Madras Agric. J., 2020; doi:10.29321/MAJ.2020.000385

RESEARCH ARTICLE

In - vitro Screening Method for Iron Use Efficient Groundnut (*Arachis hypogaea* L.) Genotypes for Calcareous Soils Based on Morpho-Physiological Responses

Reddy Kiran Kalyan V S, Meena S* and Jawahar D
*Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore-3.

Received : 12th August, 2020
Revised : 25th August, 2020
Revised : 03rd September, 2020
Accepted : 23rd September, 2020

ABSTRACT

The most promising remedy for iron chlorosis in calcareous soils is genetic variability in the crop for iron absorption efficiency. A screening technique capable of simulating the realistic situation and promoting year-round screening for the selection of iron-efficient genotypes is strongly warranted from the point of time and manpower. The present work was aimed to screen groundnut genotypes for calcareous tolerance, based on morpho-physiological parameters with 40 groundnut genotypes in solution culture. The genotypes were grown for 21 days in full strength nutrient solution with and without iron (Fe) and 15 mM $KHCO_3$. Chlorophyll content, catalase, peroxidase, NRase activity, active and total Fe concentrations, shoot weight, root weight and root volume per plant were recorded for each genotype on 21st day. Cluster analysis of the collected data showed that TAG 24, CO 7, VRI 13113, VRI 16086, VRI 13149, JL 24 and TMV 1 were among the Fe efficient genotypes with Fe tolerance index value of 94.6, 94.0, 93.6, 93.0, 91.8, 91.4 and 90.5 respectively while TMV 13, AMABC 2017-8, VRI 16075 and VRI 16154 (81.3, 80.8, 80.7 and 79.3 respectively) were among the most Fe inefficient genotypes for calcareous soils.

Keywords*: Arachis hypogaea L.; Chlorosis; Calcareous soil; Solution culture; Morpho-physiological; Fe tolerance index*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown worldwide on 28.51 million ha with a production of 45.95 million tonnes (INDIASTAT 2017-18). In India, groundnut (4.88 m ha, 9.25 m t) is the second most important oilseed next to soybean (10.33 m ha, 10.93 m t) (INDIASTAT 2017-18). Of the essential nutrients, iron (Fe) plays an important role in photosynthesis, respiration, nitrogen fixation, DNA synthesis, hormone production, chlorophyll formation, and is also a component of various redox and iron-sulphur enzymes Zheng (2010). Though the total Fe content of the soils are high, iron deficiency chlorosis (IDC) is common worldwide among crops grown in calcareous, alkaline, coarse-textured, eroded, and low organic matter-containing and cold region soils as iron is less available for uptake in these soils. High pH and bicarbonate ion concentration in calcareous soils lead to IDC by suppressing iron uptake and/or translocation in plants (Ren *et al*. 2005).

In India, more than one-third of the soils are calcareous and spread mostly in the low rainfall areas of the western and central parts of the country, where groundnut is a major crop. Hence, IDC is more prevalent in Gujarat, Maharashtra, Rajasthan, Tamil Nadu, and Karnataka states in India, causing a considerable reduction in pod yield (16-32%) (Singh. 2001; Singh *et al.,* 1995). IDC is also a common-problem in groundnut-producing areas with calcareous soils in northern China (Li *et al.,* 2015) and Pakistan (Akhtar *et al.*, 2013; Imtiaz *et al.,* 2010), causing a significant reduction in yield. Severity of IDC will be usually quite high after excessive rainfall and also for groundnut grown under irrigation due to high bicarbonate ion concentration in the rhizosphere (Singh *et al.,* 1995 ; Zuo *et al.,* 2007).

Although Fe is commonly found in soil due to its low solubility and dissolution kinetics, the availability of Fe for plants is very limited, particularly in aerated alkaline and calcareous soils. (Mengel, 1994; Shenker and Chen, 2005). Fe is essential for chlorophyll synthesis; thus, insufficient Fe in leaves causes low chlorophyll levels and results in yellowing of younger leaves (Lucena, 2000; Chatterjee *et al*., 2017). Consequently, many crop yields are negatively affected and impaired by low Fe bioavailability in soils (Martins *et al.,* 2017).

*Corresponding author's e-mail: smeenash@gmail.com

Management reform methods such as soil amendment and foliar applications are often used, but they are short-term and uneconomic. Multidimensional solutions to problems such as nutrient deficiency stress are required instead of sticking to the conventionally available high-input approach (Morgan and Connolly, 2013). In this regard, the development / identification of crop species and varieties that are adaptable to nutrient-deficient soils is a promising method for the maintenance of crop yields in resource-poor environments (Foy 1993). The cultivation of an iron-efficient plant on iron-deficient soils or on soil with slightly sufficient iron for plants illustrates the technique of "tailoring the plant to fit the soil" as opposed to the old method of "setting the soil to fit the plant" (Foy, 1983). Such techniques are used to boost tolerant genotypes adaptable to iron-deficient situations and/or improved iron-use performance.

Soil application of Fe as ferrous sulphate is often recommended to alleviate the problem of iron chlorosis and also concomitant loss in yield (Irmak *et al.,* 2012; Singh and Dayal 1992). However, this is of little benefit to the crop as iron ionizes and is converted into insoluble ferric (Fe^{3+}) compounds, which are unavailable to plants. Foliar application of ferrous sulphate has been often suggested by Frenkel *et al.* (2004) and Singh *et al.* (1993), but the major problem is poor translocation of applied Fe within the plant (Hüve *et al.,* 2003). Although foliar spray of chelated form provides Fe in available form, their use is not popular and economically not feasible. Cultivation of IDC resistant cultivars in calcareous soils is economically feasible and sustainable approach compared to application of iron containing fertilizers through soil or foliar spray. The groundnut cultivars are called 'iron efficient' if they respond to iron deficiency stress by inducing biochemical reactions that make Fe available.

MATERIALS AND METHODS

Forty groundnut genotypes which include 5 groundnut genotypes and 10 pre-released cultures from Regional Research Station, Vridhachalam, 4 genotypes from Oilseed Research Station, Tindivanam and 15 genotypes from Department of Oilseeds, Coimbatore and 3 genotypes from Coconut research station, Aliyar nagar and 3 genotypes from Regional Agricultural Research Station, Tirupathi were evaluated for their reaction to calcium induced iron chlorosis and list is furnished below:.

CO 1 (G1)	CO 2 (G2)	CO 4 (G3)	CO 6 (G4)	CO 7 (G5)
ALR 1 (G6)	ALR 2 (G7)	ALR 3 (G8)	VRI 2 (G9)	VRI 5 (G10)
VRI 6 (G11)	VRI 7 (G12)	VRI 8 (G13)	TMV 1 (G14)	TMV 2 (G15)
TMV 7 (G16)	TMV 13 (G17)	AVK2015-3 (G18)	ALG 320 (G19)	AMABC 2017-8 (G20)
INS - 2016 -10 (G21)	AMABC 2017-2 (G22)	AMABC 2017-1(G23)	GPBD - 4 (G24)	TAG 24 (G 25)
JL 24 (G26)	ICGV 07772 (G27)	NARAYANI (G28)	ABHAYA (G 29)	DHARANI (G 30)
VRI- 13110 (G31)	VRI- 16083 (G32)	VRI- 13149 (G33)	VRI- 16075(G34)	VRI- 13154 (G35)
VRI- 16084 (G36)	VRI- 13159 (G37)	VRI- 13153 (G38)	VRI- 13113 (G39)	VRI- 16086 (G40)

A solution culture experiment was conducted during 2018 to evaluate the groundnut genotypes for lime induced chlorosis tolerance in the Tamil Nadu Agricultural University, Coimbatore. Forty groundnut genotypes and were assessed for calcareous stress tolerance *in vivo* experiment.

Plastic trays of 15 L capacity were used for conducting hydroponic culture, which had been surface sterilized by wiping their surfaces with 70% ethanol to remove nutritional contamination. To aerate the solution, aqua pore tubing hooked in the side of tanks and connected via 4 mm plastic tubing to aquarium pump was used.

Fifteen litres of basal nutrient solution was taken in each tank. The concentrations of nutrients in the final solution were as follows (μM): CaCl2 (2400); K_2SO_4 (2400); $Ca(NO_3)_2$ (1600); $MgSO_4$ (800); NH_4NO_3 (400); KH_2PO_4(80); FeEDTA (40); H_3BO3 (20); $MnSO_4$ (4); $ZnSO_4$ (3); $CuSO_4$ (0.8); Na_2MoO_4 (0.12) (Tang *et al*., 1996). The pH of the nutrient solution was maintained at about 6.0 by adjusting with either dilute HCL or NaOH every two to three days. The nutrient solutions were changed for every 7 days after transplanting (DAT). A $KHCO_3$ concentration of 15 mM, buffered with 1.5 g l^{-1} $CaCO_3$ was used to create bicarbonate concentration mimicking calcareous condition. There were four treatments (Nutrient solution, Nutrient solution (-Fe), Nutrient solution + $KHCO_3$ buffered with $CaCO_3$ + Fe, Nutrient solution + $KHCO_3$ buffered with $CaCO_3$ – Fe).

Seeds were treated with Propicanazole (0.2 %) for 2 minutes, shade dried and grown in coir pith wetted with half the strength of modified Hoagland solution. After 7 days of germination of groundnut, uniform seedlings were randomly assigned to the four treatments, and transferred to the test nutrient solutions. Fifteen seedlings per container was used, which were replicated three times. The treatments were arranged in completely randomized design blocks. The experiment was conducted for duration of 21 days.

All the following observations were recorded on standard leaf (Third fully opened leaf from the top on main stem) of five plants each in every treatment to estimate mean. Such means were estimated among four replications, each in normal and deficit conditions. The methodology followed for recording various observations is presented below.

Root volume was determined by water displacement method. The actual volume displacement, measures the volume of water displaced when plant tissue is submerged in a vessel of water (Novoselov 1960).

SPAD readings were recorded using Chlorophyll Meter (SPAD 502) designed by the Soil Plant Analytical Development (SPAD) section, Minolta, Japan. The data were recorded as described by Peng *et al.* (1993). Nitrate reductase activity was estimated in fully expanded functional leaves following the method of Nicholas *et al.* (1976) and the enzyme activity was expressed as $\mu g\ NO_2\ g^{-1}\ h^{-1}$. Peroxidase activity was assayed, according to Perur (1962) and Angelini *et al.* (1990) and expressed as Δ OD 430 nm $min^{-1}\ g^{-1}$. Catalase activity was estimated as per the procedure adopted by Gopalachari (1963) and expressed as $\mu g\ H_2O_2\ g^{-1}min^{-1}$. Active iron content was analyzed as described by Katyal *et al.* (1980). After drying the root and shoot samples at 60°C for 72 h, shoot and root dry weights were recorded. Samples were ground with an agate grinder and digested with triple acid solution (nitric: sulphuric: perchloric 9:2:1v/v) for nutrient extraction (Grusak, 1995). Iron concentration was determined by an atomic absorption spectrophotometer (VARIAN CARY 50 C). Plants were harvested 21 DAT, and measured the root volume, shoot and root weight of all the groundnut genotypes.

Ranking of genotype for Fe efficiency

Cluster analysis is useful to analyse genotypes on the basis of multiple parameters simultaneously. All the obtained data were converted to relative values, i.e. Fe tolerance indexes before cluster analysis. Fe tolerance index was defined as the observations under Fe deficiency divided by the means of the controls (Fe sufficient). Cluster analysis was performed and cluster group rankings were obtained based on Ward's minimum variance cluster analysis on the means of the Fe tolerance indexes for different morphological and physiological parameters including, root weight, shoot weight, root volume, active Fe and chlorophyll content (SPAD values), catalase and peroxidase. The distance between two clusters was calculated as the ANOVA sum of squares between the two clusters in all the parameters analysed. The cluster groups were identified in dendrograms. The number of cluster groups was determined by calculating the pseudo t2 which reached a local maximum. The cluster group rankings were obtained from the averages of means over multiple parameters in each cluster group, i.e., cluster mean, in order from highest to lowest averages. A sum was obtained by adding the numbers of cluster group ranking of each parameter in each genotype. The genotypes were finally ranked based on the sums in order that those with the largest sums were ranked as the most tolerant and those with the smallest sums were ranked as the least tolerant in terms of relative Fe tolerance Zeng *et al.* (2002).

Statistical analysis

Statistical analyses were performed by using two-way ANOVA. Differences between mean values of treatments were evaluated using significant difference (SD) at a 0.05 significance level. Cluster Analysis was realized with ward's method, using Euclidean Distance (SPSS 16.0 for Windows).

RESULTS AND DISCUSSION

Marked differences in shoot dry weight was observed in the groundnut genotypes in response to Fe deficiency when they are grown on bicarbonate (calcareous) solution culture. The maximum shoot weight (0.84 g $plant^{-1}$) was recorded in VRI- 13149 under $KHCO_3$ followed by 0.77 g $plant^{-1}$ (ABHAYA) (Figure 1). Similar to our findings, Puangbut *et al.* (2009) proved that higher biomass improved yield. TMV 2, CO 2, ALR 2, VRI 2, VRI 13154 and VRI 16084 showed lower shoot weight in Fe induced deficiency conditions nearly around 0.28 g $plant^{-1}$. However, some genotypes showed deviations from the trend and produced higher shoot weight in AVK 2015-3, ALG 320, AMABC 2017-2, TAG 24, VRI- 13110 and VRI- 16084 under Fe deficient conditions as compared to Fe sufficient conditions. Higher dry matter was observed in groundnut genotypes TCGS-273, TCGS-2, TCGS 37, and Kadiri 3 and evaluated them as iron efficient genotypes (Reddy *et al*., 1993). Similar findings were reported by Krishnasamy *et al*. (2005) on sorghum genotypes and they stated that iron-efficient genotype should not only be able to absorb Fe from deficient conditions but should also produce more dry matter.

The maximum root weight under stress condition (with bicarbonate +Fe) were recorded with VRI 2, CO 7 and VRI 8 (0.53, 0.50 and 0.44 g $plant^{-1}$ respectively), at the same time these genotypes gave 0.70, 0.52 and 0.48 g $plant^{-1}$ of root weight under Fe deficient conditions and calcareousness induced nutrient solution. The minimum shoot weight (0.17 g $plant^{-1}$) was recorded with both CO 2 and VRI 16084 under calcareousness induced nutrient solution. Similarly, most of the genotypes recorded lower shoot weight with calcareousness induced nutrient solution. Irrespective of genotypes, modified full strength Hoagland

Table 1. Efficiency of different groundnut genotypes in nutrient solution culture experiment

GENOTYPES	Root volume (cc)				Nitrate Reductase (μM of NO_2 $g^{-1}hr^{-1}$)			
	With Carbonate		Without Carbonate		With Carbonate		Without Carbonate	
	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe
CO 1	0.75	0.60	0.85	0.80	2.02	1.78	2.50	2.14
CO 2	0.85	0.70	0.90	0.85	1.78	1.67	1.89	1.28
CO 4	1.70	1.40	1.75	1.65	2.22	2.17	2.28	2.06
CO 6	1.70	0.85	1.95	1.60	2.11	2.11	2.11	2.06
CO 7	1.70	1.40	1.80	1.75	2.06	1.94	2.17	1.89
ALR 1	1.15	0.90	1.15	1.05	2.22	2.06	2.39	1.94
ALR 2	0.75	0.70	0.90	0.70	1.67	1.61	1.72	1.67
ALR 3	0.60	0.50	0.70	0.45	1.44	1.44	1.44	1.50
VRI 2	1.40	1.00	1.50	1.45	1.17	1.11	1.22	1.44
VRI 5	0.85	0.90	0.70	0.90	1.50	1.33	1.67	1.50
VRI 6	0.75	0.70	0.60	0.95	1.61	1.50	1.72	1.50
VRI 7	1.00	0.70	1.30	1.00	1.56	1.39	1.72	1.61
VRI 8	1.40	1.30	1.55	1.30	1.94	1.94	1.94	1.89
TMV 1	1.20	0.50	1.50	0.45	1.53	1.39	1.67	1.50
TMV 2	1.30	1.55	1.60	0.88	2.06	2.00	2.11	2.00
TMV 7	0.65	0.55	0.80	0.55	1.50	1.44	1.50	1.44
TMV 13	1.35	1.20	1.50	1.55	1.56	1.50	1.56	1.44
AVK 2015-3	0.72	0.70	0.80	0.60	1.11	1.11	1.11	1.50
ALG 320	0.84	0.70	0.90	0.90	1.33	1.33	1.33	1.33
AMABC 2017-8	1.25	1.10	1.35	1.25	1.22	1.44	1.22	1.39
INS - 2016 -10	1.35	0.90	1.40	1.35	1.39	1.50	1.39	1.39
AMABC 2017-2	1.20	1.00	1.30	1.20	1.44	1.50	1.44	1.44
AMABC 2017-1	1.25	1.10	1.35	1.20	2.39	2.28	2.50	2.11
GPBD – 4	0.85	0.70	0.85	0.85	1.72	1.61	1.83	1.56
TAG 24	1.10	0.80	1.30	1.15	2.03	1.89	2.17	2.00
JL 24	0.95	0.70	1.10	1.00	1.58	1.44	1.72	1.33
ICGV 07772	0.85	0.70	0.95	0.90	1.44	1.39	1.50	1.44
NARAYANI	0.75	0.50	0.80	1.00	1.33	1.28	1.39	1.39
ABHAYA	0.66	0.60	0.70	0.70	2.50	2.44	2.56	2.28
DHARANI	1.45	1.10	1.70	1.55	2.28	2.11	2.44	2.00
VRI- 13110	0.65	0.50	0.70	0.70	1.33	1.17	1.50	1.44
VRI- 16083	0.85	0.70	1.10	0.90	2.22	1.94	2.50	2.06
VRI- 13149	1.45	1.20	1.70	1.40	2.19	1.89	2.50	2.28
VRI- 16075	0.80	0.70	0.90	0.90	1.58	1.44	1.72	1.33
VRI- 13154	0.70	0.60	0.80	0.70	1.50	1.50	1.50	1.39
VRI- 16084	0.55	0.50	0.70	0.50	1.14	1.11	1.17	1.44
VRI- 13159	1.15	1.10	1.30	1.10	2.03	1.96	2.11	1.94
VRI- 13153	0.70	0.50	0.90	0.70	1.67	1.61	1.72	1.17
VRI- 13113	1.00	0.70	1.30	0.90	2.14	1.94	2.33	2.17
VRI- 16086	1.05	0.80	1.10	1.00	2.11	1.94	2.28	2.17
Mean	41.17	32.5	46.05	37.38	1.67	1.53	1.84	1.59

Sources	Root volume (cc)		Nitrate Reductase (μg of NO_2 $g^{-1}hr^{-1}$)	
	SEd	CD (p=0.05)	SEd	CD (p=0.05)
Genotypes (G)	0.042	0.082	0.08	0.16
HCO_3 (B)	0.009	0.018	0.02	0.04
Iron (Fe)	0.009	0.018	0.02	0.04
G*B	0.059	0.116	NS	NS
B*Fe	0.013	0.026	0.03	0.05
G*Fe	0.059	0.116	0.11	0.22
G*B*Fe	0.083	0.164	NS	NS

solution treatment registered the highest root volume compared to other treatments (Table 1). Rukam singh *et al.,* (2007) results indicated that, genotypes showing high values dry matter considered as tolerant genotypes and may be used for further breeding programme. In NaCl tolerant lines of citrus sinensis, the growth medium inhibited root cell growth under salt stress (Ben-Hayyim and Kochba, 1983).

The data pertaining to Nitrate reductase activity content of groundnut genotypes as influenced by bicarbonate and conditions were recorded and presented in Table 1. The Nitrate reductase activity was maintained highest in iron sufficient conditions (control) compared to iron-deficient conditions with bicarbonate. The genotypes Abhaya and AMABC 2017 -1 registered the higher values of NRase both

Table 2. Efficiency of different groundnut genotypes in nutrient solution culture experiment

GENOTYPES	Catalase (μ M of H_2O_2 g^{-1} min^{-1})				Peroxidase activity (Δ OD/min/g of FW)			
	With Carbonate		Without Carbonate		With Carbonate		Without Carbonate	
	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe
CO 1	1.35	1.14	1.25	1.08	12.0	9.13	9.51	6.80
CO 2	0.98	0.87	0.93	0.83	8.8	6.12	6.53	4.00
CO 4	1.53	1.46	1.48	1.43	11.2	8.18	8.89	6.36
CO 6	1.50	1.42	1.43	1.41	11.4	8.48	9.04	6.64
CO 7	1.73	1.52	1.50	1.46	11.4	9.73	9.81	7.96
ALR 1	1.75	1.48	1.58	1.44	11.9	8.57	9.29	6.72
ALR 2	1.50	1.48	1.50	1.47	8.3	5.51	6.03	3.80
ALR 3	1.08	0.86	0.88	0.90	8.5	5.90	6.42	4.00
VRI 2	1.03	0.85	0.90	0.80	10.0	7.20	7.72	5.36
VRI 5	1.10	0.95	1.00	0.90	10.0	6.93	7.54	5.08
VRI 6	1.13	1.04	1.05	1.06	9.3	6.69	7.13	4.93
VRI 7	1.65	1.47	1.53	1.41	10.3	7.56	8.00	5.70
VRI 8	1.65	1.32	1.33	1.21	12.4	10.30	10.4	8.00
TMV 1	1.58	1.42	1.48	1.37	6.3	3.77	4.00	1.78
TMV 2	1.83	1.43	1.50	1.41	11.4	9.12	9.39	7.23
TMV 7	0.93	0.75	0.80	0.70	7.6	4.37	5.02	2.51
TMV 13	1.20	1.05	1.08	1.00	10.7	8.14	8.42	6.19
AVK 2015-3	1.48	1.33	1.40	1.28	9.0	6.36	6.90	4.71
ALG 320	1.40	1.22	1.28	1.20	9.8	7.21	7.61	5.39
AMABC 2017-8	1.43	1.30	1.33	1.28	7.5	5.54	5.73	3.76
INS - 2016 -10	1.18	1.05	1.08	1.01	9.8	7.61	7.92	5.86
AMABC 2017-2	0.73	0.55	0.55	0.50	8.9	5.98	6.58	4.23
AMABC 2017-1	1.13	1.04	1.03	1.01	11.6	8.45	9.23	6.71
GPBD – 4	1.10	0.88	0.90	0.81	10.1	7.84	7.98	5.95
TAG 24	1.65	1.50	1.55	1.45	12.3	10.1	10.8	7.96
JL 24	1.68	1.53	1.60	1.50	10.6	7.84	8.70	5.85
ICGV 07772	1.15	0.97	1.00	0.91	10.3	7.65	8.37	5.66
NARAYANI	1.33	1.12	1.15	1.05	9.3	6.43	7.20	4.27
ABHAYA	1.28	1.14	1.20	1.10	12.0	9.02	10.04	7.13
DHARANI	1.85	1.65	1.80	1.49	11.4	9.38	10.00	7.54
VRI- 13110	1.10	0.90	0.95	0.89	9.5	6.79	7.64	4.88
VRI- 16083	1.65	1.45	1.50	1.43	12.1	10.25	10.73	7.95
VRI- 13149	1.83	1.64	1.68	1.58	12.0	9.51	10.20	7.55
VRI- 16075	1.18	0.95	1.00	0.87	9.9	7.05	8.07	5.25
VRI- 13154	1.10	0.84	0.90	0.82	7.7	4.84	5.84	2.99
VRI- 16084	1.50	1.34	1.39	1.31	10.2	7.53	8.40	5.79
VRI- 13159	1.83	1.65	1.73	1.59	11.5	9.07	9.89	7.19
VRI- 13153	1.50	1.20	1.28	1.10	10.0	8.03	8.53	6.18
VRI- 13113	1.80	1.52	1.65	1.43	12.0	10.10	10.69	7.99
VRI- 16086	1.88	1.66	1.70	1.62	11.5	8.65	9.70	6.92
MEAN	1.41	1.22	1.27	1.17	10.26	7.77	8.67	6.00

Sources	Catalase (μM of H_2O_2 g^{-1} min^{-1})		Peroxidase activity (OD/min/g of FW)	
	SEd	CD (p=0.05)	SEd	CD (p=0.05)
Genotypes (G)	0.04	0.08	0.41	0.81
HCO_3 (B)	0.01	0.02	0.09	0.18
Iron (Fe)	0.01	0.02	0.09	0.18
G*B	NS	NS	0.58	1.15
B*Fe	0.01	0.03	NS	NS
G*Fe	NS	NS	0.58	1.15
G*B*Fe	NS	NS	0.82	1.63

Table 3. Efficiency of different groundnut genotypes in nutrient solution culture experiment

GENOTYPES	Active Iron iron (mg kg^{-1})				SPAD			
	With Carbonate		Without Carbonate		With Carbonate		Without Carbonate	
	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe
CO 1	27.8	23.7	32.0	25.0	36.1	34.4	37.9	35.7
CO 2	29.6	24.0	33.8	26.7	30.6	24.0	33.8	26.7
CO 4	29.0	22.6	32.0	25.0	30.8	26.4	32.0	28.0
CO 6	31.0	27.0	35.2	28.2	34.0	27.0	35.2	28.9
CO 7	26.7	22.3	28.5	24.0	27.3	22.3	28.5	24.0
ALR 1	33.4	31.5	37.6	30.4	36.4	31.5	37.6	30.4
ALR 2	23.6	18.9	25.2	18.1	32.5	32.2	35.9	32.8
ALR 3	28.6	23.6	32.8	25.8	26.3	22.1	32.8	25.8
VRI 2	23.1	21.1	27.3	20.3	24.5	19.7	27.3	20.3
VRI 5	30.1	28.0	34.4	27.3	30.2	28.0	34.4	27.3
VRI 6	32.3	35.2	36.7	31.6	27.9	24.9	31.7	25.5
VRI 7	32.4	28.7	35.3	28.2	33.9	29.1	35.3	30.0
VRI 8	38.4	29.3	42.1	29.9	38.2	36.5	42.1	38.0
TMV 1	26.8	24.2	29.6	23.8	28.8	32.2	35.7	34.1
TMV 2	34.8	26.3	38.4	30.7	34.5	31.2	38.4	33.6
TMV 7	26.5	25.0	30.1	24.4	26.5	24.0	30.1	24.4
TMV 13	15.9	13.3	20.4	13.0	17.9	13.3	20.4	13.0
AVK 2015-3	17.3	14.9	21.6	14.9	18.3	14.9	21.6	14.9
ALG 320	29.4	27.2	33.6	26.4	30.4	26.3	33.6	26.4
AMABC 2017-8	21.0	18.6	25.2	18.0	19.4	18.6	25.2	18.0
INS - 2016 -10	25.8	22.1	29.9	22.7	24.6	22.1	29.9	22.7
AMABC 2017-2	29.1	26.8	33.4	26.3	28.4	26.8	33.4	26.3
AMABC 2017-1	38.5	23.7	42.7	30.5	28.4	23.7	32.2	26.0
GPBD - 4	21.5	19.3	25.7	18.5	22.2	19.3	25.7	18.5
TAG 24	38.7	34.7	42.6	35.5	40.7	34.7	42.6	35.5
JL 24	24.9	19.6	27.1	19.9	28.8	24.0	32.9	27.1
ICGV 07772	20.3	18.1	24.4	16.7	20.3	15.9	24.4	16.7
NARAYANI	21.0	18.7	25.3	18.2	22.0	18.7	25.3	18.2
ABHAYA	33.4	27.0	37.7	29.9	32.4	27.0	37.7	29.9
DHARANI	38.0	32.0	42.5	35.1	41.0	35.8	42.5	35.1
VRI- 13110	19.0	15.2	23.2	14.8	31.2	27.6	34.7	27.5
VRI- 16083	33.9	30.3	37.1	30.0	35.9	30.3	37.1	30.0
VRI- 13149	24.2	21.8	28.5	21.6	27.2	21.8	31.3	27.1
VRI- 16075	40.8	28.6	45.0	29.8	22.9	22.8	29.9	22.2
VRI- 13154	22.4	18.8	28.0	19.0	24.4	18.8	28.0	19.0
VRI- 16084	27.3	24.2	32.3	23.0	30.3	24.2	32.3	23.0
VRI- 13159	35.0	30.8	39.8	32.6	38.0	35.8	39.8	32.6
VRI- 13153	27.0	20.9	31.6	24.3	28.0	20.9	31.6	24.3
VRI- 13113	31.6	29.9	36.3	29.8	34.6	29.9	36.3	29.8
VRI- 16086	39.8	35.7	44.8	35.1	39.0	33.8	41.3	35.1
MEAN	28.7	24.6	32.7	25.1	29.6	25.81	32.96	26.61

Sources	SEd	CD (p=0.05)	SEd	CD (p=0.05)
Genotypes (G)	0.41	0.81	0.49	0.97
HCO_3 (B)	0.09	0.18	0.11	0.22
Iron (Fe)	0.09	0.18	0.11	0.22
G*B	0.58	1.15	0.70	1.38
B*Fe	0.13	0.26	0.16	0.31
G*Fe	0.58	1.15	0.70	1.38
G*B*Fe	0.82	1.63	0.99	1.95

under bicarbonate + Fe (2.50 and 2.39 µM of NO_2 $g^{-1}hr^{-1}$) and without bicarbonate + Fe (2.56 and 2.50 µM of NO_2 $g^{-1}hr^{-1}$) treatment (Table 1). In the present study, irrespective of treatment conditions the genotype VRI 16086 recorded the highest mean catalase activity (1.88, 1.66, 1.70 and 1.62 µM of

Table 4. Ranking of genotypes based on Fe tolerance indexes in a cluster analysis (Ward's minimum variance analysis). All the data was presented in relative values (percentage) calculated per plant.

GENOTYPES	Sht Wt	Root Wt	Root Vol	NRASE	CAT	POD	Act Fe	SPAD	Mean	cum avg	cluster grouping	Gen ranking
CO 4	85.2	94.1	97.1	97.4	96.7	82.4	90.6	96.3	92.5			
ALR 1	90.9	87.5	100	92.9	90.3	86.6	88.8	96.8	91.7	91.26	1	3
CO 1	92.5	84.2	94.1	93.6	92.6	86.7	86.9	95.3	90.7			
VRI- 16083	71.0	91.9	95.5	92.8	90.9	90.9	91.4	96.8	90.1			
AMABC 2017-1	88.9	81.8	92.6	88.4	85.0	79.6	90.2	88.2	86.8			
VRI 6	84.4	81.8	91.7	88.4	92.0	76.7	88.0	88.0	86.4			
ABHAYA	83.1	71.7	85.7	87.1	89.8	83.7	88.6	85.9	84.5			
VRI 2	85.3	75.7	86.7	87.7	87.4	77.2	84.6	89.7	84.3	84.59	2	4
GPBD - 4	85.7	78.3	88.2	88.5	81.8	79.0	83.7	86.4	84.0			
CO 2	77.8	73.9	88.9	83.6	91.8	74.2	87.6	90.5	83.5			
ALR 3	88.2	73.1	85.7	90.3	81.5	75.5	87.2	80.2	82.7			
VRI 8	90.9	91.7	90.3	100.0	97.0	92.7	91.2	90.7	93.1			
TMV 2	96.3	92.9	93.8	97.6	92.4	91.2	90.6	89.8	93.1			
ALR 2	94.4	88.9	83.3	97.1	100	92.8	93.7	90.5	92.6			
CO 6	92.2	94.4	87.2	100.0	95.3	85.5	88.1	96.6	92.4	92.36	3	2
DHARANI	93.8	85.7	85.3	93.4	97.3	96.5	89.4	96.5	92.2			
VRI- 13159	92.9	93.9	84.6	96.2	94.5	90.4	87.9	95.5	92.0			
VRI 7	90.9	89.5	84.6	90.7	92.7	93.2	91.8	96.0	91.2			
TAG 24	107	90.9	92.3	93.6	93.9	92.7	90.9	95.5	94.6			
CO 7	92.9	96.2	94.4	94.9	91.9	92.1	93.7	95.8	94.0			
VRI- 13113	93.8	100	96.2	91.9	91.7	93.3	87.1	95.3	93.6			
VRI- 16086	93.3	93.5	95.5	92.5	90.4	95.7	88.8	94.4	93.0	92.70	4	1
VRI- 13149	98.8	91.7	94.1	91.6	91.8	94.2	84.9	86.9	91.8			
JL 24	93.2	90.2	90.9	91.9	95.2	90.6	91.9	87.5	91.4			
TMV 1	90.7	91.4	93.3	91.6	93.7	92.1	90.5	80.7	90.5			
VRI- 16084	100	83.3	78.6	88.0	87.3	82.4	84.5	93.8	87.2			
VRI- 13110	87.7	82.1	78.6	88.7	86.4	80.4	81.9	89.9	84.5			
VRI- 13153	82.1	81.3	77.8	87.2	85.3	85.3	85.4	88.6	84.1	84.36	5	6
TMV 7	82.9	84.2	81.3	93.3	86.0	66.1	88.0	88.0	83.7			
AMABC 2017-2	87.2	87.5	84.6	86.8	75.3	73.9	87.1	85.0	83.4			
VRI 5	87.3	79.0	78.6	83.8	86.4	75.4	87.5	87.8	83.2			
TMV 13	54.6	84.6	90.0	87.2	90.0	78.7	77.9	87.8	81.3			
AMABC 2017-8	62.8	84.6	88.9	84.4	88.8	76.4	83.3	77.0	80.8	80.51	6	7
VRI- 16075	55.6	81.5	88.9	86.1	84.8	81.5	90.7	76.6	80.7			
VRI- 13154	53.9	81.3	87.5	86.7	81.8	75.8	80.0	87.1	79.3			
ALG 320	75.7	86.2	88.9	85.7	87.9	77.7	87.5	90.5	85.0			
AVK 2015-3	79.1	87.1	90.0	83.8	87.8	76.7	80.1	84.7	83.7			
INS - 2016 -10	83.3	88.5	85.7	80.6	91.5	80.8	86.3	82.3	84.9	84.51	7	5
ICGV 07772	82.4	83.9	89.5	87.3	87.0	81.3	83.2	83.2	84.7			
NARAYANI	78.7	84.6	87.5	89.9	86.5	77.4	83.0	87.0	84.3			

(Sht wt – Shoot weight; Root wt - Root weight, Root vol - Root volume, CAT – Catalase; POD – Peroxidase, ACT FE – Active iron; SPAD - Soil Plant Analysis Development (SPAD) chlorophyll meter; cum avg – cumulative average; gen ranking – genotype ranking

H_2O_2 g^{-1} min^{-1}) followed by Dharani compared to other genotypes in all the treatments respectively and lowest was recorded in TMV 7 (0.70 µM of H_2O_2 g^{-1} min^{-1}) under iron-deficient conditions (Table 2). Iron acts as an enzyme activator or cofactor in chlorophyll synthesis and activates several other enzymes, including catalase, peroxidase, nitrate reductase and nitrogenase. Iron deficiency has

been found to reduce the activity of oxidative stress-related enzymes like catalase and peroxidase in several plant species that is attributed to less Fe concentration in Fe- deficient leaves M'sehli *et al.* (2014) and Boodi *et al.* (2016). Mahurkar *et al*. (1992) finding also states that the ability of enzymes *viz.*, CAT, POD and nitrate reductase decreased with the increase in degree of chlorosis in groundnut leave. Under iron deficiency conditions, the activity of both peroxidase and catalase enzyme decline. Our findings are also coping with Machold and Stephan (1964) as about 80 per cent and 50 percent decrease in the activity of catalase and peroxidase enzymes, respectively due to iron deficiency.

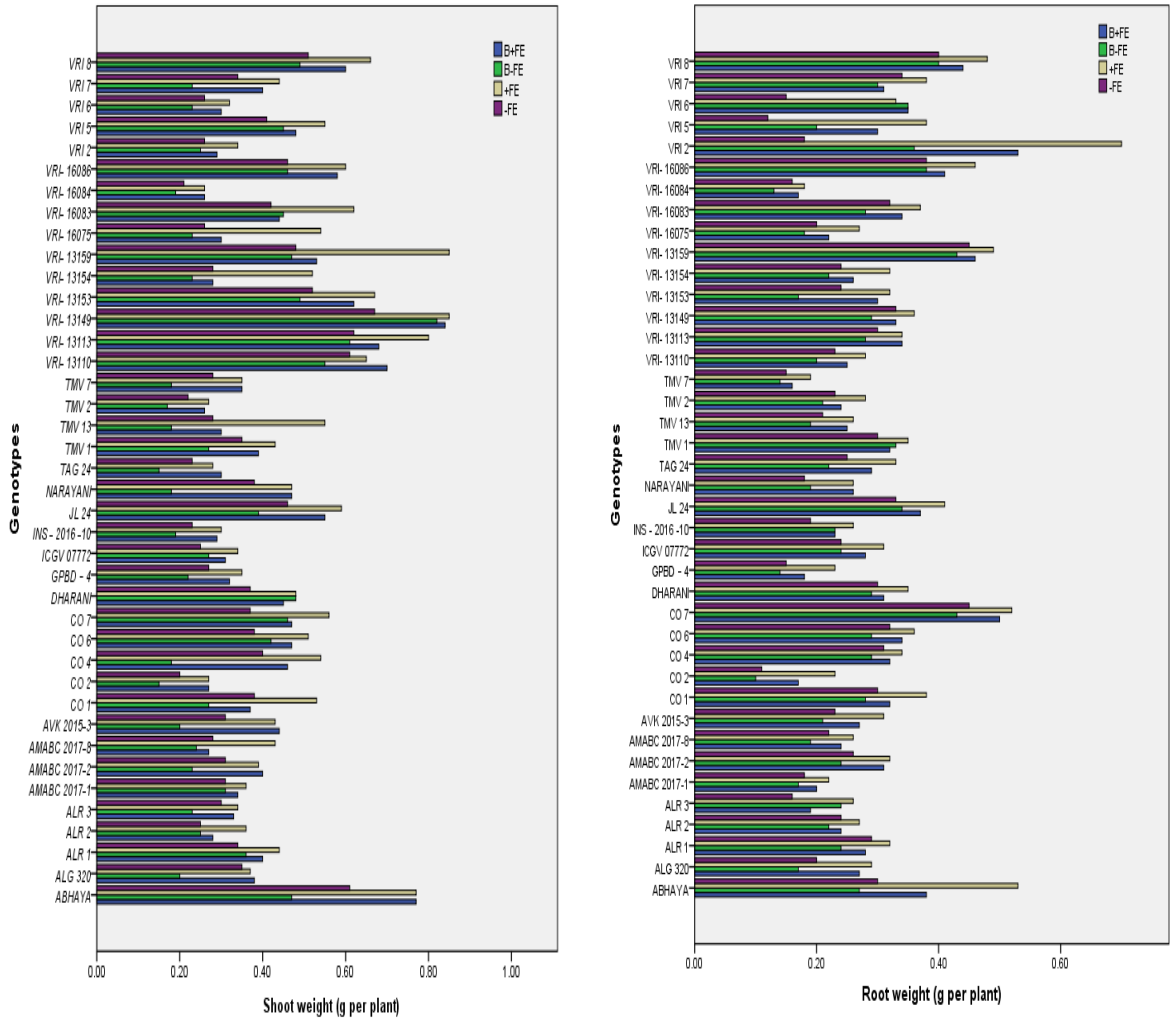

Figure 1. Efficiency of different groundnut genotypes in nutrient solution culture experiment

The mean peroxidase activity was significantly higher under iron sufficient conditions compared to iron-deficient soil conditions and $KHCO_3$ treated nutrient solution. The mean POD activity was decreased by 2.19 and 3.3%, respectively, in $KHCO_3$ induced and iron-deficient conditions over sufficient nutrient conditions. Irrespective of the genotypes, VRI 8, Abhaya and VRI 13113 recorded highest POD activity (12.4, 12.0 and 12.0 OD/min/g of FW, respectively) and the lowest was recorded in TMV 1 (3.77 OD/min/g of FW) (Table 2). Iron sufficient and iron deficient conditions results in variation of antioxidant enzymes (peroxidase and catalase) in all the groundnut genotypes. Boodi *et al.* (2016) observed a reduction in peroxidase activity among all groundnut genotypes in iron-deficient soil conditions compared to iron sufficient soil conditions. However, a lower reduction was observed among resistant genotypes compared to susceptible ones, probably due to comparatively higher active-Fe maintained in leaves under Fe-stress conditions. Similar results were obtained in the previous works of Sanjana (2004) in soybean genotypes and Nagarathnamma (2006) in groundnut genotypes. It turned out that the active involvement of this antioxidant enzyme was related, at least in part, to the tolerance to Fe-deficiency-induced oxidative stress Essa *et al.* (2015).

Maximum SPAD (41.0 and 40.7) value was given by Dharani and TAG 24 respectively under with calcareous induced treatment with Fe, while the

same genotypes recorded higher SPAD value in Fe suficient condition as 42.5 and 42.6 respectively. Irrespective of genotypes, the lower SPAD value average was given with bicarbonate – Fe treatment (25.8) and highest under control (32.9). Under calcareous induced treatment with Fe and without Fe, genotypes viz., AMABC 2017-8 and TMV 13 gave SPAD value below 20 and these genotypes were most sensitive to calcareousness (Table 3). Similar results were also reported by Song *et al.* (2017), where lower SPAD values were recorded in Fe deficient plants as compared to the plants treated with foliar applications. High CaCO3 level and soil water content decreased the chlorophyll concentration (SPAD value) (Zuo *et al.*, 2007). Li and Yanxi, (2007) reported that the physiological parameters *i.e.*, SPAD and active Fe value are related to crop yield. Similar results were reported by Akhtar *et al*. (2013) and concluded that genotypes with more active Fe and SPAD values produced a higher yield.

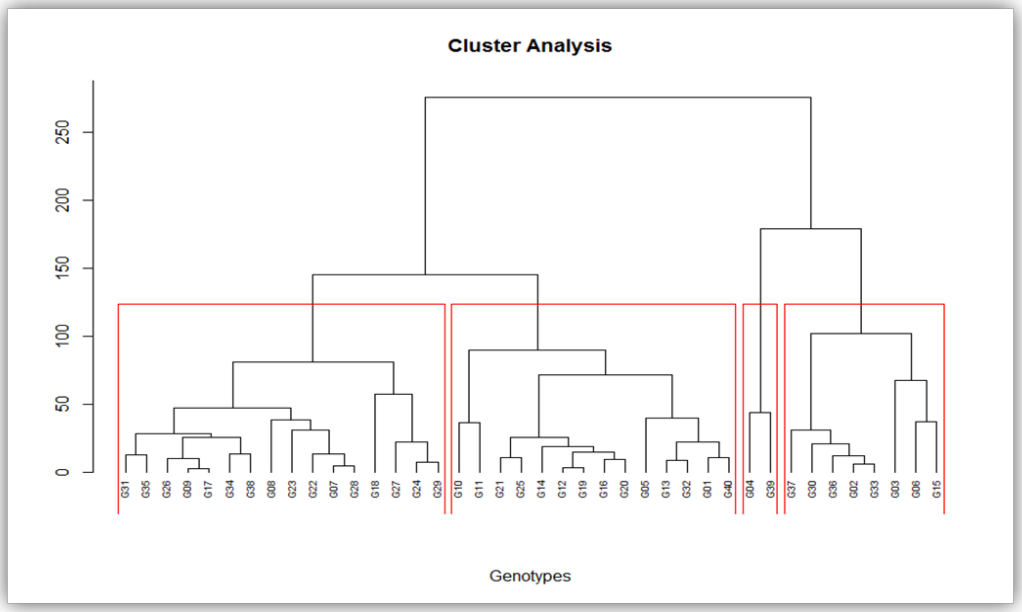

Figure 2. Multivariate analysis using Fe tolerance indexes in morphological and physiological parameters using Ward's minimum variance cluster analysis.

The maximum active Fe concentration (45.0 and 44.8 mg kg^{-1}) was given by VRI- 16075 and VRI - 16086 respectively under Fe sufficient conditions; at the same time, these genotypes gave active Fe concentration values of 29.8 and 35.1 respectively, under Fe deficient conditions. The maximum active Fe concentration (40.8 and 39.8 mg kg^{-1}) was given by VRI- 16075 and VRI – 16086 respectively under calcareousness induced conditions, whereas minimum (13.3 and 14.9 mg kg^{-1}) active Fe in case of Fe deficient and calcareousness induced conditions was obtained from TMV 13 and AVK 2015-3 (Table 3). Active Fe concentration is an important parameter in Fe deficiency. As the parameter is related to chlorophyll content, both parameters affected each other. Active Fe concentration is directly correlated with the chlorophyll content. Similar results were proved by previous work Singh *et al.* (1990); Gao and Shi (2007). Ohwaki and Sugahara (1993) also reported that the genotypic differences of sensitive and resistant cultivars of chickpea to Fe-deficiency were attributed to the active iron in the leaves when grown under Fe-stress conditions. In the same line, the active iron content of leaves of iron efficient groundnut genotypes was more than in iron inefficient groundnut genotypes in calcareous soil was reported by Kulkarni *et al*., 1995.

Based on multivariate analysis using Fe tolerance indexes in morphological and physiological parameters using Ward's minimum variance cluster analysis (Figure 2), the genotypes were divided into seven cluster groups (Table 4). Based on this analysis varieties, TAG 24, CO 7, VRI 13113, VRI 16086, VRI 13149, JL 24 and TMV 1 ranked first falling in clusters ranked first with an averaged cumulative Fe tolerance indexes of 92.7 %, whereas TMV 13, AMABC 2017-8, VRI 16075 and VRI 13154 were among the stress-sensitive genotypes with averaged Fe tolerance indexes of 80.51 %. Loop and Finck (1984) advocated the usefulness of total Fe, generally total Fe concentration in plant tissue is not related to the occurrence of chlorosis Rashid *et al.* (1997).

CONCLUSION

Physiological and morphological attributes were affected more under iron-deficient conditions

compared to iron sufficient conditions in groundnut genotypes. Antioxidant enzymes like peroxidase, catalase were profoundly reduced in inefficient genotypes compared to efficient genotypes under iron-deficient conditions. Based on the morpho-physiological parameters studied, it can be suggested to assess Fe efficiency of groundnut genotypes. TAG 24, CO 7, VRI 13113, VRI 16086, VRI 13149 and JL 24 are the best five genotypes as these were screened out to be Fe deficiency tolerant genotypes based on Fe tolerance index values . The method demonstrated in this study, *i.e.,* cluster group ranking of genotypes based on multiple morpho-physiological characters can be applied in calcareous tolerance breeding to evaluate calcareous tolerance among genotypes with a great advantage over conventional methods.

Funding and Acknowledgment

The financial help by Science and Engineering Research Board (SERB) as Core Research Grant for the research is gratefully acknowledged

REFERENCES

Akhtar, S., Shahzad, A., Arshad, M. and Hassan, F. 2013. Morpho-physiological evaluation of groundnut (Arachis hypogaea L.) genotypes for iron deficiency tolerance. *Pak. J. Bot*, **45(3):** 893-899.

Angelini, R., F. Manes. and R. Federico. 1990. "Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems." *Planta.* **182(1):** 89-96.

Ben-Hayyim, G. and Kochba, J. 1983. Aspects of salt tolerance in a NaCl selected stable cell lines of Citrus sinensis. *Plant Physiol*. **71**: 685-690.

Boodi, I. H., Pattanashetti, S. K., Biradar, B. D., Naidu, G. K., Chimmad, V. P., Kanatti, A. and Debnath, M. K. 2016. Morpho-physiological parameters associated with iron deficiency chlorosis resistance and their effect on yield and its related traits in groundnut. *J. crop sci. biotech*. **19(2):** 177-187

Chatterjee, A., Lovas, S., Rasmussena, H., and Goos, R. J. 2017. Foliar application of iron fertilizers to control iron deficiency chlorosis of soybean. *Crop. Forage Turfgrass Manag*. 3, 0.

Essa, E. M., Zaghloul, S. M., Salama, Z. A., Rakha, F. A., Mabrouk, Y., El-Bendary, A. A. and El-Fouly, M. M. 2015. Physiological and Biochemical Evaluation of Fe-efficiency in Fe-Deficient Maize Genotypes. *American J. Agric. Biol Sci.*, **10(1):** 55.

Fageria, N. K. 1994. *Deficiência nutricionais na cultura do arroz*. EMBRAPA-SPI.

Frenkel, C., Hadar, Y. and Chen, Y. 2004. Peanut plants based bioassay for iron deficiency and its remediation. *Soil sci.plant nutrition.*, **50(70):** 1063-1070.

Foy CD. 1983. The physiology of plant adaptation to mineral stress. *Iowa State J. Res.* **54:**355-391.

Foy CD. 1993 Role of Soil Scientists in Genetic Improvement of Plants for Problems Soils. In: Adaptation of Plants to Soil Stresses. INTSORMIL Pub., No. 94-2, University of Nebraska, Lincoln, USA.185-206.

Gao, L. and Shi, Y. 2007. Genetic differences in resistance to iron deficiency chlorosis in peanut. *J. plant nutrition*, **30(1):** 37-52

Gopalachari, N. C. 1963. Changes in the activities of certain oxidizing enzymes during germination and seedling development of Phaseolus mungo and Sorghum vulgare (No. RESEARCH)

Grusak MA. 1995. Whole-root iron (III)-reductase activity throughout the life cycle of iron-grown *Pisum sativum* L. (Fabaceae): relevance to the iron nutrition of developing seeds. *Planta.* **197(1):** 111-117.

Habib, A. F. and Joshi, M. S. 1982. Combating iron chlorosis in black soils of Malaprabha and Ghataprabha Project Area. *Current Sci.*, **11:** 51-54.

Hartzook, A. 1975. Lime-induced iron chlorosis in groundnut: treatment and prevention. *FAO Plant Protection Bulletin*

Hartzook, A. 1982. The problem op iron deficiency in peanuts (arachis hypogaea L.) on basic and calcareous soils in Israel. *J. plant nutrition*, **5(4-7):** 923-926.

Hüve, K., Remus, R., Lüttschwager, D., and Merbach, W. 2003. Transport of foliar applied iron (59Fe) in Vicia faba. *J. plant nutrition*, **26(10-11):** 2231-2242.

Imtiaz, M., Rashid, A., Khan, P., Memon, M. Y. and Aslam, M. 2010. The role of micronutrients in crop production and human health. *Pak. J. Bot.*, **42(4):** 2565-2578.

Irmak, Seyyid, Ayse Nuran Çıl, H. Yücel, and Zulkuf Kaya. 2012. "The effects of iron application to soil and foliarly on agronomic properties and yield of peanut (Arachis hypogaea)." *J Food, Agric and Environ.,* **10(3/4):** 417-422

Katyal, J. C. and Sharma, B. D. 1980. A new technique of plant analysis to resolve iron chlorosis. *Plant and Soil*, **55(1):** 105-119

Kim, S. A. and Guerinot, M. L. 2007. Mining iron: iron uptake and transport in plants. *FEBS letters*. **581(12):** 2273-2280

Krishnasamy, R., Jegadeeswari, D., Surendran U and Sudhalakshmi, C. 2005. Screening of sorghum (*Sorghum bicolor*) genotypes for their iron efficiency. *World J. Agric. Sci.,* **1:** 98-100.

Kulkarni, V. N., Gowda, M. V. C., Nadaf, H. L and Manjunathaiah. 1995. An evaluation of screening method for iron absorption efficiency in groundnut. *Karnataka J. of Agrl.Science*. **8(2):** 214-219.

Land FAO and Plant Nutrition Management. 2000. Prosoil - problem soil database. FAO, Rome, Italy. http://www.fao.org/ag/AGL/agll/prosoil/default.html.

Li, G and Yanxi, S. 2007. Genetic differences in resistance to iron deficiency chlorosis in peanut. *Journal of Plant Nutri.*, **30(1):** 37-52.

Li, X., Yang, Y., Gao, B. and Zhang, M. 2015. Stimulation of peanut seedling development and growth by zero-valent iron nanoparticles at low concentrations. *PLoS One*, **10(4):** 1-12.

Loop, E. A. and Finck, A. 1984. Total iron as a useful index of the Fe-status of crops. *J. Plant Nutrition.* ***7*****(1-5):** 69-79.

Lucena, J. J. 2000. Effects of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis. A review. *J. Plant Nutr.* **23:** 1591–1606.

M'sehli, W., Houmani, H., Donnini, S., Zocchi, G., Abdelly, C. and Gharsalli, M. 2014. Iron deficiency tolerance at leaf level in Medicago ciliaris plants. *American J. Plant Sci.,* **5:** 2541-2553.

Machold, O and Stephan, W. W. 1964. The function of iron in porphyrin and chlorophyll biosynthesis. *Phytochemistry*. **8:** 2189-2192.

Mahurkar, D.G., Phadnawis, B. N., Deshmukh, D. P., Debre, W.M and Takzure, S.C. 1992. Effect of chlorosis on enzyme activities in leaves of groundnut cultivars. *Journal of Soils and Crops*. **2(1):** 5-7.

Marschner, H ., Römheld, V., Horst, W. J. and Martin, P. 1986. Root-induced changes in the rhizosphere: Importance for the mineral nutrition of plants. *J. Plant Sci. Soil Sci.,* **149(4):** 441-456

Martins, J. G., Ferreira, C. M. H., Dey, S. S., Barros, M. T., and Soares, H. M. V. M. 2017. N,N -Dihydroxy-N,N -diisopropylhexanediamide, a siderophore analogue, as a possible iron chelating agent for hydroponic conditions: metal equilibrium studies. *J. Iran. Chem. Soc.* **14**: 1079-1088.

Mengel, K. 1994. Iron availability in plant tissues-iron chlorosis on calcareous soils. *Plant Soil.* **165**: 275–283.

Morgan JB, Connolly EL.2013.Plant-soil interactions: nutrient uptake. Nature Education Knowl. 4(8):2

Nagarathnamma, R. 2006. *Evaluation of groundnut genotypes for lime induced chlorosis tolerance.* UAS, Dharwad.

Nicholas, J. C., Harper, J. E. and Hageman, R. H. 1976. Nitrate reductase activity in soybeans (*Glycine max* [L.] *Merr.*): I. effects of light and temperature. *Plant Physiol.* **58(6):** 731-735.

Novoselov VS.1960. A closed volumeter for plant root systems. *Fiziologiya Rastenii*. **7**: 243-244.

Ohwaki, Y and Sugahara, K. 1993. Genotypical differences in responses to iron deficiency between sensitive and resistant cultivars of chickpea (*Cicer arietinum*). *Plant and Soil*. **155:** 473-476.

Panchaksharaiah, S. 1982. *Investigations on the effect of nitrogen, iron, gypsum, sulphur and calcium on growth and yield of groundnut (Arachis hypogaea L.) and iron efficiency of genotypes on black soil under irrigation.* Ph. D. Thesis, Uni. Agric. Sci. Bangalore.

Peng, S., García, F. V., Laza, R. C., and Cassman, K. G. 1993. Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. *Agron. J.*, ***85*****(5)**: 987-990

Perur, N. G. 1962. Measurement of peroxidase activity in plant leaf tissue. *Curr.t Sci.*, **31(1)**: 17-18

Puangbut, D., Jogloy, S., Vorasoot, N., Akkasaeng, C., Kesmalac, T., and Patanothai, A. 2009. Variability in yield responses of peanut (Arachis hypogaea L.) genotypes under early season drought. *Asian J. Plant Sci.,* **8(4):** 254-264

Rashid, A., Rafique, E. and Ali, N. 1997. Micronutrient deficiencies in rainfed calcareous soils of Pakistan. II. Boron nutrition of the peanut plant. *Commun. soil sci.plant analysis.*, **28(1-2)**: 149-159

Reddy, K.B., Asalatha, M and Venkaiah, K. 1993. Differential Response of groundnut genotypes to iron deficiency stress. *J. Plant Nutr.*, **16(3):**523-531.

Ren, L., Zuo, Y.M., Jiang, Rong-feng., Zhang, F.S. 2005. Mechanisms of bicarbonate induced iron-deficiency chlorosis of peanut on calcareous soils. *Acta Ecologica Sin*. **25**: 795-801.

Rukam singh., Deepak Issar., PV Zala., and Nautiyal P.C., 2007. Variation in sensitivity to salinity in groundnut cultivars during seed germination and early seedling growth.*ejournal.icrisat.org*.**5**:1-7.

Sanjana, K. 2004. Role of manganese and boron on physiology and biochemistry of rust incidence in soybean *(Glycine max (L.) Merrill).* University of Agricultural Sciences GKVK, Bangalore.

Shenker, M., and Chen, Y. 2005. Increasing iron availability to crops: Fertilizers, organo-fertilizers, and biological approaches. *Soil Sci. Plant Nutr.,* **51:** 1–17.

Singh, A.L. 2001. Yield losses in groundnut due to micronutrient deficiencies in calcareous soils of India. In *Plant Nutrition pp*. 838-839. Springer, Dordrecht

Singh, A. L., and Dayal, D. 1992. Foliar application of iron for recovering groundnut plants from lime-induced iron deficiency chlorosis and accompanying losses in yields. *J. of Plant Nutri.,* **15(9):** 1421-1433

Singh, A. L., Chaudhari, V., and Koradia, V. G. 1993. Spray schedule of multimicronutrients to overcome chlorosis in groundnut. *Indian J. Plant Physiol.*, **36:** 35-35

Singh, A. L., Chaudhari, V., Koradia, V. G., and Zala, P. V. 1995. Effect of excess irrigation and iron and sulphur fertilizers on the chlorosis, dry matter production, yield and nutrient uptake by groundnut in calcareous soil. *Agrochimic*. **39:** 184-198.

Singh, A. L., Joshi, Y. C., Chaudhari, V., and Zala, P. V. 1990. Effect of different sources of iron and sulphur on leaf chlorosis, nutrient uptake and yield of groundnut. *Fertilizer Res.*, **24(2):** 85-96

Song, Y., Dong, Y., Kong, J., Tian, X., Bai, X., and Xu, L. 2017. Effects of root addition and foliar application of nitric oxide and salicylic acid in alleviating iron deficiency induced chlorosis of peanut seedlings. *J. Plant Nutr.*, **40(1):** 63-81

Zeng, L., Shannon, M. C., and Grieve, C. M. 2002. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. *Euphytic*. **127(2):** 235-245

Zheng, S. J. 2010. Iron homeostasis and iron acquisition in plants: maintenance, functions and consequences. *Ann. Bot.*, **105(5)**: 799-800

Zuo, Y., Ren, L., Zhang, F., and Jiang, R. F. 2007. Bicarbonate concentration as affected by soil water content controls iron nutrition of peanut plants in a calcareous soil. *Plant Physiol. Biochem.*, **45(5):** 357-364