	280	226
8	Co 88028	24.80	33.13	28.97	0.638	1.632	1.135	210	296	253
9	Co 91010	14.87	27.37	21.12	0.377	1.419	0.898	132	250	191
10	Co 94005	31.50	36.43	33.97	0.742	0.993	0.868	207	241	224
11	Co 94008	26.07	29.40	27.74	0.797	1.180	0.989	196	250	223
12	Co 94012	31.27	36.37	33.82	0.817	1.451	1.134	200	231	216
13	Co 97001	21.77	31.80	26.79	0.439	1.072	0.756	165	265	215
14	Co 97009	24.83	33.27	29.05	0.706	1.388	1.047	180	265	223
15	971862	16.03	25.37	20.70	0.399	0.995	0.697	160	240	200
	Mean	24.34	31.78	28.06	0.649	1.348	0.999	187	254	221
	SE		CD		SE	CD		SE	CD	
	Varieties	2.621	4.765		0.063	0.132		9.28	18.59	
	Treatment	1.753	7.544		0.059	0.254		15.91	68.45	

C – Control plot, A – Amelioration plot, SE - Standard Error, CD - Critical Difference

Table 3. Cane yield and sugar yield of fifteen sugarcane varieties

S. No.	Clones / Varieties	Cane yield (t ha <sup>-1</sup> )			Sugar yield (t ha <sup>-1</sup> )		
		C	A	Mean	C	A	Mean
1	Co 7219	32.3	85.3	58.8	3.82	11.87	7.85
2	Co 8021	91.3	108.7	100.0	13.98	16.88	15.43
3	Co 85019	89.7	96.7	93.2	12.70	14.27	13.49
4	Co 86032	94.3	129.7	112.0	15.19	21.17	18.18
5	Co 86249	91.7	128.0	109.9	12.85	18.38	15.62
6	Co 87025	14.0	43.3	28.7	1.63	6.03	3.83
7	Co 88025	56.3	86.7	71.5	7.89	12.93	10.41
8	Co 88028	68.7	103.3	86.0	9.02	14.51	11.77
9	Co 91010	26.7	67.7	47.2	3.37	10.31	6.84
10	Co 94005	95.3	117.0	106.2	13.93	17.51	15.72
11	Co 94008	64.0	108.3	86.2	9.07	15.94	12.51
12	Co 94012	103.3	116.3	109.8	15.79	18.86	17.33
13	Co 97001	48.7	103.3	76.0	6.38	14.69	10.54
14	Co 97009	61.0	65.3	63.2	8.14	9.52	8.83
15	971862	51.7	101.0	76.4	6.29	14.72	10.51
	Mean	65.9	97.4	81.7	9.34	14.51	11.93
	SE		CD		SE	CD	
	Varieties	7.21	14.45		1.06	2.12	
	Treatment	3.35	14.40		0.32	1.39	

Table 4. Effect of ameliorative treatment on soil characters

S.No.	Soil character	Control	Treated
1	pH	8.47	7.36
2	EC (dS m <sup>-1</sup> )	0.58	0.74
3	Organic carbon (%)	0.63	0.70
4	Available N (kg ha <sup>-1</sup> )	244	260
5	Available P (kg ha <sup>-1</sup> )	52	60
6	Available K (kg ha <sup>-1</sup> )	796	808
7	Available Fe (ppm)	3.35	5.11
8	Available Zn (ppm)	2.82	3.46
9	Available Cu (ppm)	2.71	2.69
10	Available Mn (ppm)	12.72	12.75

Table 5. Interrelationship (r) among SPAD reading and other parameters of fifteen sugarcane varieties

	SPAD	Total chlorophyll	Active iron	Cane yield	Sugar Yield
SPAD	1	0.816**	0.860**	0.853**	0.859**
Total chlorophyll		1	0.853**	0.761**	0.736**
Active Iron			1	0.776**	0.769**
Cane yield				1	0.984**
Sugar Yield					1

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