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Telephone : 91-0285-2673041/ 2672461

Fax: : 91-0285-2672550/ 267382

E-mail : Alsingh16@gmail.com

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Cover photo : Field view of screening trial on iron- chlorosis in calcareous soil

Backpage photo : Field showing severe iron-chlorosis (Top) and reaction of tolerant and susceptible lines at peak growth stages (bottom)

Preface

Groundnut (*Arachis hypogaea* L) is an important food legume and oilseed crop of tropical and subtropical areas of the world and grown on about 25 million hectares of land. Though it is now being cultivated in about 100 countries in different agro-climatic regions, India, China, USA, Senegal, Indonesia, Nigeria, Brazil and Argentina are the major groundnut producing countries. As groundnut require only about 400-500 mm (500-700 mm evapo-transpiration) of water its cultivation has been extended on almost all soil types in tropical countries, however, due to its underground fruiting habit, drought tolerance, high calcium requirement and tremendous nitrogen fixing capacity, it is mainly grown on light textured poor fertility soils of semiarid region where soils are mostly calcareous and alkaline and occurrence of iron-chlorosis, is very common. Though the average yield of groundnut in the world is around 1300 kg ha^{-1}, about 70% of the world groundnut production occurs in the semi-arid tropics with average yield around 800 kg ha^{-1}.

In India, more than one-third of the soil is calcareous and spread mostly in the low rainfall areas of the western (Gujarat, Maharashtra, Rajasthan and Karnataka) and central (M.P.,U.P.) parts of the country where groundnut is a major crop. The calcareous soils is most suitable for groundnut cultivation mainly due to its high Ca and cation contents and their easy accessibility by pods, good soil aeration and peg penetration due to looseness of soil. However, iron-chlorosis is of common occurrence in these soil causing considerable yield reductions in groundnut. The intensity of iron-chlorosis is more during rabi-summer than kharif season.

Looking to the severity of this malady and survival of groundnut crop on calcareous soil, the National Research Centre for Groundnut at Junagadh, took a lead in the country and continuously worked for about 20 years to find out the causes, diagnosis of symptoms and preventive measures through applications of iron containing fertilizers and chelates, time and their mode of application, and selection of iron-efficient genotypes and published the finding in various research papers and reports. In this bulletin, an effort was made to synthesize the causes, diagnosis of symptoms and all the practical and cost effective solutions for the prevention and correction of iron-chlorosis in groundnut. The implementation of which will increase its productivity, quality and availability. As, visible symptoms are often used to help identify the disorder, the photograph described were taken either directly from field or developed in sand culture experiment.

We, thankfully, acknowledge the contribution of scientists and researchers, situated mainly in western parts of the country and engaged in iron nutrition of groundnut who have worked very hard and generated valuable information's on iron-chlorosis of groundnut, which has been compiled in this bulletin. The help received from the mineral nutrition group of NRCG specially Mr Y.C. Joshi, Mrs. Vidya Chaudhari, Mr V.G. Koradia, and Mr P.V. Zala in the various ways is acknowledged.

We hope that this bulletin will prove valuable for managing the iron-chlorosis in groundnut and the physiologist, agronomists, soil scientists and extension workers using it will in turn pass on this knowledge to the farmers to increase their pod yield.

M.S. Basu A. L. Singh N. B. Singh

Contents

1. Introduction

The lime-induced iron-deficiency chlorosis (LIIC), commonly known as iron-chlorosis, is of common occurrence in groundnut, in calcareous and alkaline soils world-wide. In India, more than one-third of the soil is calcareous and spread mostly in the low rainfall areas of the western (Gujarat, Maharashtra, Rajasthan and Karnataka) and central (M.P., U.P.) parts of the country where groundnut is a major crop. However, this deficiency is more prevalent in Saurashtra region of Gujarat, Marathwada region of Maharashtra, and part of Rajasthan, Tamil Nadu and Karnataka, causing considerable yield reductions. This Fe-deficiency chlorosis occurs as an interveinal to complete chlorosis in young and emerging groundnut leaves, which depending upon the intensity of the chlorosis, may recover with crop growth stages or in extreme cases, may result in death of plant causing little yield losses to complete crop failure. Also in India, approximately 7 % population is facing iron deficiency and groundnut is a good source of iron.

This calls for the attention of the researchers to find out the cost effective measures for amelioration of iron-chlorosis of groundnut. Looking to the severity of the problem, National Research Centre for Groundnut took a lead and continuously worked for about 20 years to find out the causes, diagnosis of symptoms and preventive measures through applications of iron containing fertilizers and chelates, time and their mode of application, and selection of iron-efficient genotypes. An effort was made in this bulletin, to synthesize all the practical and cost effective solutions for the prevention and correction of this deficiency in groundnut.

2. Occurrence, causes and yield losses

Though iron is the fourth most abundant element in the soil, still its deficiency in plant is most widespread. In Saurashtra region and many other part of India where groundnut is grown on calcareous soils, chlorosis due to iron deficiency is a major problem and is of common occurrence (Plate 1). Under unfavourable soil condition, this chlorosis appears 15 days after emergence (DAE) of seedlings and continues to occur on the young developing leaves throughout the crop growth period, however, its maximum intensity in the field was noticed during 30-70 DAE, the peak vegetative growth period (Singh et al., 1987, 1995). Under favourable soil condition, there is automatic recovery of this chlorosis with crop growth as the leaves become older, but under adverse conditions the newly emerging leaves continue to show chlorosis. Under severe deficiency, this may sometimes result in death of leaves and plant causing crop failure.

The groundnut is grown during kharif as well as during rabi-summer seasons. Survey in the farmer's fields indicated severe to mild chlorosis, in groundnut leaves, the intensity of which was more during rabi-summer than that during kharif season. In calcareous soils of Saurashtra, Gujarat more than 60 % groundnut field showed chlorosis during rabi-summer season compared with less than 20 % during kharif season. Kharif is the main cropping season of groundnut in Saurashtra, where groundnut crop is grown as rain fed, and rest of the time the field lies fallow and hence the intensity of chlorosis was less during kharif season due to mono-cropping. Only on a limited area, depending upon the irrigation facility, the second crop of groundnut is also grown as rabi-summer season crop. In this season, most of the farmers give frequent irrigation mainly due to the high evapo-transpiration demand of the crop. As a result, majority of the groundnut fields show chlorosis and remain chlorotic throughout the cropping season.

a

b

Plate 1. Groundnut crop showing iron-chlorosis in calcareous soil of Saurashtra, India during rabi-summer (a) and kharif seasons (b).

Also, the groundnut-groundnut cropping sequence leads to multi-micronutrient deficiencies during both the seasons and groundnut-wheat sequence shows multi-micronutrient deficiencies in groundnut during kharif season in many parts of India. In some of the fields, the chlorosis becomes so severe that there is no development of peg and pod and finally plant dies. The visual symptoms and elemental analysis indicated that more than 70 % of the chlorotic plants showed Fe-deficiency and only 30 % plant S-deficiency during rabi-summer season. In contrast, during kharif season about 60 % of the chlorotic plants showed S-deficiency and only 40 % Fe-deficiency. In Andhra Pradesh, though the soil is rich in iron and chlorosis is mainly due to S-deficiency, yet the iron-chlorosis occurs in groundnut during kharif season in a few areas.

Though, high free $CaCO_3$, HCO_3^-, moisture, pH, poor aeration, and root damage and intensive cropping enhance the deficiency, the principal soil factors causing Fe-deficiency chlorosis in India are, the presence of excess lime (15-40% $CaCO_3$) hence high soil pH (7.5 to 9.0), excess irrigation and a few places high organic matter and P content of the soil. In some places, the chlorosis was also due to the S-deficiency as high lime content precipitates S as $CaSO_4$ and finally leaching of SO_{4-}-S during excessive rain. In Saurashtra, Gujarat, there is a lime rocks below soil surface varying from only 15 cm soil depth to as much as 3 meter due to which there is high amount of $CaCO_3$ in the soil. The supply of mineral N as NH_4NO_3 also induces Fe-chlorosis in groundnuts in Vertisols (Sahrawat et al., 1990).

In a series of study, it was noticed that at normal irrigation (irrigation at –0.6 bar) in the calcareous soil, 15-27 % of plants showed chlorosis which mainly due to calcareous nature of the soil, however, this chlorosis increased to 36 % or even more when the irrigation frequency was increased (irrigation at -0.3 bar) (Singh et al., 1987, 1995). Thus, when compared the normal irrigation reduced the chlorosis of groundnut considerably and increased upto 29 % pod yield over its excess irrigation (Singh et al., 1995). In these soils whenever irrigation is given, the water comes into contact with lime releasing CO_2 and bicarbonate thereby increasing their concentrations. When the soil moisture increases, microbial respiration increases, gas exchange decreases and partial pressure of CO_2 increases, which results in

a

b

Plate 2. Groundnut showing iron-chlorosis symptoms under various level of Fe in sand culture (a) and excess irrigation in soil (b).

the increase of biocarbonate concentration in soil and reduce the availability of Fe. Also, as the root respiration uses O_2 and produces CO_2, the gas exchange between air and in the soil's pore space and the atmosphere is needed which is partially blocked by excess irrigation.

To quantify groundnut yield loss due to iron-chlorosis, diagnostic on-farm trials were carried out at two village sites (Umri and Katneswar) prone to iron-chlorosis in Maharashtra, India, by ICRISAT during post-rainy season, in the alkaline (pH >8) soil and rich in lime (>16% $CaCO_3$) where severe chlorosis occurred as early as the seedling stage (Potedar et al., 1995). The groundnut genotypes SB XI and ICGS 11 were grown on flatbed and ridge, and foliar sprays with Fe sources ($FeSO_4$ or FeEDTA) where the yield (pod, haulm and total rabi-summer matter), final plant stand, harvest index and oil content increased significantly and chlorosis rating decreased significantly with ridge planting or foliar Fe sprays. A strong negative relationship was also observed between yield (pod, haulm, total dry matter) and oil content, and visual chlorosis rating, thus indicating the yield losses due to iron-chlorosis.

Though, chlorosis occurred throughout the cropping period, its intensity and duration was more important. In general, crop showing less than 10% chlorotic plant did not affect the yield much. But crop showing more than 10 % chlorotic plant caused significant yield losses and chlorosis during 30-70 DAE was identified as the critical period causing maximum yield losses in groundnut (Singh et al., 1987, 1995). Chlorosis during early stages till 20 DAE and also during maturity (80 DAE onwards) did not affect the yield. However, under extreme cases, many a times early severity of iron-chlorosis caused complete crop failure. Depending upon the management practices, the yield losses due to iron-chlorosis in groundnut, in India, was 20 to 41 %, which is a very high amount and has to be looked seriously (Singh and Joshi, 1997).

3. Deficiency symptoms and their diagnosis

3.1. Visual symptoms, chlorophyll and chlorotic rating

Among all the micronutrients, iron is the most important nutrient as it is a component of cytochrome oxidase, ferredoxin protein, chlorophyll and several enzyme systems and Fe-deficiency is most commonly observed symptoms in groundnut. The Fe-deficiency symptoms, in groundnut, first appear as interveinal chlorosis of young rapidly expanding leaves (Plate 3). Under sever condition veins also may become chlorotic with white papery leaves. These areas later become brown and necrotic leading to death of the leaves and plant (Fig. 4). Under favourable soil condition, many times, there is automatic recovery of this chlorosis as the leaves become older, but under adverse conditions, this chlorosis continues to occur and, some times may result in death of plant causing crop failure.

To study the intensity of iron-deficiency chlorosis, the percentage chlorotic plants and visual chlorotic trtating score (VCR) of the crop have been established (Singh and chaudhuri, 1991, 1993) which were also found having positive correlation with chlorophyll content and chlorophyll meter (SPAD reading (Singh et al., 1990; Samdur et al., 2000). As the chlorosis symptoms continue to occur, groundnut crop showing interveinal chlorosis in their top 5 leaves are rated for these parameters at various growth stages (mainly at 30, 50 and 70 DAE) as follows:

- Percent chlorosis: 100 (Number of chlorotic plants / total plants).
- Visual chlorotic rating (VCR) score on 1-5 scale, (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)

On an average, the chlorotic and green plants, showed 2 and 7.2 mg chlorophyll g^{-1} dry wt of leaves and produced 5 and 9 g pod yield and 13 and 16 g haulm yield $plant^{-1}$, respectively (Singh et al., 1987). In a response study, the nutrient efficient genotypes showed more chlorophyll (Chl a and b), carotenoid and better growth and yield than the nutrient-inefficient ones.

a

b

Plate 3. Typical Fe-deficiency symptoms in groundnut appear as interveinal chlorosis of young rapidly expanding leaves (a) and under sever conditions veins also become chlorotic (b). Under favourable condition there is automatic recovery of this chlorosis in newly emerging leaves (a).

a

b

Plate 4. Groundnut plant showing, sever iron-chlorosis with chlorotic white papery leaves, brown and necrotic spots on chlorotic leaves leading to death of plant (a), and recovery of iron-chlorosis (regreening) in leaves due to foliar application of iron-sulphate (b).

3.2. Plant and soil analysis

Iron deficiency is most widespread in the world mainly due to its availability in root zone rather than abundance. About one third of the soil in the world is calcareous where iron-deficiency is most common problem. In groundnut, the Fe-deficiency is most severe when grown on calcareous and alkaline soils containing less than 5 ppm DTPA extractable Fe and high soil moisture (Singh, 1994).

Although, the total Fe was more in the green leaves than the chlorotic one, this was not true in some of the cases. The critical limit of Fe in groundnut leaves is 40 ppm and sufficiency level is 50-300 ppm, but in the standing crop the Fe-deficiency is visible only when leaves concentration falls below 30 ppm. However, the active iron (Ferrous iron) should be taken as criterion, as total iron may not, sometimes, shows differences between chlorotic and healthy plants. Also the critical level of Fe content in leaves vary with soils and groundnut genotypes. The chlorotic leaves showed less than 6 ppm ferrous iron in red soil (Rao et al., 1987), but less than 12 ppm in calcareous soils (Singh, 1994). Iron deficient leaves of groundnut always had less active iron (ferrous iron) than the normal one. Though the normal concentration of Ca in groundnut leaves varies from 1.2 to 2.5 %, the chlorotic leaves of groundnut, when grown in calcareous soil, contained very high amount of Ca (3.8 to 6.1 %) which block the uptake and translocation of Fe and many other nutrients causing alteration in the nutrient absorption and concentration.

To find out the differences in groundnut genotypes, the green Fe-efficient (GG 2, PKVG 8 and TG 17) and chlorotic, Fe-inefficient (NRCG Acc. 162, 7472 and NcAc 17090) groundnut genotypes were grown in the same field on calcareous soil and subjected to the estimation of total and active iron (Fe^{++}) content, chlorophyll, total nutrient uptake and their concentration, at different growth stages. The Fe-efficient (iron-chlorosis tolerant) genotypes showed 2-3 times more active iron content, and higher uptake of all the nutrient, except Ca, than the Fe-inefficient ones.

The Virginia runner groundnut genotypes, because of their high nutrient absorption capacities and better tolerance of iron-chlorosis, always remain green throughout the cropping season. The erect type Spanish and

Valencia bunch genotypes, on the otherhand showed yellowish canopy most of the time and are more prune to iron-chlorosis.

3.3. Metallo-enzymes

The activities of metallo-enzymes ie. peroxidase, ascorbic acid oxidase and nitrate reductase (NR) were studied at different growth stages to find out the differences between the green (Fe-efficient) and chlorotic (Fe-inefficient) groundnut genotypes grown on the calcareous soil under similar conditions. It was noticed that alongwith chlorophyll content, the activities of peroxidase and nitrate reductase (NR) in leaves and peroxidase in root of green plant was appreciably higher than the chlorotic one. Peroxidase activity in the leaf, stem and root tissues of green genotypes were 1.5-3.0, 1.5-2.0 and 2.0-4.0 folds respectively, that in the respective tissues of the chlorotic genotypes. The activity of NR in young leaves of green genotypes was 1.2-2.5 folds that in the chlorotic. The peroxidase activity (Δ OD g^{-1} s^{-1}) in leaves, stems and roots of tolerant genotypes was always more than 0.15, 0.3 and 0.6, respectively, and the genotypes showing lower activities were susceptible to iron-chlorosis. The NR activity in young expanded leaves was 8 μmol NO_2 g^{-1} fr.wt. h^{-1} and above for Fe-efficient genotypes and below 4 for Fe-inefficient genotypes. Interestingly, both the peroxidase and nitrate reductase activities increased with the external application of iron. However, the respiration rate of root was lower in Fe-efficient genotype than inefficient one. As the Fe-deficient plant showed less peroxidase activity and the effect of which was more pronounced in root tissues, root peroxidase may act as an indicator of diagnosing iron-chlorosis in groundnut.

4. Remedies

The iron-chlorosis is of common occurrence in calcareous soil and hence efforts are being made at global level to find out its remedies through soil and foliar application of iron containing fertilizers and also through selection of Fe-efficient groundnut varieties. Also this iron-deficiency has become so severe that globally an international symposium is organized every alternate year to review the progress and chalk out the new strategies. Some of the successful attempts made for the prevention and correction of iron-chlorosis in field grown groundnut are discussed below.

4.1. Soil amelioration

For the prevention of occurrence of iron-chlorosis in the field, the soil ameliorative measures were tried. Repeated experimentation with iron and non-iron containing chemicals for several years revealed that soil application of iron sulphate, gypsum, phosphogypsum, elemental sulphur and pyrite could ameliorate the iron-chlorosis of groundnut, increased chlorophyll, pod yield and Fe uptake by groundnut (Table 1).

Table 1. Chlorosis[a], Chlorophyll[a] content (mg g^{-1} dw of leaves), pod yield (kg ha^{-1}) and Fe uptake (g Fe ha^{-1}) in groundnut cv. J 11 grown with various soil amendments in calcareous soil (Singh and Joshi, 1997).

Treatments	% chlorosis	Chl content	Pod yield	Fe uptake	
				60DAE	Maturity
Control	21.8	4.12	1520	2840	6490
Iron sulphate (50 kg ha^{-1})	5.2	6.40	1930	4140	8990
Gypsum (110 kg ha^{-1})	10.6	4.85	1740	3510	6830
Phospho-gypsum(125 kg ha^{-1})	10.3	4.90	1795	3440	6890
Elemental S (20 kg ha^{-1})	8.8	5.30	1826	3750	7570
Pyrite (43 kg ha^{-1})	6.4	6.20	1910	4102	8701
L.S.D. (0.05)	1.2	0.30	82	110	280

[a]data are the average of three observations at 35, 55 and 75 DAE.

The recovery was best achieved with iron sulphate and pyrite and 20 kg Fe ha^{-1} soil application of as pyrite (43 kg pyrite ha^{-1}) or 10 kg Fe as iron sulphate (50 kg ha^{-1} iron sulphate) completely prevented the occurrence of iron-chlorosis in calcareous soil. Gypsum, phosphogypsum, and elemental S are sulphur sources, these also recovered iron-chlorosis and increased pod yield (Singh and Chaudhari, 1995).

The pyrite, iron sulphate and elemental S were most effective when applied to soil, half as basal and remaining half in two equal doses at 25 and 50 DAE. The iron sulphate directly releases Fe^{2+} and SO_4^{2-} after coming in contact with moist soil which are absorbed by the plant. However, pyrite and elemental S are slow releasing and first oxidize to SO_4^{2-} before becoming available to the plant. In presence of moisture and air, pyrite is first oxidized to $FeSO_4$ and sulfuric acid and the $FeSO_4$ further oxidizes to sulfuric acid and ferric hydroxide. This sulfuric acid helps soil to reduce pH and also acts as a S source. Agricultural grade pyrite is a cheap source of Fe and S which is present as reserves at Amjhore, Bihar in plenty and being marketed by Pyrites, Phosphates and Chemicals LTD. This can be effectively used for growing groundnut in the alkaline and calcareous soils.

The experiments on management of iron-chlorosis due to excessive irrigation in calcareous soil, have indicated that the yield losses could not be fully offset by providing the external iron sources. Because, the excess irrigation affected air exchange between the soil and the atmosphere and accumulated high bicarbonate in the root zone during its respiration induced iron chlorosis (Singh et al 1990). In such cases either application of iron sources and withholding the irrigation or growing the groundnut on raised bed, are essential. The soil applications of iron sulphate and iron citrate could recover the plant from chlorosis caused due to excess irrigation, increased chlorophyll content and pod yield and Fe uptake (Table 2). Besides these, soil application of FYM, decomposed organic matter, FYM + iron containing chemicals, sulphuric acid (0.5% applied with irrigation water) also ameliorate the iron chlorosis of groundnut in calcareous soil.

The chelated Fe such as FeEDDHA (ethylene diamine di -(O-hydroxy phenyl acetate), is most effective as soil application but are costly and not available to the groundnut grower. In Israel, 50% yield increase was reported by 10 kg Fe-EDDHA ha^{-1}. Looking to the potential of iron chelates, various iron containing compounds and chelates namely Fe-EDTA, Fe-EDDHA, $FeSO_4$ and iron citrate were compared for alleviating lime induced iron-chlorosis in calcareous soils. It was noticed that all these sources of iron when applied in soil reduced occurrence of iron-chlorosis and excessive vegetative growth and increased chlorophyll and Fe^{2+} contents of leaves and pod yield. The study also reveals that at 5 kg Fe ha^{-1} (lower doses), beneficial effects of iron sources on groundnut were more pronounced with chelates FeEDDHA and iron citrate than other iron sources, but at higher doses (20 kg Fe ha^{-1}) the $FeSO_4$ and iron citrate did well and increase the pod yield over their lower doses. As the FeEDDHA, though very effective, is a costly chemical and is not availabile to the farmer, application of higher doses of commercial grade $FeSO_4$ available in plenty, is recommended. To observe the interaction of genotypes with soil amendments, both the Fe-efficient (GG 2, CSMG 84-1, TAG 24 and TG 26) and Fe-inefficient (PBS 3, PBDR 36, VRI 3 and I_2) groundnut genotypes when studied, the appreciable responses on both the genotypes was observed, but the Fe-inefficient genotypes showed better response to iron sources than the Fe-efficient one.

Table 2. Effects of various iron sources and excess irrigation on the chlorosis, chlorophyll content (mg g^{-1} dw of leaves) and pod yield of GAUG 1 groundnut variety (average of two seasons).

Treatments	Chlorosis (%)	Total Chl. (mg g^{-1})	Pod yield (kg ha^{-1})	Fe uptake (g Fe ha^{-1})
Excess irrigation (E)	36.0	2.97	1110	1251
Iron sulphate + E	11.7	5.68	1393 (25.4)	2961
Iron citrate + E	15.8	5.39	1373 (23.7)	2824
Normal irrigation (N)	26.9	3.60	1433 (29.1)	1561
Iron sulphate + N	6.8	6.20	1873 (68.7)	3812
Iron citrate + N	10.1	5.80	1710 (54.1)	3522
LSD (0.05)	3.2	0.28	58	290

The percent chlorosis and chlorophyll contents are the average of three observations recorded in three replicates at 35, 55 and 75 DAE (days after emergence of seedlings). Figures in parentheses indicate percent increase over E (excess irrigation).

While standardization of field doses, following Fe and S fertilizers, their doses and mode of application were recommended for effective amelioration of iron-chlorosis in groundnut:

Fertilizers	Nutrient dose (kg ha^{-1})	Amount of fertilizers (kg ha^{-1})	Mode of application
Iron sulphate	10-20 kg Fe	50-100	Soil, 50% as a basal, 25% at 30 DAE and 25% at 50 DAE
FeEDDHA	5-10 kg Fe	25-50	As above
Iron sulphate	2 kg Fe	10	Foliar sprays of 0.5% aqueous suspension thrice at 30, 50 and 70 DAE.
Iron sulphate	3 kg Fe	15	7.5 kg ha^{-1} as basal in the soil and 7.5 kg ha^{-1} on the foliage (three foliar sprays)
Iron citrate	2-3 kg Fe	9- 13	Foliar sprays of 0.5% aqueous suspension thrice rat 30, 50 and 70 DAE.
Gypsum	20-40 kg S	100-200	Soil, 50% as a basal, 25% at 30 DAE and 25% at 50 DAE,
Phosphogypsum	20-40 kg S	125-250	As above
Elemental S	20 kg S	20	As above
Pyrite	20 kg Fe	43	As above

Results of a field experiment to assess the effects of phosphate carriers, S application (as gypsum), Fe and IAA on the Fe nutrition of groundnut grown in a calcareous soil revealed that single superphosphate (SSP) was more effective than diammonium phosphate in improving Fe nutrition and chlorophyll synthesis (Singh and Sahu, 1993). Increased P and Fe contents in chlorotic leaves showing symptoms of Fe deficiency suggested that Fe, despite absorption and uptake, was subjected to inactivation, and thus Fe content per se was not the cause of chlorosis. Better amelioration of chlorosis with SSP application compared with DAP indicated a role of S in preventing inactivation of Fe, possibly caused by excessive P accumulation.

Thus, for the prevention of Fe-deficiency chlorosis to occur, the soil amelioration with pyrite and iron sulphate are recommended in India.

4.2. Foliar application

Foilar application of water soluble iron containing chemicals is the another approach to ameliorate the Fe-deficiency in standing crop (Fig 4b). The available iron sources are ferrous sulphate, ferrous ammonium sulphate, ferric citrate, and Fe-EDTA which can be used as foliar spray. Experiments on the foliar application of various iron sources were conducted on groundnut and the results are summarized in Table 3. Foliar application of 0.1% Fe either of iron sulphate, Fe-EDTA or iron citrate could recover the groundnut plant from iron chlorosis in the standing crop (Singh et al., 1995). Though all the Fe sources were effective in recovering the groundnut plant from iron-chlorosis, increasing chlorophyll and pod yield when applied as aqueous solution containing 0.1% Fe, the mixed application of 0.5 % iron sulphate + 0.01 % citric acid was the best. To maintain the plant green, throughout the cropping season, 3-4 such sprays are required at 15-20 days intervals (Singh and Devi dayal, 1992).

Table 3. The recovery of chlorosis, and increase in chlorophyll content and pod yield of Girnar 1 groundnut variety as affected by foliar application of various iron sources (Singh and Dayal, 1992).

Treatments	% chlorosis		Total Chl (mg g^{-1} d.w. of leaves)		Pod yield (kg ha^{-1})
	50DAE	70DAE	60 DAE	80 DAE	
Control	38.2	24.6	5.6	5.2	1090
Iron sulphate	8.0	7.0	7.9	6.6	1330 (22.0)
Iron sulphate + Citric acid	7.2	6.3	8.1	6.8	1410 (29.4)
Iron citrate	7.6	6.8	8.0	6.7	1300 (19.3)
Fe-EDTA	7.8	6.9	7.8	6.6	1280 (17.4)
LSD (0.05)	3.2	4.2	0.3	0.5	80

Figures in parentheses indicate percent increase over control.

In an on farm trial, conducted by ICRISAT, foliar sprays of iron sulphate was the most effective for correcting Fe-chlorosis (Potedar et al., 1995). Foliar spray of FeEDDHA and IAA corrected the chlorosis, however the highest pod yield of 1.94 t ha^{-1} was obtained from foliar application of 0.2% FeEDDHA followed by 1.81 t ha^{-1} from 60 kg P_2O_5 as SSP or 45 kg S ha^{-1} gypsum (Singh and Sahu, 1993).

Due to low quantity, the micronutrients are applied either through seed or through foliar applications for groundnut. As iron has to be absorbed by the plant leaves, the foliar application is very effective during wet and humid weather conditions, but not during dry weather. Thus, to make the absorption effective, the spray should be done in the evening hours.

4.3. Drip application

As the foliar application is not very effective during dry weather conditions prevailing in the arid and semi-arid region, the application of iron fertilizer through drip is the alternative. Now-a-days, looking to the water economy, the drip irrigation is becoming very popular in the semi-arid regions, and hence to utilize this system, a series of field experiments were conducted to find out the possibility of iron, application through drip irrigation in groundnut. The iron sulphate at 3 kg Fe ha^{-1} were applied through soil application, foliar sprays and drip irrigation and chlorophyll content in leaves, plant growth, yield and yield attributes were recorded. The drip application of Fe, increased pod yield by 31-36, %, over control. However, soil and foliar applications could increase the pod yield by 11-21, 25 %, over control respectively. Also, application of Fe through drip irrigation increased the shelling out-turn by 5-8 %, seed mass by 9-26 % and sound mature seeds (SMS) by 15 % and nutrient use efficiency over their soil and foliar applications. Interestingly, drip application of water alone also increased 8-17 % pod yield, 13-17% haulm yield, 12-26% 100-seed mass, and 17% SMS over flood irrigation.Thus, for better nutrient utilization and high groundnut yield, the iron should be applied through drip irrigation in semi-arid region if the facility for the same exists.

4.4. Seed dressing with iron fertilizer and biofertilizers

The seed dressing of iron sources were also tried to test the effectiveness of this method of nutrient application in groundnut crop. The iron containing salts such as iron sulphate, ferric chloride, at a rate of 5 kg ha^{-1}, were compared by applying as seed dressing, and also in the furrows as soil application. It was noticed that only ferrous sulphate could increase the yield and other parameters as seed dressing. The ferric chloride caused damage to seed and showed reduction in field germination. However, both these sources did well if placed in the furrow along with other fertilizers. Interestingly, response of ferrous sulphate as seed dressing was very good and hence recommended for its use to avoid early chlorosis in groundnut at seedling stages (Singh, 2003).

The induced chlorosis was also partially corrected by inoculation with *Bradyrhizobium* strains NC 92 which produced siderophore bound iron. Inoculation with *Bradyrhizobium* and *Pseudomonas* improves iron nutrition by synthesis of chelates (siderophores) that keep iron in soluble form (Jurkevitch et al., 1988; O Hara et al., 1988). Inoculation of nodulating groundnuts with Bradyrhizobium strain NC 43.3 enhanced whole plant DM production and O-phenanthroline extractable Fe and N contents of the plants (Sahrawat et al., 1990).

Rhizobox and field experiments were conducted in China to investigate nutritional interactions between groundnut and maize in intercropping systems for Fe acquisition. The iron-chlorosis symptoms in groundnut grown in monoculture were more severe and widespread compared to those of groundnuts intercropped with maize indicating a marked improvement in the iron nutrition of groundnut intercropped with maize in the field (Zuo et al., 2000). To confirm the same the rhizoboxes experiments were conducted where roots of maize and groundnut were either allowed to interact with each other or prevented from making contact by inserting a solid plate between the root systems of the two species. The results showed that the chlorophyll and HCl-extractable Fe concentrations in young leaves of groundnut in the intercropping system with unrestricted interactions of the roots of both plant species were much higher than those of groundnut in monoculture. It is suggested that the improvement in the Fe nutrition of groundnut intercropped with maize was mainly caused by rhizosphere interactions between groundnut and maize (Zuo et al., 2000).

4.5. Selection for Fe-efficient genotypes

The above discussed methods have some major limitations. The soil application requires large amount of iron fertilizer or chelates, whereas foliar method requires frequent applications. The drip irrigation requires a high amount of investment leading to high cost of cultivation.

a

b

Plate 5. Field view of a screening trial on tolerance of iron chlorosis (a) and close view of some iron-chlorosis tolerant and susceptible lines (B) of groundnut in calcareous soil at NRCG, Junagadh, India.

a

b

Plate 6. Reaction of iron-chlorosis tolerant and susceptible groundnut genotypes during early 30-40DAE (a) and peak (60-70 DAE) growth stages (b) in calcareous soil at NRCG, Junagadh, India.

As all these may not be viable solutions on long-term basis, selection of iron-efficient genotypes, which can tolerate iron-chlorosis, could be the potential alternative solution to this malady. The results of an on-farm trial conducted by ICRISAT indicated that foliar sprays of $FeSO_4$ were the most effective for correcting Fe-chlorosis, however the response varied with groundnut varieties and that prompted ICRISAT's groundnut breeding programme to initiate screening for Fe-efficient genotypes (Potedar et al., 1995). As enough genotypic differences exist in groundnut germplasm, and calcareous soils of Saurashtra, Gujarat is a hot spot for occurrence of iron-chlorosis, a pilot project was initiated at NRCG, Junagadh wherein investigations were made to screen all the released groundnut cultivars of India and all the germplasm and advanced breeding lines available with this center, and to classify them in various categories of tolerance of iron-chlorosis so that these genotypes could be used, either directly in the targeted areas or could be used as donor parent in breeding for developing iron-chlorosis tolerant cultivars (Plates 5 and 6).

4.5.1. Screening for their tolerance of lime-induced iron-chlorosis

The germplasm lines, advance breeding lines and groundnut cultivars were screened separately in a series of *in situ* field trials conducted at NRCG, Junagadh from 1985-2001 and a total of 5100 germplasm lines, 200 advance-breeding lines and 120 Indian groundnut cultivars were screened and classified into various categories according to their degree of tolerance/susceptibility. The groundnut crop was monitored for their occurrence of iron-chlorosis and visual chlorotic rating scores (VCR) of top 5 leaves recorded at 30, 50 and 70 DAE (Plate 5 and 6) and based on the average value of VCR during the active crop growth stages, the genotypes were judged for their tolerance of iron-chlorosis (Singh 1994, 2000; Singh and Chaudhari, 1991, 1993). The susceptibility of groundnut to iron deficiency is very sensitive to genetic control which appears to be located in the root behavior and the rate of efficiency of iron absorption from the soil. Gowda et al. (1993) reported that the iron absorption efficiency is dominant and inefficiency is recessive. The chlorophyll content was positively correlated with active iron content (Kulkarni et al 1994). As there is a strong association between iron-efficiency and productivity, selection of iron-efficiency in early generations is suggested (Kulkarni et al., 1995).

As we have to screen a number of genotypes, initially the groundnut genotypes were classified into the different categories of their tolerance of iron-chlorosis based on the VCR of top 5 leaves. However, later on the chlorophyll content and direct reading with chlorophyll meter (SPAD-502, Minola, Japan) in top 5 leaves were also assessed with limited genotypes and found doing well (Singh, 1994; Samdur et al., 2000). Also, in a limited genotypes, the active iron content and activities of peroxidase in leaves, roots and stem and nitrate reductase in leaves were assessed and found working well. Of these, root peroxide was identified as an indicator for Fe-efficient lines. The values of various parameters published by our laboratory in different articles are synthesized in Table 4 based on which further screening is being done in most of the laboratory in India and abroad.

As the VCR does not require any equipment, the same in the top 5 leaves in 1-5 scale (1, green leaves with no chlorosis and 5, severe chlorosis on all leaves as mentioned in diagnosis part), were observed during various growth stages (30, 50 and 70 DAE) and based on these, the groundnut cultivars were classified into various categories of their tolerance of iron-chlorosis such as tolerant (Fe-efficient), moderately tolerant, normal (neutral), and susceptible (Fe-inefficient) following Singh and Chaudhari (1991; 1993,) and Samdur et al. (1999). However, the list being very exhaustive for normal category genotypes, only the tolerant, moderately tolerant, and susceptible genotypes were mentioned in most of the papers.

Also, an effort was made to find out the iron nutrition behavior of a few groundnut genotypes in red laterite soil by screening the groundnut varieties and germplasm lines at NRCG-Outreach Research Centre, Bhubaneswar during 1996 where the Fe-efficient groundnut genotypes did well in red soil where iron is in plenty. However, the Fe-inefficient genotypes showed chlorosis in this red soil. The tolerant (Fe-efficient), moderately tolerant and highly susceptible (Fe-inefficient) genotypes are listed in Table 5.

Table 4. Various parameters used in classifying the categories of tolerance of iron-chlorosis.

Parameters	Tolerant	Moderately tolerant	Normal	Susceptible
Visual appearance of crop and leaves	Dark green leaves, rare appearance of chlorosis	Dark green to light green, with slight chlorosis	Green to light-green with chlorotic leaves	Yellow to whitish yellow
VCR (average of season)	1 in >95 % plant, < 2 in <5% plant	1 in >90 % plant, 2 in <10% plant	2-3 in <20 % plant, 1 in >80 % plant,	3-5 in >20% plant, 2 in <80 % plant,
Total Chlorophyll content (mg g^{-1} dry. wt. leaves)	> 7.5	7.0 - 7.5	6.0-7.0	<6.0
SPAD Chlorophyll meter reading	>35	30-35	25-30	<25
Peroxidase activity (ΔOD g^{-1} s^{-1}) in root	>0.6	0.5-0.6	0.2-0.5	<0.2
NR activity in young leaves (µmol NO_2 g^{-1} fr. wt. h^{-1})	>8	6-8	4-6	<4

4.5.2. Utilization of Fe-efficient genotypes

To utilize these identified tolerant genotypes, effort was made for yield evaluation of the tolerant genotypes at NRCG and AICRPG centres to introduce these directly as cultivar. Also breeding programme was initiated at BARC, Mumbai, ICRISAT, Patancheru and at NRCG, Junagadh to use these as donor parents and some of the promising lines are about to be released for cultivation.

Table 5. Groundnut genotypes showing tolerance of iron-chlorosis.

Groundnut	Tolerant	Moderately tolerant	Highly susceptible
Varieties	GG 2, JL 24, MH 2, MH 4, TG 17, VRI 2, M 13, ICGV 86522, 86590, 86031, TAG 24, TG 26, SG 84, MH 2, MH 4, CSMG 9101, CSMG 84-1, GAUG 10, GG 11, Punjab1, ICG(FDRS)10,	Somnath, TG 1, M 37, Chandra, G 201, UF 70-103, Jyoti, Jawan, TMV 7, MA 10, MA 16, ICGV 86008, CSMG 884, ICGS 76, J(E) 2, DH 8, Kopergaon 1, Tifspan, S 206, DH-3-30, GG 4, GG 5, GG 6, K 134	VRI 3, ICGS 11, ICGS 44, ICGS 65, Chico, Robut 33-1, GAUG 1, ICGV 87276, Kadiri 2, TG 3, Co 1, Latur 33,
Advanced breeding lines	PKVG 8, Akola Sel. I_1, PBS 70, 89, 189, PBDR 41, 4-9-1, 7-6-13 B, 7-6-26	CGC 3, NDN 19, PBS 13, 145, 90, 91, PBDR 39, 7-6-17	AK NRCG 1, I_2 PBDR 13, 36, 2-21
Germplasm accessions	NRCGs 389, 1114, 1308, 2588, 3498, 4255, 5389, 5513, 6450, 6820, 6919, 7027, 7085, 7258, 7267, 7347, 7417, 7607, 7599,	NRCGs 4015, 4659, 5118, 7110	NCAc 17090, NRCGs 7472, 162

Yield evaluation of Fe-efficient genotypes and AICORP(G) testing

After screening of more than 5000 germplasm accessions during 1985 to 1990, about forty groundnut genotypes were identified as tolerant of iron-chlorosis. Of these, 25 iron-chlorosis tolerant genotypes having high yields, were evaluated for their yield and yield attributes during 1990-1991 and in the next year, only ten high yielding Fe-efficient groundnut genotypes were picked up and further tested for their yield and yield attributes for two-three years (during 1992-1994) along with national (JL 24 and SB XI) and zonal (GG 2) cultivars as control. The pod and haulm yields, shelling and oil percent and 100 seed wt. of these genotypes are given in Table 6.

Table 6. Evaluation of yield and other characters of groundnut genotypes.

Groundnut Genotypes		Pod yield (Kg ha^{-1})				Haulm yield (Kg ha^{-1})		
Iron-chlorosis tolerant	NRCG Acc. Nos.	K 1992	RS 1993	K 1993	K 1994	K 1992	RS 1993	K 1993
FeESG10-1	7085-1	2256	2772	1013	1158	3355	3655	1287
FeESG10-3	7085-3	2612	2815	636	1323	3415	3741	1133
FeESG8	6919	1608	2339	717	1311	3215	3216	1293
FeEVG6	3498	-	1583	663	958	-	3625	1373
FeEVG5	1308	-	2333	547	382	-	3834	1720
FeESG17	7607	2060	3667	-	-	2742	4538	-
FeEVG17	7599	2136	3185	699	-	4017	4567	1573
FeESG1	389	1772	-	-	-	3557	-	-
FeESG22	5118	1554	-	-	-	1220	-	-
FeESG2	2588	-	-	-	1330	-	-	-
GG 2	(ZC)	2229	2520	640	983	3499	3100	1267
SB XI	(NC)	-	-	1011	483	-	-	1640
JL 24	(NC)	-	-	733	903	-	-	1787
LSD (0.05)		110	151	78	146	160	175	134

	Shelling (%)				100-kernal wt (g)				% oil
Iron-chlorosis tolerant	K 1992	RS 1993	K 1993	K 1994	K 1992	RS 1993	K 1993	K 1994	K 1994
FeESG10-1	63	65	67	67	37.0	35.0	25.0	32	52.9
FeESG10-3	69	66	70	72	32.0	32.0	23.3	32	50.0
FeESG8	72	68	70	69	38.5	36.0	30.2	32	44.9
FeEVG6	-	64	67	70	-	35.6	28.6	40	47.1
FeEVG5	-	65	66	64	-	38.6	36.4	31	45.9
FeESG17	70	70	-	-	35.9	36.0	-	-	-
FeEVG17	58	58	62	-	44.9	44.0	37.3	-	-
FeESG1	63	-	-	-	35.0	-	-	-	-
FeESG22	66	-	-	-	33.1	-	-	-	-
FeESG2	-	-	-	73	-	-	-	34	-
GG 2	66	65	70	72	42.3	40.3	27.6	35	51.4
SB XI		-	73	68		-	27.5	32	49.8
JL 24		-	69	68		-	38.1	35	
LSD (0.05)	2	1.5	2	-	2.6	2.8	2.2	-	-

K stands for Kharif and R as Rabi-summer, the codes stands FeESG for Fe-efficient Spanish Groundnut and FeEVG for Fe-efficient Valencia Groundnut. The ZC and NC are zonal and national checks, respectively.

Over all, it was observed that the Fe-efficient genotypes FeESG 8, FeESG 10-1, 10-3, and FeESG 2 out yielded GG 2. The shelling percent of these genotypes were in between 64-73, except FeEVG 17 which showed 58 and 62%. Genotype FeESG 10-1 showed high oil content (52.9%) and FeESG 8 contained less (44.9%). The genotypes FeESG 8, FeESG 10-1, 10-3, are early duration also with a maturity period of 85-90 days during kharif season and 95-100 days during rabi/summer season.

The results of field experimentation for consecutive four years revealed that the genotypes FeESG 8, FeESG 10-1, 10-3 of Spanish group and FeEVG 17 of Valencia group have good yield potential and hence can be released directly for their cultivation in the area where iron-chlorosis is a major problem causing severe yield loss. After thorough evaluation for three years at NRCG, two entries FeESG 8 and FeESG 10-1, having short duration, high yield besides having tolerance of iron chlorosis and many other characters such as tolerant of drought, salinity and diseases, were entered into the AICRP(G) system during 1999 to test these entries for two years at the centres and areas having iron-deficiency problem. These genotypes when tested at hot spots out-yielded the checks during two consecutive years 1999 and 2000 in AICRP(G) system. Based on their performance, these entries were promoted for AVT testing in zone II during 2001. These genotypes need to used further in breeding programme.

Multiplication of iron-efficient and iron-inefficient lines:

As screening for iron chlorosis is a continuous process and tolerant groundnut genotypes are required, 20 Fe-efficient and 5 Fe-inefficient groundnut genotypes are being maintained through field multiplications at NRCG, Junagadh and supplied to the scientist for making crosses and further experimentation on basic and applied research.

The categorization of groundnut cultivars, germplasm and advanced breeding lines described here may serve as a reference for selection of suitable genotypes for their cultivation in calcareous soils and thus help to avoid yield losses due to iron-chlorosis. For, red and laterite soils, yield potential of the cultivar may be considered as the principal criterion irrespective of genotype being tolerant or susceptible to iron chlorosis. The list also may serve as a reference for further taking breeding and identifying iron-chlorosis tolerant cultivars for calcareous soils in many other countries.

5. Strategies

To manage the iron-chlorosis of groundnut in field, following strategies need to be adopted in the areas where such problem is of common occurrence and causing yield reductions.

- Grow identified iron-chlorosis tolerant genotypes only.
- Keep the crop free from iron-chlorosis during 30-70 DAE to avoid any yield losses.
- For prevention of iron-chlorosis apply 10-20 kg Fe ha^{-1} either as pyrite, iron sulphate or 10 kg Fe ha^{-1} FeEDDHA in soil as basal.
- Seed dressing of 5 kg ha^{-1} iron sulphate 100 kg^{-1} seed prevent iron chlorosis during early growth stages.
- In the standing crop foliar spray of 0.5% $FeSO_4$ + 0.02% citric acid at 500, 500 and 1000 l ha^{-1} at 30, 50 and 70 DAS, respectively may alleviate iron-chlorosis.
- Grow groundnut on raised bed in the high moisture areas
- In semi-arid region, if facility exists, apply iron-fertilizer through drip.

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