RESEARCH ARTICLE Legume Research- An International Journal, Volume 49 Issue 5: 806-813 (May 2026)

Photosynthetic and Molecular Responses of Groundnut Genotypes to Iron Deficiency

P. Latha[1], K.V. Naga Madhuri[1], T. Anitha[1], E. Aparna[1], M. Swethasree[1], K. John[1], S. Shalini Devi[2]

10.18805/LR-5389

ABSTRACT

Background: The challenge of iron deficiency in calcareous soils significantly inhibits crop growth, causing chlorosis and yield reductions. Screening for promising iron-deficiency-tolerant groundnut genotype for calcareous soils, offer insights into crop stress adaptation and productivity enhancement in challenging environments.

Methods: An experiment aimed at understanding the responses of six chosen groundnut genotypes, with one iron-deficiency-tolerant genotype (ICGV 86031) as a control (6+1), was conducted at Acharya N G Ranga Agricultural University, Regional Agricultural Research Station in Tirupati. The investigation encompassed both phenotypic and molecular analyses under conditions of iron sufficiency and deficiency. The selection of these six genotypes was based on their phenotypic traits, emphasizing high physiological performance under iron deficiency stress.

Result: Notably, TCGS-2018 and ICGV 86031 displayed elevated chlorophyll levels and photosynthetic rates compared to other genotypes, regardless of iron availability. Also, ICGV-86031, TCGS-1862 and TCGS-2018 exhibited higher total iron content and demonstrated superior tolerance to iron deficiency stress. Gene analysis of four selected genotypes highlighted higher expression levels of NRAMP family genes in TCGS-2018 under iron deficiency, indicating their role in iron balance. Distinct patterns were observed in the expression of ZIP1 and AhIRT1 genes, which are relevant to iron and zinc balance and varied among genotypes. Overall, these findings underscore TCGS-2018 as a promising iron-deficiency-chlorosis tolerant groundnut genotype offering enhanced productivity for calcareous soils.

Key words: Genes, Groundnut, Iron chlorosis, Phenotypic, Yield.

INTRODUCTION

Groundnut (*Arachis hypogaea* L., 2n = 4x = 40) is cultivated extensively worldwide, providing approximately 20% of the annual global supply of edible oil and 11% of food protein. However, the cultivation of groundnuts in calcareous soils often leads to deficiencies in iron (Fe) and/or zinc, which can significantly hinder groundnut productivity.

Groundnut, renowned for its resilience to various weather conditions, is often cultivated in marginal to poor soil types where micronutrient deficiency poses a significant challenge. In calcareous soils, iron uptake is notably deficient, exacerbated by a pH exceeding 7.5, poor drainage, low organic matter and high bicarbonate ion concentrations, leading to Iron Deficiency Chlorosis (IDC). Globally, approximately 800 million hectares of calcareous soils exist, predominantly in regions with temperate and hot climates. Iron deficiency chlorosis (IDC) is a prevalent condition affecting crops cultivated in these soil types (Land, FAO and Plant Nutrition Management, 2000).

In the plant, iron (Fe) is a constituent or a cofactor of many oxidant enzymes which plays a major role in crucial metabolic processes (Cakmak *et al.,* 2023). Fe as a cofactor of different anti oxidant enzymes *viz.* super oxide dismutase, catalase, peroxidise, detoxifies the cells by scavenging the free radicals (Volodyaev and Vladimirov, 2023). Uptake of Fe is scantily available in the soils of more than 7.5 pH, high calcium carbonate soils, poorly drained soils and soils with low organic matter. In the world, calcareous soils occupy an approximate area of 800 m ha, primarily concentrated in regions with temperate and hot climates. Iron deficiency chlorosis (IDC) is a common condition among crops grown in these soil types (Land, FAO and Plant Nutrition Management, 2000).

Iron (Fe) within plants serves as a constituent or cofactor for numerous oxidant enzymes, crucially contributing to metabolic processes (Cakmak *et al.,* 2023). Fe functions as a cofactor for various antioxidant enzymes, including superoxide dismutase, catalase and peroxidase, aiding in cell detoxification by neutralizing free radicals (Volodyaev and Vladimirov, 2023). In groundnut plants, iron deficiency symptoms manifest initially as interveinal chlorosis on young

[1]Regional Agricultural Research Station, Acharya NG Ranga Agricultural University, Tirupati-517 502, Andhra Pradesh, India. [2]Department of Microbiology, Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad-500 094, Telangana, India.

Corresponding Author: P. Latha, Regional Agricultural Research Station, Acharya NG Ranga Agricultural University, Tirupati-517 502, Andhra Pradesh, India. Email: p.latha@angrau.ac.in

How to cite this article: Latha, P., Naga Madhuri, K.V., Anitha, T., Aparna, E., Swethasree, M., John, K. and Devi, S.S. (2026). Photosynthetic and Molecular Responses of Groundnut Genotypes to Iron Deficiency. *Legume Research.* **49(5):** 806-813. doi: 10.18805/LR-5389.

Submitted: 21-07-2024 **Accepted:** 29-01-2025 **Online:** 01-05-2025

leaves. With severe iron deficiency, chlorosis extends to the veins, causing leaves to turn white, then brown and eventually become necrotic. This progression stunts plant growth, reducing both yield and fodder production, especially in cases of severe deficiency. Acute iron deficiencies can even lead to plant death and complete crop failure (Irmak *et al.,* 2012;).

The identification of resilient genotypes against micro-nutrient deficiencies offers a promising solution for soils with low nutritional value and contributes to improved human health (Johnson *et al.,* 2023; Chitdeshwari and Brindhavani 2023). Earlier studies by Tayade *et al.* (2022) and Kumar *et al.* (2022) have reported the response of groundnut genotypes to iron deficiency chlorosis (IDC), highlighting higher pod yields in IDC-tolerant genotypes compared to susceptible lines (Kumar *et al.,* 2022; Naidu *et al.,* 2021). Therefore, the current study aimed to identify groundnut genotypes developed at the Tirupati center for their tolerance to iron deficiency chlorosis in calcareous soils and validate certain known candidate genes responsible for iron uptake and transportation.

MATERIALS AND METHODS

The experiment was conducted during the *kharif* season of the year 2020 at Plant Breeding Department, Regional Agricultural Research Station (RARS) in Tirupati, Andhra Pradesh. A total of nineteen groundnut genotypes, along with a control (19+1), underwent phenotypic assessment for tolerance to iron deficiency chlorosis (IDC). Among these, six distinct lines, along with the control, underwent further screening for IDC tolerance based on both physiological and molecular criteria. Seeds of six groundnut genotypes-TCGS 1399, TCGS 1416, TCGS 1789, TCGS 1792, TCGS 1862, TCGS 2018-as well as the iron deficiency-tolerant genotype ICGV 86031 (used as a control) were planted in pots during the *kharif* season of 2021 under controlled conditions (temperature: 25 ± 2°C; relative humidity: 95%). The experiment was conducted in three replications, with soils deficient in iron (Fe-D) and calcareous ($CaCO_3$ content: 22%) and soils sufficient in iron (Fe-S) (iron content: 2.3 ppm in Fe-D soils and 8.5 ppm in Fe).

SPAD Chlorophyll Meter Reading (SCMR) was recorded for the third leaf from the top of the stem as a mean of four random plants in each genotype using the SPAD meter of Minolta Company, NJ, USA (SPAD-502). Chlorophyll 'a' and 'b', total chlorophyll and carotenoid content were estimated as per the method given by Arnon (1949). The units were mentioned in mg per gram fresh weight (FW) basis. Small pieces of 100 milligrams (mg) leaf tissue were incubated for 30 minutes (min) in 7 millilitres (ml) of dimethyl sulfoxide at 65°C. After the incubation period, the supernatant was separated, made up to 10 ml and the absorbance was recorded at 645 nm and 663 nm for measurement of chlorophyll pigments and total carotenoids measured at 652 nm using Eppendorf Biospectrophotometer.

Peroxidase activity (Mahadevan and Sridhar, 1986) was estimated at a wavelength of 436 nm using Eppendorf Biospectrophotometer. Fresh leaf tissue of one gram (g) was grinded using 0.1 M phosphate buffer (3 ml) of pH 6.0 in a pre-cooled mortar and pestle, centrifuged at 3,000 rpm at 5°C for 15 min. The supernatant was collected for estimation of enzyme activity. Then, 0.1 ml of the centrifuged aliquot mixed with 3 ml buffer solution, 0.05 ml guaiacol solution and 0.03 ml hydrogen peroxide solution in a cuvette, mixed well and estimated for peroxidise activity.

Gas exchange traits *viz.,* photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), intercellular CO_2 concentrations (Ci) and instantaneous water use efficiency (WUEi) were measured at 90 days in Fe sufficient and Fe deficient soils (Six plants of each genotype per treatment) using the LI-6400 gas exchange portable infrared CO_2 analyzer (IRGA) (ADC, Bio scientific Ltd, Hoddesdon, UK).

Active Fe content was assessed at 510 nm using an Eppendorf Biospectrophotometer. To prepare the solution, 15 g of o-phenanthroline was dissolved in 850 ml of deionized water and the mixture was continuously stirred while 1 N HCl was added drop by drop until the pH reached approximately 5.5. The final volume was adjusted to one litre. Leaves, specifically the third leaf from the top of the main stem of plants, were collected and washed with tap water, followed by rinsing with 0.1 N HCl and then double distilled water. Without delay, two grams of small leaf pieces were placed in glass bottles and 20 ml of the ophenanthroline solution was added. The mixture was stirred with the extractant and left at ambient temperature for 16 hours. Afterwards, it was filtered through grade 1 filter paper to measure the active Fe content in the filtrate, expressed as mg kg^{-1} on a fresh weight basis (Katyal and Sharma, 1980).

Analysis of variance (ANOVA) is employed to assess the research findings utilizing SPSS (Version 20, IBM SPSS). To examine mean differences, the Duncan's multiple range test was conducted at a significance level of 5%.

For the molecular investigation, leaf tissue from three biological replicates was collected in the morning between 10-11:30 AM and immediately snap-frozen. This was done for the purpose of isolating total RNA from seven groundnut genotypes subjected to both Fe-sufficient and Fe-deficient soil conditions. Total RNA extraction was performed using RNAlso Plus reagent (Takara Bio Inc) according to the manufacturer's instructions. Following extraction, RNA was treated with the TURBO DNA-freeTM kit (Life Technologies) and its quality was assessed using 1% agarose gel electrophoresis. Subsequently, one microgram (µg) of RNA was treated with DNaseI and reverse-transcribed using the iScriptTM cDNA synthesis kit (Biorad) with oligodT primer. The resulting cDNA was then utilized for semi-qPCR analysis in a 10 ml reaction mix comprising 2 µl of template cDNA (100 ng/µl), 0.5 µl (5 pmol) of forward and reverse gene-specific primers, 1 µl of Biolabs Taq buffer, 1 µl of $MgCl_2$, 0.2 µl of Biolabs Taq DNA polymerase, 1 µl of 10 mM

dNTPs and 4.8 µl of Milli-Q water. The actin (5′AAGCTG GCTTACATTGCCCT3′; 3′TGACCTGTCCATCAGGCAAC5′) gene was used as the reference control and the gene-specific primers were used for relative expression of selected genes (Supplementary Table 1). The gels were quantified for intensity difference by employing ImageJ software tool (https://imagej.nih.gov/ij) (Wahab *et al.,* 2020).

RESULTS AND DISCUSSION

Phenotypic studies

Phenotypic studies aid in classification of groundnut genotypes. Previously, Mann *et al.* (2015) and Zanjareetal (2023) utilizedphenotypic characteristics to identify groundnut genotypes tolerant to iron deficiency induced chlorosis and leaf spot disease respectively.

Phenotypic analysis revealed significant differences in chlorophyll content and gas exchange parameters-including photosynthetic rate, stomatal conductance and transpiration rate-under iron-deficient conditions. Iron (Fe) deficiency stress stands out as a critical nutritional stress factor among various abiotic stresses, particularly affecting chlorophyll levels in leaves and often leading to plant death, particularly in calcareous soils. Morphological and physiological traits play a crucial role in characterizing plant responses to such stresses. Building upon previous

Supplementary Table 1: Primer details for gene expression analysis.

Gene	Primer sequence 5′ 3′		Mer
NRAMP 1	Forward	5′-CCTCATCACTGCCTTCGT-3′	18
	Reverse	5′-ATTGCTGTGTTATCCTTGGTC-3′	21
NRAMP 3	Forward	5′-AGGTTGAAAAAATGGATGAGAGC-3′	23
	Reverse	5′-TCAGAACATCTAACGATTGCTCAG-3′	24
NRAMP 5	Forward	5′-TTACTCCCAAACTCAGTGGTCAAG-3′	20
	Reverse	5′-GTGGAGGAAGAGGTTGTGCG-3′	20
IRT 1	Forward	5′-GTTCTCTGCCTTATTCACGCTCAT-3′	24
	Reverse	5′-GCCAACACTAACAACAACACCCAT-3′	24
YSL 3	Forward	5′-TATTTGGAGAACCAGAGGCAGC-3′	22
	Reverse	5′-CGCCAACGATACTGGAATGC-3′	20
ZIP 1	Forward	5′-ATGAGATTGCGGCGGTGT-3′	18
	Reverse	5′-GCATAGTTCACATAGTCCTCTCCAG-3′	25
Actin	Forward	5′-CTGAAAGATTCCGATGCCCTGA-3′	22
	Reverse	5′-AACCACCACTCAAGACAATGTTACCA-3′	26

Table 1: Mean performance of genotypes for morpho-physiological and biochemical parameters in Fe-sufficient (Fe-S) and Fe-deficient (Fe-D) soils at 90 DAS.

Groundnut	Treatment	SCMR	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})	Shoot peroxidase activity (Units min^{-1} g^{-1})	Root peroxidase activity (Units min^{-1} g^{-1})
TCGS- 1399	Fe- S	39.43^{bc}	0.58^{d}	0.22^{bc}	0.64^{d}	0.055^{a}	1.67^{e}	3.89^{d}
	Fe- D	17.57^{c}	0.61^{bc}	0.11^{a}	0.38^{c}	0.048^{b}	2.04^{d}	3.39^{e}
TCGS- 1416	Fe- S	40.27^{b}	1.68^{ab}	0.32^{bc}	1.71^{c}	0.079^{bc}	3.85^{c}	2.58^{e}
	Fe- D	22.03^{b}	0.73^{bc}	0.14^{a}	0.88^{bc}	0.045^{b}	3.60^{c}	2.83^{f}
TCGS- 1789	Fe- S	33.53^{c}	0.93^{cd}	0.14^{b}	1.15^{dc}	0.074^{bc}	3.85^{c}	3.88^{d}
	Fe- D	22.27^{b}	0.45^{c}	0.13^{bc}	0.78^{bc}	0.040^{b}	3.33^{c}	3.66^{d}
TCGS- 1792	Fe- S	35.23^{d}	1.35^{bc}	0.26^{bc}	1.61^{c}	0.075^{bc}	1.45^{e}	2.09^{f}
	Fe- D	21.23^{b}	0.83^{bc}	0.15^{a}	0.92^{bc}	0.048^{b}	1.78^{d}	2.82^{f}
TCGS- 1862	Fe- S	37.80^{c}	1.40^{bc}	0.34^{a}	2.02^{b}	0.091^{bc}	2.43^{d}	7.76^{a}
	Fe- D	20.27^{b}	0.71^{bc}	0.13^{a}	0.85^{bc}	0.043^{b}	3.50^{c}	7.57^{a}
TCGS- 2018	Fe- S	45.47^{a}	1.98^{a}	0.40^{a}	2.38^{a}	0.118^{a}	7.20^{a}	6.41^{b}
	Fe- D	28.40^{a}	1.31^{a}	0.25^{a}	1.55^{a}	0.074^{a}	8.19^{a}	6.50^{b}
ICGV- 86031	Fe- S	29.80^{f}	1.79^{ab}	0.36^{a}	2.14^{ab}	0.106^{ab}	6.35^{b}	5.35^{c}
	Fe- D	21.90^{b}	1.06^{ab}	0.20^{a}	1.26^{ab}	0.067^{ab}	6.72^{b}	6.09^{c}

Note: Data represent means ± SD (n = 15). Different letters above the tabulated values indicate significant difference (DMRT, $p<0.05$). Values marked with same alphabets are not significantly different for Fe-sufficient and Fe-deficient separately.

research, six distinct genotypes were chosen from a pool of 19 groundnut lines. Genotypic variations in morpho-physiological traits were evaluated for their tolerance to iron deficiency chlorosis (IDC) at different growth stages, as demonstrated by Ishwar *et al.* (2016). Similarly, in the present study, significant genotypic differences were observed among the selected groundnut genotypes (6+1), particularly in their response to Fe deficiency stress in soils with high calcium content, in comparison to Fe-sufficient soils. This genotypic variability was further investigated to assess RNA expression patterns related to IDC tolerance.

SCMR was employed to measure relative chlorophyll content, revealing significant differences in unit-less values across the leaves (specifically the third leaf) of all genotypes at 90 days after sowing (DAS), indicating genotype responses to SCMR under Fe-deficient stress conditions. This investigation highlighted significantly higher SCMR values in genotype TCGS 2018 under both Fe-sufficient (45.47) and Fe-deficient (28.40) conditions, while the lowest SCMR

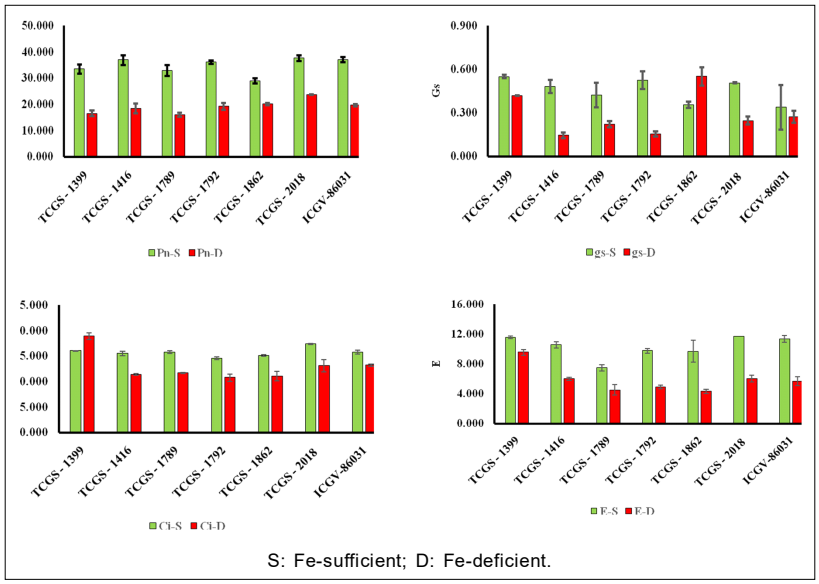

Fig 1: Effect of Fe deficiency on photosynthetic parameters: Net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (gs) and intercellular CO_2 concentration (Ci) in leaves of seven peanut genotypes grown in Fe-sufficient (Fe-S) and Fe-deficient (Fe-D) condition.

Table 2: Mean performance of genotypes for morpho-physiological and biochemical parameters in Fe-sufficient (Fe-S)) and Fe-deficient (Fe-D) soils at 90 DAS.

Groundnut	Treatment	Haulm weight (g plant^{-1})	Pod weight (g plant^{-1})	Active Fe (ppm)	Total Fe (ppm)
TCGS- 1399	Fe- S	5.64^{b}	5.37^{cd}	14.20^{ab}	126.50^{ab}
	Fe- D	2.35^{c}	1.97^{d}	10.07^{bc}	48.17^{cd}
TCGS- 1416	Fe- S	6.54^{b}	5.03^{d}	12.13^{bc}	121.00^{b}
	Fe- D	3.77^{b}	2.87^{bc}	7.00^{bc}	39.60^{de}
TCGS- 1789	Fe- S	5.77^{b}	4.87^{d}	14.20^{ab}	130.00^{ab}
	Fe- D	3.83^{b}	3.28^{b}	8.60^{cd}	46.40^{cde}
TCGS- 1792	Fe- S	6.43^{b}	5.90b^{cd}	9.13^{c}	69.13^{c}
	Fe- D	3.66^{b}	2.40^{cd}	5.07^{e}	36.93^{e}
TCGS- 1862	Fe- S	10.34^{a}	6.72^{b}	13.40^{ab}	140.93^{ab}
	Fe- D	3.81^{b}	2.77^{bc}	10.5^{abc}	74.17^{b}
TCGS- 2018	Fe- S	9.96^{a}	7.79^{a}	16.60^{a}	142.27^{ab}
	Fe- D	4.95^{a}	3.69^{a}	12.73^{ab}	55.70^{c}
ICGV- 86031	Fe- S	6.52^{b}	5.98^{bc}	16.13^{a}	149.57^{a}
	Fe- D	3.75^{b}	2.76^{bc}	13.00^{a}	102.47^{a}

Note: Data represent means ± SD (n = 15). Different letters above the tabulated values indicate significant difference (DMRT, $p < 0.05$). Values marked with same alphabets are not significantly different for Fe-sufficient and Fe-deficient separately.

values were observed in TCGS 1399 (17.57) under Fe-D Table 1. Ishwar *et al.* (2016) emphasized the utility of SCMR for large-scale phenotyping of groundnut germplasm for IDC tolerance, noting its correlation with leaves exhibiting more chlorotic symptoms and lower SPAD values.

The chlorophyll pigment contents were assessed in leaves exposed to Fe-sufficient (Fe-S) and Fe-deficient (Fe-D) conditions across all genotypes. The findings indicated a significant reduction in all measured pigments-chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids-in Fe-D leaves at 90 days after sowing (DAS) (Table 1). Notably, chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids exhibited significantly higher values in TCGS-2018, followed by the ICGV 86031 genotypes, compared to other genotypes under both Fe-S and Fe-D conditions at 90 DAS. This suggests the critical role of iron levels in pigment synthesis in groundnut leaves during the reproductive stage. Across all genotypes, a significant decrease in chlorophyll 'a', 'b' and total chlorophyll levels was observed in Fe-D plants compared to Fe-S plants. TCGS-1399 displayed the lowest levels of total chlorophyll, measuring 0.64 mg g^{-1} FW in Fe-S and 0.38 mg g^{-1} FW in Fe-D plants. Importantly, SCMR values and the severity of chlorosis are directly linked to the chlorophyll content of the leaves.

Iron deficiency primarily affects the key photosynthetic apparatus within chloroplasts, thereby impeding electron transfer complexes such as PSI, PSII, cytochrome b6f and ferredoxins, as well as biosynthesis pathways for chlorophyll (Curie *et al.,* 2003). Gas exchange traits, including Photosynthetic rate (Pn), stomatal conductance (gs), intercellular carbon dioxide (Ci) and transpiration rate (E), were notably lower in the leaves of genotypes cultivated in Fe-deficient soils compared to Fe-sufficient conditions at 60 days after sowing (DAS), with few exceptions observed across all genotypes (Fig 1). Significant genotype × Fe interactions were observed for Pn, gs, Ci and E, indicating cultivar-specific responses of gas exchange to Fe-deficient stress. Notably, the greatest increase in Pn was recorded in TCGS-2018 under both Fe-sufficient (37 µmol CO_2 m^{-2} s^{-1}) and Fe-deficient (23.65 µmol CO_2 m^{-2} s^{-1}) conditions. Ci levels were elevated under Fe-deficient stress in TCGS-1399 (283 µ mole CO_2 $mole^{-1}$), while they decreased in other genotypes. These findings highlight the significant impact of Fe deficiency on photosynthetic rates in groundnut. Stomatal conductance (gs) and transpiration rate (E) were significantly reduced in all genotypes under Fe-deficient conditions, with TCGS-1399 exhibiting the highest E (9.54 mmol H_2O m^{-2} s^{-1}) and TCGS-1862 displaying higher gs (0.55 mol H_2O m^{-2} s^{-1}) compared to others, respectively (Fig 1).

Fe deficiency stress disrupts the equilibrium of the reactive oxygen metabolism system. Peroxidase (POD) activity exhibited varied trends among genotypes at 90 days after sowing (DAS) (Table 1). The genotype TCGS-2018 displayed significantly elevated peroxidase enzyme activity in shoots under both Fe-sufficient (7.20) and Fe-deficient (8.19) conditions. Conversely, TCGS 1399 exhibited the lowest POD activity in both Fe-sufficient (1.67) and Fe-deficient (2.04) shoot samples, while TCGS-1792 recorded the lowest values in root samples (2.09 under Fe-sufficient and 2.82 under Fe-deficient conditions). Root peroxidase activity was higher in TCGS 1862 under both Fe-sufficient (7.76) and Fe-deficient (7.57) conditions. Additionally, across several

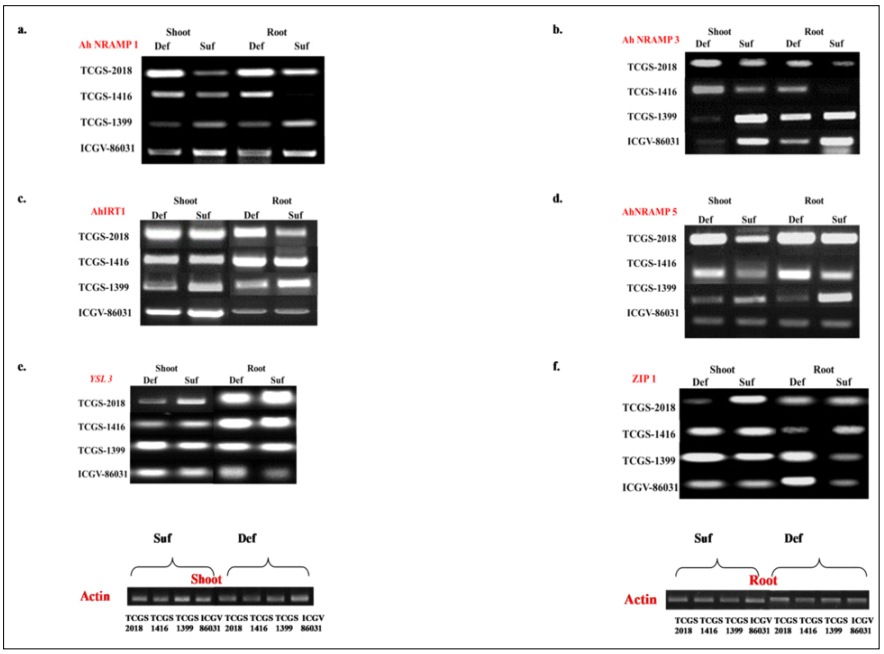

Fig 2: Candidate genes showed differential gene expression between groundnut genotypes under iron sufficient (Suf) and iron deficient (Def) conditions

Table 3: Relative gene expression density of seven DEGs measured among four genotypes by employing Image J Software tool.

Tissue type	Genotypes	Condition	AhNRAMP1	AhNRAMP3	AhIRT1	AhNRAMP5	YSL3	ZIP1
Shoot	TCGS-2018	Fe- S	0.51±0.02	1.01±0.06	2.06±0.01	1.23±0.01	0.96±0.06	1.50±0.02
		Fe- D	1.53±0.06	1.27±0.05	2.18±0.03	2.22±0.04	0.25±0.01	0.21±0.01
	TCGS-1416	Fe- S	1.04±0.04	0.98±0.05	2.19±0.02	0.84±0.01	1.68±0.02	1.98±0.03
		Fe- D	1.02±0.02	1.47±0.06	1.68±0.05	1.42±0.01	1.05±0.04	1.44±0.01
	TCGS-1399	Fe- S	0.89±0.03	2.61±0.06	1.99±0.11	0.97±0.05	2.07±0.03	1.86±0.02
		Fe- D	0.56±0.03	0.14±0.01	1.07±0.05	0.40±0.02	2.50±0.05	2.41±0.01
	ICGV86031	Fe- S	1.27±0.05	2.48±0.01	2.13±0.13	0.65±0.01	2.02±0.01	1.53±0.02
		Fe- D	0.62±0.02	0.09±0.01	1.35±0.05	0.39±0.00	1.61±0.02	1.13±0.02
Root	TCGS-2018	Fe- S	0.72±0.02	0.29±0.01	0.78±0.03	1.86±0.03	2.68±0.03	0.99±0.4
		Fe- D	0.97±0.07	0.55±0.02	1.11±0.01	1.49±0.09	1.59±0.01	0.66±0.02
	TCGS-1416	Fe- S	0.16±0.02	0.07±0.01	1.16±0.01	0.96±0.05	1.71±0.02	0.67±0.02
		Fe- D	0.96±0.05	0.73±0.01	2.11±0.05	2.08±0.02	2.58±0.01	0.37±.02
	TCGS-1399	Fe- S	1.03±0.08	1.99±0.03	1.53±0.02	1.90±0.04	2.29±0.06	0.61±0.02
		Fe- D	0.85±0.04	2.31±0.01	1.30±0.04	0.27±0.00	3.01±0.01	2.95±0.05
	ICGV86031	Fe- S	1.08±0.06	1.74±0.04	0.52±0.01	0.29±0.06	0.55±0.05	0.50±0.03
		Fe- D	0.51±0.03	0.89±0.08	0.53±0.04	0.32±0.00	1.33±0.04	1.95±0.05

genotypes, root POD activity surpassed that of shoots. The observed increase in root POD activity under Fe deficiency is attributed to the roots' ability to tolerate reactive oxygen species generated by Fe deficiency stress, thereby triggering a protective response against cellular membrane damage induced by reactive oxygen, thus safeguarding the plant from further harm.

The concentration of iron (Fe) in leaves serves as a crucial plant parameter for evaluating genotypes' tolerance to Fe deficiency. Plants supplied with sufficient Fe exhibit markedly higher Fe concentrations in leaves compared to those experiencing Fe deficiency. Under Fe-sufficient (Fe-S) conditions, TCGS-2018 and the reference genotype ICGV-86031 demonstrated significantly higher active Fe content (16.60 ppm and 16.13 ppm, respectively) compared to other genotypes. Conversely, under Fe-deficient (Fe-D) conditions, ICGV-86031 (13 ppm) followed by TCGS-2018 (12.73 ppm) exhibited significantly higher active Fe content at 90 days after sowing (DAS). Total iron content was notably higher in ICGV-86031 (149.57 ppm and 102.47 ppm) followed by TCGS-1862 (140.93 ppm and 74.17 ppm) and TCGS-2018 (142.27 ppm and 55.70 ppm) genotypes under Fe-S and Fe-D conditions, respectively (Table 2). Groundnut genotypes classified as tolerant or susceptible to iron deficiency exhibit variability in their iron reduction capacity.

The results regarding the correlation between chlorophyll content and iron concentration is varied and decreased SCMR values in reduced total iron content from our results are in accordance with Kumar *et al.* (2022), where seems to be incomplete sentence.

Under iron deficiency stress, all physiological functions are compromised, leading to diminished growth and yield (Nsiri and Krouma, 2023). This trend is reflected in the yield attributes of all genotypes, with haulm and pod weights reduced in Fe-deficient (Fe-D) samples compared to Fe-sufficient (Fe-S) ones. However, TCGS-2018 exhibited the highest pod yield (7.79 g plant^{-1} under Fe-S and 3.69 g plant^{-1} under Fe-D conditions). Conversely, TCGS-1399 displayed the lowest values for both haulm and pod yield parameters under both Fe-S and Fe-D conditions (2.35 g plant^{-1} and 1.97 g plant^{-1}, respectively).

Gene expression study

Iron uptake and transport are regulated by transporters encoded by IRT genes (Rahman *et al.,* 2022). In later stages of iron deficiency, the NRAMP1 response is induced to enhance ferrous ion uptake in roots (Chen *et al.,* 2019). Similar results were obtained in the present study, wherein NRAMP family genes *viz.*, AhNRAMP1, AhNRAMP3, AhNRAMP5 (Table 3, Fig 2), which showed differential expression in four groundnut genotypes. The study aimed to investigate the expression patterns of six genes associated with iron uptake and transport-AhNRAMP1 (Natural resistance-associated macrophage Protein), AhNRAMP3, AhNRAMP5, AhYSL3, ZIP1 (Zinc/Iron-Regulated Transporter-Like Protein) and AhIRT1-in various groundnut genotypes. Semi-quantitative RT-PCR was employed under iron deficiency stress conditions to assess the transcription levels of these genes in both roots and young leaves of groundnut genotypes. The semi-quantitative RT-PCR analysis using gene-specific primers revealed distinct expression patterns for all six genes-AhNRAMP1, AhNRAMP3, AhNRAMP5, AhIRT1, AhYSL3 and ZIP1. Further analysis involved evaluating the expression levels of these genes across different genotypes for variations in intensity, measured in pixels, using the ImageJ tool.

In genotype TCGS-2018, the expression of AhNRAMP1, AhNRAMP3 and AhNRAMP5 (1.53, 1.27, 2.22, respectively) was notably high under Fe-D conditions in shoot tissues. Conversely, in root tissue of TCGS-2018 under Fe-D conditions,

the expression of the AhNRAMP3 gene was relatively low (0.55). In the reference genotype ICGV-86031, AhNRAMP1, AhNRAMP3 and AhNRAMP5 genes showed moderate expression levels under Fe-D conditions compared to Fe-S conditions in both tissues, albeit lower than those observed in the TCGS-2018 genotype.

Prior research has indicated that overexpression of YSL3 in Arabidopsis contributes to the translocation and detoxification of cadmium (Cd), enhancing Cd translocation from roots to shoots (Chen *et al*., 2019). In the current study, YSL3 was found to be down-regulated in groundnut shoots under Fe-deficient stress conditions, except in the case of TCGS-1399 genotype, as shown in Table 3 and Fig 2. Conversely, in roots, YSL3 was up-regulated under Fe-deficient stress conditions, indicating its importance in roots experiencing Fe deficiency.

Previous studies have suggested that ZIP1 is involved in both iron and zinc homeostasis (Boonyaves, 2015). In the present study, the TCGS-1399 genotype exhibited higher expression levels of ZIP and AhIRT1 genes under Fe-deficient conditions in both root and shoot tissues compared to other genotypes. These findings align with a notable decrease in total Fe and active Fe content observed in this genotype. Earlier research has implicated the OsIRT1 gene in Fe homeostasis, uptake, transport and translocation mechanisms (Pradhan *et al.,* 2020). The AhIRT1 gene encodes a functional iron transporter and is induced by iron deficiency stress in roots (Ding *et al.,* 2010). In this study, up-regulation of the AhIRT1 gene was observed in shoot tissue under Fe-deficient conditions, while decreased expression was observed in roots, except for the TCGS-1416 genotype under Fe deficit conditions, suggesting the involvement of alternative mechanisms in Fe uptake alongside the known AhIRT1 mechanism.

CONCLUSION

Phenotypic analysis of groundnut genotypes, including the reference, revealed significant differences in chlorophyll content and gas exchange traits under iron-deficient conditions. TCGS-2018 and ICGV 86031 displayed higher chlorophyll levels and photosynthetic rates compared to other genotypes, regardless of iron availability. Further analysis revealed that TCGS-2018 exhibited significantly higher peroxidase enzyme activity in root and shoot tissues under both soil conditions. The up-regulation of AhNRAMP1 and AhNRAMP 5 family genes in both shoot and root tissues suggested that TCGS-2018 exhibits tolerance against Fe-deficiency stress. Moreover, molecular assessment of candidate genes confirmed their involvement in iron transportation within tissues, with increased expression under Fe deficiency stress, particularly showing upregulation in tolerant genotypes.

ACKNOWLEDGEMENT

The author is highly thankful for funding supportby Acharya N G Ranga Agricultural University, Lam, Guntur, Andhra Pradesh.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology.* **24(1):** 1-15.

Boonyaves, K. (2015). Expression of IRT1 combined with NAS and ferritin genes led to enhanced iron accumulation in the rice grains (Doctoral dissertation, ETH Zurich).

Cakmak, I., Brown, P., Colmenero-Flores, J.M., Husted, S., Kutman, B.Y., Nikolic, M. and Zhao, F.J. (2023). Micronutrients. In: Marschner's Mineral Nutrition of Plants. [Rengel, Z., Cakmak, I. and White, P. (Eds.)]. 4th edition. Cambridge, MA: Academic Press. pp 283-385.

Chen, C., Cao, Q., Jiang, Q., Li, J., Yu, R. and Shi, G. (2019). Comparative transcriptome analysis reveals gene network regulating cadmium uptake and translocation in peanut roots under iron deficiency. *BMC Plant Biology.* **19:** 1-14.

Chitdeshwari T., Brindhavani P.M. (2023). Differential response of groundnut genotypes for varying $CaCO_3$ concentrations. *Legume Research.* **46(3):** 316-323. doi: 10.18805/LR-4724.

Curie, C. and Briat, J.F. (2003). Iron transport and signaling in plants. *Annual Reviews of Plant Biology*. **54:** 183-206. doi: 10.1146/annurev.arplant.54.031902.135018.

Ding, H., Duan, L., Li, J., Yan, H., Zhao, M., Zhang, F. and Li, W.X. (2010). Cloning and functional analysis of the peanut iron transporter AhIRT1 during iron deficiency stress and in tercropping with maize. *Journal of Plant Physiology*. **167(12):** 996-1002.

Irmak, S., CIL AN, Yücel H., Kaya, Z. (2012). The effects of iron application to soil and foliarly on agronomic properties and yield of peanut (*Arachis hypogaea*). *Journal of Food Agriculture and Environment.* **10(3/4):** 417.

Ishwar Boodi, 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. *Journal of Crop Science and Biotechnology*. **19:** 177-187.

Johnson, K.H., Krishna, T.A., Dash, M., Thiyageshwari, S., Ceasar, S.A. and Selvi, D. (2023). Food and nutritional security: Innovative approaches for improving micronutrient use efficiency in soybean [*Glycine max* (L.) Merrill] under hostile soils. *Journal of Soil Science and Plant Nutrition.* **23(1):** 56-70.

Katyal, J.C. and Sharma, B.D. (1980). New technique to resolve iron chlorosis. *Plant Soil.* **55(1):** 105-109.

Kumar, O., Choudhary, M., Sridevi, O. and Naidu, G.K. (2022). GGE biplot analysis for SCMR and yield of groundnut geno types under Iron deficient calcareous soil. *The Pharma Innovation Journal.* **SP-11(5):** 910-915.

Kumar, O., Sridevi, O., Naidu, G.K. and Patil, B.C. (2022). Association between productivity parameters and iron deficiency chlorosis in groundnut (*Arachis hypogaea* L.). *The Pharma Innovation Journal*. **11(5):** 1123-1127.

Kumar, O., Sridevi, O., Naidu, G.K., Patil, B.C. and Choudhary, M. (2022). Genetic variability for resistance to iron Chlorosis, yield and yield related traits in segregating population of groundnut. *The Pharma Innovation Journal*. **11(5):** 1128-1133.

Land, FAO and Plant Nutrition Management (2000). Prosoil - Problem Soil Database. FAO, Rome, Italy.

Mahadevan, A. and Sridhar, R. (1986). Methods in Physiological Plant Pathology. 3rd Edn. Sivakami Publication. Madras. India. p 316.

Mann, A., Singh, A.L., Oza, S., Goswami, N., Mehta, D. and Chaudhari, V. (2015). Effect of iron source on iron deficiency induced chlorosis in groundnut. *Legume Research*. **40(2):** 241-249. doi: 10.18805/lr.v0iOF.6849.

Naidu, G.S.K., Pattanashetti, S.K, Omesh, K., Sridevi, O. and Patil, B. (2021). Differential response of groundnut geno types for iron (Fe) deficiency chlorosis tolerance and productivity traits under Fe-supplemented and Fe-non-supplemented conditions. *Indian Journal of Genetics and Plant Breeding*. **81:** 74-86.

Nsiri, K. and Krouma, A. (2023). The key physiological and bio chemical traits underlying common bean (*Phaseolus vulgaris* L.) response to iron deficiency and related interrelationships. *Agronomy*. **13(8):** 2148. https://doi.org/10.3390/agronomy13082148.

Pradhan, S.K., Pandit, E., Pawar, S., Pradhan, A., Behera, L., Das, S.R. and Pathak, H. (2020). Genetic regulation of homeo stasis, uptake, bio-fortification and efficiency enhancement of iron in rice. *Environmental and Experimental Botany*. **177:** 104066.

Rahman, M.A., Bagchi, R., El-Shehawi, A.M., Elseehy, M.M., Anee, S.A., Lee, K.W. and Kabir, A.H. (2022). Physiological and molecular characterization of strategy-I responses and expression of Fe-transporters in Fe-deficient soybean. *South African Journal of Botany*. **147:** 942-950.

Tayade, A.D., Motagi, B.N., Jadhav, M.P., Nadaf, A.S., Koti, R.V., Gangurde, S.S. and Bhat, R.S. (2022). Genetic mapping of tolerance to iron deficiency chlorosis in peanut (*Arachis hypogaea* L.). *Euphytica*. **218(4):** 46.

Volodyaev, I. and Vladimirov, Y.A. (2023). Enzymatic Sources of Free Radicals. In Ultra-weak Photon Emission from Biological Systems: Endogenous Biophotonics and Intrinsic Biolumi nescence, Cham: Springer International Publishing. pp 219-261.

Zanjare, S.R., Suryawanshi, A.V., Zanjare, S. Snehal, Shelar, V.R. and Balgude, Y.S. (2023). Screening of groundnut (*Arachis hypogaea* L.) genotypes for identification of sources of resistance against leaf spot disease. *Legume Research*. **46(3):** 288-294. doi: 10.18805/LR-4370.