Available online at www.elixirpublishers.com (Elixir International Journal)

Food Science

Elixir Food Science 113 (2017) 49282-49286

ISSN: 2229-712X

Genotypic Variation of Leaf Chlorophyll and Yield in Relation to Severity of Chlorosis in Sugarcane

Radhamani, R.[1,*], R. Kannan[1], A. Manohar[1] and P. Rakkiyappan[2]
[1]College of Science, Eritrea Institute of Technology, Eritrea, N.E.Africa.
[2]Sugarcane Breeding Institute (ICAR), Coimbatore - 641007, Tamilnadu, India.

ARTICLE INFO

Article history:
Received: 30 October 2017;
Received in revised form:
5 December 2017;
Accepted: 16 December 2017;

Keywords
Genotypic variation,
SPAD meter,
Chlorophyll content,
Active iron,
Yield and quality.

ABSTRACT

A field experiment was conducted to evaluate the resistance and susceptibility of twenty four sugarcane varieties to iron chlorosis in an iron deficient soil (Typic Haplustert). Marked differences were observed among the investigated varieties in respect of their sensitivity to Fe chlorosis. Among the varieties Co 8021, Co 86032, Co 86249, Co 94005 and Co 94012 recorded higher cane yield and were identified as most tolerant, and recommended for cultivation. Using resistant genotypes proved to be the most effective treatment in reducing chlorosis severity. Occurrence of the chlorosis was found to be associated with SPAD readings, chlorophyll content, and active Fe content of leaves, cane yield and sugar yield.

Introduction

Sugarcane (*Saccharum spp*. Hybrid) 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 (Anon., 2008). In India, the deficiencies of some micronutrients in sugarcane have been observed; 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 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. Sugarcane crop is known to suffer from Fe deficiency under widely varying soil situations (Shrivastava *et al.*, 2000). Iron chlorosis is more frequently noticed in sugarcane crop than in others due to higher removal of iron (Rakkiyappan, 1987). 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 identify tolerant varieties to this malady.

Materials and Methods

An investigation was carried out at Sugarcane Breeding Institute, Coimbatore to identify the tolerant varieties to iron deficiency in a sandy loam iron deficient soil (Typic Haplustert) by planting twenty four sugarcane clones/varieties with normal cultivation practices under field conditions.

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} and CEC of 15.4 mmol(p+)ha^{-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 with 3.60 per cent $CaCO_3$. The soil was deficient in available iron (3.20 ppm) while the other parameters such as available zinc (2.10 ppm), manganese (14.20 ppm) and copper (1.58 ppm) were above critical level.

The chlorophyll meter readings were taken in the first fully expanded leaf from the top at three different stages of plant growth viz., tillering, grand growth and maturity stages by using SPAD 502 (Minolta, Japan) chlorophyll meter (Plate 1). Ten SPAD readings were taken around the midpoint of each leaf and averaged the mean 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 (Katyal and Sharma, 1980). The crop was harvested at maturity (12^{th} month) and cane yield and NMC were recorded for each plot and sugar yield was computed using commercial cane sugar percent with cane yield.

Results and Discussion

Among the 24 investigated varieties, Co 8021, Co 86032, Co 86249, Co 88025, Co 88028, Co 92020, Co 94005 and Co 94012 did not show any chlorotic symptoms while varieties Co 7219 and Co 87025 showed severe chlorosis at tillering stage (Plate 1). The SPAD meter reading, chlorophyll content and metabolically active iron content of plant, cane yield,

Tele:
E-mail address: radhamtp@rediffmail.com

NMC and sugar yield of sugarcane (Table 2-3) can be used as an index of iron chlorosis.

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	CEC (mmol(p+)ha^{-1}	15.4
5	Organic carbon (%)	0.59
6	Available N (kg ha^{-1})	264
7	Available P (kg ha^{-1})	40
8	Available K (kg ha^{-1})	816
9	Available Fe (ppm)	3.20
10	Available Zn (ppm)	2.10
11	Available Cu (ppm)	1.58
12	Available Mn (ppm)	14.20
13	$CaCO_3$ (%)	3.60

Plant characteristics

The varieties showed significant effect on SPAD meter reading, total chlorophyll and metabolically active iron content of leaves at different stages of plant growth.

SPAD meter reading

Chlorophyll meter readings recorded at tillering, grand growth and maturity stages were 15.9, 15.1 and 15.2 respectively. At tillering stage, varieties Co 8021, Co 86032, Co 88025, Co 88028, Co 92020 and Co 94005 recorded higher chlorophyll meter reading (>18.8) while Co 419, Co 740, Co 7219, Co 85019, Co 86010, Co 87025, Co 94003 and 971862 registered lower chlorophyll meter reading (<13.2). The chlorophyll meter reading varied from 6.2 to 28.6 with the mean of 15.9. At grand growth stage, varieties Co 8021, o 86027, Co 86032, Co 86249, Co 88028, Co 89010, Co 94005, Co 94012 and Co 97008 recorded higher chlorophyll meter reading (>17.2) while Co 419, Co 740, Co 7219, Co 85019, Co 86016, Co 87025, Co 91010, Co 94003 and Co 97001 registered lower chlorophyll meter reading (<13.1). The chlorophyll meter reading varied from 4.5 to 21.8 with the mean of 15.1.

Plate 1. Recording SPAD meter readings

a. SPAD reading of chlorotic variety.

b. SPAD reading of Non-chlorotic variety.

At maturity stage, varieties Co 8021, Co 86032, Co 88025, Co 88028, Co 89010, Co 94005 and Co 97008 recorded higher chlorophyll meter reading (>18.2) while Co 419, Co 740, Co 7219, Co 86010, Co 86027, Co 86249, Co 87025, Co 92020, Co 93009, Co 94003, Co 97009 and 971862 registered lower chlorophyll meter reading (<12.3). The chlorophyll meter reading varied from 5.1 to 33.9 with the mean of 15.2.

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 iron availability and concentration of active iron in leaf blade as well as chlorophyll content. Significant correlations at tillering stage 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 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; Abadıa *et al*., 1991; Morales *et al*., 1991 and Radhamani *et al*., 2016).

Total chlorophyll content

Total chlorophyll content of leaves followed the same trend as that of SPAD readings at tillering stage. Around 70 % of the varieties showed varying degree of chlorosis indicating differential varietal response to iron deficiency except Co 8021, Co 86032, Co 86249, Co 88025, Co 88028, Co 92020, Co 94005 and Co 94012. Total chlorophyll content at tillering stage ranged from 0.139 to 0.993 mg g^{-1} with the mean of 0.376 mg g^{-1}. With respect to varieties, Co 419, Co 740, Co 86010, Co 87025, Co 91010, Co 94003 and Co 94008 registered lower chlorophyll content (<0.248 mg g^{-1}), and were more susceptible to iron deficiency and inefficient utilizer of soil iron. Varieties Co 8021, Co 86032, Co 88025, Co 92020 and Co 94005 recorded higher chlorophyll content of more than 0.620 mg g^{-1} revealed the resistance to iron deficiency being an efficient utilizer of soil iron. At grand growth stage, the total chlorophyll content ranged from 0.145 (Co 94003) to 1.070 mg g^{-1} (Co 86032) with the mean of 0.443 mg g^{-1}. At maturity stage, Co 86032 recorded the highest total chlorophyll content of 0.935 mg g-1 and the lowest chlorophyll content was registered in Co 87025(0.075 mg g^{-1}) with the mean of 0.334 mg g^{-1}.

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 = 0.933**) at tillering stage. Marsh *et al*. (1963) and Terry and Low (1982) also reported close correlation between chlorophyll content of leaves and iron content. Marsh *et al*., (1963) pointed out the importance of iron in the formation of chlorophyll due to its role in the formation of α–aminoleveulinic acid. The soil of the experimental field was deficient in iron. 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 was 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 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 the tolerant varieties increased the active iron content and the plants were free from chlorosis.The metabolically active iron content at tillering stage varied from 131 to 313 with a mean of 209 ppm. Among the varieties, Co 8021, Co 86032, Co 88028, Co 92020 and Co 94005 recorded higher active iron content (> 269 ppm).The lower active iron content was noticed in varieties, Co 740, Co 86027, Co 87025, Co 91010, Co 93009, Co 94008, Co 97001 and Co 97008 (< 181 ppm). At grand growth stage, the mean active iron content was decreased and it ranged from 81 (Co 91010 and Co 94003) to 201 ppm (Co 8021) with the mean of 122 ppm.The mean metabolically active iron content was increased from 122 ppm to 160 ppm at maturity stage, in which the lowest active iron content of 113 ppm was recorded in 971862 and the highest was observed in Co 89010 (238 ppm).

Chlorotic plants should be evaluated by the quantification of active iron (Katyal and Sharma, 1980; Zohlen, 2000). Active iron (Fe^{2+}) is fundamental in the synthesis of protoporphirine 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, in 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**) at tillering stage.

Yield attributes:

The varieties had significant influence on cane yield, sugar yield and number of millable canes (NMC).

Cane yield

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. The cane yield ranged from 15 to 99 t ha^{-1} with the mean of 56.2 t ha^{-1}. Varieties/clones viz., Co 8021, Co 86032, Co 86249, Co 94005 and Co 94012 were found relatively tolerant to iron deficiency and recorded fairly good yield (> 90 t ha^{-1}). These varieties could be recommended for cultivation in iron deficient soils. Varieties Co 7219, Co 87025 and Co 91010 were highly susceptible to this malady and recorded less than 20 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 at tillering stage, 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 = 0.598**).The SPAD reading and chlorophyll content also showed significant relationship (r = 0.623** and r = 0.653**) 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).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).

Table 2. Varietal differences on SPAD reading, chlorophyll content (mg g^{-1}) and active Fe content (ppm) of 24 sugarcane varieties.

S. No.	Clones / Varieties	Tillering stage			Grand growth stage			Maturity stage		
		SPAD	Chlorophyll	Active Fe	SPAD	Chlorophyll	Active Fe	SPAD	Chlorophyll	Active Fe
1	Co 419	9.1	0.146	188	12.8	0.351	88	12.1	0.222	163
2	Co 740	11.4	0.224	169	12.1	0.297	88	8.8	0.367	150
3	Co 7219	13.2	0.414	206	10.0	0.327	152	8.9	0.429	138
4	Co 8021	26	0.859	306	18.7	0.654	201	24.5	0.438	156
5	Co 85019	10.7	0.312	256	10.5	0.323	148	13.9	0.367	156
6	Co 86010	9.5	0.182	188	12.3	0.246	149	9.5	0.180	138
7	Co 86027	15.9	0.282	181	20.5	0.460	119	9.2	0.262	200
8	Co 86032	28.4	0.758	281	21.8	1.070	153	33.9	0.935	231
9	Co 86249	14.6	0.276	189	17.4	0.739	197	10.6	0.172	131
10	Co 87025	10.7	0.220	143	13.1	0.254	102	10.0	0.075	144
11	Co 88025	22.6	0.637	244	21.7	0.692	99	20.4	0.271	156
12	Co 88028	22.7	0.374	269	21.8	0.570	101	23.2	0.272	144
13	Co 89010	15.8	0.304	188	18.5	0.654	113	22.4	0.454	238
14	Co 91010	16.1	0.248	150	9	0.264	81	14.2	0.293	175
15	Co 92020	18.8	0.620	313	15.3	0.436	163	9.8	0.161	125
16	Co 93009	15.6	0.279	181	14.1	0.294	151	11.5	0.205	138
17	Co 94003	6.2	0.139	188	9.5	0.145	81	5.1	0.211	150
18	Co 94005	28.6	0.993	313	18.5	0.371	101	28.3	0.369	163
19	Co 94008	13.7	0.207	170	13.7	0.453	98	14.8	0.290	181
20	Co 94012	13.6	0.325	193	19.9	0.323	105	16.3	0.274	125
21	Co 97001	13.7	0.291	175	6.6	0.209	125	14.1	0.527	144
22	Co 97008	18.4	0.333	131	21	0.771	95	23.6	0.558	200
23	Co 97009	16.3	0.305	219	14.9	0.435	94	11.4	0.429	175
24	971862	11.3	0.303	188	9.7	0.276	125	8.6	0.254	113
	Mean	15.9	0.376	209	15.1	0.443	122	15.2	0.334	160
	SD	5.99	0.23	53	5.08	0.22	35	7.33	0.18	31.8
	CI	2.40	0.09	21	2.03	0.09	14	2.93	0.07	12.7
	UCI	18.4	0.47	231	17.2	0.53	136	18.2	0.40	172
	LCI	13.6	0.29	188	13.1	0.36	108	12.3	0.26	147

Varietal difference in cane yield was reported by Osman *et al*. (2006) and Radhamani *et al*. (2016).

Sugar yield and NMC

The varieties Co 8021, Co 85019, Co 86032, Co 86249, Co 88028, Co 94005 and Co 94012 recorded relatively higher sugar yield.The lowest sugar yield was recorded in the varieties Co 419, Co 740, Co 7219, Co 86010, Co 86027, Co 87025,Co 91010, Co 92020, Co 94003 and Co 97001. 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}. 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 = 0.611**).

Table 3. Varietal differences on cane yield, sugar yield and NMC of 24 sugarcane varieties.

S. No.	Clones / Varieties	Cane yield (t ha^{-1})	Sugar yield (t ha^{-1})	NMC (lakh ha^{-1})
1	Co 419	42	5.08	0.60
2	Co 740	42	4.64	0.65
3	Co 7219	19	1.98	0.76
4	Co 8021	97	13.84	1.12
5	Co 85019	81	10.06	0.92
6	Co 86010	42	4.83	0.44
7	Co 86027	26	2.13	0.46
8	Co 86032	99	13.53	1.21
9	Co 86249	96	13.83	1.10
10	Co 87025	15	1.55	0.43
11	Co 88025	67	8.28	0.50
12	Co 88028	82	11.03	0.86
13	Co 89010	64	8.57	0.62
14	Co 91010	20	2.51	0.62
15	Co 92020	22	2.53	0.73
16	Co 93009	40	5.89	0.85
17	Co 94003	34	4.2	0.71
18	Co 94005	97	12.25	1.03
19	Co 94008	81	9.19	0.96
20	Co 94012	98	13.75	1.01
21	Co 97001	21	2.34	0.82
22	Co 97008	48	5.51	0.70
23	Co 97009	54	6.45	0.81
24	971862	61	7.88	0.92
	Mean	**56.2**	**7.16**	**0.78**
	SD	30	4.22	0.20
	CI	12	1.69	0.08
	UCI	68	8.85	0.87
	LCI	44	5.47	0.70

SD - Standard deviation, CI - Confidence interval, UCI - Upper confidence interval, LCI - Lower confidence interval.

For number of millable canes (NMC), the varieties Co 8021,Co 86249, Co 94012,Co 94005 and Co 91010 registered the higher NMC of more than 1.00 lakh ha^{-1}. The variety Co 86027 recorded lowest NMC of 0.215 lakh ha^{-1} followed by Co 740 (0.252 lakh ha^{-1}). The NMC ranged from 0.215 to 1.11 lakh ha^{-1} with the mean of 0.75 lakh ha^{-1}.The varieties recorded higher cane and sugar yield also gave higher NMC as could be seen from the positive association (r = 0.909** and r = 0.895**) with cane yield and sugar yield. The NMC is a genetical character (Osman et al., 2006), although influenced by other factors. Such varietal difference was also reported by Shrivastava *et al*. (2000).The present investigation showed significant varietal variation in NMC, cane yield and sugar yield.This agrees with the results of other workers, Goos and Johnson (2000) and Naeve and Rehm (2006).

Correlation between parameters of sugarcane varieties

At tillering stage, the cane yield was significantly correlated with SPAD readings, chlorophyll content, active iron and sugar yield (Table 4) more than at grand growth and maturity stages.This result suggests that analysis of plant characters at early stage may be suitable to identify the tolerant varieties to iron deficiency.

Table 4. Correlation between parameters of sugarcane varieties.

	SPAD	Total chlorophyll	Active Iron	Cane yield	Sugar Yield
SPAD	1	0.900**	0.860**	0.623**	0.611**
Total chlorophyll		1	0.933**	0.653**	0.647**
ActiveIron			1	0.598**	0.574**
Cane yield				1	0.971**
Sugar Yield					1

** - significant at 1 % level

Conclusion

In the present work, it can be concluded that the varieties, Co 8021, Co 86032, Co 86249, Co 94005 and Co 94012 were found to give higher cane yield revealing their tolerant nature to iron deficiency and were recommended for cultivation. Using resistant genotypes proved to be the most effective treatment in reducing chlorosis severity.The varieties such as Co 7219 and Co 87025 recorded relatively lower cane yield indicating the susceptible nature of these varieties and could serve as indicator varieties to detect iron deficiency in soils.

Acknowledgement

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

References

Anonynous. (2008). Sugar statistics. *Co-op. Sug*., **39(5)**: 65.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoxidase in *Beta vulgaris*. *Pl. Physiol*., **24**: 1-5.

Chhibba, I.M., V.K. Nayyar, M.L. Kapur and J. Singh. (2004). Varietal differences in chlorosis, yield and nutrient composition in sugarcane grown on a typic Ustochrept. *Indian J. Sug. Cane Technol*., **19(1&2)**: 78-82.

Gupta, V.K., A.K. Singh and R.C. Pant. (2004). The effect of iron deficiency on root morphology, ethylene content and uptake of other micronutrients in vitro grown cultivars of sugarcane. *Indian J. Pl. Physiol*., **9(2)**: 124-131.

Katyal, J.C. and B.D. Sharma. (1980). A new technique of plant analysis to resolve iron chlorosis. *Pl. Soil*, **55**: 105-119.

Lingenfelser, J.E., W.T. Jr., Schapaugh, J.P. Schmidt and J.J. Higgins. (2005). Comparison of genotype and cultural practices to control iron deficiency chlorosis in soybean. *Commun. Soil Sci. Pl. Anal.*, **36**: 1047–1062.

Marsh,H.V.,H.J. Evans and G. Matrone. 1963. Investigations of the role of iron in chlorophyll metabolism. II. Effect of iron deficiency on chlorophyll synthesis. *Pl. Physiol.,* **38**: 638-642.

Miller, G.W., A. Denny, J. Pushnik and M.H. Yu. 1982. The formulation of delta aminolevulinate: A precursor of chlorophyll in barley and the role of iron. *J. Pl. Nutri*., **5**: 289-300.

Naeve,S.L. and G.W. Rehm. (2006). Genotype 3 environment interactions within iron deficiency chlorosis tolerant soybean genotypes. *Agron. J.* **98**: 808–814.

Osman, A.M., G. Vijaya Kumar, U.S. Natarajan and C. Babu. (2006). Investigations on sugar cane families for ratooning ability and iron chlorosis tolerance under field conditions. *Indian Sug*. **56(8)**: 25-30.

Radhamani, R, R. Kannan and P. Rakkiyappan. 2016. Leaf chlorophyll meter readings as an indicator for sugarcane yield under iron deficient Typic Haplustert. *Sugar Tech.* 18(1): 61-66.

Rakkiyappan, P. (1987). *Effect of soil types and levels of nitrogen and potassium on yield, quality and nutrient uptake of two sugarcane varieties (CoC 671 and Co 6304)*. Ph.D. Thesis, Tamil Nadu Agric. Univ., Coimbatore, India.

Rakkiyappan, P., S. Thangavelu and R. Radhamani. (2002). Effect of ferrous sulphate on sugarcane varieties grown in iron deficient soil. *Sugar Tech*., **4(1-2)**: 33-37.

Shrivastava, A.K., H.N. Shahi and D.V. Yadav. (2000). Chlorosis in sugarcane. *Indian J. Sug. Cane Technol*., **15**: 68-79.

Singh, R.G. (1972). The microelement nutrition of sugarcane. *Co-op. Sug*., **4(1)**: 5-11.

Terry, N. and G. Low. 1982. Leaf chlorophyll content and its relationship to the intercellular localization of iron. *J. Pl. Nutr*., **5**: 301-310.

Westerveld,S.M.,A.W.Mckeown, D.Scott and M.R.Mc Donald.(2004). Assessment of chlorophyll and nitrate meters as field tissue nitrogen tests for cabbages, onions and carrots. *Hort. Technology* **14(2)**: 179-188.

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. of Pl. Nutri.*, **29**: 1207-1217.

Zohlen, A. 2000.The use of 1,10 o-phenanthroline in estimating metabolically active Fe in plants.*Commun. Soil Sci. Pl. Anal*., **31**: 481–500.