Effect of iron source on iron deficiency induced chlorosis in groundnut

Anita Mann*, A.L. Singh, Shubhangi Oza, Nisha Goswami, Deepti Mehta and Vidya Chaudhari

ICAR-Directorate of Groundnut Research,
Junagadh-362 001, India.

ABSTRACT

In a field study, the effect of iron source through foliar as well as basal application was studied on lime induced iron-deficiency chlorosis (LIIC), chlorophyll content, nitrate reductase activity, available Fe and micronutrients in groundnut. The visual chlorotic rating screen of various Fe-efficient and Fe-inefficient cultivars clearly identified Fe-efficient and Fe-inefficient. Tirupati-4 was showing symptoms of chlorosis and was Fe-inefficient. Fe absorption capacity varied among cultivars. Applications of iron increased active Fe content in LGN-2 by 5.6 % and 163.18% in CSMG-84-1 respectively. A significant increase in chlorophyll content (10%) and nitrate reductase (110%) was observed with foliar spray of $FeSO_4$. A significant damage of lipid peroxidation was observed in absence of iron which was improved by 37% in Tirupati-4 and 16.67% in CSMG-84-1 by foliar and basal supplementation of Fe, respectively. A strong correlation among the Fe, Mn, Zn and K depicted ionomic interaction with different treatments. Based on the ion absorption capacity and the level of chlorosis, the groundnut genotypes were grouped as tolerant, moderately tolerant and sensitive to iron chlorosis.

Key words: Active Fe, Chlorosis, Iron deficiency, Ionomics, Nitrate reductase, Peroxidise.

INTRODUCTION

Iron deficiency is a widespread agricultural problem in many crops, especially in groundnut in calcareous and alkaline soils (Singh, 2004; Singh *et al*., 2003). In these soils, total Fe is high but occurs in chemical forms not available to plant root (Lindsay and Schwab, 1982). Plants respond to Fe limitation by inducing a series of physiological and morphological changes in the roots to facilitate the mobilization of sparingly soluble Fe compounds in the root environment (Singh and Mann, 2012). Alkaline pH tends to accentuate chlorosis problems and under these conditions, soil applications of iron sulphate are often ineffective in correcting this disorder (Singh, 2004). The narrow limit between phytotoxicity and deficiency of iron brings the need for defining appropriate rates to be used. Even on the world scale, it is estimated that Fe deficiency is widespread occurring in about 30 to 50% of cultivated soils (Cakmak, 2002).Using different chelates that are obtained by reaction between metal salts and artificial and natural complexes is the most important way for preserving iron against increasing precipitation of iron in soil with increasing pH (Koksal *et al*., 1998) and prevent LIIC. Iron chelates such as Fe-EDDHA have been shown to be highly effective in correcting Fe chlorosis in a wide range of horticultural plants growing in alkali soils. Foliar fertilization with Fe fertilizers also has been used to correct Fe chlorosis in some crops where high pH or other environmental factors may reduce root uptake of soil-applied Fe. Application of iron chelates such as

Fe-EDDHA were found highly effective in correcting Fe chlorosis in groundnut in alkali soils (Singh and Dayal 1992) while foliar application of Fe fertilizers correct Fe chlorosis in standing crop (Singh *et al*., 2003). Soil and foliar application of iron containing chelates and acidic fertilizers have been recommended as remedies for iron-deficiency chlorosis (Tagliavini *et al*., 2000; Toselli *et al*., 1997). To be effective, soil applications of Fe require either synthetic chelates or large amounts of inorganic iron fertilizers that lead to high production costs. Most of commercial chelate products contain Fe-EDDHA or Fe-EDDHMA, but their efficacy can differ (Hernandez *et al*., 1995).

A great deal of research have been conducted over the past 50 years to determine the most effective and economical methods of correcting LIIC in varoius crops (Mortvedt, 1991; Sanz *et al*., 1992; Singh *et al*. 1993; Singh 2004). Many Fe sources and methods of application have been tested during this time. However, effective treatments have not been found, yet foliar application of water soluble iron fertilizers appears to be one of the most cost-effective remedies to control Fe deficiency (Singh, 2004; Singh and Chaudhari, 1993). Unfortunately, there is no easy, inexpensive, or long-term correction for iron chlorosis. Treatments may be rather expensive and give disappointing results. Because plant and soil conditions vary greatly, there is no single approach that is consistently best. Iron is one of the essential elements but low use and less mobility for plants.

*Corresponding author's e-mail: anitadgr13@gmail.com.

Among all the micronutrients plants need iron more than others (Taiz and Zeiger, 2002). Iron (Fe) is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions (Brittenham, 1994). When active iron (Fe) is low in leaves chlorosis occurs because Fe is required by several enzymes involved in the formation of chlorophyll. The soil usually has a large amount of iron but it is not in the soluble form needed by the plant. The most soluble form in oxidized (aerated) soils is Fe $(OH)_3$, where Fe is in the Fe^{3+} form. This iron becomes less soluble at higher soil pH especially when the soil has large amounts of calcium carbonate. Plants prefer to take up the reduced form of iron Fe^{2+}. Fe uptake is genotype dependent in most of plants and thus the plants are classified as Fe-efficient and Fe-inefficient. Plants have adapted mechanisms to help extract iron from the soil. Type I plants, such as soybean, azaleas, and blueberries, excrete acids and chemical reductants from their roots. The acids make the Fe $(OH)_3$ more soluble and the reductants change insoluble Fe (III) to more soluble Fe (II). Type II plants, such as corn and grasses excrete iron chelators that bind Fe (III) and the plants are able to absorb the iron through the root. Plants do vary in their ability to get Fe out of the soil. High lime IDC (iron deficiency chlorosis) is quite common for soybean, peanut, grapes, citrus and peaches. The use of soil test for iron will generally not indicate these problems. High lime IDC is more severe in soils with finely divided lime (calcium carbonate). The fine calcium carbonate particles contact the plant root and slowly neutralize the excreted acid that is meant to solubilize iron in the soil. The effect is that the plants cannot take up iron that is in the soil and thus yellowing of leaf or chlorosis symptoms appear. Survival and productivity of crop plants exposed to environmental stresses are dependent on their ability to develop adaptive mechanisms to avoid or tolerate stress. Accumulating evidence suggest that mineral nutritional status of plants greatly affects their ability to adapt to their adverse environmental conditions.

Thus, the study was planned to evaluate the effectiveness of iron source in correcting Fe chlorosis and to determine the relationships between chlorosis symptoms and Fe source in groundnut genotypes.

MATERIALS AND METHODS

The experiment was conducted in the experimental fields of Plant Physiology section of Directorate of Groundnut Research, Junagadh during *rabi*-summer 2013. Choice of material used in this study was based on preliminary studies that were conducted to study the yield potential of nutrient efficient groundnut genotypes. Five groundnut genotypes SG- 99, GG -20, LGN -2, Tirupati-4 and CSMG-84-1 were selected for the study. The spacing was 45 x 10 cm between rows with row length of 5 m and one row per genotype. Two sets of experiments were conducted as below.

Experiment-I: Control - No fertilizer; T_1 - Recommended fertilizer application (basal application of iron source).
Experiment-II: Control - fertilization application along with micronutrients except iron source; T_2: Foliar spray of 0.5 % $FeSO_4$ at 50 DAS.

The first fully opened leaf of the main axis from 10 randomly selected plants of each genotype was taken to measure chlorophyll. The recovery from iron chlorosis has been carried out by the pigment measurement technique SPAD-502 (total index), total active Fe content in leaves, and the measurements of minerals with atomic absorption spectroscopy for Fe, Mn, Zn and flame photometry for K.

The leaves of different genotypes were observed on different stages during the cropping season to determine their VCR. 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. The VCR and percentage of chlorotic leaves were recorded at three different plant stages (40, 60, 80 days after sowing) and based on these observations, the groundnut genotypes were classified as (i) tolerant (genotypes showing dark green leaves) ii) moderately tolerant (green plants with light green young leaves) (iii) susceptible (light green to yellow plants having moderate intervenial to complete chlorosis). Average data were taken for chlorosis (%) observed at different days after emergence. For each treatment, the first fully opened leaf of the main axis from 10 randomly selected plants of each genotype was taken for all observations in the morning, placed in ice-bags and transferred to the laboratory.

Active Fe content in leaves was measured using standard spectrophotometric method of o-phenanthroline (pH 3.0). Two gram fresh chopped green and chlorotic leaves were incubated in 20 ml O-phenanthroline solution for at least 16 hrs at RT. The supernatant was filtered through whatman filter paper no 1 and Fe^{+2} activity was measured at 510 nm following the procedure of Katyal and Sharma (1955).

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

Chlorophyll (*a*) = [12.7 × A (663) – 2.69 × A (645)]xVmg/g
Chlorophyll (*b*) = [22.9 × A (645) – 4.68 × A (663)]xVmg/g

Nitrate reductase activity: 200 mg of fresh chopped leaf tissue was suspended in 5.0 ml reaction mixture containing 5.0% propanol and 0.02 M potassium nitrate in 0.1 M phosphate buffer (pH 7.5). The leaf samples were incubated in dark at 30°C. After 2 hrs, 0.4 ml of reaction mixture was mixed with 0.2 ml of 1.0% sulphanilamide (prepared in 3N HCl and 0.2 ml of 0.02 % N-napthylene diamine dihydro chloride) and kept for 20 min. Four ml of distilled water was added before measuring absorbance at 540 nm. Nitrate reductase activity was expressed as $\mu g\ NO_2\ g^{-1}h^{-1}$(McNamara *et al*, 1971).

Total protein content was measured by Bradford's assay using BSA as standard and expressed as mg/g F.W (Bradford, 1976). Peroxidase was assayed using guaiacol and enzyme activity was measured as units $min^{-1}mg^{-1}$ F.W. at 470 nm (Dias and Costa, 1983). The membrane damage (Heath and Packer, 1968) was measured in terms of lipid peroxidation (n mol g^{-1} F.W.).

Statistical analysis: All the data were subjected to variance analysis using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). Duncan's multiplication range test was applied at 5 per cent probability level to compare the mean differences. Correlation analysis was performed to determine the relationship between the traits using the Pearson coefficient.

RESULTS AND DISCUSSION

Morpho-physiological studies: The appearance of yellowing of leaves was more in control conditions, improves with foliar spray but no difference in yellowing was observed with fertilization application. In SG-99 and CSMG-84-1, VCR was 1 (green leaves) in control as well as with basal application of iron source. Similarly, in Tirupati-4, VCR was 3 (yellow leaves) in both the cases (Table 1). With foliar spray of $FeSO_4$, chlororsis was reduced in genotypes, CSMG-84-1, LGN-2 and in Tirupati-4 whereas in SG-99 and GG-20, no change in leaf colour was seen. Generally, the genotypes are classified as tolerant and sensitive to iron deficiency induced chlorosis (IDC) based on yellowing of leaves. Thus, based on VCR value, SG-99 and LGN-2 can be grouped as tolerant; GG-20 and CSMG-84-1 as moderately tolerant and Tirupati-4 as sensitive to chlorosis (Table 1). When looking at SPAD values, it can be seen that there was variability in

Table 1: Visual Chlorotic Rating (VCR) value of groundnut genotypes.

	VCR value				
	C (without fertilizer)	C (with fertilizer)	Foliar spray of $FeSO_4$	Basal Fe	Group
SG 99	1.0	1.4	1.5	1.0	T
GG-20	2.0	2.0	2.0	2.0	MT
LGN-2	1.0	1.7	1.5	1.0	MT
Tirupati 4	3.0	2.8	2.5	3.0	S
CSMG 84-1	1.0	2.1	1.6	1.0	T

the SPAD values of the first trifoliates when plants were grown under high or low Fe conditions. The highly susceptible line Tirupati-4, which showed low SPAD values in control conditions, also showed the lowest SPAD values in the first trifoliate leaves, with both sources of iron application (Fig. 1b). The remaining lines showed intermediate SPAD reading values. Generally the VCR are negatively correlated with SPAD reading, hence, in this study, no correlation was found between SPAD values relative to visual chlorosis rating values. Vasconcelos and Grusak (2013) also found that soybean lines that had shown higher IDC tolerance in the field also showed higher SPAD values when grown in laboratorial Fe-limiting conditions. Fe is important in chlorophyll formation, photosynthesis, enzyme systems, chloroplast development and respiration of plants (Miller *et al*., 1995; Halvin *et al*., 1999; Singh 2004; Singh and Chaudhari 1991). Under iron deficiency loss of chlorophyll in the younger leaves is the common visible symptom. El-Gharbi *et al*. (1994) suggested that the measurement of chlorophyll concentration is the best method for assessing iron chlorosis. In comparison to control, both iron supplementations (foliar spray of $FeSO_4$ as well as basal application) brought significant increased chlorophyll content in all the groundnut genotypes except Tirupati-4 (basal application, Fig. 1A). In case of basal iron application, marginal increase was observed in Tirupati 4 followed by GG-20 while other genotypes showed increase in chlorophyll content. Maximum increase was observed in LGN-2 i.e., 39.46 per cent increase in chlorophyll content as compared to control (without fertilizer application). With foliar application, maximum content was found in SG 99 but maximum increase was observed in Tirupati-4 (Fig., 1A). Iron is directly or indirectly involved in the production of chlorophyll and deficiency of iron irreversibly damages chlorophyll synthesis (Jacobson and Oertli, 1956). The common precursor for chlorophyll and heme synthesis is δ-aminolevulinic acid (ALA) and the rate of ALA formation is controlled by iron (Miller *et al*. 1995). Iron application increased chlorophyll contents as compared with the control, but there was no marked difference between iron rates on these traits. That was perhaps due to the association of Fe with chlorophyll formation (Mazaherinia *et al*., 2010). This result was in agreement with (Borowski and Michalek, 2011; Ai-Qing *et al*., 2011; Amanullah *et al*. 2012; Mohsen, 2013 and Kobraee *et al*., 2011) who demonstrated that adding Fe alone or in combination with other micronutrients increased chlorophyll content of plants.

Nitrate reductase is a complex enzyme that contains iron in one of its subunits (Crawford *et al*., 1992) and lack of iron can reduce NR activity (Marschner, 1995). Nitrate reductase activity was less under both the control conditions i.e. (without fertilizer and with fertilizer) which increased with supply of iron source. A significant increase in NR was
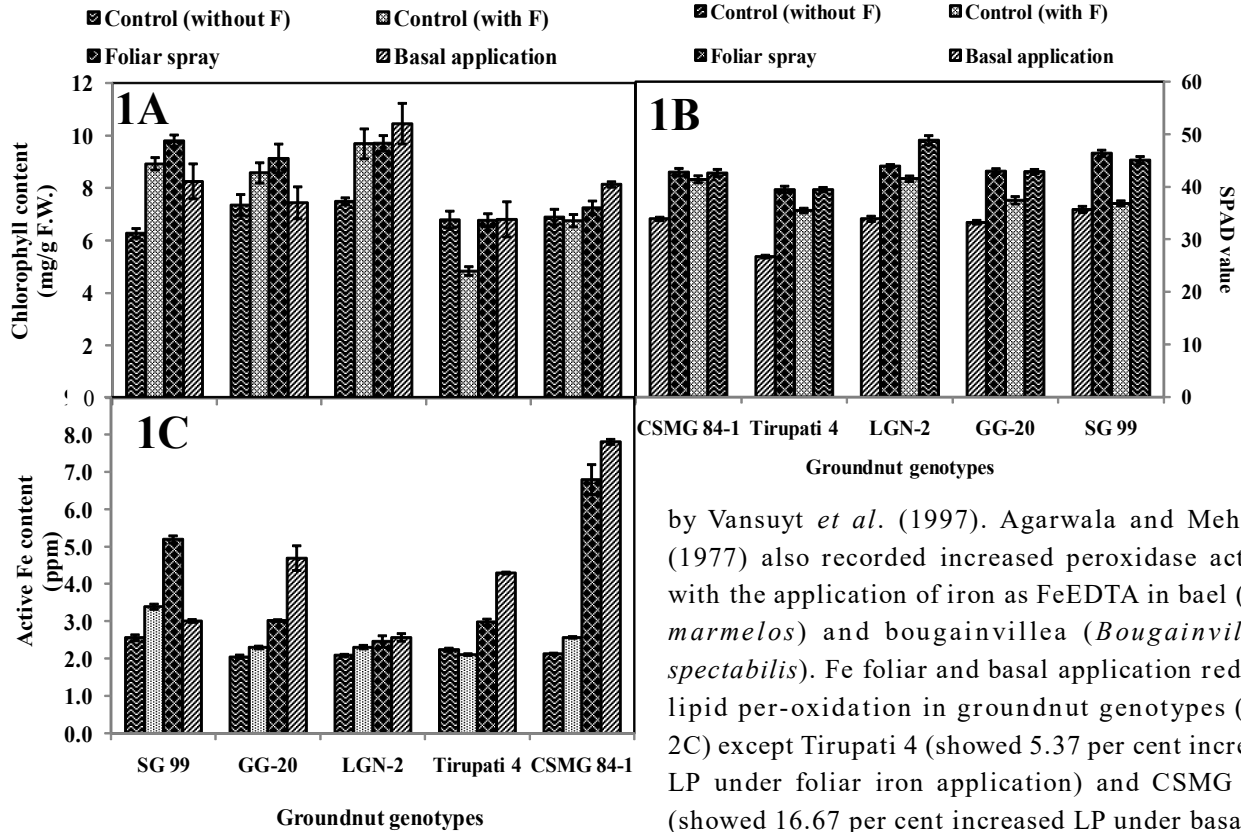
observed with foliar spray of $FeSO_4$ than with basal application. The increase in enzyme activity was more in resistant check than the susceptible one. Under foliar application (Fig., 2D), SG -99 showed maximum increase (48.19 µg NO_2 g^{-1} hr^{-1}) in NR activity from the control (9.65 µg NO_2 g^{-1} h^{-1}) while genotype Tirupati 4 showed the least increase in NR activity (22.79 µg NO_2 g^{-1} h^{-1}) than their respective control (21.91 (48.19 µg NO_2 g^{-1} hr^{-1}). In our earlier studies also, groundnut leaves showed lesser nitrate reductase activities at deficient levels of Fe, Mn, Zn, Cu and Mo and toxic levels of Zn and Cu (Singh *et al*., 1995). Iron directly influences the activity of nitrate reductase enzyme as it constituted the prosthetic group (4Fe-4S) of this enzyme. Iron is also a part of ferredoxin, which on reduction provides the required reductant for the activity of nitrate reductase. Hence, reduction in nitrate reductase activity was observed when the genotypes grown in iron limiting conditions (Kaushik and Mishra, 2015). In case of basal application, mean NR activity increases from 34.42 µg NO_2 g^{-1} hr^{-1} to 39.67 µg NO_2 g^{-1} hr^{-1} (Fig., 2D). The reduction of NR activity under iron limiting conditions might be the result of a decrease in ferrodoxin, the electron donor for the NR, under iron deficiency. The decrease in ferrodoxin may down-regulate NR activity to prevent nitrite accumulation, which is toxic to the plant. The ferrodoxin content and NR activity are restored upon iron resupply (Marschner, 1995). The increase in NR activity after iron was resumed to nutrient solutions in the present experiment also supports this idea. Poonnachit and Darnell (2004) have found in *Vaccinium* species that iron concentration had a significant effect on root NR activity during the -Fe treatment period. NR activity was greater under iron-deficient compared with iron-sufficient conditions at week 3. However, by week 5, NR activity under iron- deficient conditions was lower than under iron sufficient conditions. When iron supply was resumed to -Fe plants at week 6, NR activities in these plants increased to rates similar to + Fe plants.

A significant increase in active Fe content was observed in all the genotypes with foliar as well as basal application of iron source. With foliar application, mean active Fe content increased from 2.55 to 4.09 and followed the trend i.e. CSMG 84-1 (163.18 %) > SG- 99 (52.94 %) >Tirupati 4 (41.04 %) > GG-20 (30.04 %) > LGN-2 (5.6 %) over their respective control (Fig., 1C). Similar, result of increase was observed with basal application of iron source. The percent increase in active Fe content differed between varieties. This can be explained by the variations between varieties in their response to the treatments (Türemifl *et al*., 1997). The difference in active Fe content among the varieties may be caused by different Fe absorption capacities through the leaves and roots (Römheld and Kramer, 1983; Marschner *et al*.1987).
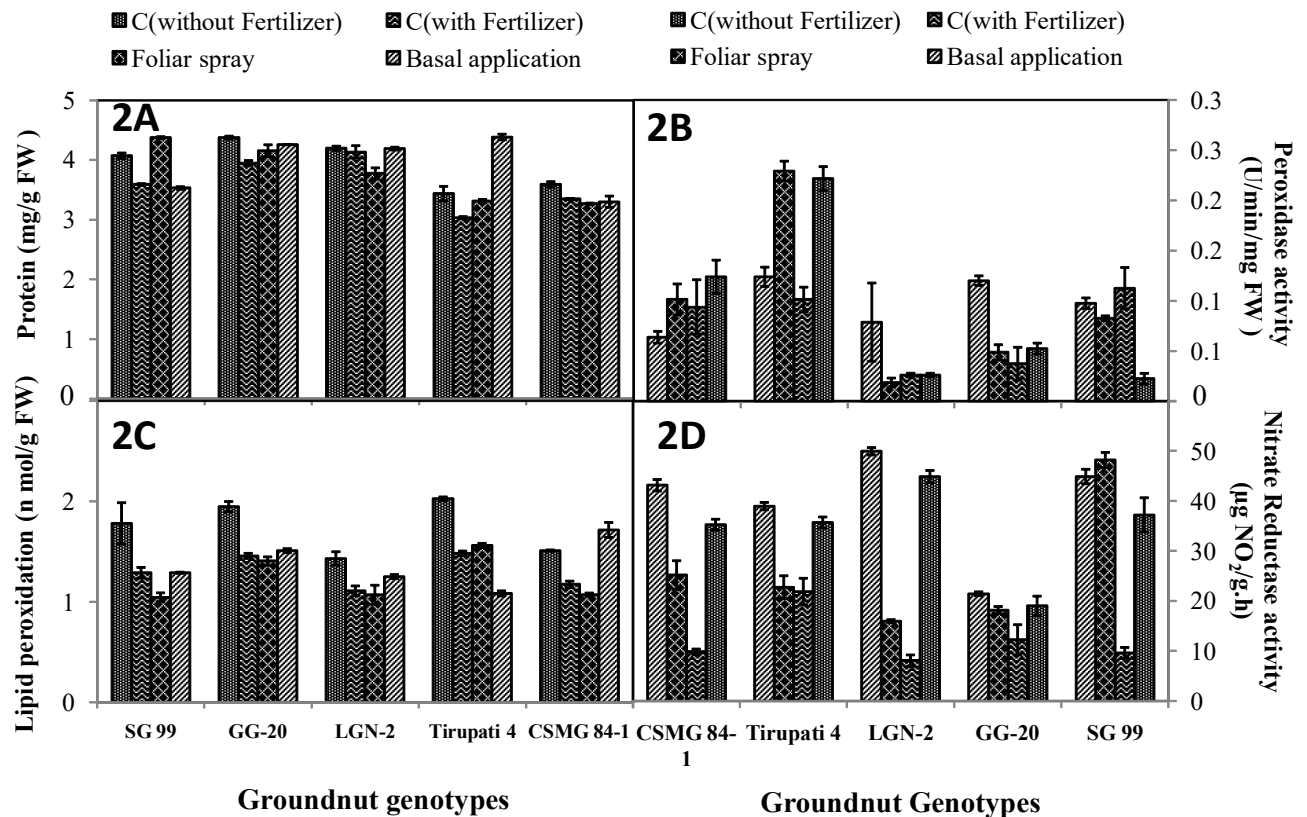
Iron foliar application caused an increase in protein content, compared with control treatment in all the groundnut genotypes except LGN-2 and CSMG 84-1 whereas no significant effect was observed with basal iron application. On the other hand, all the genotypes showed decreased protein content except Tirupati 4 (29.41 per cent increase) under basal iron application (Fig., 2A). This may be due to the importance of iron for chlorophyll formation, photosynthesis and enzyme systems and respiration of plants (Havlin *et al*, 1999). Also, Iron plays role in biological redox system, enzyme activation and oxygen carrier in nitrogen fixation (Romheld and Marschner, 1991). Zeidan *et al*. (2010) reported that application of 1% $FeSO_4$ increased wheat grain protein and Fe contents. A strong correlation was found in total protein content and chlorophyll (Table 2). In accordance with our results, Monsef-Afshar *et al*. (2012) reported that application of 1 per 1000 nano-iron chelate increased Fe and protein content of cowpea seed.

Iron is the main part of a cofactor for many antioxidant enzymes. On the other hand, it can function as a prooxidant since it catalyzes free radicals through Fenton reaction. It has been shown that many enzymes such as catalase and superoxide dismutase in order to perform well play a role in controlling reactive oxygen species (ROS). Production of ROS, like superoxide onion radical (O^{-}_{2}), singlet oxygen ($^{1}O_2$), hydrogen peroxide (H_2O_2) and hydroxide radicals (OH^{-}) can damage many cellular compounds such as proteins, membrane lipids, and nucleic acids. Plant cells respond to ROS formation by increased production of metaloenzymes such as superoxide dismutase, catalase, peroxidase, and specially ascorbate peroxidase which protect them against oxidative damage causing many oxidative stresses (Halliwell and Gutteridge, 1987). The effect of iron deficiency on ROS system was studied in terms of peroxidase enzyme and lipid peroxidation. For peroxidase activity, no significant effect was noticed under both the iron supplementation in all the genotypes. The highest activity of peroxidase enzyme was obtained in Tirupati 4 (0.237 U/min/mg FW) when foliar iron application was used (Fig., 2B) and genotypes GG-20 and LGN-2 showed maximum activity (0.117 and 0.116 U/min/mg FW) when basal iron application was used whereas the lowest activity of peroxidase enzyme was obtained in LGN-2 (0.016 U/min/mg FW) with foliar spraying and in CSMG 84-1 (0.058 U/min/mg FW) with basal application in comparison with their respective control. Micronutrients, especially Fe act as metal components of various enzymes and also Fe is associated with saccharide metabolism, photosynthesis and protein synthesis. Iron has important functions in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase, as well as in photorespiration, the glycolate metabolism and chlorophyll content (Marschner, 1995; Sharma, 2002). The mean activity of POX was significantly increased by both iron applications (Fig., 2B). The increase in the activity of enzyme might be due to triggering induction of POX genes expression by iron application as reported in *Brassica napus*

■ Control (without F)   ▨ Control (with F)   ▨ Control (without F)   ▨ Control (with F)
▨ Foliar spray   ▨ Basal application   ▨ Foliar spray   ▨ Basal application

by Vansuyt *et al.* (1997). Agarwala and Mehrotra (1977) also recorded increased peroxidase activity with the application of iron as FeEDTA in bael (*Agle marmelos*) and bougainvillea (*Bougainvillaea spectabilis*). Fe foliar and basal application reduced lipid per-oxidation in groundnut genotypes (Fig., 2C) except Tirupati 4 (showed 5.37 per cent increased LP under foliar iron application) and CSMG 84-1 (showed 16.67 per cent increased LP under basal

■ C(without Fertilizer)   ▨ C(with Fertilizer)   ■ C(without Fertilizer)   ▨ C(with Fertilizer)
▨ Foliar spray   ▨ Basal application   ▨ Foliar spray   ▨ Basal application

iron application). This significant decrease in lipid peroxidation was in favour for growth and could be attributed to the protection of polysaturated fatty acids of cell membrane from oxidative damage (Asada, 1999). A significant correlation between peroxidase activity and lipid peroxidation (Table 3) depicts the scavenging system of ROS.

The micronutrients viz., Fe, Mn, Zn and K were analyzed on atomic absorption spectrophotometer (AAS) after triacid digestion of dried leaves. The concentration of Fe in dry matter was increased with foliar source of iron but no significant increase in total Fe concentration was found with basal application of fertilizer whereas Mn concentration increased with foliar spray (Table 2). Kobraee et al (2011) have found in soybean that high soil concentration of manganese and iron had negative effects on zinc absorption. There was a significant and negative correlation between manganese and iron concentration in seed and Mn and Fe

concentration in leaf. Similarly, in present investigation, Mn and Zn concentration was more in leaves without any treatment but it decreased with fertilizer application. On the other hand, with foliar application of $FeSO_4$, Mn and Zn concentration increased in leaves. This shows that the absorption of these two micronutrients was inhibited by the presence of iron in soil but in the leaves this inhibitory effect was not present. Ghasemi-Fasaei et al. (2003) reported that application of iron decreased mean Mn concentration by 91% in soybean. Admittedly, transportation of iron and manganese from roots to shoots affected by antagonistic effects of these elements and thus it differ from the uptake by roots from the soil. Interactions among micronutrients affect their uptake, distribution, and utilization in plants (Zhao et al, 2011). Many studies have examined these interactive effects, especially between Fe and Zn. For example, Sliman (1990) reported antagonism between Fe and Zn in soybean. Other studies have found that Fe reduced Mn concentrations in Indian

TABLE 2. Pearson Correlation Coefficient for trait association in groundnut genotypes.

Traits	Protein content	Peroxidase activity	Lipid peroxidation	Nitrate reductase activity	Active Fe content	Chlorophyll content	SPAD value
Protein content	1	-0.531**	-0.324	0.219	0.011	0.477**	0.231
Peroxidase activity		1	0.454*	0.230	0.204	-0.185	-0.616**
Lipid peroxidation			1	-0.196	-0.469**	-0.416*	-0.435*
Nitrate reductase activity				1	0.609**	-0.162	-0.392*
Active Fe content					1	-0.100	-0.222
Chlorophyll content						1	0.330
SPAD value							1

TABLE 3. Effect of iron source on mineral nutrient contents in groundnut genotypes.

Foliar Spray of iron source (FeSO₄)

Genotypes	Fe (mg g⁻¹dw)		Mn (ppm)		Zn (ppm)		K (%)	
	C	T	C	T	C	T	C	T
SG 99	8.47	9.69	46.00	54.66	69.33	58.66	1.113	1.143
GG-20	6.26	8.46	48.66	57.00	54.33	63.33	1.030	0.927
LGN-2	8.26	9.03	53.66	51.66	37.00	46.33	0.787	0.940
Tirupati 4	6.83	7.78	45.66	51.66	52.66	51.66	0.627	0.770
CSMG 84-1	7.56	13.12	53.00	69.33	38.00	41.33	0.947	1.040
Mean	7.48	9.62	49.40	56.86	50.26	52.26	0.901	0.964
	T = 0.024 ± 0.008		T = 1.37 ± 0.461		T = 1.07 ± 0.35		T = 0.44 ± 0.015	
CD @ 5%	G = 0.038 ± 0.012		G = 2.16 ± 0.73		G = 1.63 ± 0.54		G = 0.07 ± 0.023	
	T × G = 0.053 ± 0.018		T × G = 3.06 ± 1.03		T × G = 2.30 ± 0.77		T × G = 0.10 ± 0.033	

Basal application of iron source

Genotypes	Fe (mg g-1dw)		Mn (ppm)		Zn (ppm)		K (%)	
	C	T	C	T	C	T	C	T
SG 99	4.86	4.86	51.66	43.66	63.00	65.00	1.27	1.21
GG-20	4.07	4.23	49.33	51.00	45.66	50.66	1.02	1.32
LGN-2	4.56	4.59	50.66	47.00	52.33	55.66	1.25	1.44
Tirupati 4	3.87	5.18	46.33	51.33	46.33	43.33	1.07	1.01
CSMG 84-1	10.36	13.67	58.33	54.33	57.66	58.67	1.14	0.92
Mean	5.548	6.507	51.26	49.46	53.00	54.66	1.15	1.18
	T = 0.069 ± 0.057		T = 1.74 ± 0.587		T = NS		T = 0.18 ± 0.006	
CD @ 5%	G = 0.267 ± 0.090		G = 2.76 ± 0.93		G = 3.05 ± 1.02		G = 0.03 ± 0.009	
	T × G = 0.038 ± 0.012		T × G = 3.90 ± 1.31		T × G = NS		T × G = 0.04 ± 0.014	

mustard (Hamlin *et al*., 2008) and in soybean leaves (Izaguirre-Mayoral and Sinclair, 2005), but increased Mn concentrations in soybean shoots. Other authors reported a negative correlation between Zn and Cu (Pearson *et al*., 2008; Kumar *et al*., 2009). Murphy *et al*. (2008) found a significant correlation between Cu and Mn in spring wheat. Based on these observations, the iron absorption capacity and its effectiveness in correcting iron deficiency induced chlorosis can be defined. In order to devise breeding and genetic transformation programs that aim at generating high-yielding and IDC-tolerant groundnut lines, it is necessary to better understand the mechanisms that enable tolerant plants to survive under Fe-limiting conditions. Since the improvement of plant foods as sources of essential mineral nutrients can be accomplished by: increasing the concentration of the nutrient(s) and maintaining bioavailability; maintaining the concentration and improving bioavailability; or increasing both concentration and availability of the selected nutrient(s). A strategy that exploits genetic variability to breed staple crops with enhanced ability to fortify themselves with micronutrients (genetic biofortification) offers a sustainable, cost-effective alternative to conventional supplementation and fortification programs. Moreover, deeper understanding of genetic control mechanisms of iron acquisition and metabolism and development of molecular markers will facilitate breeding programs for developing iron rich crops.

CONCLUSION

This work showed that foliar application of iron source can be used to increase leaf iron concentration in groundnut plants suffering from lime induced iron deficiency chlorosis at initial stages of chlorosis but for a longer treatment to correct the iron deficiency disorder basal application of iron source is more beneficial. These studies can be helpful in developing iron rich crops or crop products.

ACKNOWLEDGEMENT

REFERENCES

Agarwala, S.C. and Mehrotra, N.K. (1977). Catalase and peroxidase in leaves of iron deficient plants. *Proc. Indian Acad. Sci,*. 86B:55–60.

Ai-Qing, Z., Qiong-Li, B., Xiao-Hong, T., Xin-Chun, L. and Gale, W.J. (2011). Combined effect of iron and zinc on micronutrient levels in wheat (*Triticum aestivum* L.). *J. Environ. Biol.*, **32:** 235-239.

Amanullah, M.M., Archana, J., Manoharan, S. and Subramanian, K. S. (2012). Influence of Iron and AM Inoculation on Metabolically Active Iron, Chlorophyll Content and Yield of Hybrid Maize in Calcareous Soil. *J. Agron.,* **1:** 27-30.

Arnon, D.I. (1949). Copper enzyme in isolated chloroplasts polyphenolooxidase in Beta vulgaris. *Plant Physiol*., **24:**1–15.

Asada, K. (1999). The water-cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Ann. Rev. Plant Physiol. Plant Mol. Biol*., **50:** 601-639.

Borowski, E. and Michalek, S. (2011). The effect of foliar fertilization of French bean with iron salts and urea on some physiological processes in plants relative to iron uptake and translocation in leaves. *Acta Sci. Pol. Hortorum. Cultus*., **10:** 183-193.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72:** 248-254.

Brittenham, G.M. (1994). New advances in iron metabolism, iron deficiency and iron overload. *Curr. Opin. Hemato.*, **1:** 549-556.

Cakmak, I. 2002. Plant nutrition research: Priorities to meet human needs for food in sustainable way. *Plant Soil*., **247:** 3-24.

Crawford, N.M., Wilkinson, J. Q. and LaBrie, S.T. (1992). Control of nitrate reduction in plants. *Aust. J. Plant Physiol*., **19:** 377-385.

Dias, A. and Costa, M. (1983). Effect of low salt concentrations on nitrate reductase and peroxidase of sugar beet leaves. *J. Exp. Bot*., **34:**537–543.

El-Gharbi, A., Jraidi, B. and Barbera, G., (1994). Performance of rootstocks of almond, peach and peach × almond hybrids with regard to iron chlorosis. *Acta Horti.*, **373:** 91–97.

Ghasemi-Fasaei, R., Ronaghi, A., Maftoun, M., Karimian, N. and Soltanpour, P.N. (2003). Influence of Fe-EDDHA on iron- manganese interaction in soybean genotypes in a calcareous soil. *J. Plant Nutr,*. **26:** 1815-1823.

Halliwell, B. and Gutteridge, J. M. (1987). In: Free Radicals in Biology and Medicine. (Halliwell, B. and Gutteridge, J. M. Eds.).'Protection against oxidants in biological system: the superoxide theory of oxygen toxicity'. P 86-123 Clarendon Press. Oxford.

Hamlin, R.L. and Barker, A.V. (2008). Nutritional alleviation of zinc-induced iron deficiency in Indian mustard and the effects on zinc phytoremediation. *J. Plant Nutr.*, **31:** 2196-2213.

Havlin, J.L., Beaton, J.D. Tisdale, S.L. and Nelson, W.L. (1999). Soil Fertility and Fertilizers-An Introduction to Nutrient Management 6th Ed. Prentice Hall, New Jersey.

Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophy.*, **125:** 189-198.

Hernandez, A., Garate, A. and Lucena, J. (1995). Efficacy of commercial Fe (III)-EDDHA and Fe (III)-EDDHMA chelates to supply iron to sunflower and corn seedlings. *J. Plant Nutr.*, **18:** 1209–1223.

Izaguirre-Mayoral, M.L. and Sinclair,T.R. (2005). Soybean genotypic difference in growth, nutrient accumulation and ultrastructure in response to manganese and iron supply in solution culture *Annl. Bot.*, **96:** 149-158.

Jacobson, L. and Oertli, J.J. (1956). The relation between iron and chlorophyll contents in chlorotic sunflower leaves. *Plant Physiol.*, **31:**199–204.

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

Kaushik, M. S. and Mishra, A. K. (2015). Iron induced modifications in physiological attributes and SDS-PAGE of whole cell proteins pattern of Anabaena PCC 7120 and its derivative ntcA mutant. *Indian J. Biotech.*,**14:** 87-93.

Kobraee, S., Mohamadi, G.N., Hosein, H.S., Kajori, F.D. and Delkhosh, B. (2011). Influence of micronutrient fertilizer on soybean nutrient composition. *Indian J. Sci. Tech.*, **4:** 763-769.

Koksal, A.L., Dumanoglu, H., Tuna, G. and Aktas, M. (1998). The effects of different amino acid chelate foliar fertilizers on yield, fruit quality, shoot growth and Fe, Zn, Cu, Mn content of leaves in Williams's pear culivar (*Phyrus communis* L.). *Turk J. Agri.* **23:** 651-358.

Kumar, R., Mehrotra, N.K., Nautiyal, B.D., Kumar, P. and Singh, P.K. (2009). Effect of copper on growth, yield and concentration of Fe, Mn, Zn and Cu in wheat plants (*Triticum aestivum* L.). *J. Environ. Biol.*, **30:** 485-488.

Lindsay, W.L. and Schwab, A.P. (1982). The chemistry of iron in soil and its availability to plants. J. *Plant Nutr.*, **4:** 821-840.

Marschner, H. (1995). Mineral nutrition of higher plants, 2nd edn. SanDiego: Academic Press.

Marschner, H., Romheld, V. and Cakmak, I. (1987). Root-induced changes of nutrient availability in the rhizosphere. *J. Plant Nutr.*, **10:** 1175-1184.

Mazaherinia, S., Astaraei, A.R., Fotovat, A., Monshi, A. (2010). Nano iron oxide particles efficiency on Fe, Mn, Zn and Cu concentrations in wheat plant. *World Applied Sci. J.*, **7:** 36-40.

McNamara, A. L., Meeke, G. B. R., Shaw, P. D. and Hageman, R. H. (1971). Use of dissimilatory nitrate reductase from *Escherichia coli* and formate as a reductive system for nitrate assays. *Agric. Food Chem.*, **19:** 229-231.

Miller, G.W., Huang, I.J., Welkie, G.W. and Pushmik, J.C. (1995). In: Iron nutrition in soils and plants. (Abadia J. Ed). Function of iron in plants with special emphasis on chloroplasts and photosynthetic activity. Kluwer Academic Publishers, Dordrecht;

Mohsen, K. (2013). Effect of Foliar Application of Iron and Zinc on Growth and Productivity of Cucumber. *Bull. Environ. Pharma. Life Sci.*, **2:** 11-14.

Monsef Afshar, R., Hadi, H. and Pirzad, A. (2012). Effect of Nano-iron foliar application on qualitative and quantitative characteristics of cowpea, under end season drought stress. *Int. Res. J. App. Basic Sci.*, **3:** 1709-1717.

Mortvedt, J. (1991). Correcting iron deficiencies in annual and perennial plants: Present technologies and future prospects. *Plant Soil.*, **130:** 273–279.

Murphy, K.M., Reeves, P.G. and Jones, S.S. (2008). Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars. *Euphytica.*, **163:** 381-390.

Murphy, L.S. and Walsh, L. M. (1972). In: Micronutrients in Agriculture (eds. J. J. Mortvedt, P.M. Giordano and W.L. Lindsay). Correction of micronutrient deficiencies with fertilizers, p. 347-387.. Soil. Sci. Amer., Madison, Wis.

Pearson, J.N., Rengel, Z., Jenner, C.F. and Graham, R.D. (2008). Manipulation of xylem transport affects Zn and Mn transport into developing wheat grains of cultured ears. *Physiol. Plant.*, **98:** 229-234.

Poonnachit and Darnell. (2004). Effect of ammonium and nitrate on ferric chelate reductase and nitrate reductase in *Vaccinium* species. *Ann. Bot.*, **93:** 399-405.

Romheld, V. and Kramer, D. (1983). Relationship between proton efflux and rhizodermal transfer cells induced by Fe deficiency. *Z. Pflanzen Physiol.*, **113:** 73-83.

Romheld, V. and Marschner, H. (1991). Genotypic differences among graminaceous species in release of phytosiderophores and uptake of iron of phytosiderophores. *Plant Soil.*, **123:** 147-153.

Sanz, M., Cavero J. and Abadia J., (1992). Iron chlorosis in the Ebro river basin, Spain. *J. Plant Nut.*, **15:** 1971–1981.

Sharma, A.K., (2002). In: Biofertilizers for Sustainable Agriculture. Agrobios, Jodhpour, India.

Singh A.L, Basu, M.S. and. Singh, N.B. (2003). Iron deficiency chlorosis and its management in groundnut. National Research Center for groundnut (ICAR), Junagadh, India. 30 p.

Singh, A. L. and Chaudhari, Vidya. (1991). Screening of groundnut varieties tolerant to iron chorosis. *Indian J. Agri. Sci*., **61:** 925-927.

Singh, A.L. (2004). Mineral nutrient requirement, their disorders and remedies in Groundnut. pp. 137-159. In: Groundnut Research in India (Eds. M.S. Basu and N. B. Singh) National Research Center for Groundnut (ICAR), Junagadh, India

Singh, A.L. and Mann, Anita (2012). Recent Advances in Plant Nutrition. In: Proc National Seminar of Plant Physiology on "Physiological and molecular approaches for development of climatic resilient crops" 12-14 Dec 2012, ANGRAU, Hyderabad, India pp. 6-22.

Singh, A.L. and Chaudhari, Vidya. (1993). Screening of groundnut germplasm collection and selection of genotypes tolerant of lime-induced iron-chlorosis. *J. Agri. Sci.*, Cambridge, **121:** 205-211.

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

Singh, A.L., Chaudhari, Vidya. and Koradia, V.G. (1993). Spray schedule of multi-micronutrients to overcome chlorosis in groundnut. *Indian J. Plant Physiol*., **36:**35-39.

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

Sliman, Z.T. (1990). Effect of zinc on iron-stress-response mechanism of two soybean genotypes. *Agric. Sci.*, **2:** 61-69.

Tagliavini, M., Abadia, J., Rombola, A., Abadia, A., Tsipouridis, C. and Marangoni, B. (2000). Agronomic means for the control of iron deficiency chlorosis in deciduous fruit trees. *J. Plant Nut.*, **23:** 11–12.

Taiz, L and Zeiger, E. (2002). Plant physiology, Sinauer Associates, Inc. Sunderland, Massachusetts.

Toselli, M., Tagliavini, M., Marangoni B. and Sansavini, S. (1997). Iron chlorosis of peaches: recognition, prevention and therapy. In: XXII Convegno Peschicolo, Cesena, Italy. P 28–30

Türemifl, N., Ozguven., A.L., Paydas S. and Idem. G. (1997). Effects of sequestrene Fe-138 as foliar and soil application on yield and earliness of some strawberry cultivars in the subtropics. ISHS Acta Horticulture 441, V Temperate Zone Fruit in the Tropics and Subtropics Adana, Turkey.

Vansuyt, G., Lopez, F., UGent, D.I. and Briat, J.F. (1997). Iron triggers a rapid induction of ascorbate peroxidase gene expression in *Brassica napus. FEBS Letters*., **410:** 195-200.

Vasconcelos, M.W. and Grusak, M.A. (2013). Morpho-physiological parameters affecting iron deficiency chlorosis in soybean (*Glycine max* L.). *Plant Soil*., **374:** 161-172.

Zeidan, M. S., Manal, F. and Hamouda, H. A. (2010). Effect of Foliar Fertilization of Fe, Mn and Zn on Wheat Yield and Quality in Low Sandy Soils Fertility. *World J. Agric. Sci*., **6:** 696-699.

Zhao, A.Q., Q.Li. B., Hong, T. X., Chun, X. L. and Gale, W.J. (2011). Combined effect of iron and zinc on micronutrient levels in wheat (*Triticum aestivum* L.). *J. Environ. Biol*., **32:** 235-239.