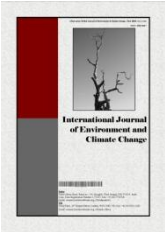

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In vitro Screening of Iron Efficient Groundnut Cultivars for Calcareous Soil

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Authors' contributions

This work was carried out in collaboration among all authors. Author VSRKK performed the field experiment, laboratory analysis, statistical analysis and wrote the first draft of the manuscript. Authors SM, DJ and SK designed the study, managed the analyses of the study, literature searches and manuscript suggestions. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In the present study twenty groundnut genotypes are evaluated for their resistance to IDC and to identify feasible indicators for screening iron (Fe) efficient groundnut genotypes in calcareous soils based on the morphophysiological parameters at 45 days after sowing (DAS).
Study Design: The experiment was replicated three times with two iron treatments (+Fe and – Fe) in a randomized block design
Place and Duration of Study: Field screening of genotypes was carried during *Kharif* 2019 at Thondamuthur block, Coimbatore district (10°59'31.9" N 76°47'15.4 E), Tamil Nadu, India.
Methodology: The randomized field experiment was comprised of two major factors, i) Fe status (with Fe, without Fe), and ii) genotypes (twenty) with differential IDC response. Seven morpho-physiological parameters associated with IDC resistance were evaluated in groundnut genotypes.
Results: Under Fe deficit conditions, IDC efficient genotypes recorded significantly higher shoot dry weight, root dry weight, root volume, SPAD values, active Fe, catalase activity, peroxidase

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activity, and higher yield compared to susceptible ones because of better Fe utilization efficiency. The various morpho-physiological parameters studied showed a significant correlation with pod yield. The four genotypes *viz*., TAG 24, CO 7, VRI 8, and VRI-16086 were efficient under both sufficient and deficit conditions under calcareousness. The stepwise multiple regression shows the peroxidase (POD) accounts for 71 % under +Fe condition and SPAD accounted for 66 percent in -Fe in predicting the pod yield. Hence, POD and SPAD can be used as indicators for selecting Fe efficient groundnut cultivars for calcareous soil.

Conclusion: The findings indicate that SPAD values are most optimal for the initial large-scale screening of groundnut genotypes for tolerance to IDC, whereas peroxidase may be used to validate established genotypes.

Keywords: Iron deficiency chlorosis (IDC); calcareous soils; iron efficient; groundnut; morpho physiological parameters.

1. INTRODUCTION

Iron (Fe) is a vital micronutrient for the survival and proliferation of all plants [1]. It is the very first element to be identified as essential for nitrogen fixation, photosynthesis, respiration, chlorophyll formation, DNA synthesis, hormone production and is also a component of various redox and iron-sulfur enzymes [2,3]. Insufficient Fe in leaves causes low chlorophyll levels and resulting in the yellowing of younger leaves [4,3]. As a result, many crop yields are adversely affected and hampered by low bioavailability in soils [5].

Calcium carbonate ($CaCO_3$), dolomite [$CaMg(CO_3)_2$], and magnesian calcite are the most common carbonate minerals that impart calcareousness in soil. They account for more than 90 percent of natural carbonates. Carbonates are common in many soils of the world, especially arid, semi-arid, and sub-humid soils [6]. Calcareous soils tend to be low in organic matter and available nitrogen. The high pH level results in unavailability of micronutrient availability e.g. zinc and iron (lime induced chlorosis). Minimal concentrations of inorganic forms in a pH range of 7.4-8.5 are common to calcareous soils which cover 30 percent of the earth's land surface, especially in arid and semiarid regions [7,8]. The combined effect of high pH, high lime content, and a high proportion of active lime is instrumental in inducing lime induced Fe deficiency in calcareous soil by suppressing ion uptake or translocation in plants [9]. Reported symptoms of insufficient nutrition in calcareous soils are chlorosis and stunted growth. This is attributed to high pH and reduced nutrient availability. Iron availability to plants from soil is most often a matter of availability rather than quantity. Marschner, H. 1995 found that improved nutritional management is required to grow crops successfully on calcareous soils. Fertilizer management on calcareous soils differs from that on non-calcareous soils because of the effect of soil pH on soil nutrient availability and chemical reactions that affect the loss or fixation of some nutrients. Therefore, new, cheaper, and more environmentally friendly approaches are needed to overcome this problem.

Groundnut (*Arachis hypogaea* L.) is an essential oilseed crop with a high nutritional value [10]. It is grown worldwide at 28.51 million ha with a production of 45.95 million tonnes [11]. 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). Though the average yield of groundnut in the world is around 1300 kg ha^{-1}, about 70% of the world groundnut production occurs in the semi-arid tropics with an average yield of around 800 kg ha^{-1} where soils are mostly calcareous and alkaline and occurrence of iron-chlorosis, is very common [12]. The maximum groundnut production is brought together in five states viz., Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, and Maharashtra. These five states represented around 86 percent of the absolute region under groundnut. The common problem observed with the cultivation of groundnut in calcareous and alkaline soils is iron deficiency which often causes substantial yield loss. The management of lime induced Fe deficiency in groundnut through chemical fertilizers may cost the farmers and not economically viable. An economically viable and sustainable approach in calcareous soil is the cultivation of iron-efficient cultivars by exploiting the genetic variability observed with regards to resistance to Iron Deficit Chlorosis (IDC) [13].

Plants follow two strategies for the acquisition and uptake of iron. Dicots and monocots, excluding grasses, are part of Strategy I Plants, where plant roots use tools for acidification and enzymatic reduction of Fe on the outer surface of roots [14]. Strategy II Plants (Graminaceous species) obtain Fe using muginic acid (MA) family phytosiderophores. Methionine is a precursor to MAs synthesis [15]. MAs are naturally synthesized in grassy plants. Groundnut belongs to Strategy I plants whose physiological reactions to Fe deficiency stress include the release of hydrogen ions and root-reducing agents and an increased reduction of Fe (III) in root-plasma [16,17]. Past studies have shown that the capacity for iron compounds to fix iron deficiencies in plants grown in alkaline and calcareous soils depends on two key factors [18]: I) the ability of iron compounds to retain soluble iron in soil solution; and (ii) the ability of plant roots to assimilate iron from the iron compounds present in soil solution.

Management reforms approach like soil amendment and foliar applications are often employed but these are short-term and uneconomical. Multidimensional solutions to problems such as nutrient deficiency stress are required instead of sticking to the conventionally available high-input approach [19]. In this regard, the development/identification of crop species and varieties that are adaptable to nutrient-deficient soils is a promising method for maintaining crop yields in resource-poor environments [20]. Cultivation of an iron-efficient plant on iron-deficient soils or on soil with slightly sufficient iron for plants reflects the technique of " tailoring the plant to fit the soil" in contrast to the older strategy of "tailoring the soil to fit the plant" [21]. Such techniques are used to improve tolerant genotypes adaptable to iron-deficient situations and/or increased iron-use efficiencies.

The development of micronutrient-efficient genotypes can be a succession method to solve micronutrient deficiencies in the soil and to improve human health [22]. IDC tolerant cultivars yields were considerably higher than those of susceptible cultivars [23,24]. The purpose of the present investigation is to study the feasibility of using morphophysiological parameters at 45 days after sowing (DAS) for screening and selection of iron efficient groundnut genotypes for calcareous soil. At 35 to 45 days after emergence (DAE) represents the mean pre-flowering stage of bambara groundnut, a growth stage sensitive to abiotic stress with strong subsequent effects on yield and yield parameters [25,26]. Therefore, early selection (i.e., 35 DAE) for greater root length density at depth can be expected to help enhance the genetic gains and yield improvement in bambara groundnut breeding efforts [27].

2. MATERIALS AND METHODS

Twenty groundnut genotypes (Table 1) which includes 10 groundnut genotypes from Regional Research Station, Vridhachalam, 4 genotypes from Oilseed Research Station, Tindivanam, and 6 genotypes from Department of Oilseeds, Coimbatore were evaluated for their reaction to calcium-induced iron chlorosis. Field screening of these genotypes was carried during Kharif 2019 at Thondamuthur block of Coimbatore district (10°59'31.9" N 76°47'15.4 E), Tamil Nadu, India. The experiment was replicated three times with two iron treatments (+Fe and –Fe) in a randomized block design. Two sets of treatments were maintained for sampling and observations at 45 DAS and at harvest stages. Iron as ferrous sulfate @ 50 kg ha^{-1} as basal. The experimental soil was low in organic carbon content (2.6 g kg^{-1}), low in available nitrogen (249 kg ha^{-1}), medium in phosphorus (12.5 kg ha^{-1}) and medium in available potassium (276 kg ha^{-1}) and also deficient concerning available iron (3.5 mg kg^{-1}). The groundnut genotypes were raised in plots of 2.5 x 2.5 m by adopting a spacing of 30 cm x 10 cm. A recommended package of practices was adopted in raising the crop.

To study the feasibility of using the morphophysiological parameters at 45 days after sowing (DAS) for screening iron efficient groundnut cultivars for calcareous soil, the observations/ analysis were carried out. Since, various reports showed that the flowering stage, 30 - 50 days after emergence was the most sensitive period to Fe deficiency within the growing season [28,29], 45 DAS was considered for recording the morpho-physiological parameters.

Table 1. List of groundnut genotypes selected to find iron efficiency in calcareous soil

TAG 24(G1)	CO 7(G2)	VRI- 13113(G3)	VRI- 16086(G4)	VRI- 13149(G5)
JL 24(G6)	TMV 1(G7)	VRI - 8(G8)	TMV 2(G9)	ALR 2(G10)
TMV 13(G11)	AMABC 2017-8(G12)	VRI- 16075 (G13)	VRI-13154(G14)	VRI- 16084 (G15)
VRI-13110(G16)	VRI- 13153(G17)	TMV 7(G18)	AMABC 2017-2(G19)	VRI 5 (G20)

2.1 Morpho-physiological Parameters

All the following observations were recorded on the standard leaf (Third fully opened leaf from the top on the main stem) of five plants each in every treatment to estimate the 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 the water displacement method. The actual volume displacement measures the volume of water displaced when plant tissue is submerged in a vessel of water [30].

SPAD Chlorophyll Meter Reading (SCMR) was employed as a measure of leaf chlorophyll level since several studies have shown that SCMR readings correlate well with the chlorophyll levels [31,32]. The SCMR value, enzyme activities, and active iron content were recorded in the third fully matured fresh leaves from the top. SCMR values were recorded using Chlorophyll Meter (SPAD 502) designed by the Soil Plant Analytical Development (SPAD) section, Minolta, Japan [33].

Peroxidase activity (change in OD value at 430 nm g^{-1} $min^{-1)}$ was determined according to Perur [34] and Angelini et al. [35]. One gram of leaf was extracted using 0.1M phosphate buffer (pH 7.0) and a known volume of the extract was added to a cuvette containing 3 ml phosphate buffer and 3 ml pyrogallol was added and the increase in absorbance at 430 nm was recorded. The change in absorbance in minutes was used to calculate the enzyme activity.

Catalase activity was assayed from the rate of H_2O_2 decomposition extinction coefficient of 39.4 mmol as measured by the decrease in the absorbance at 240 nm, following the procedure of Aebi [36]. The reaction mixture contains 50 mmol potassium phosphate buffer (pH 7.0) and the appropriate volume of extract. The reaction was initiated by adding 10 mmol of H_2O_2. One unit of catalase is defined as the amount of enzyme that liberated half of the peroxide oxygen from 10 mmol H_2O_2 solutions in 100 sec at 25°C and expressed as µg H_2O_2 $g^{-1}min^{-1}$.

Active Fe (Fe^{2+}, ferrous) content was estimated as per the procedure of Katyal and Sharma [37]. Ophenanthroline solution required for estimation of active Fe was prepared by adding 15 g of o-phenanthroline to 850 ml of distilled water; to this continuously stirring solution, 1 N HCl was added drop-wise until the last traces of the salt were soluble and pH was around 5.5, and the final volume was made up to 1 liter. The standard leaves of plants collected were washed with tap water followed by 0.1 N HCl and then rinsed with double distilled water. Fresh leaves (2 g) were chopped with a stainless steel knife and immediately transferred to glass bottles and 20 ml of o-phenanthroline solution was added and stirred gently to embathe the plant sample with the extractant. The bottles were stopped and allowed to stand for about 16 h at room temperature. The contents were filtered through Whatman No. 1 filter paper and active Fe was directly estimated in the filtrate by measuring the transmittance at 510 nm in UV-Vis spectrophotometer and expressed as mg kg^{-1} on a fresh weight basis.

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 a triple acid solution (nitric: sulphuric: Perchloric 9:2:1 v/v) for nutrient extraction [38]. Iron concentration was determined by an atomic absorption spectrophotometer (VARIAN A220).

The crop was harvested at maturity and the pod yield was recorded. The genotypes were grouped into inefficient if the varietal mean yield is less than median - standard deviation and efficient if the mean yield is more than the median + standard deviation [39]. Diseases and insect pests were adequately controlled throughout the study. Plants were maintained weed-free by hand weeding.

2.2 Data Analysis

The collected data were subjected to analysis of variance. To study the relationship of morphophysiological parameters recorded at 45 DAS with pod yield and to identify the desirable traits for screening Fe efficient genotypes, the Pearson correlation and multiple regression were made using SPSS 16.0 package.

3. RESULTS AND DISCUSSION

3.1 Shoot and Root Dry Matter

The shoot dry weight in +Fe treated genotypes ranged from 3.01 g per plant for VRI 13154 to 6.73 g per plant for JL 24 (Fig. 1). In the -Fe treatment plant growth was comparatively lower and the inhibition has differed among genotypes. The lower Dry Matter Production (DMP) recorded in minus iron treatment might be due to lower chlorophyll content in the leaves which reflected on the photosynthetic efficiency, nutrient acquisition, and utilization efficiency of the crops. This is revealed in the present investigation by the lower SCMR values recorded in – Fe treatment and the highly positive correlation of SCMR with DMP (45 DAS) and pod yield. A strong positive correlation was observed between shoot dry weight (SDW) recorded at 45 DAS and pod yield as 0.832 ** (+Fe) and 0.795** (-Fe) (Table 4).

The genotype and treatment were the sources of variation for all the parameters including root dry weight (RDW). The interaction between the two factors also resulted in significant differences. The lack of Fe supplement and calcareousness in the soil resulted in a progressive decrease in root dry matter in all the genotypes and seemed to be less affected for efficient genotypes even under Fe starvation. The root dry weight values varied between 0.7 and 1.52 g per plant irrespective of the treatment and genotypes (Fig. 1). However, it is noteworthy that the -Fe treatment recorded lower RDW compared to the +Fe treatment and the reduction differed based on the efficiency of the genotype.

The Dry Matter Production (DMP) of a crop reflects its efficiency in utilizing the available resources and there is always a positive relationship between DMP and the yield, as it forms the basis for translocation. Dry matter recorded at 45 DAS showed significant variation among the genotypes. Jogloy et al. [40] reported a significant difference in shoot dry weight among groundnut cultivars.

3.2 Root Volume

Different trends between the genotypes were detected for different genotypes; in fact, the efficient genotypes showed a slight decrease in root volume as a consequence of its greater Fe efficiency. On the contrary, in inefficient, these root volume showed the highest reduction in the minus Fe treatment. The response of the tolerant genotypes to iron deficiency is often associated with an increased number of primary and secondary roots [41] leading to a more efficient exploration of the soil. The genotype TMV 2 recorded the highest value 12.2 CC concerning root volume at 45 DAS followed by CO 7 and TMV 1 (12.15 and 12.15 cc) (Fig. 1). The association between root volume and SDW (45 DAS) was highly related to an r-value of 0.965** (Table 3). Also, a significant positive correlation was noticed between root volumes (45 DAS) with pod yield as 0.634** and 0.638** for +Fe and -Fe treatments respectively (Table 4).

3.3 SPAD Chlorophyll Meter Reading

SPAD meter measures the difference in light absorption at 430 and 750 nm [42]. The reduction in SPAD values in the –Fe treatment was not uniform among the genotypes and it ranged from 0.63 % (CO 7) to 15.7 % (AMABC 2017-2). Irrespective of the genotypes, iron treatment resulted in higher SPAD values. SPAD values were significantly higher among efficient genotypes compared to inefficient genotypes, but significantly lesser among inefficient ones without Fe treatment. Comparison between genotypes shows a lesser reduction in SPAD values among efficient genotypes, while significant reduction among inefficient ones for SPAD. SPAD reading recorded by the genotypes was in the order of VRI -16086 (42.55) > VRI 8 (40.1) TAG 24 (40.03) > VRI -13113 (39.85) and CO 7 (39.80) under +Fe and CO 7 > TAG 24 > VRI 16086 > VRI 8 > TMV 1 under -Fe treatment (Fig. 1).

Significant variation in SCMR values among groundnut cultivars grown in calcareous soil was reported by (Gao and Shi, [29]). Pattanashetti et al. [43] also observed enormous genetic variation in SCMR reading of groundnut mini core collection across five growth stages. Similar results were also reported by Mann et al. [44] and Song et al. [45], where lower SPAD values were recorded in no Fe received plants (-Fe) as compared to the plants treated with iron. This might be due to the association of iron in the biosynthesis of the porphyrin ring of chlorophyll. The impairment of chlorophyll biosynthesis and chloroplast development might be the reason for the decreased SPAD value under – Fe plants. A positive correlation was observed between SPAD reading recorded at 45 DAS and pod yield both under + Fe and – Fe treatments (Table 4). Similar, to our findings, Puangbut et al. [46] proved that higher SPAD values, resulted in higher biomass, hence improved yield.

3.4 Peroxidase and Catalase Activity

The mean peroxidase and catalase activity were significantly higher under iron treatment compared to without iron treatment. The mean

POD activity was decreased by 15.25 % in the treatment without iron application. Machold and Stephan [47] reported a 50 % reduction in peroxidase activity due to Fe deficiency. Ishwar et al. [48,49] also observed a reduction in peroxidase activity among all groundnut genotypes in iron-deficient soil conditions compared to iron-sufficient soil conditions. The lowest activities of catalase and peroxidase observed in control were due to lesser defensive mechanisms operating in the plant [50]. However, a reduction in peroxidase activity was low for the genotypes VRI -16086 (5.79) > CO 7 (5.84) > VRI 5 (6.31) > TAG 24 (9.83) probably due to comparatively higher active-Fe maintained in leaves by these genotypes under Fe-stress conditions (Fig. 1).

Variation among the genotypes concerning catalase activity was observed both under – Fe and +Fe conditions. Similar results were obtained in the previous works of Sanjana and Koti [51] in soybean genotypes; groundnut genotypes [52,53], and *Medicago ciliaris* by M'Sehli et al. [54]. It turned out that the active involvement of these antioxidant enzymes was related, at least in part, to the tolerance to Fe-deficiency-induced oxidative stress [55].

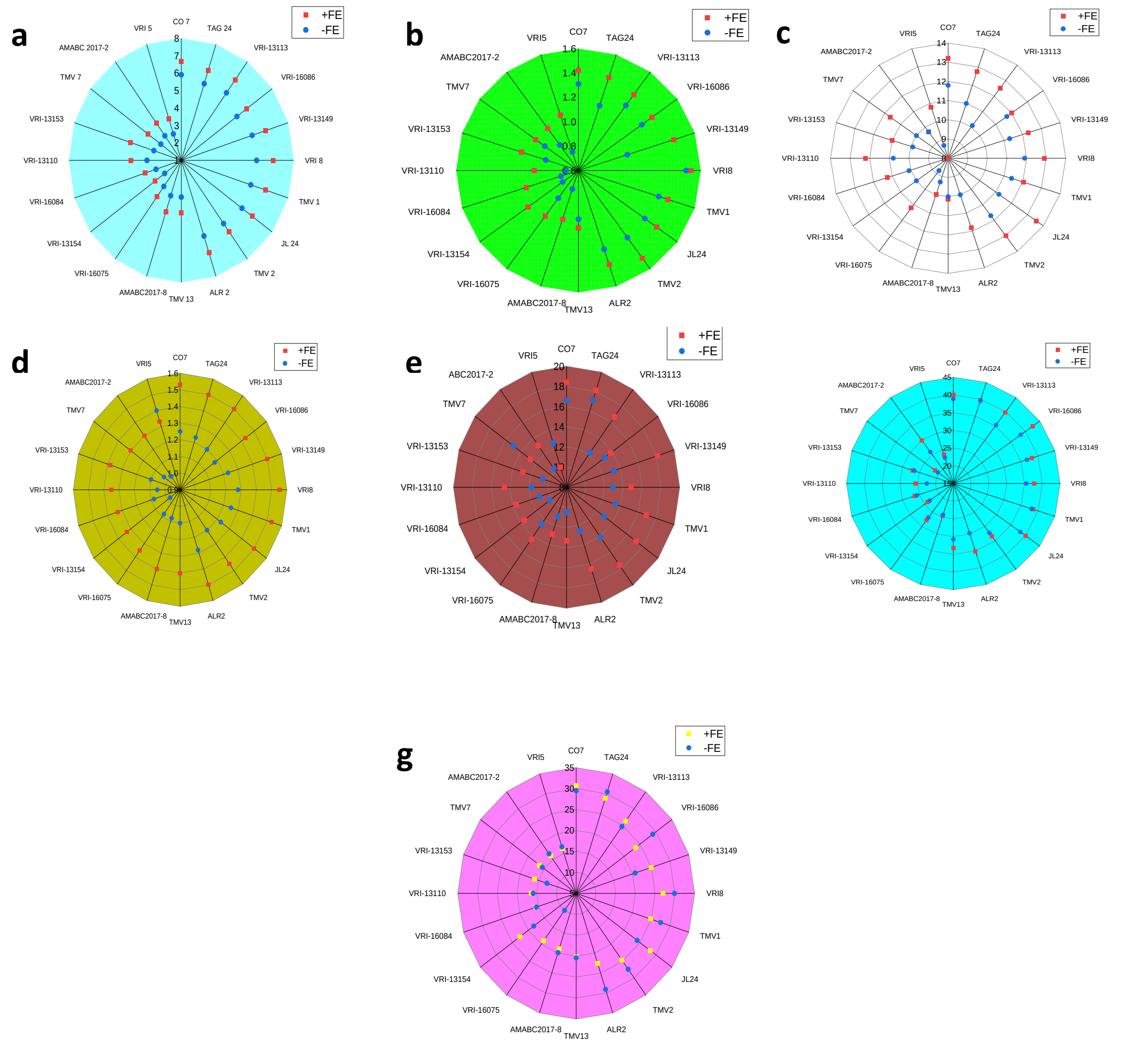

Fig. 1. Differential response of groundnut genotypes for morpho-physiological parameters under normal and deficit Fe conditions: (a) Shoot dry weight (SDW) (g per plant) (b) Root dry weight (RDW) (g per plant) (c) Root volume (RV) (cc) (d) Catalase activity (μM of H_2O_2 g^{-1} min^{-1}) (e) Peroxidase activity (OD min^{-1} mg^{-1} protein) (f) SPAD values (g) Active Fe content (mg kg-1 fresh weight)

3.5 Active Iron Content

Singh [56] has reported that active iron is taken as a criterion and observed lower active iron in chlorotic plants. An increase in active iron content with the application of iron was observed in all the genotypes and was in the order of VRI 8, TAG 24, JL 24, CO7, and TMV 1. The genotypes VRI 8 (33.15), TMV 2 (32.75), and TAG 24 (32.73) maintained higher active Fe content even under Fe stress (-Fe) (Fig. 1) showing the differences in Fe supply level among the groundnut cultivars grown on the same soil and exhibiting the efficiency of these genotypes to acquire Fe even under Fe deficient condition.

The difference in active iron content across the genotypes might be attributed to the variation in Fe absorption capacities through leaves and roots [57]. Generally the genotypes VRI 16084 (42.55), VRI 8 (40.1), and TAG 24 (40.05) which recorded higher SPAD reading contained much higher active Fe in leaves than with chlorotic ones (lower SPAD). This showed the differences in Fe acquisition across the groundnut cultivars grown on the same soil. A strong and positive correlation was observed between SPAD reading, peroxidase, catalase activity, and leaf active iron content. Hence, higher active iron content, SPAD value, catalase, and peroxidase activity are the probable factors responsible for higher iron absorption efficiency in efficient genotypes.

3.6 Pod Yield

There was considerable variation in pod yield among different peanut cultivars grown on calcareous soil. The pod yield ranged from 1309 to 2942 kg ha $^{-1}$ with Fe application and from 1178 to 2647 kg ha $^{-1}$ without Fe application (Fig. 2). These results indicate the genetic variability of the groundnut genotypes for Fe efficiency under calcareous conditions. Increased pod yield with the iron application conforms with the findings of (Guruprasad et al. [58]). The efficient groundnut genotypes (TAG 24, CO 7, VRI 13113, VRI 8, and VRI-16086) recorded 82.6 higher pod yield over inefficient genotypes under + Fe condition (Table 2). In the case of without Fe application, efficient genotypes recorded 70.1 percentage increased pod yield over inefficient genotypes.

Grouping of the genotypes for iron efficiency based on mean and standard deviation [39] showed that TAG 24, CO 7, VRI 13113, VRI 8, VRI-16086 as efficient genotypes (with Fe application) and TAG 24, CO 7, VRI 8, TMV 1, VRI-16086, ALR 2 as efficient genotypes (without Fe application). (Table 2). Under both the +Fe and –Fe applied conditions, the genotypes TAG 24, CO 7, VRI 8, and VRI-16086 were efficient in Fe acquisition from the soil. These results confirm that Fe deficiency is a grain yield-limiting factor in the studied soils. Therefore, to achieve greater crop yields, correction of Fe deficiency through appropriate approaches is necessary. The yield data of treatment lacking Fe showed that there was a significant loss of pod yield in the treatment than Fe added. This indicated that micronutrient deficiencies resulted in the loss of yield and this loss could be recovered if the relevant micronutrient was applied. Moreover, in calcareous soils, there is high uptake of Ca by plants that restricts the uptake of other micronutrients [59] and thus constrains the plant to suffer from micronutrient deficiencies.

3.7 Associations

Without Fe treatment, a highly significant (P < 0.001) positive correlation was observed between SDW and SPAD (0.973).SDW showed a highly significant (P < 0.01) positive correlation with all other parameters, while CAT and POD showed a significant (P < 0.01) lower degree positive correlation with parameters like RV, SPAD, and ACTFE (Table 3). Pod yield showed highly significant (P < 0.01) positive correlation to SDW and SPAD in +Fe and - Fe treatments (0.832, 0.795 and 0.832, 0.813 respectively), while active Fe and root volume showed a lower degree of positive correlation compared to other parameters viz., SDW, RDW, CAT, POD, and SPAD (Table 4) in both conditions. The stepwise multiple regression indicated the feasibility of using SDW and SPAD for the screening of iron efficient groundnut genotypes for calcareous soil as they accounted for 70 percent (+Fe) and 66 percent (-Fe) for predicting the pod yield (Table 5).

Studies show that selection based on drought tolerance indices SPAD, RWL, SLA, TDM, and HI will result in the identification of drought-tolerant genotypes [60]. Initial large-scale screening of groundnut genotypes for IDC resistance, SPAD values are most ideal while active Fe could be utilized for confirmation of identified lines [53]. SPAD value has been earlier indicated as a feasible screening indicator to select iron-resistant groundnut cultivars (Li et al. 2009). SPAD is an indirect indicator of chlorophyll content as significant correlations have been established across different stages in the present study as well as by other researchers [13]. The utility of peroxidase activity as an indicator to identify IDC-resistant groundnut genotypes [53]. Hence, SPAD and POD are suitable for large-scale screening of groundnut genotypes for IDC response.

Table 2. Grouping of genotypes for their iron efficiency in calcareous soil

	With Fe application	Without Fe application
Efficient	TAG 24, CO 7, VRI 13113, VRI 8, VRI-16086	TAG 24, CO 7, VRI 8, TMV 1, VRI-16086, ALR 2
Inefficient	TMV 13, AMABC - 2017- 8, VRI 5, VRI 13153	VRI 5, VRI 13153, TMV 13, AMABC - 2017-8,VRI -16075

Table 3. Associations between morpho-physiological parameters considering at 45 DAS in deficit-Fe condition

Trait[a]	RDW	RV	CAT	POD	SPAD	ACTFE
SDW	0.965**	0.934**	0.951**	0.850**	0.973**	0.795**
RDW		0.926**	0.904**	0.779**	0.954**	0.808**
RV			0.904**	0.725**	0.911**	0.824**
CAT				0.796**	0.899**	0.707**
POD					0.811**	0.619**
SPAD						0.795**

SDW- Shoot dry weight; RDW - Root dry weight; RV – Root volume; CAT - Catalase; POD – Peroxidase; SPAD - Soil Plant Analysis Development and ACTFE – Active iron

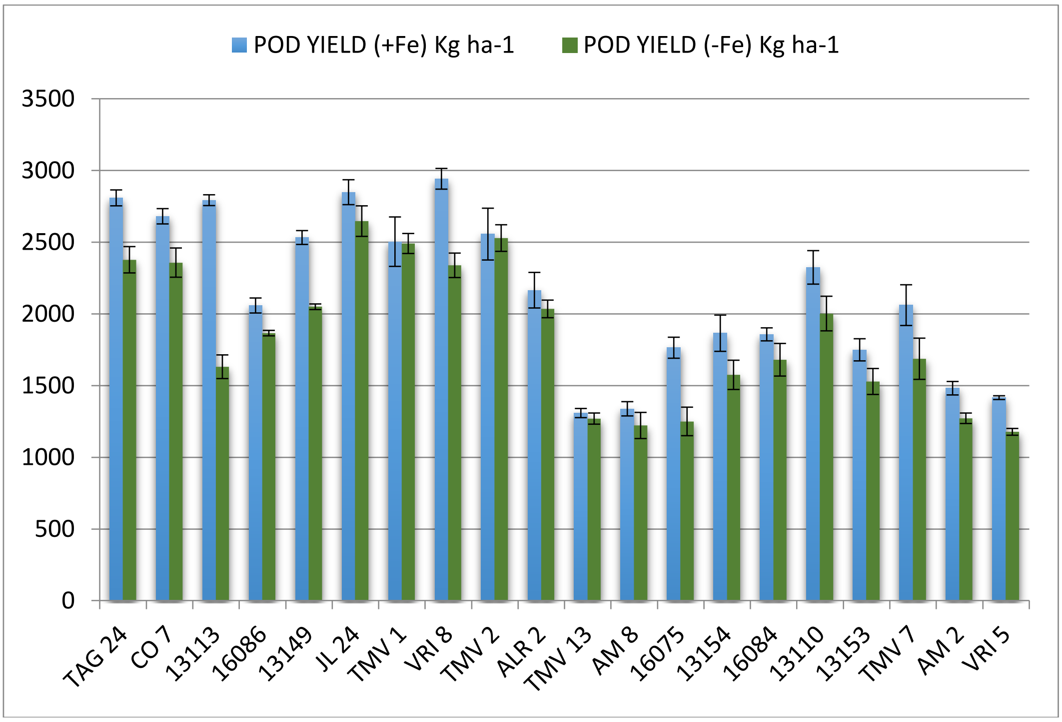

Fig. 2. Pod yield of groundnut genotypes in a calcareous soil under with and without by iron application

Table 4. Associations between mean of morpho-physiological parameters and pod yield (+Fe) and pod yield (-Fe)

Trait[a]	POD YIELD (+Fe)	POD YIELD (-Fe)
SDW	0.832**	0.795**
RDW	0.787**	0.772**
RV	0.634**	0.638**
CAT	0.761**	0.785**
POD	0.785**	0.774**
SPAD	0.832**	0.813**
ACTFE	0.548*	0.653**

SDW- Shoot dry weight; RDW - Root dry weight; RV – Root volume; CAT - Catalase; POD – Peroxidase; SPAD - Soil Plant Analysis Development and ACTFE – Active iron

Table 5. Multiple regression equation for predicting pod yield with morphophysiological parameters at 45 DAS

Treatment	Prediction equation (Y)	R^2	Adjusted R^2
With iron (+Fe)	203.89 (POD) - 967.45	0.716	0.700
Without iron (-Fe)	85.97 (SPAD) - 379.48	0.660	0.641

4. CONCLUSION

Twenty groundnut genotypes were screened for their iron efficiency in calcareous soil which exhibited a wide variation in the morphophysiological parameters viz., root dry weight, shoot dry weight, root volume, catalase activity, peroxidase activity, SPAD value, and active iron content at 45 DAS both under iron application and Fe stress condition. Grouping of the genotypes for iron efficiency in calcareous soil showed that TAG 24, CO 7, VRI 13113, VRI 8, VRI-16086 are efficient genotypes with Fe application. The genotypes TAG 24, CO 7, VRI 8, TMV 1, VRI-16086, and ALR 2 are efficient under minus Fe application.

A significant and positive correlation of the studied morphological and physiological parameters at 45 DAS with pod yield showed the dependability of the parameters in selecting efficient groundnut genotypes for calcareous soil. The stepwise multiple regression indicated that POD and SPAD accounted for 71 percent (+Fe) and 66 percent (-Fe) correlation in predicting the pod yield respectively. Hence, POD and SPAD can be used as indicators for selecting Fe efficient groundnut cultivars for calcareous soil.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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