Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotypes tolerant to iron-deficiency chlorosis

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Groundnut, an important oilseed crop of India, frequently suffers from iron-deficiency chlorosis which may become so severe that the entire plant becomes yellow, papery-white, and may even die resulting in severe yield losses. As groundnut genotypes differ in their ability to utilize iron, selection of Fe-efficient genotypes which can tolerate Fe-deficiency chlorosis is the solution to this problem. Presently no field instrument is used to measure the intensity of chlorosis and only visual diagnosis, using visual chlorotic rating (VCR) and chlorophyll content determination method is widely used for selecting genotypes tolerant to iron-deficiency chlorosis in the field. Therefore, the present investigation was carried out to explore the possibility of using a chlorophyll meter (SPAD) for rapid and *in situ* screening of groundnut genotypes for their tolerance to iron chlorosis. The study showed that correlation between SPAD readings and chlorophyll content was positive and highly significant with *r* value of 0.94 for chlorophyll *a*, 0.90** for chlorophyll *b*, and 0.93** for total chlorophyll. This has clearly indicated a closer relationship of these traits with SPAD reading, i.e. higher the SPAD reading, higher will be the chlorophyll pigments and vice versa. The regression lines showed that these variables have a linear relationship with each other. On the basis of the linear relationship regression equations were developed from which it is possible to predict the chlorophyll content and VCR of the leaves. Thus the chlorophyll meter is an efficient and speedy equipment for screening genotypes for their tolerance to iron-deficiency chlorosis.**

PHOTOSYNTHESIS is the most important biochemical process occurring in plants and chlorophyll is the key pigment involved in it. Groundnut, an important oilseed crop, frequently suffers from iron-deficiency chlorosis in most parts of India, particularly in calcareous, alkaline and marginal soils[1–3]. Singh and Joshi[4] reported mild to severe yield losses depending upon the intensity of chlorosis. Many times chlorosis becomes so severe that the entire plant becomes yellow, papery-white, with reduced photosynthetic activity and ultimate death. Soil and foliar application of iron containing fertilizers helps the plants recover from chlorosis but their effect does not persist for long in the field and requires frequent applications[5]. The selection of Fe-efficient genotypes which can tolerate Fe-deficiency chlorosis could be a potential alternative solution to this problem. As groundnut genotypes differ in their response to Fe stress[2,6], visual chlorotic rating (VCR) method followed by chlorophyll estimation is used for measuring the

Figure 1. ***a***, Resistant and susceptible checks; ***b***, Susceptible genotype PBS 14021; ***c***, Resistant genotype PBS 24004 with susceptible check PBS 20101.

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Table1. Mean SPAD reading, visual chlorotic rating, chlorophyll *a*, chlorophyll *b* and total chlorophyll contents (mg/g dry weight basis), and pod yield in advanced breeding genotypes of groundnut

Entry name	Pedigree of the genotypes	SPAD reading	VCR	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Pod yield (g/plant)
PBS 12115	Kisan × NCAc 17133	32.54	1.75	5.72	1.83	7.55	8.86
PBS 12118	VG(E)5 × B 227	25.09	2.50	5.01	1.46	6.47	10.61
PBS 12120	VG(E)5 × B 227	26.06	2.50	4.39	1.23	5.62	8.86
PBS 12124	TMV 2 × PI 337409	28.96	2.42	5.04	1.47	6.51	7.27
PBS 12126	Latur 33 × PI 405132	27.40	2.17	5.42	1.60	7.02	13.30
PBS 14016	CGC 3 × JL 24	22.01	3.08	3.96	1.19	5.15	13.87
PBS 14021	TMV 7 × Chico	25.78	3.17	4.45	1.30	5.75	9.14
PBS 19003	M 13 × PI 314817	25.45	3.17	4.28	1.25	5.53	9.47
PBS 21031	TMV 10 × PI 405132	29.63	1.83	5.69	1.76	7.45	16.54
PBS 21052	C1 IV (Selection)	24.61	3.08	3.95	1.22	5.17	5.71
PBS 21063	M 13 × NCAc 17278	37.85	1.08	6.94	2.27	9.21	5.13
PBS 22015	Latur 33 × PI 405132	23.46	2.83	4.07	1.20	5.27	6.96
PBS 22017	Latur 33 × PI 405132	27.43	2.50	5.17	1.51	6.68	9.88
PBS 22020	M 13 × PI 215696	25.23	2.50	4.18	1.25	5.43	11.73
PBS 22023	GAUG 10 × CGC 4018	25.10	3.00	4.45	1.30	5.75	4.45
PBS 23003	RSB 87 × CGS 101	31.98	1.50	6.12	1.88	8.00	9.17
PBS 24001	Chandra × Chico	29.40	2.33	5.41	1.73	7.14	5.52
PBS 24002	GG 11 × Robut 33-1	27.58	2.50	5.68	1.86	7.54	5.69
PBS 24003	GG 11 × Robut 33-1	31.98	1.83	5.86	1.78	7.64	11.54
PBS 24004	Latur 33 × Tifrun	32.59	1.25	6.42	2.13	8.55	11.26
PBS 24006	M 13 × Robut 33-1	36.35	2.00	6.04	1.85	7.89	5.91
PBS 24030	M 13 × Robut 33-1	29.93	2.17	5.48	1.43	6.91	5.52
PBS 24040	Latur 33 × Tifrun	35.71	1.92	6.54	1.94	8.48	7.96
PBS 29030	M 13 × NCAc 17278	33.96	2.00	6.04	1.88	7.92	9.54
Sel 28 a	Dh 3-30 × NCAc 2214	26.01	2.50	4.67	1.35	6.02	13.32
FSD-1	GAUG 1 × GG 2	29.75	2.75	5.15	1.54	6.68	10.15
FSD-22	GAUG 1 × GG 2	29.66	1.75	4.85	1.48	6.33	10.62
FSD-37	GAUG 1 × GG 2	25.22	2.58	4.17	1.25	5.42	6.80
FSD-61	GAUG 1 × GG 2	31.16	1.91	5.80	1.75	7.55	8.20
FSD-66	GAUG 1 × GG 2	33.25	1.83	6.23	2.06	8.29	10.99
PBS 20100 (RC)	I_1	41.83	1.00	7.67	2.19	9.86	9.31
PBS 20101 (SC)	I_2	24.28	3.00	4.26	1.26	5.52	8.20
PBS20511 (SC)	VRI 3	24.05	3.16	4.06	1.18	5.24	6.79
PBS 20055 (SC)	ICG 7887	21.53	3.58	4.17	1.25	5.42	10.22

RC, Resistant check; SC, Susceptible check.

intensity of chlorosis[5–7]. As the estimation of chlorophyll content is laborious and time consuming, now-a-days the chlorophyll meter is being used for fast estimation of chlorophyll in the field[8,9]. The present investigation was carried out to explore the possibility of using the chlorophyll meter for rapid field screening of groundnut genotypes for their tolerance to iron chlorosis.

Thirty advanced breeding genotypes of groundnut were grown in a randomized block design with three replications during rabi–summer 1999 at the National Research Centre for Groundnut (NRCG), Junagadh. The plot size for each genotype was 5 m × 0.45 m. The soil of the experimental plot had a pH of 7.9, contained 29.6% calcium carbonate, 0.8% organic carbon, 0.06% total nitrogen, 6 ppm available P (Olsen's), and 1.35 ppm available Fe. This soil was thus suitable for screening groundnut germplasm to ascertain their tolerance for iron-deficiency chlorosis. For comparison, one iron chlorosis tolerant (I_1) and three iron chlorosis susceptible checks (I_2, VRI 3 and ICG 7887; Figure 1) were included in the experiment based on the results of earlier studies[2,7]. The recommended package of practice was followed to grow the crop. The first fully opened leaf of the main axis, from 10 randomly selected plants of each genotype was collected and read for chlorophyll using the chlorophyll meter (SPAD-502 Minolta, Japan) and estimated for chlorophylls *a* and *b* and total chlorophyll (TC) content following Arnon[10] at 30, 45, 60, and 75 days after emergence (DAE). Chlorophyll contents and SPAD reading recorded at various stages were averaged. VCR on 1–5 scale (1 = tolerant, 5 = highly susceptible) of iron deficiency was also recorded for the first 5 leaves from the top on the same date, following Singh and Chaudhari[6]. Based on chlorophyll contents and VCR as per Singh and Chaudhari[6], the genotypes were put into three categories: (i) Tolerant – showing dark green leaves, rare appearance of chlorotic plants with VCR less than 2.00 and TC content more than 8.00 mg/g dry weight of leaves; (ii) Moderately tolerant – green leaves with VCR ranging between 2.01 and

Table 2. Correlation coefficients and regression equations between SPAD reading and chlorophyll *a*, chlorophyll *b*, total chlorophyll, and visual chlorotic rating

Characters	Correlations coefficients (*r*) at four samplings					Regression equations
	30 DAE	45 DAE	60 DAE	75 DAE	Over means	
Chlorophyll *a*	0.77**	0.89**	0.77**	0.92**	0.94**	$Y = -0.30 + 0.190X$
Chlorophyll *b*	0.69**	0.62**	0.77**	0.87**	0.90**	$Y = -0.23 + 0.062X$
Total chlorophyll	0.75**	0.79**	0.78**	0.92**	0.93**	$Y = -0.53 + 0.253X$
VCR	–0.67**	–0.64**	–0.77**	–0.60**	–0.87**	$Y = 5.50 - 0.110X$

**Significant at 1 per cent level; *Y*, Predicted value of chlorophylls *a* and *b*, TC, and VCR; *X*, Value of chlorophyll meter (SPAD reading).

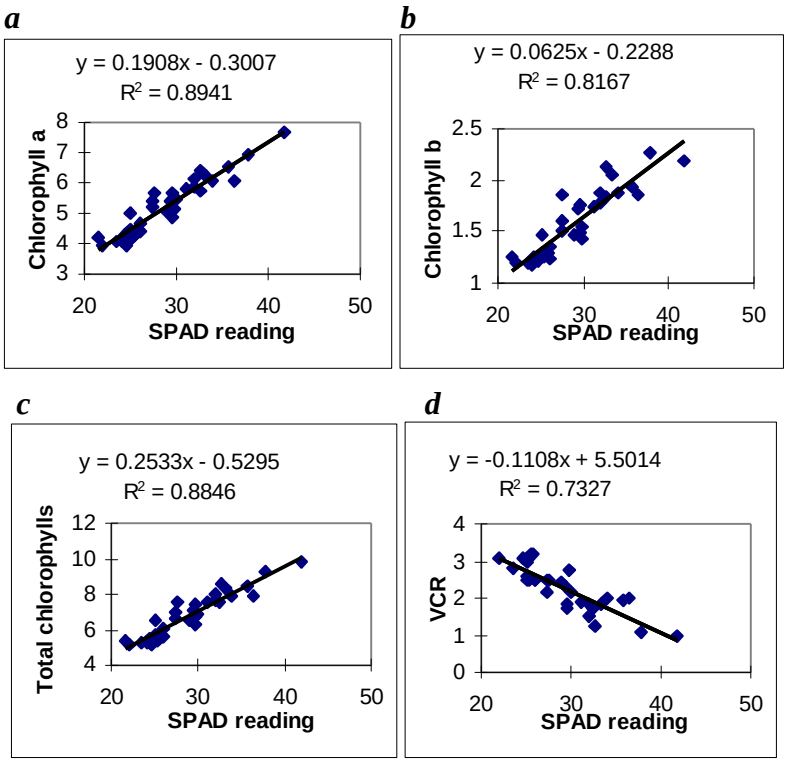

Figure 2. Relationship between ***a***, SPAD reading and chlorophyll *a* (mg/g); ***b***, SPAD reading and chlorophyll *b* (mg/g); ***c***, SPAD reading and total chlorophyll (mg/g); ***d***, SPAD reading and visual chlorotic rating. R^2, Coefficient of determination.

2.75 and TC content 6.5-8 mg/g, and (iii) susceptible – light green to yellow, VCR more than 2.75 and TC content less than 6.00 mg/g, and plant showing some interveinal to complete chlorosis (a typical symptom of Fe-deficiency). The crop was harvested at maturity and pod and haulm yields were recorded. Data were analysed statistically.

The analysis of variance revealed significant mean squares for all the traits under study, viz. chlorophyll *a*, chlorophyll *b*, TC content, VCR and SPAD readings, recorded at various crop growth stages and also for pod and haulm yields. This indicated the substantial genetic variability present in the test materials for their response to Fe deficiency chlorosis related traits. The VCR scores, chlorophyll contents, SPAD reading, pod and haulm yields for various groundnut genotypes are presented in Table 1.

The TC content and SPAD reading were highest at 9.86 mg/g dry weight of leaves and 41.83, respectively in the tolerant check I_1 (PBS 20100); the lowest SPAD reading of 21.53 was recorded in one of the susceptible checks ICG 7887 (PBS 20055). The genotypes 'PBS 21063', '23003', '24004', '24040', and 'FSD 66' which fell under the tolerant category showed 9.21, 8.00, 8.55, 8.48, and 8.29 mg/g TC and 37.85, 31.98, 32.59, 35.71 and 33.25 SPAD reading, respectively. All these genotypes showed a SPAD reading of more than 30. The genotypes 'PBS 12115' '12124', '12126', '21031', '22017', '24001', '24002', '24003', '24006', '24030', '29030', 'FSD-1', and 'FSD-61' were categorized as moderately tolerant. The genotypes 'PBS '14016', '14021', '22023' and FSD-37 which were highly sensitive to chlorosis showed SPAD

reading between 22.0 and 25.8 and chlorophyll content below 6 mg/g. The lowest TC content of 5.15 mg/g was recorded in 'PBS 14016' with a SPAD reading 22.01.

Correlation coefficients (r) and regression equations between SPAD reading and chlorophyll contents and VCR are presented in Table 2. Correlation coefficients between the SPAD reading and chlorophyll contents were highly significant at all the stages of sampling. Over the mean of four samples recorded at 30, 45, 60 and 75 DAE, the r values between the SPAD reading and chlorophyll *a* (0.94**), chlorophyll *b* (0.90**), and TC content (0.93**) were very high, positive and significant, indicating closer relationship of these traits with the SPAD reading, i.e. higher the SPAD reading higher will be the chlorophyll pigments and vice versa. The regression lines (Figure 2 *a–d*) showed that these variables are linearly related with each other. On the basis of the linear relationship, regression equations were developed (Table 2). Further, to test the consistency in ranking of genotypes with respect to the SPAD reading and estimated chlorophyll contents, the rank correlation coefficients (r_s) were calculated. It was found that the value of r_s between SPAD and chlorophylls *a*, *b* and TC content was very high and positive (0.94, 0.91 and 0.94, respectively), showing similar rankings of different genotypes. In the present investigation, the tolerant genotypes showed SPAD reading more than 30 and total chlorophyll content more than 8.0 mg/g dry weight of leaves and VCR below 2.0. On the other hand, genotypes with SPAD reading below 25, TC content less than 6.0 mg/g, and VCR more than 2.75 were found to be sensitive to iron-chlorosis. On the basis of the regression equations given here, it is convenient for researchers to predict chlorophyll content and VCR in the plants for categorization of genotypes.

The use of the SPAD meter in nitrogen management of tall fescue[8] (*Festuca arundinaceae*) and switch grass[9] (*Panicum virgatum*) has been demonstrated. The present study clearly shows that the chlorophyll meter (SPAD) is an efficient and speedy equipment for chlorophyll estimation in groundnut and can be used for screening genotypes having higher efficiency of iron utilization and thus selecting iron-chlorosis tolerant genotypes.

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