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

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ARTICLE INFO

Article history:
Received: 11 April 2015;
Accepted: 3 June 2015;
Received in revised form: 25 May 2015;

Keywords
Sugarcane,
Spad Meter,
Chlorophyll,
Chlorosis,
Active Iron.

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.

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 sulphate and enzymatic activities. Iron exists in soils 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 (Fe2+) 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 m-1 and organic carbon content of 0.52 per cent. The soil was low in available nitrogen (211 kg ha-1) with high available phosphorus (39 kg ha-1) and potassium (764 kg 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 12th 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 (Aron, 1949) and active iron content estimation (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, Zn, Mn and Cu). The crop was harvested at maturity (12th month) and cane yield was recorded for each

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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 91100 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 91100 (21.12) and 971862 (20.70).

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, Abadía 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 mg g⁻¹ than the control (0.649 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 mg g⁻¹, which was significantly higher than other varieties and the lowest was recorded in 971862 (0.697 mg g⁻¹), which was on par with Co 97001 (0.756 mg g⁻¹). Such varietal difference was reported by Shrivastava et al. (2000); Goos and Jofson (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 α-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 971862 (0.697 mg g⁻¹). Such varietal difference was reported by Shrivastava et al. (2000); Goos and Jofson (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 α-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.
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 (Fe⁴⁺) is fundamental in the synthesis of protoporphyrine IX, the precursor of chlorophylls, the close relationship of Fe²⁺ to chlorophylls and chlorosis makes the determination of Fe²⁺ a good indicator of the nutrient status of crops. Orthophenanthrolinel 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 (Fe³⁺) 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 t ha⁻¹ over control. The control treatment registered the mean cane yield of 65.9 t ha⁻¹ and ameliorative treatment recorded the cane yield of 97.4 t ha⁻¹ with a mean of 81.7 t ha⁻¹. Among the varieties, the highest cane yield of 112.0 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 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.

Varieties/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 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 t ha⁻¹ in control and less than 50 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 t ha⁻¹ than the control plot (9.34 t ha⁻¹). The ameliorated plot had improved the sugar yield of more than 5 t ha⁻¹ over control. Among the varieties, Co 86032 registered the highest sugar yield of 18.18 t ha⁻¹, which was on par with Co 94012 (17.33 t ha⁻¹). The variety Co 87025 recorded significantly lowest sugar yield of 3.83 t ha⁻¹. The sugar yield ranged from 3.83 to 18.18 t ha⁻¹ with the mean of 11.93 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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Soil Character</th>
<th>pH</th>
<th>EC (dS m⁻¹)</th>
<th>Organic carbon (%)</th>
<th>Available N (kg ha⁻¹)</th>
<th>Available P (kg ha⁻¹)</th>
<th>Available K (kg ha⁻¹)</th>
<th>Available Fe (ppm)</th>
<th>Available Zn (ppm)</th>
<th>Available Mn (ppm)</th>
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Table 2. SPAD meter readings, total chlorophyll and active Fe contents of fifteen sugarcane varieties

<table>
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<th>S.No.</th>
<th>Clones / Varieties</th>
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C – Control plot, A – Amelioration plot, SE – Standard Error, CD - Critical Difference

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

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<th>S.No.</th>
<th>Clones / Varieties</th>
<th>Cane yield (t ha⁻¹)</th>
<th>Sugar yield (t ha⁻¹)</th>
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Table 4. Effect of ameliorative treatment on soil characters

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

<table>
<thead>
<tr>
<th></th>
<th>SPAD</th>
<th>Total chlorophyll</th>
<th>Active iron yield</th>
<th>Sugar Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPAD</td>
<td>1</td>
<td>0.816**</td>
<td>0.860**</td>
<td>0.850**</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td>1</td>
<td>0.853**</td>
<td>0.761**</td>
<td>0.736**</td>
</tr>
<tr>
<td>Active iron</td>
<td>1</td>
<td>0.776**</td>
<td>0.769**</td>
<td></td>
</tr>
<tr>
<td>Cane yield</td>
<td>1</td>
<td>0.984**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar Yield</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgement

Authors are grateful to Director, Sugarcane Breeding Institute (ICAR), Coimbatore for providing permission and facilities for taking up this study.

References


Yan-Ju Liu, Yi-Ping Tong, Yong-Guan Zhu, Hui Ding and F. Andrew Smith. 2006. Leaf chlorophyll readings as an indicator for spinach yield and nutritional quality with different nitrogen fertilizer applications. *J. Pl. Nutr.,* **29**: 1207-1217.
