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Exploiting genetic variation for lime-induced iron-deficiency chlorosis in groundnut (*Arachis hypogaea*)

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ABSTRACT

The screening of 114 groundnut (*Arachis hypogaea* L.) genotypes (61 advanced breeding lines and 53 released cultivars) was undertaken in the screening plots to identify the iron-efficient genotypes tolerant to lime-induced iron-chlorosis. The crop was grown and the intensity of chlorosis of top five leaves was rated for visual chlorotic rating (VCR) score on a 1-5 scale and the percentage of plants showing deficiency symptoms at 10, 20, 30 and 65 days after emergence during the cropping season. The tolerant genotypes had shown significantly lower VCR, higher Soil Plant Analysis Development (SPAD) chlorophyll meter reading and chlorophyll values, active Fe, and high yield compared to the sensitive ones. The correlation of visual chlorosis range with SPAD, chlorophyll and available iron content along with the mineral nutrients like, Fe, Zn, Mn, K, and P clearly identified the groundnut genotypes as tolerant, moderately tolerant and sensitive to iron chlorosis. Based on various parameters, out of 114 genotypes, 22 were grouped as tolerant, 48 moderately tolerant, 32 normal and 12 sensitive. Maximum variability was obtained in groundnut genotypes for Fe and fodder weight.

Key words: Chlorophyll, Groundnut, Iron chlorosis, Mineral nutrients, SPAD

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop with high nutritious value (Bishi *et al.* 2015) a worldwide production of 45 million tonnes coming from 25 million ha area in more than 100 countries mostly grown under vagaries of weather condition on poor fertility soil (FAOSTAT 2015). In India, it is grown mostly in the low rainfall areas of Gujarat, Rajasthan, Maharashtra, Karnatka in calcareous and alkaline soils where iron deficiency is a widespread problem of groundnut called as lime induced iron-deficiency chlorosis (LIIC) causing considerable yield reductions (Singh 2004, Singh *et al.* 2003) and is one of the most susceptible crops to LIIC (Gholizadeh *et al.* 2007). This chlorosis is caused by higher concentration of carbonates and bicarbonates of Ca in presence of excess moisture. Since iron is largely assimilated by plants in the form of ferrous ions, the most calcerous soils which are alkaline in reaction having higher concentration of carbonate and bicarbonate would not favour its availability to plants thereby causing iron chlorosis.In calcareous and alkaline soils, Fe is mainly found as poorly soluble oxides and/or hydroxides, and therefore, the amount of Fe available to plants is very low and despite the ubiquitous presence of Fe in the earth's crust, the low solubility of Fe compounds prevents plant Fe uptake and induces development of Fe deficiency symptoms. As a result, majority of the groundnut fields show chlorosis and remain chlorotic throughout the cropping season.

The physiological and morphological modifications in the roots facilitate mobilization of Fe compounds in the root environment (Singh and Mann 2012). Alkaline pH accentuate chlorosis problems and soil applications of iron sulphate are often ineffective (Singh 2004, Singh *et al.* 1995) however iron chelates preserve iron from its precipitation in soil with increasing pH (Koksal *et al.* 1998) and prevent LIIC (Singh and Dayal 1992). Similarly, the foliar application of fertilizers has a small influence on plant growth and a low mobility in the plant tissues, thus demanding frequent applications during the plant cycle. As the crop genotypes differ greatly in their response to iron availability and have been designated as iron-efficient (tolerant of iron chlorosis) and iron-inefficient (sensitive to iron chlorosis) cultivars (Brown and Jolley 1989, Singh and Chaudhari 1993), the best way to reduce iron chlorosis problems in groundnut could be the use of tolerant cultivars with a very high capability of absorbing iron from the soil. Iron efficient genotypes have already been identified for old groundnut cultivars and several genotypes, but the information is lacking for the latest cultivars and advanced breeding lines. Therefore, the present investigation was

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planned to screen recent cultivars advanced breeding lines for their tolerance to LIIC.

MATERIALS AND METHODS

One hundred fourteen genotypes (61 advanced breeding lines and 53 groundnut cultivars) (Table 1) were screened for iron chlorosis during *kharif* 2012 at ICAR-Directorate of Groundnut Research, Junagadh on calcareous soil having low available iron with pH 8.71 (alkaline).The field was prepared and 8-10 cm deep furrows were opened 45 cm apart. Diammonium phosphate (DAP) at 110 kg/ha was applied uniformly in the furrows and mixed with soil before sowing. Cultivations, irrigation and plant protection procedures were carried out as and when required. All the groundnut genotypes were evaluated in Augmented Block Design in which 60 plants of each genotype were grown in adjacent rows each 5 m long. The row to row and seed to seed spacing were 45 cm and 10 cm respectively. ICGV 86031and NRCG 7472 were taken as resistant and susceptible checks respectively.

The leaves of different genotypes were observed at different stages during the cropping season to determine their visual chlorotic rating. The groundnut crop showed intervenial chlorosis, a typical symptom of iron deficiency, in the young and emerging leaves. The intensity of chlorosis of top five leaves was scored by determining the visual chlorotic rating (VCR) score on a 1-5 scale (i.e. 1=normal green leaves with no chlorosis, 2= green leaves but with slight chlorosis on some leaves, 3= moderate chlorosis on several leaves, 4= moderate chlorosis on most of the leaves, 5= severe chlorosis on all leaves) and the percentage of plants showing deficiency symptoms (Singh and Chaudhari 1993). The VCR and the percentage of chlorotic leaves were recorded at three different plant growth stages (40, 60, 80 days after sowing) during *kharif* season and based on these observations, the groundnut genotypes were classified as (i) tolerant (genotypes showing dark green leaves having VCR value 1 in >95% and <2 in <5% plants): (ii) moderately tolerant (green plants with VCR value 1 in >90% and 2 in <10% plants); (iii) Normal (plants with green with chlorotic leaves, VCR values 2-3 in <20%, 1 in >80% plants) and (iv) Susceptible (plants with yellow to whitish yellow leaves, VCR value 3-5 in >20% and 2 in <80% plants). Average data were taken for chlorosis (%) observed at different days after emergence during the growing season. The individual screening of groundnut lines and final categorization was done at the end of cropping season.

Active Fe content in leaves was measured using o-phenanthroline (pH 3.0) following the method given by Katyal and Sharma (1980).Two gram fresh chopped green and chlorotic leaves were incubated in 20 ml O-phenanthroline solution for atleast 16 hr at room temperature and the OD of the supernatant was measured at 510 nm.

SPAD 502 Plus meter was used to measure the SPAD reading of individual plants taking the first fully opened leaf of the main axis from 10 randomly selected plants of each genotype.

Table 1 List of groundnut genotypes and advanced breeding lines

Genotype	Genotype	Genotype
PBS 12009	PBS 18055	JGN 3
PBS 12018	PBS 18057	TG 37 A
PBS 12029	PBS 18062	JGN 23
PBS 12032	PBS 18064	GG 13
PBS 12038	PBS 19018	LGN 1
PBS 12066	PBS 19020	MH 2
PBS 12067	PBS 19021	LGN 2
PBS 12074	PBS 19022	ICGV 00350
PBS 12092	PBS 19023	Kadri 9
PBS 12116	PBS 19024	GG 16
PBS 12163	PBS 19027	Kadri 6
PBS 12167	PBS 14135	Chintamani
PBS 12168	CS 280	DG 4-3
PBS 12169	CS 287	Kokan Tapora
PBS 12171	CS 291	Kadri Harithandra
PBS 12172	CS 296	TMV 13
PBS 12175	CS 298	SG 99
PBS 12180	CS 332	GPBD-4
PBS 12181	CS 349	JL 286
PBS 12183	CS 369	ICGV 00351
PBS 12185	CS 401	TMV 1
PBS 12186	CS 240	GG 5
PBS 13003	Girnar-2	VJ 9521
PBS 13020	Girnar-3	AK 159
PBS 13021	G-G-8	NRCG 162
PBS 13022	Mallika	CSMG 9510
PBS 14042	ICGV 00348	VRI 3
PBS 14083	TMV 4	GG 20
PBS 16035	T 28	GG 7
PBS 16040	RG 425	CSMG 84-1
PBS 16044	VG 9816	M 37
PBS 18004	TMV 3	ICGV 91114
PBS 18006	RG 510	DRG 17
PBS 18029	R 2001-3	JCG 88
PBS 18033	TPG 41	NRCG 162
PBS 18035	HNG 69	CSMG 84-1
PBS 18037	GPBD 5	
PBS 18038	JL 501	ICGV 86031
PBS 18045	VRI 16	NRCG 7472

Chlorophyll estimation was done using Acetone method of Arnon (1949) for which 0.2 g chopped leaves were incubated overnight in 80 % acetone. Chlorophyll (*a* and *b*) was measured using a spectrophotometer and was estimated by the equations:

Chlorophyll (*a*) = 13.7 ×A(663) – 576 × A(645)mg/g;

Table 2 Descriptive analysis of all variables with mean values

Variable	Mean	Std Dev	CV
VCR	1.96±0.04	0.47	23.72
SPAD	30.16±0.46	4.94	16.38
Chl	5.08±0.16	1.71	33.67
Chl. conc	10.21±0.33	3.55	34.81
Active Fe	78.47±1.49	16.08	20.49
Fe	404.56±17.90	192.75	47.64
Mn	77.30±1.71	18.44	23.85
Zn	38.28±0.99	10.71	27.97
Cd	45.53±2.30	24.76	54.38
K	115.74±3.07	33.07	28.57
P	0.254±0.05	0.05	18.88
Pod wt	30.16±0.79	8.46	28.05
Fodder wt	50.13±1.76	18.93	37.75
Seed yield	66.48±0.73	7.90	11.89
100-seed wt	33.61±0.74	7.95	23.66

Chlorophyll (*b*) = 25.8 ×A(645) – 7.60 × A(663) mg/g.

Tri-acid digestion of 1.0 g oven dried tissue was done for macro and micronutrients analysis. Fe, Zn and Mn were analyzed directly on Atomic Absorption Spectrometer (AAS) at absorption mode and K at emission mode. Phosphorus was analyzed using sulfate molybdate following the method given by Fiske and Subbarao (1925). The 5 ml of plant digest aliquot was diluted with 40 ml distilled water to which 2 ml of sulfate molybdate solution was added, swirled and cooled in ice water to a temperature below 20ºC. Then 2 ml of amino-naphthol- 4 sulfuric acid was added and volume was made to 50 ml with distilled water with continuous shaking. After 10 min, the blue colour was measured at 660 nm.

Fodder weight, seed yield and 100 seed weight were measured and expressed as g/plant.

Data were analysed using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA).Correlation analysis was performed to determine the relationship between the traits using the Pearson coefficient procedure. Histogram was prepared using the software STAR.

RESULTS AND DISCUSSION

Visual Chlorotic Rate (VCR)

The mean value for VCR was 1.96±0.04 with range value of 2.40. Thirty five groundnut genotypes had VCR value equal to or less than the tolerant check while 80 lines had VCR value more than the tolerant check. PBS 12172, PBS 12180, PBS 12183, PBS 13021, PBS 19020 and PBS 19022 had VCR value equal to the tolerant check. The interveinal chlorosis showing Fe deficiency appeared in the young leaves of susceptible varieties, 20 days after emergence. The plants partly recovered from chlorosis when the young leaf became older. But the appearance of chlorosis in the emerging young leaves continued till 10-15 days after emergence, depending on the duration of the variety. Based on the percentage of chlorotic plants and general appearance of Fe deficiency symptoms, the groundnut genotypes were categorized into tolerant, moderately tolerant, normal and susceptible (Table 4) to lime induced iron chlorosis. The VCR values were correlated with other physiological parameters like chlorophyll, SPAD, available Fe and P and pod weight for their categorization for iron deficiency induced iron chlorosis symptoms. A significant negative correlation was observed between VCR, SPAD and Chlorophyll (Table 3) which clearly indicates that with

Table 3 Pearson's Correlation among various parameters studied in groundnut

	SPAD	Chl	Chl conc	Active Fe	Fe	Mn	Zn	K	P	Pod wt	Fodder wt	Seed yield	100-seed wt
VCR	-0.590**	-0.560**	-0.544**	-0.610**	-0.374**	-0.013	-0.108	-0.151	-0.244**	-0.197*	-0.169	0.125	-0.139
SPAD		0.353**	0.340**	0.390**	0.307**	-0.065	0.099	0.149	0.194*	0.081	0.177	-0.019	0.173
Chl			0.991**	0.351**	0.332**	0.028	-0.007	0.269**	0.146	0.096	0.195*	-0.138	-0.038
Chl conc				0.357**	0.352**	0.046	-0.004	0.277**	0.150	0.068	0.165	-0.126	-0.056
Active Fe					0.448**	0.109	0.255**	0.055	0.111	-0.035	0.005	-0.105	0.107
Fe						0.177	0.349**	0.323**	0.314**	0.018	0.024	0.051	0.082
Mn							0.193*	0.323**	0.302**	0.018	-0.076	0.119	0.143
Zn								0.009	0.045	-0.141	-0.072	0.056	0.090
K									0.633**	0.119	0.232*	0.025	0.230*
P										0.110	0.042	0.117	0.139
Pod wt											0.474**	-0.004	0.002
Fodder wt												-0.167	0.166
Seed yield													0.314**

*Significant at (P=0.05); ** Significant at (P=0.01)

Table 4 Categorization of groundnut cultivars for their tolerance of iron-chlorosis

Tolerant	Moderately tolerant	Normal	Sensitive
PBS 12169, 12171, 12175, 12183, 13021, 16040, 16044, 19020, 19021, 19024, CS 349, CS 369, Girnar-2, ICGV 00348, RG 425, RG 510, TPG 41, HNG 69, SG 99, GPBD-4, VJ 9521, CSMG 9510	PBS 12032, 12163, 12167, 12168, , 12172, 12180 bold, PBS 13003, 13020, 13022, 14083, 16035, 18037, 18045, 18062, PBS 19018, PBS 19022, PBS 19023, PBS 19027,CS 280, CS 287, CS 291, CS 298, CS 401, CS 240, Girnar-3, MallikaT 28, TMV 3, JL 501,TG 37 A, GG 13, MH 2, LGN 2, Kadri 9, GG 16, Chintamani, DG 4-3, Kokan Tapora, Kadri, Harithandra, ICGV 00351, TMV 1, GG 5, GG 20,CSMG 84-1, M 37, DRG 17, JCG 88	PBS 12009, 12018, 12029, 12038, 12066, 12067, 12074, 12092, 12116, 12181, 12185, 12186, 18029, 18033, 18035, 18038, 18057, 14135, CS 296, G-G-8, TMV 4, VG 9816, R 2001-3, PBD 5, VRI 16,JGN 23, LGN 1, ICGV 0350, TMV-13, AK 159, VRI 3, GG-7	NRCG 162, NRCG 7472, ICGV 91114, JL 286, Kadri 6, JGN 3, CS 332, PBS 18064, PBS 18055, PBS18004, PBS18006, PBS 14042

yellowing of leaves, greenness of leaves decreases and hence the SPAD and chlorophyll content. Sánchez-Rodríguez *et al.* (2014) showed high levels of P aggravated Fe chlorosis in chickpea, groundnut, lupine and sorghum grown on artificial substrates prepared with mixtures of Fe oxide (ferrihydrite)-coated, calcium carbonate and quartz sand.

SPAD and chlorophyll content

The mean SPAD reading was 30.16±0.46 with a range value of 26.75.The maximum SPAD reading was in Kadiri 9, whereas minimum SPAD reading was observed in advanced breeding line, PBS 12092. Twenty four groundnut lines had SPAD between 15-25, 82 genotypes showed SPAD reading between 25-35 and only 17 lines had SPAD reading between 35-45. The genotype having the highest SPAD value of 43 has the total chlorophyll content (TC) 8.53 mg/g fresh wt, whereas the genotype PBS 12092 with lowest SPAD value of 16.25 has TC of 7.64 mg/g fresh wt. The maximum TC (9.17 mg/g fw) was noticed in PBS 13171. Mostly Fe-efficient genotypes have TC ranging from 5-10 mg/g fw while the Fe-inefficient genotypes have TC from as low as 1.7 mg/g fw. Correlation coefficients between SPAD reading and chlorophyll contents were highly significant which indicates their association with each other.

Zuo *et al.* (2007) have reported that the severity of iron deficiency chlorosis in young leaves of groundnut was closely related to the increased soil water content (SWC) and $CaCO_3$ supply. In addition, the chlorophyll concentration (the SPAD value) decreased with increasing soil water supply. In comparison with the control, high $CaCO_3$ level generally decreased chlorophyll concentration. Rengel and Römheld (2000) reported that wheat genotypes grown under Fe deficiency developed severe chlorosis with the duration of the deficiency and chlorophyll content decreasing drastically. Gholizadeh *et al.* (2007) found that the chlorophyll content of the groundnut leaves was influenced by the Fe concentration in culture media. Comparison of the contents of chlorophyll *a*, *b*, chl and total in the seventh leaves of all Fe treatments showed at the same time the lowest amount of chlorophyll in Fe-free medium grown plants. Similar results have been observed by Boodi *et al.* (2016) in groundnut under deficit-Fe conditions. They have found that iron deficiency chlorosis (IDC) resistant genotypes recorded significantly lower visual chlorosis rating (VCR), higher SPAD values, active Fe, chlorophyll content, peroxidase activity, and high yield compared to susceptible ones.Under deficit-Fe conditions, high yield among resistant genotypes could be attributed to higher seed weight, number of pods and haulm yield, while contrasting reduction in main stem height and number of primaries.

Active Fe content

Active Fe content measures the available active form of iron which a plant is able to uptake. Two genotypes, Chintamani and JL 286 had available Fe content less than the sensitive genotype while nine genotypes had between sensitive to tolerant check values. Five genotypes, PBS 19018, PBS 16035, TPG 41, MH 2 and RG 510 had active Fe content more than 100 ppm which shows that these groundnut lines are Fe-efficient and able to uptake the available iron from soil. Eight genotypes, CS 332, GG 20, NRCG 162, ICGV 91114, Kadiri 6, JGN 3, JL 502 and GG 5 had Fe content less than 50 ppm which shows the inefficiency of these genotypes for Fe uptake. The concentration of active iron (HCl-extractable iron concentration) in young leaves of groundnut decreased with increasing SWC at 10 days and 20 days after soil water treatment; specifically, it decreased remarkably with increasing SWC and $CaCO_3$ supply (Zuo *et al.* 2007). Compared with low $CaCO_3$ level, higher content depressed the active iron concentration. The concentration of active iron in primary leaves, generally increased marginally with increasing SWC at 20 and 35 days. $CaCO_3$ combined with soil water content decreased significantly total iron concentration in young leaves. $CaCO_3$ combined with soil water content decreased total iron concentration in groundnut root. Notably, total iron concentration in root of groundnut increased with increasing soil water content, and $CaCO_3$ combined with soil water content enhanced the accumulation of iron in root of groundnut at 20 and 35 days. Earlier we have reported that application of iron source to iron deficit groundnut plants increases the active Fe content and improves the iron chlorosis deficiency symptoms (Mann *et al.* 2017). This same behaviour was observed in oats (McDaniel and Brown 1982), chickpea (Saxena and Sheldrake 1980), beans (Zaitner *et al.* 1982), groundnuts (Samdur *et al.* 2000), and pear (Ma *et al.* 2005). Romheld

(2000) has observed that the total iron concentration in chlorotic leaves is similar to that in green leaves. Further, a significant correlation of available Fe with total Fe and Zn was seen under iron chlorosis conditions in the present study, which may give insights into the rapid transcriptional responses to Fe shortage in plants, which is important for understanding how changes in nutrient availability are translated into responses that help to avoid imbalances in ion distribution.

Mineral nutrient analysis

Maximum variation was observed in total Fe content depicting a wide range (129-706 ppm) with mean value of 404.56±17.90. Sixty two genotypes had uptake of Fe more than the tolerant check, whereas three breeding lines had Fe content less than the sensitive check. Rest of the genotypes had Fe uptake within the mean range value. The mean range for Zn was 17-100 ppm with mean value of 38.28±0.99. Fifty genotypes had Zn content more than the tolerant check (39 ppm) while the rest had below it. None of the genotypes had Zn content less than the sensitive check (17 ppm). The range of Mn varied from 30-130 ppm with one genotype only having Mn content less than the sensitive check. Most of the lines had Mn content more than the tolerant check and only 17 genotypes had Mn content comparable to the checks. A significant correlation of Mn with Zn, K and P depicts the ionomic interaction under iron chlorosis conditions. Total phosphorus concentration varied from 0.082-0.372 %. Tolerant check had 0.253 % with 0.132 % in sensitive check. Fifty eight genotypes showed P more than the tolerant check while one line PBS 16035 had P (%) lower than the sensitive check. Just like Fe content, maximum variation was seen in K concentration where the range varied from 0.37-1.99 %. Seventy two genotypes had K content more than the tolerant check (1.05%) with the lowest concentration in sensitive check only. A significant correlation of K with P, fodder weight and 100 seed weight was observed. Mean pod weight was 30.16±0.79 with range value of 45-95 while the mean fodder weight was 50.13±1.76. Maximum variability was observed in fodder weight which clearly shows the effect of iron chlorosis on plant growth and yield. Mean of seed yield was 66.48±0.73, whereas the mean value of seed weight was 33.61±0.74. A significant correlation was observed between seed yield and 100 seed weight. All the morphological and physiological values in the whole population show a relatively normal distribution around the mean value that lays between the check values. Maximum frequency of SPAD value ranges between 25-35 in approximately 82 lines (Fig 2). However for chlorophyll content, the maximum individuals (70 genotypes) retain the total chlorophyll content between 4-6 mg/g FW. Similarly, the frequency distribution for active Fe content was in a range of 80-90 ppm covering 38 genotypes while the frequency distribution for potassium (K) content was 1-1.3 % shown by 58 genotypes/lines. Impairment of the mineral nutrition of plants can be accompanied by an enhanced potential for photo-oxidative damage, and this threat can be especially serious when plants are simultaneously exposed to an environmental stress. Mineral nutrients, such as N, K, Mg, Ca, Fe and Zn, supplied at adequate levels are an essential requirement for the maintenance of photosynthesis activities and utilization of light energy in CO_2 fixation (Cakmak 2006). Therefore, the improvement of mineral nutrition of plants becomes a major contributing factor in protecting them from photo-oxidative damage under marginal environmental conditions. The widespread problem of iron deficiency chlorosis in dicotyledonous plants in calcareous soils was reflected in the groundnuts grown in our experiment. Iron deficiency mostly occurs in high pH soils, especially in calcareous soils with marginal levels of available iron. Moreover, the relative high soil water content combined with high $CaCO_3$ supply, leads to high HCO_3^- concentration and, consequently, the available iron concentration in groundnut rhizosphere is decreased. Iron uptake will result, either directly or indirectly, in iron chlorosis of iron inefficient groundnut plants. The groundnuts display 'Strategy I' mechanisms for responding to iron stress, in which increased reductase activity occurs on the surface of the plant roots in addition to release of protons and reductants from the roots (Bienfait 1988). The Fe(III) reducing capacity and apoplasmic iron pool of groundnut roots are closely related to iron uptake. It has been demonstrated that the apoplasmic iron pool is more easily reduced than ferric precipitates for plants grown in calcareous soil, and only when this iron pool was exhausted the plants become increasingly chlorotic. Thus, it is possible that groundnut plants grown in high $CaCO_3$ supplied soil may have had high HCO_3 concentration in the rhizosphere and that this led to the accumulation of additional iron in an apoplasmic iron pool, which also inhibited iron transport to the shoots. Benke *et al.* (2014) identified QTLs with new putative candidate genes involved in Fe homeostasis under a deficient or adequate Fe nutritional status which have so far not been considered as relevant for efficient Fe homeostasis under both, low or high Fe concentrations. Iron-deficiency-induced transcriptional changes were studied in *Arabidopsis* roots by microarray analyses (Buckhout *et al.* 2009) which revealed that the expression of 60 genes were changed after 6 hr of Fe deficiency and 65% of these were found to overlap with a group of 79 genes that were altered after 24 hr. A high number of transcripts encoding ion transport proteins were found which function to increase the Fe concentration and decrease the zinc (Zn) concentration in the cytosol. It appeared that with Fe deficiency, Zn uptake increases, most probably due to low specificity of the Fe transporter IRT1 which is induced upon Fe deficiency. In our studies also, a significant correlation of available Fe with total Fe and Zn was seen under iron chlorosis conditions. Iron in the root surroundings of calcareous soils occurs mostly in an oxidized form (Fe^{3+}) which is not available to the plants. This Fe^{3+} must be reduced to Fe^{2+} before it can be taken up by the plant. The secretions of root modify the chemical composition of the root atmosphere making the iron available to the plant. In calcareous soils, where

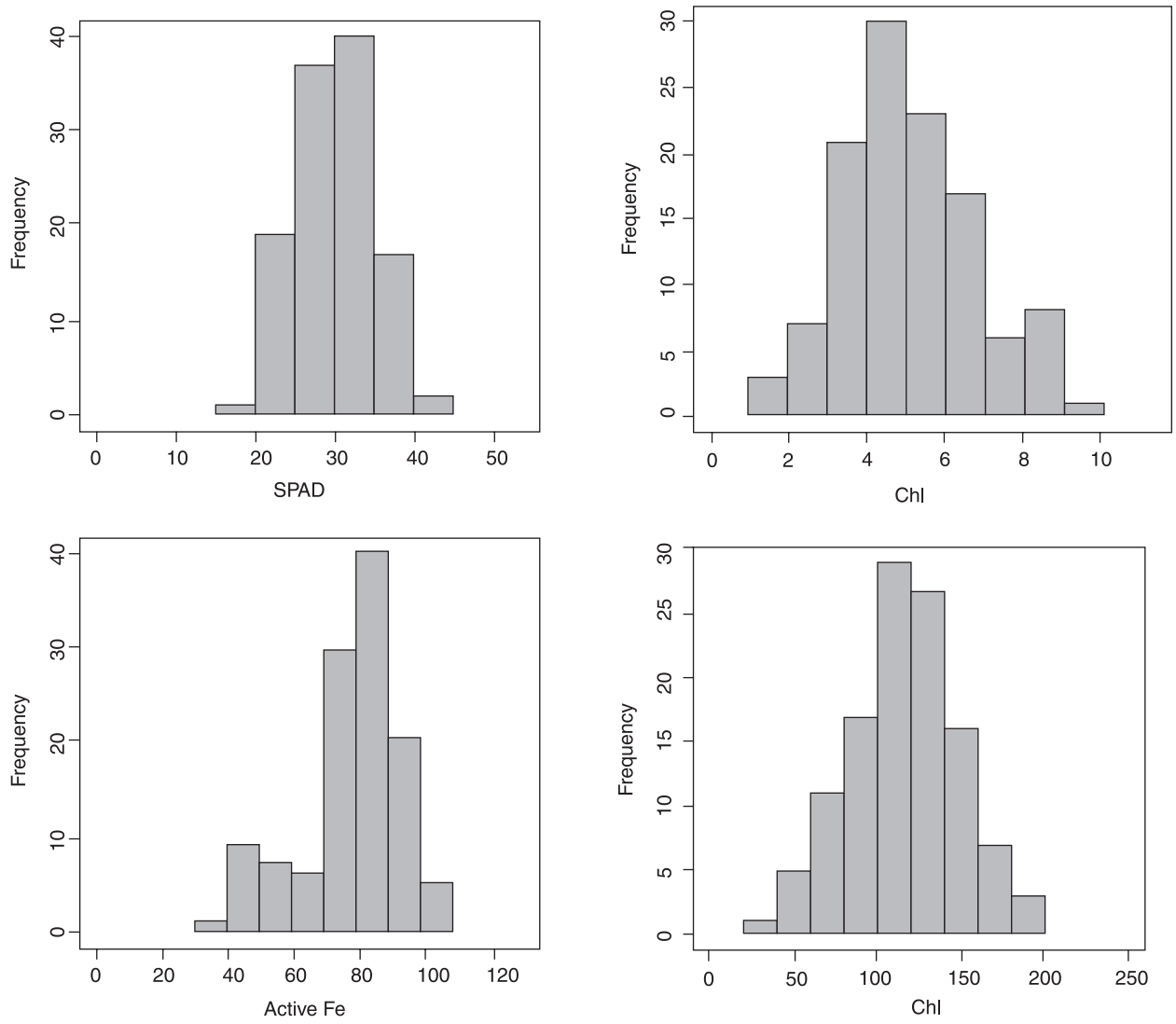

Fig 1 Histogram of SPAD, chlorophyll (chl), active Fe content and potassium content (K) in groundnut genotypes.

lime induced iron chlorosis is a major problem causing yield reduction in groundnut, iron chlorosis-tolerant or iron efficient genotypes should be grown to prevent further yield losses. These genotypes can be crossed with other high-yielding genotypes to develop new iron efficient high-yielding groundnut genotypes. The cultivars tolerant to iron chlorosis in this study showed higher efficiency of iron utilization, which may be due to higher capacity of roots to reduce iron from ferric to ferrous form and to produce iron-chelating compounds in the root exudates which made iron available to plant. Majority of the soils in Gujarat are naturally high in lime (calcium carbonate and other calcium compounds) driving the soil pH above 7.5. On these calcareous soils, iron chlorosis is common on susceptible plants. Identified genotypes with tolerance to iron chlorosis can be used in the groundnut improvement programmes especially for the alkaline calcareous soils. Groundnut cultivar development would greatly benefit from simplified access to the genetic diversity available in related diploid species of *Arachis*. Genomic research is also being used to enhance the amount of genetic diversity available for use in conventional breeding through the development of transgenic groundnut, and the creation of TILLING populations and synthetic allotetraploids. Marker assisted selection (MAS) is becoming more common in groundnut cultivar development programs, and several cultivar releases are anticipated in the near future.

The present investigation puts insights into the adverse effect of yellowing of leaves and thus identification of tolerant lines for LIIC. Identification of tolerant groundnut genotypes for their entry into gene pool for crop improvement programmes along with other contributing factors is generally the effective way for problem in calcareous soils.

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