INVESTIGATIONS INTO NUTRITIONAL DISORDERS CAUSING CHLOROSIS OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) AT ICRISAT CENTER

A thesis submitted to the
Andhra Pradesh Agricultural University
in part fulfilment of the requirements
for the award of the degree of

MASTER OF SCIENCE IN AGRICULTURE

by

J. KOTESHWAR RAO, B.Sc(Ag.)

Dept. of Soil Science & Agril Chemistry
College of Agriculture
Andhra Pradesh Agricultural University
Rajendranagar, Hyderabad 500 030

Soil Fertility & Chemistry Sub-program
Farming Systems Research Program
ICRISAT Center
ICRISAT Patancheru P.O.
A.P. 502 324.

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CERTIFICATE

Shri. J. KOTESHWAR RAO has satisfactorily prosecuted the course of research, and the thesis entitled "INVESTIGATIONS INTO NUTRITIONAL DISORDERS CAUSING CHLOROSIS OF GROUNDNUT (Arachis hypogaea L.) AT ICRISAT CENTER" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. We also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

Date:

Co-Chairman:
Dr. J.R. Burford
Principal Soil Chemist
International Crops Research
Institute for the Semi-Arid
Tropics

Chairman:
Dr. T.M. Vithal Rao
Professor of Soil Science &
Agril. Chemistry
Andhra Pradesh Agricultural
University

CERTIFICATE

This is to certify that the thesis entitled "Investigations into nutritional disorders causing chlorosis of groundnut (Arachis hypogaea L.) at ICRISAT Centre" submitted in partial fulfilment of the requirements for the degree of Master of Science in Agriculture in the major subject of Soil Science and Agricultural Chemistry of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by Sri. J. Koteshwar Rao under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The major findings have been already submitted for publication. All assistance and help received during the course of the investigations have been duly acknowledged by him.

Co-chairman of the Advisory Committee

Chairman of the Advisory Committee

Thesis approved by the Student Advisory Committee.

Chairman (DR. T.M. VITHAL RAO)

Co-chairman (DR. J.R. BURFORD)

Member (DR. R.L. NARASIMHAM)

Member (DR. A. NARAYANAN)

Member (DR. A. SHIV RAJ)

TABLE OF CONTENTS

LIST OF TABLES

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(J. KOTESHWAR RAO)

ABSTRACT

Title: Investigations into nutritional disorders causing chlorosis of groundnut (Arachis hypogaea L.) at ICRISAT Center.

Name: J. Koteshwar Rao

Chairman: Dr. T.M. Vithal Rao Professor & Head
Department of Soil Science & Agricultural Chemistry
College of Agriculture, Rajendranagar.

Co-Chairman: Dr. J.R. Burford Principal Soil Chemist, ICRISAT.

Degree: Master of Science in Agriculture

Major field of study: Soil Science and Agricultural Chemistry

Andhra Pradesh Agricultural University

1982

Groundnut (Arachis hypogaea L.) at ICRISAT Center often becomes chlorotic for a short period during its growth. The cause was suspected to be iron deficiency, but verification was not simple, because of the lack of reliable diagnostic tests. Verification of the cause was therefore sought in this study by monitoring the iron content of foliage of cv TMV 2 throughout the season, by comparing the iron contents of breeding entries showing tolerance or susceptibility to the nutrient stress, and by pot experiments.

The monitoring of the iron content of leaves in the field showed that chlorosis was associated with low levels of o-phenanthroline extractable iron (an estimate of ferrous iron). Similarly, four breeding entries exhibiting marked chlorosis contained significantly lower extractable iron than four entries in which chlorosis was only mild or not evident. The extractable iron contents of the buds or first unfolded leaf of chlorotic plants were always less than 6 µg/g fresh tissue. The youngest leaf tissue was selected as the most appropriate plant part for analysis because chlorosis usually occurs only in the young leaves, and preliminary testing showed that extractable iron contents were lowest in the youngest leaves.

On the basis of these results, the recently developed assay for ferrous iron content of leaves (the o-phenanthroline extractable iron) offers much promise as an index of the iron status of groundnut plants, whereas the total iron content of leaves was unsuitable as such an index. Total iron contents were not related to the occurrence of chlorosis. Available iron content of the soil, as assessed by the DTPA extractable iron content, was also unsuitable for predicting the occurrence of deficiency because all soils on which chlorosis occurred contained significantly more DTPA extractable iron than the critical levels reported in India. The failure of the predictive soil test was attributed to the primary cause of the deficiency, which appears to be due to lack of iron in a physiologically active form within the plant, rather than to unavailability of iron in the soil.

The pot experiment attempted to create reproducible conditions for studying iron deficiency in groundnut, by inducing the deficiency through additions of sodium carbonate or borewell water. The need for this arose because of the variability in occurrence of the deficiency in the field. Both treatments caused chlorosis, but this could not be attributed to iron deficiency, because additions of iron chelate did not amend the chlorosis although these did increase the levels of available iron in the soil. Further studies are recommended to investigate the importance of other nutrient deficiencies on ICRISAT soils.

LIST OF SYMBOLS AND ABBREVIATIONS

N	nitrogen	pH	hydrogen ion activity
P	phosphorus	ppm	parts per million
K	potassium	meq/l	milliequivalents per litre
Ca	calcium	t/ha	tonnes per hectare
Mg	magnesium	cv	cultivar
S	sulfur	DTPA	Diethylene triaminepentaacetic acid
Fe	iron	$CaCl_2$	calcium chloride
Mn	manganese	o-Ph	orthophenanthroline
Zn	zinc	CaO	calcium oxide
Cu	copper	Fe-EDDHA	sodium ferric ethylene diamine di (o-hydroxy phenyl acetate)
Mo	molybdenum	$FeSO_4$	ferrous sulphate
Co	cobalt	OH^-	hydroxyl ion
Ni	nickel	$ZnSO_4.7H_2O$	zinc sulphate
Cr	chromium	K_2SO_4	potassium sulphate
Si	silica	H_2SO_4	sulphuric acid
Fe^{2+}	ferrous	g	gram
Fe^{3+}	ferric	ml	millilitres
Mn^{2+}	manganous	conc.	concentration
Mn^{4+}	manganic	%	percentage
CO_2	carbon dioxide	nm	nanometre
HCO_3^-	bicarbonate	cm	centimetres
CO_3^{2-}	carbonate	kg/ha	kilograms per hectare
EC	electrical conductivity	µg/g	micrograms per gram
ICG	ICRISAT Groundnut		

^{o}C	degree centigrade	viz.	namely
mg	milligrams	am	before noon
w/w	weight by weight	pm	after noon
AR	analytical reagent	SE	standard error
OD	oven dried	Mb	mainbud
D	deionised	Lb	lateral bud
B	borewell	L-1	first fully opened leaf
max	maximum	L-2	2nd fully opened leaf
min	minimum	L-3	3rd fully opened leaf
DW	dry weight	L-4	4th fully opened leaf
km	kilometres	L-5	5th fully opened leaf
mm	millimetres	$<$	less than
m^2	square metre	$>$	greater than
e.g.	for example	$\leq$	less than or equal to
		$\geq$	greater than or equal to

INTRODUCTION

Groundnut (Arachis hypogaea L.) is an important oilseed crop of tropical and sub-tropical regions of the world. The semi-arid tropics produce two-thirds of the world's groundnut (ICRISAT, 1979); it is the major oilseed crop of India, which accounts for 42 and 35 percent of the world acreage and production respectively. India ranks first in both area and production among the groundnut growing countries in the world (F.A.O. 1980); production was 5.0 million tonnes from an area of 6.2 million ha in 1981. Of the states of India, Andhra Pradesh ranked second in both area (1.1 million ha) and production (0.8 million tonnes); the average yield in Andhra Pradesh of 0.7 t/ha was slightly lower than the Indian average of 0.8 t/ha.

Little or no fertilizer was used in traditional systems of groundnut culture, which involved both sole and inter cropping. The newer cultivars, with their higher yield potential,frequently require additional nutrients such as phosphorus, calcium, sulfur, zinc and iron. Iron is essential for plant growth because of its involvement with the activation of several enzyme systems including chlorophyll formation. A continuing supply of iron is essential for good plant health; any factor that interferes for only a short time with absorption of iron by plant roots or utilization within the plant may cause the plant to rapidly develop symptoms of severe iron deficiency (Brown, 1961).

The severity and incidence of chlorosis in groundnut at ICRISAT Center appears to be increasing on alfisols which are intensively cropped, heavily fertilized, and frequently irrigated. The chlorosis is very similar to that caused by iron deficiency, but investigations have been hindered by the intermittent appearance of chlorosis, and its occurrence only in irregular patches. Its occurrence is sometimes associated with heavy rainfall or irrigation. The cause is suspected to be primarily due to increasing pH of soil, due to heavy irrigation with water containing bicarbonates and carbonates. Investigations have also been hindered by the variable success of attempts to correct iron deficiency, and the lack of an established satisfactory diagnostic tissue test. (Katyal and Sharma, 1980). The total iron content of plant tissue does not provide a reliable index of iron deficiency (Singh 1970; Patel et al. 1977). However, recent work has indicated that the orthophenanthroline extractable iron content of chlorotic leaves may be suitable as a diagnotic test for iron deficiency. (Katyal and Sharma, 1980).

Any attempt to investigate this chlorotic disorder must attempt to answer the following questions:

i) Is the disorder due to iron deficiency per se or are other nutrients or environmental factors also involved?

ii) What is the cause of the disorder?

iii) What is the effect of a transient appearance of the disorder on yield?

iv) How can we correct the disorder after its appearance in the field?

v) How can we prevent the development of the disorder, either by fertilizer/soil amendments at or prior to seeding, or by changes in agronomic practices.

Initial investigations were made on the following aspects:

1. In field studies, to monitor iron content in groundnut leaves throughout a season to determine whether the orthophenanthroline extractable iron content was related to the incidence of chlorosis, and to investigate the relationship between the incidence of chlorosis and environmental conditions.

2. To conduct pot experiments to determine whether
   a) the chlorosis was due to iron deficiency
   b) chlorosis could be initiated by use of the center's bore-well water, or by artifically increasing the alkalinity of the soil.

II. REVIEW OF LITERATURE

II.1. IRON AS AN ESSENTIAL ELEMENT FOR PLANT GROWTH

The essentiality of iron for plant growth was discovered over a century ago after Gris (1843) showed that foliar application of iron salts was beneficial to chlorotic grapevines. Gris showed that plants which were deprived of an adequate supply of iron failed to develop chlorophyll and became chlorotic. Sachs (1860) is credited as having been the first to establish,through solution culture experiments, that iron is an essential element for the growth of higher plants.

Iron is essential for plant growth because of its involvement in many biochemical pathways. It is a constituent of many compounds but two are of particular importance: the cytochromes, and leghaemoglobin. Iron thus plays a vital role in electron transport and nitrogen fixation. It is also important as an activator of a number of enzymes (Agarwala and Sharma, 1976).

Some metabolic consequences of iron deficiency are a decrease in sugars (particularly reducing sugars), organic acids (e.g. malic and citric acids), and vitamins (riboflavin and flavin mononucleotide). These effects demonstrate the involvement of iron in synthesis of chloroplast proteins,carbohydrates, organic acids and vitamins (Agarwala and Sharma 1976).

The involvement of iron in photosynthetic activity is reflected by the visual symptoms of iron deficiency. Iron is essential for the formation of chlorophyll, although its precise role in chlorophyll synthesis has not yet been established (Agarwala and Sharma, 1976). Of the different proteins in plants, the chloroplast proteins are the most severely affected by iron deficiency, which can cause a decrease in the size of chloroplasts and also their disintegration (Agarwala and Sharma, 1976).

II.2. DEFICIENCY SYMPTOMS

The visual symptoms of deficiency are usually interveinal chlorosis, with the youngest leaves being first affected. Under a more severe deficiency stress, the entire leaf, the veins and the interveinal areas become pale yellow in color, and it may be bleached white in the most severe instances. These symptoms reflect the vital role of iron in several enzyme systems especially those involved in the formation of chlorophyll (Mengel and Kirkby, 1979).

Seeds usually contain sufficient iron to supply the requirements of a plant in the early stages of growth (Brown, 1961). In soybeans enough iron was supplied from the cotyledons to maintain a green plant up to the first trifoliate leaf stage (Brown and Holmes, 1955a). However, older plants require a continuous supply of iron. For example, if the supply of iron is suddenly restricted, mobility in the plant ceases and

the new growth very quickly becomes chlorotic (Brown and Holmes,1955b)

II.3. CAUSES OF IRON CHLOROSIS

II.3.1 Soil factors

II.3.1.1 Reaction: Of the various factors known to cause iron chlorosis in plants, pH of the soil and plant system is one of the most important, because an increase in pH causes the solubility of iron in solution to decrease. As pH increases, ferrous ion is converted into the hydroxide form, which is insoluble and unavailable for use by plants (Brady, 1974).

$$Fe^{2+} + 2OH^{-} \longrightarrow Fe(OH)_2$$

Chlorosis is therefore common in upland crops on calcareous soils, which have a high pH (Brown et al. 1955).

The transport of iron within the plant is also affected by the pH of the conducting tissue. Rogers and Shive (1932) reported that the iron which accumulated in parts of the plant with high pH was not available for plant processes. On the other hand, tissues with low pH did not show any accumulation of iron.

II.3.1.2 Phosphorus: High phosphorus concentrations in plant tissue may promote the development of iron deficiency. For soybean, the causes were partly due to the precipitation of iron by phosphate within the conducting tissue as well as in the leaves;Brown (1961) observed that increasing both the phosphorus and the calcium concentration in solution culture

increased the translocation to the tops of phosphorus and calcium which induced iron deficiency. Increasing the concentration of only one without the other did not induce iron deficiency.

Because of the precipitation of iron by high phosphorus concentrations in the plant, Dekock and Stremecki (1954) suggested that the P:Fe ratio might in fact be a better index of the iron status of a plant than the iron content alone. However, phosphorus also interacts with pH in inducing iron chlorosis in beans. Biddulph (1951) reported that bean plants were healthy when grown in water culture with a phosphate concentration of 10^{-4}M at pH 4.0. With 10^{-3}M phosphate at pH 7.0 the plants were chlorotic; although iron was still absorbed by the roots, but it precipitated on the surface of the roots, within them, and also in the leaves.

II.3.1.3 Calcium, carbonate and bicarbonate: The characteristic symptoms of iron deficiency are commonly referred to as "lime-induced chlorosis", because iron deficiency commonly occurs on calcareous soils (Brown 1961). Many investigations on the iron nutrition of plants indicated that one or more of calcium, bicarbonate, carbonate and pH were major factors causing the onset of deficiency or a reduction in iron status. However, these factors are often not truly independent. It is therefore difficult to infer that an iron deficiency was primarily caused by one factor. Examples of some of the main conclusions reported are given in the following paragraphs.

Juritz (1912) was the first scientist to relate the incidence of the chlorosis to the calcium carbonate content of the soil. Gartel (1974) also stated that iron chlorosis commonly occurs in French vineyards where soils high in carbonate suffer from poor aeration.

Boxma (1972) reported that a high bicarbonate content (200-300 ppm) in soil was the main cause of lime induced chlorosis in apple orchards; he found a significant correlation between lime-induced chlorosis and the bicarbonate content of the soil in the spring under field conditions. Harley and Linder (1945) reported that irrigation water relatively high in bicarbonates (> 200 ppm) induced iron chlorosis in apple and pear orchards. Saglio (1969) reported that lime-induced iron chlorosis in grapes was aggravated by high bicarbonate levels in soil solution. Wadleigh and Brown (1952) showed that 8 meq/1 of sodium bicarbonate in nutrient solution caused chlorosis of dwarf red kidney beans and reduced their growth by one third. Growth ceased completely when the concentration was increased to 32 meq/1. The effect of bicarbonate on iron nutrition was attributed to reduced iron availability at the root surface.

Porter and Thorne (1955) demonstrated that chlorosis assumed in beans at high bicarbonate levels, regardless of the pH of the solution. Miller et al. (1960) suggested that bicarbonate perhaps induced chlorosis by an indirect effect of increasing soluble phosphorus levels, which in fact was shown to occur.

Taper and Leach (1957) reported that increasing the calcium level in the nutrient solution reduced the uptake of both iron and manganese and narrowed the ratio of iron to manganese in solution required for healthy growth of kidneybean. The effect of Ca is difficult to separate from the effect of soil pH on iron availability. The effect of excess calcium in a soil is generally associated with high pH and consequent unavailability of iron (Dekock, 1955).

II.3.1.4 Micronutrient inter-relationships in the development of iron chlorosis: Apart from the effects of calcium, phosphorus and bicarbonate on the development of chlorosis, Wallace and Lunt (1960) have reported that iron deficiency can easily be induced in the plants by high levels of Cu, Mn, Zn, Mo, Ni, Cr and Co in soil.

Somers and Shive (1942) showed the importance of the ratio of iron to manganese in plant tissues; they clearly demonstrated that the manganese:iron ratio was closely related to the appearance of chlorosis in soybean plants. Chlorosis appeared when the ratio was either too high (manganese toxicity) or too low (manganese deficiency i.e. Fe toxicity). Brown et al. (1959) by using a split-root medium technique did not observe any chlorosis in soybeans at high levels of manganese and other micronutrients.

In a series of papers, Brown et al. (1955 and 1959) have reported that an excess of heavy metals easily induced iron chlorosis. Copper may

effectively reduce iron translocation in plants. Reuther and Smith (1952), using sand culture, demonstrated that an excess of copper caused iron chlorosis in citrus. Brown (1967) has reported that copper decreased translocation of iron in corn.

Wallace et al. (1976b) reported that excess zinc induced iron deficiency in soybeans. One cultivar (PI 54619-5-1) was iron deficient when the zinc concentration in solution culture was 10^{-4}M. Iron contents in leaves were reduced to a greater extent by the high zinc level in the PI 54619-5-1 cultivar than in another cultivar (Hawkeye). The high zinc level resulted in depressed iron contents in leaves, stems, and roots of both cultivars.

II.3.1.5 Moisture extremes: Wallace et al. (1976a) studied the effects of different soil moisture levels on the growth and nutrition of iron inefficient cultivars of soybean (PI 54619-5-1) when grown in calcareous soil; those in dry soil were very small and green (non-chlorotic), whereas those in very wet soil were larger and severely chlorotic. The chlorotic plants had higher levels of Mn, Si, Mg, and K in leaves; this effect is typical of lime-induced chlorosis. The increase in chlorosis at higher moisture contents is common and contrasts with the expected effect of an increase in the incidence of Fe (and Mn) deficiency with drying of the soil due to oxidation of Fe^{2+} to Fe^{3+} and Mn^{2+} to Mn^{4+}, and a decrease in incidence of deficiency due to reduction from oxidised to reduced state (Ponnamperuma, 1972).

II.3.1.6 High levels of nitrate nitrogen: Cain (1954) observed that acidloving plants may become chlorotic even in acid soils and when supplied with nitrate nitrogen. This effect was attributed to an increased pH of tissues due to uptake of nitrate nitrogen. North and Wallace (1959) concluded that nitrate nitrogen is an important factor in the induction of chlorosis in Macadamia spp. in Southern California.

II.3.1.7 Additions of organic matter to the soil: Brown (1961) stated that "soil iron" available to plants is affected markedly by reactions with soil. Additions of organic matter in an acid soil normally increase the available iron content, because the carbonic acid formed from the carbon dioxide of the decomposing organic material enhanced the solubility of iron compounds. The reverse was true in calcareous soil. Green manure crops disked into a calcareous soil and then followed by irrigation, had often caused severe iron chlorosis in deciduous trees.

II.3.2 Plant factors

II.3.2.1 Genotype: Plant genotypes differ considerably in their performance under iron stress (Brown, 1978). Most of the work in this field has been done by Brown (1961) and his associates on the reaction of soybean cultivars to iron stress; they have shown that there are three mechanisms by which plants may differ in their utilization of iron:

(i) Absorption by the root system

(ii) Translocation within the plant

(iii) Utilization of iron within the leaf.

Brown and his co-workers have studied mainly the absorption of iron by roots using soybean as test crop (Brown,1978). Root stocks of plant species or cultivars within species differed in their ability to use iron in alkaline soils (Brown,1978). Plants were classified as iron-efficient, if they respond to iron stress and induce biochemical reactions that make iron available for use in the plant, and iron-inefficient if they do not.

Weiss (1943) was the first scientist to establish differences in the performance of plants to iron stress; he showed that a single gene controlled the differing susceptibility to iron status of 2 soybean cultivars: "PI soybeans" (susceptible) and "Hawkeye" (tolerant).

Brown et al. (1958) found through reciprocal grafting that the root stocks were responsible for this differential "iron efficiency" of the HA and the PI cultivars under iron stress. Further work by Brown and Bell (1969) and Tiffin and Brown (1961) showed that the cultivars differed in their absorption of iron because of different efficiencies in reduction of iron prior to its uptake.

The Plant Nutrition Group of the Botany Department, Lucknow University have reported marked differences in the susceptibility of some high yielding cultivars of Gardenpea, Chickpea, Greengram, Black gram to iron stress (Agarwala and Sharma, 1974). Agarwala and Sharma (1974) studied iron uptake and iron reduction in chlorosis susceptible

and non-susceptible cultivars using radioactive ^{59}Fe; their results supported Brown's contention (Brown, 1978) that apparent differences in iron-efficiency reduction may be due to differences in the uptake of iron by the susceptible and the non-susceptible cultivars (Agarwala and Sharma, 1974).

Table 1a: Difference in susceptibility and predominant symptoms of iron stress in some high yielding and important cultivars of legumes (Agarwala and Sharma, 1974).

Crop plant	Main visual symptoms other than chlorosis of young leaves	Susceptible cultivars	Non-susceptible cultivars
Pea (*Pisum sativum* L.)	Leaf margins necrotic, curled and ragged; white necrotic regions on chlorotic leaves; reduction in size of leaves; premature shedding of flowers; suppression of pod formation.	T-56 T-61	T-163
Chickpea (*Cicer arietinum* L.)	Necrosis, drying and premature shedding of chlorotic foliage, white lesions, necrosis, distortion and curling of young leaves; suppression of flowering and fruits.	BG 1 G 130 Pusa 53 H.208 T.3	C.235 T.1 GWL.2 N-59
Green gram (*Vigna radiata* Verd.)	Tissue necrosis, death of growing point of the shoot; development of axillary branches.	BG 1 T.51 T.1 T.2	T.44 305
Black gram (*Vigna mungo* Verd.)	Necrosis and scorching of prophylls; development of axillary buds and necrosis of young leaves.	BG 369 T-9	T.69 K.63

An iron-efficient plant may respond to iron stress without having shown any visual iron deficiency symptoms such as chlorosis. When plants respond to iron stress, the following products or biochemical

reactions occur, more in iron-efficient than in iron-inefficient plants (Brown, 1978):

i) Hydrogen ions are released from the roots (Olsen, 1958).

ii) Reducing compounds are released from the roots (Brown et al. 1961).

iii) Organic acids (particularly citrate) increase in roots (Iljin, 1952).

iv) Ferric iron is reduced at the roots (Ambler et al. 1971).

v) The plant remains tolerant of relatively high phosphorus in the growth medium (Brown, 1972).

Each of these factors is associated with more efficient uptake and utilization of iron by the plant.

This response mechanism to iron stress is adaptive in several plant species (e.g. Soybean, Maize), and is known to be genetically controlled in several plant species (e.g. Soybean, Maize, Tomato) (Bell et al. 1958; Wann and Hills, 1973; Weiss, 1943).

Work with soybean (Weiss, 1943) and maize (Bell et al. 1958) cultivars indicated that cultivar performance under nutritional stress was determined by the genetic make up of the cultivars.

11.3.2.2 Viruses, soil microorganisms and nematodes - as factors in inducing iron chlorosis: Crawford (1939) reported that viruses can produce symptoms in plants that can be corrected or masked by amendments of iron.

A possible implication of this finding is that plants and viruses compete for iron and perhaps other micronutrients. When iron is insufficient, symptoms may be more severe than when iron is not in a critical level (Wallace and Lunt,1960). Martin et al. (1956) found that the presence of certain fungi, nematodes or other organisms can induce iron disorders.

Thorne and Wann (1950) found that the decomposition of organic matter by microorganisms can increase iron chlorosis by increasing the amount of carbondioxide and bicarbonate in soil solution.

II.3.3 Atmospheric factors

II.3.3.1 Temperature extremes: Temperature may affect the uptake of iron by influencing the rate of growth of the plants and the activities of microflora in the soil. Jones (1938) and Millikan (1945) noted that cool temperatures enhanced chlorosis of gardenias and flax.

Burtch et al. (1948) have reported that extremes in soil temperature promote the development of chlorosis; a high moisture level together with low soil temperature is the condition most conducive to the development of lime-induced chlorosis.

II.4 DIAGNOSIS AND PREDICTION OF IRON DEFICIENCY THROUGH SOIL AND PLANT ANALYSI

II.4.1 Soil analysis

Several factors have stimulated the need for research on the development of soil tests for micronutrients. Cox and Kamprath (1972) have

discussed these factors. Increased crop yields have resulted in more attention being given to the need for these elements. As yields have risen, the incidence of micronutrient deficiencies has become more frequent, because high yields cause greater removal of micronutrients from the soil. This factor, coupled with a lesser addition of micronutrients as contaminants in the more concentrated fertilizers in use today, has caused concern about the depletion of micronutrients in the soil. One of the most effective means of determining whether a particular nutrient is limiting or not is the soil test.

The objectives of micronutrient soil tests are:

(i) To identify the soils in a region or in a farmers' field that are deficient. This information is important for determining whether a soil can supply adequate micronutrients for optimum crop production, as well as for adequate nutrition of animals that may feed upon the produce.

(ii) To estimate the probability of a profitable response to the application of micronutrients (Cox and Kamprath, 1972).

Very few calibrations of soil test-crop response have been reported for iron in the literature. Several methods, though, have been devised to extract iron from soil, on the assumption that the techniques might be useful. Olson (1965) mentioned a number of these, yet concluded that no

one method had received wide usage or become accepted as a standard.

II.4.1.1 Acidic extractants

(i) Exchangeable: Extraction with acidic 1N amonium acetate (NH_4COOCH_3) has been shown to be of some use by Olson and Carlson (1950) and Randhawa et al. (1967). Olson and Carlson (1950) calibrated their method by comparing soil analysis values using ammonium acetate of pH 4.8 with the degree of deficiency symptoms observed in plants growing on the soil. At iron levels between 0.01 and 0.3 ppm chlorosis was moderate to severe. Between 0.3 and 2.2 ppm iron chlorosis was slight to moderate; and plants grown on soils ranging between 2.0 and 32.0 ppm iron were not chlorotic. From these results, it appeared that the critical level would be 2.0 ppm iron by this method for plants sensitive to iron deficiency, for example sorghum.

Randhawa et al. (1967) found 1N ammonium acetate (pH 3.0) as a useful extractant; he proposed that 15 ppm of extractable iron was the critical limit, below which crop responses were observed in wheat and maize.

II.4.1.2 Chelating extractants: Lindsay and Norvell (1969) developed the micronutrient soil test based on diethylene triamine penta acetic acid (DTPA). They used a mixture of 0.005M DTPA, 0.01M $CaCl_2$ and 0.01M triethanol amine, adjusted to pH 7.3. The soil test successfully ranked

the responsiveness of sorghum grown on 77 Colorado soils to zinc, iron and manganese fertilizers. The better acceptability of DTPA than other extractants or chelating agents appears to be related to the convenience of an extractant suitable for simultaneously assessing four micronutrient cations, viz; zinc, iron, manganese and copper.

The critical range for sorghum was 2.5 to 4.5 ppm extractable iron (Lindsay and Norwell, 1978). This method is presently in use in most parts of the world.

However, the occurrence of deficiency seems to be dependent on many factors, apart from an "available" amount in the soil. Some of these factors are even inherent in the plant. It is doubtful that any iron soil test will be very reliable until the more important of these factors are understood (Cox and Kamprath, 1972).

II.4.2 Plant analysis

Plant analysis indicates the accessibility to the plant of the nutrients in the soil. According to Aldrich (1967), it is used for the following purposes:

i) Diagnosis, or confirming the diagnosis of visible symptoms.
ii) Locating areas of incipient deficiencies.
iii) Indicating whether an applied nutrient entered the plant.
iv) Indicating interactions among nutrients.
v) Understanding the internal functioning of the plants.

Iron analyses are probably invalid unless the leaf material has been washed in dilute acid or detergent because the leaves may carry some dust containing iron (Bennett, 1945). Jacobson (1945) also found it necessary to wash leaves in order to properly evaluate the iron status of pear and citrus trees.

The prediction of micronutrient deficiencies based on diagnostic tissue analysis has been reasonably successful for all the micronutrient elements except iron (Cox and Kamprath,1972). For example, total iron content in the plant was not associated with the occurrence of chlorosis. (Hoffer and Carr, 1920; Milad, 1924). Also, chlorotic tissue or plants were found to have higher concentrations of iron than healthy, for corn stalks (Hoffer and Carr, 1920), pear leaves (Milad, 1924), soybean plants (Somers and Shive, 1942), pea leaves (Singh,1970) and rice plants (Patel et al. 1977). It was therefore inferred that much of the iron present in chlorotic plants is in an insoluble form, and is physiologically inactive.

Since iron deficiency may be associated with an imbalance with other plant nutrients. Several workers (Bennett, 1945; Dekock, 1958; Mehrotra et al. 1976) have suggested the use of Fe:P, Fe:Ca, and Fe:Mn nutrient ratios for diagnosing iron chlorosis. However, several workers have indicated that these ratios are not universally applicable. (Lindner and Harley, 1944; Wallace and Hewitt, 1946; Agarwala and Kumar, 1962).

Because iron is linked with a number of enzyme systems (Price 1968), a change in the activities of enzymes (such as catalase, peroxidase) has been investigated for use as an index of iron deficiency in plants (Del Rio et al. 1978).

Oserkowsky (1933) suggested that extraction of plant tissue with dilute acid could estimate the active iron. Wallace (1971) and Patel et al. (1977) have also proposed analysis of plants for a fraction of iron which correlates with the occurrence of chlorosis.

In some instances, the acid extractable iron correlated well with the incidence of chlorosis in chlorotic potato plants (Bolle Jones, 1955), or with chlorophyll contents and iron deficiency symptoms (Jacobson, 1945); but in others it did not (Oserkowsky, 1933). The lack of acceptance of this technique was attributed to lack of specificity in the form of iron being extracted (Katyal and Sharma, 1980). According to Machold (1968), ferrous iron is the "active fraction" of iron in the plant.

Katyal and Sharma (1980) have further developed the idea of analysing tissue for active iron. They extracted tissue with o-phenanthroline; this technique estimates the ferrous iron (Gupta, 1968) which is assumed to be that fraction of iron which is more important for synthesis of chlorophyll and consequently occurrence of chlorosis.

The choice of 1-10 o-phenanthroline (O-Ph) as an extractant for Fe^{2+} was based on its remarkably higher stability constant for Fe^{2+} than Fe^{3+}.

On this basis, it preferentially chelates Fe^{2+} (Gupta, 1968). The highly specific orange colour of the Fe^{2+} phenanthroline complex makes possible the determination of Fe^{2+} by the simple procedure of reading the transmittancy at 510 nm in a spectrophotometer.

II.5. IRON DEFICIENCY IN GROUNDNUT

There are only a few published papers that specifically involve research on the iron nutrition of groundnuts. Some general symptoms of iron deficiency in groundnut have been reported by Gopalakrishnan et al. (1962) and Verma and Bajpai (1964). These symptoms were: chlorosis of younger leaves, reduction in leaf size, highly stunted plant growth, and in acutely deficient plants, drying and dropping of leaves. Lachover and Ebercon (1972) also reported that severe iron deficiency of groundnut caused the entire surface of the young leaflets to appear whitish-yellow, often with the development of red spots, followed by necrosis of the margins.

Young (1967)also reported that irrigated "Starr" spanish-type groundnuts commonly showed marked chlorosis when grown on the more calcareous soils in his fields; he found that the soil contained 3 to 4 tons of available CaO per acre under the chlorotic areas. Mild chlorosis did not cause any detectable decrease in groundnut yields; severe chlorosis decreased groundnut yield by about 50%.

Hartzook et al. (1971) reported that iron chlorosis and growth retardation in groundnut plants was associated with high soil pH (7.6 to 8.3), high levels of lime (upto 23%), phosphorus and bicarbonate in soil and/or irrigation water. He attributed the induced deficiency to the effect of these factors in causing inactivation of iron in both soil and plant systems. In subsequent work, Hartzook et al. (1972a, 1974) reported that there was considerable genetic variability among groundnut cultivars with respect to differential utilization of iron on calcareous soils. Three iron-inefficient commercial cultivars and five efficient experimental cultivars of groundnuts were compared under iron treated (Fe-EDDHA) and untreated conditions for yield and market quality of pods. The gain in yield for chelate treatment ranged from 22 to 210% for the inefficient commercial cultivars but only 8 to 18% for the efficient cultivars. Lachover and Ebercon (1968) reported groundnut grown on a loess-like Negev soil had yields reduced due to iron chlorosis. Hartzook et al. (1972b) suggested growing of iron-efficient cultivars on calcareous and alkaline soils, instead of applying costly iron compounds to the field; they have isolated genetic variants of groundnuts with differential iron absorption.

Gopalakrishnan and Srinivasan (1976) compared chlorosis of groundnut caused by poor drainage with that caused by mineral deficiencies of nitrogen, sulfur, iron and observed that

a) chlorotic symptoms of the foliage developed on the 60th day;

b) poor drainage resulted in a reduction of pod yield by 30 percent, and oil content by 3.2 percent.

c) sulfur, iron and nitrogen deficiency reduced the yield by 34, 38 and 41 percent respectively.

There have been several recent reports of research on iron chlorosis in India. "Lime-induced chlorosis" was stated to be one of the major factors in limiting the yields of groundnut under irrigation on black clay soils (Chandrasekhar Reddy, 1979). Patel et al. (1982) reported that chlorosis was acute during prolonged drizzling rain in groundnut grown on medium-black calcareous soils of the Saurashtra region of Gujarat; the typical yellowing of the leaves was attributed to a combination of poor drainage and lime-induced iron deficiency.

Lime-induced chlorosis is not necessarily due to non-availability of iron in soil but it may be due to restricted translocation of iron from root to shoot or inactivation of iron within the plant tissues. Patel et al. (1982) have also reported that iron chlorosis occurred under wet soil conditions, especially where a heavy downpour was followed by prolonged light rain. They attributed this effect to migration of clay particles from the surface soil to a depth of 1 or 2 cm resulting in the formation of a layer (pan) which restricted the permeability of air to the rootzone: they further stated that subsequent drying of the soil with formation of

cracks permitted free movement of air to the rhizosphere which alleviated the chlorosis. However,they did not provide any proof of these suggested mechanisms.

II.6 REMEDIES FOR IRON CHLOROSIS

Iron chlorosis is one of the most difficult micronutrient disorders to correct in the field. For correcting iron deficiency in plants, it is first necessary to understand the conditions which cause the deficiency. Some of the methods proposed are:

i) Correction of soil reaction and/or decreasing carbonate concentration by acidifying the soil:

This approach is not practicable as it is very expensive (Kanwar, 1976).

ii) Drainage improvement

Drainage improvement, restricted irrigation, and exposing the trees to a dry period were found to be effective for the control of chlorosis in citrus, when the chlorosis was due to wetness of the soil (Kanwar, 1976).

iii) Use of iron-efficient cultivars

Genetic variability among groundnut and soybean cultivars for efficient utilization of iron on calcareous soils have been

reported by Harzook et al. (1972a, 1974) and Weiss (1943). Although application of iron compounds to the soil or plant may effectively overcome the deficiency, the most efficient "treatment" would be the breeding of cultivars adapted to soil conditions that promote the deficiency.

iv) Application of iron compounds to soil or plants

a) Application of iron compounds to soil: Soil amendments with either inorganic or synthetic organic sources of iron have been extremely variable in their effectiveness due to the reactions that occur between the applied iron and soil components (Murphy and Walsh, 1972). Under some conditions, the application of inorganic salts containing iron (particularly $FeSO_4$) to the soil has given good results, but generally this method is very wasteful, because the ferrous ion oxidized quickly to the ferric form and thus becomes inactivated (Wallihan, 1965; Kanwar, 1976).

Because many difficulties have been encountered with soil applications of inorganic iron salts, a considerable amount of attention has been given to application of chelates to the soil. The iron chelates have the property of keeping iron in solution by protecting it from the ordinary reactions that form insoluble compounds such as iron hydroxide, iron phosphate, and iron carbonate (Wallihan, 1965).

Schneider et al. (1968) pointed out that iron deficiency must be corrected in early stages of plant growth to obtain maximum yield responses.

They suggested that soil applications of iron chelate should be made before or at planting with Fertilizer-N applications also increased the efficiency of iron uptake. Lachover and Ebercon (1969) tried several chelating agents to control iron chlorosis. Of the iron chelates tested, the most effective has been Sequestrene 138 Fe (Commercial Fe EDDHA, i.e. sodium ferric ethylenediamine di-(O-hydroxy phenyl acetate).

Promising results were also obtained by coating seeds with chelate as an iron starter, followed by an additional top dressing. Lachover and Ebercon (1969) concluded that groundnuts could be grown economically in their highly calcareous soils using Sequestrene 138 Fe at 10 to 15 kg/ha in two dressings (10 and 46 days after emergence); this produced the high yield of 4315 kg/ha of pods and 4350 kg/ha of haulms.

b. Application of iron compounds to plants

Applications of ferrous sulphate or other soluble salts of iron to plants, by spraying onto leaves, have differed widely in their effectiveness, and this has been related to the species of the plant. The plants which do not respond well present a practical difficulty (Wallihan, 1965).

i) The entry of iron is localised i.e. the iron that enters the leaves gets quickly immobilized and does not benefit leaves which develop later on.

Wallace et al. (1957) suggested spraying of chelates onto plant leaves would be more economical than sprays of other ferrous compounds. They suggested the following advantages of foliar applications in general over applications of iron to soil:

(i) Elimination of uncertainty due to the complexity of the iron soil reactions.

ii) Irrigation is not required to move the compounds into the root zone for absorption by plants.

iii) Economy of materials is effected by foliar applications, because of removal of iron soil interactions.

iv) More rapid responses of applied iron.

On the other hand, Wallace et al. (1957) pointed out that disadvantages of foliar applications of iron also exist: which are

i) greater chance of toxicity

ii) incomplete coverage of plant leaves and therefore a subsequent uneven response.

iii) Need for repeated applications.

Young (1967) reported that sprays of 1.5 or 2% iron chelate or iron polyflavonoid with triton spreader caused moderately chlorotic

groundnut leaves to turn green within a week. About three sprays per season are needed to control chlorosis. Lachover and Ebercon (1969) applied iron polyflavonoid spray on leaves. At first, two sprays of 0.2% solution were applied and found a slight and temporary improvement. However, when two more sprays (at the age of 46 and 67 days) with a higher concentration (2% solution) were used, there was a marked improvement in color and the plants started to grow.

Khatri and Singh (1968) tried a number of inorganic, organic and polyflavonoid forms of iron carriers for controlling iron chlorosis in groundnuts. On the basis of their observations, compounds tested can be arranged in the following order based on their effectiveness.

Rayplex-Fe > Ferrous ammonium sulphate > Ferrous sulphate > Ferrous tartrate > Ferric citrate. Results from these studies are summarised in Table 1b.

Lachover et al. (1970) reported that Sequestrene 138 applied at 4000 g/acre in two equal split dressings at 22 and 45 days after seeding was very effective. This treatment increased the pod yield by 50% and the haulm yield by 40% over the control yield (no iron added). Hartzook et al. (1971) reported that Sequestrene 138 when applied at the rate of 10 kg/ha gave an increase of 39 percent in pod yield. The Sequestrene was dissolved in water as a 10% solution and injected into the soil with special equipment on both sides of the row, at a distance of 5 cm from the plants and at a depth of 3-5 cm.

Table 1b: Effect of spray application* of different iron compounds on chlorotic groundnut plants. Khatri and Singh (1968).

Observations recorded 15 days after application

Compound	Response
Ferrous ammonium sulphate	Very little scorching. Irregular greening with bigger spots, localised at margins, involving 50-60 percent of area - Increased growth observed. Greening started 2 days after the application.
Ferrous sulphate	Little scorching. Irregular greening with smaller spots localized at margin, involving 50-60% of leaf area. Greening started 2 days after the application.
Ferrous tartrate	No scorching. Irregular greening with small dots, localised all over the leaf surface, involving 20-30 percent leaf area. No appreciable increase in plant growth was recorded. Greening started 2 days after the application.
Ferric citrate	Scorching noticeable in very minute dots spreading irregularly all over the leaf surface. Greening was recorded in very minute dots, involving hardly 20-30 percent of leaf area. No appreciable increase in growth was observed. Greening started 2 days after the application.
Rayplex-Fe	Greening with large coalescing spots, involving 70-80 percent leaf area. No scorching. Appreciable increase in plant growth was also recorded. Greening started 3 days after the application.

Containing 0.1% Fe for each compound.

In pot trials with sandy loam soil, conducted by Lachover and Ebercon (1972), groundnut seedlings exhibited symptoms of mild chlorosis in younger leaves. Among the various iron compounds used to rectify the

deficiency, application of Sequestrene 138 (containing 6% Fe) at the rate of 10 to 15 kg/ha at 10 and 46 days after seedling emergence was found effective. Several chelates were examined, and all decreased leaf chlorosis and increased leaf peroxidase activity. Yields of unshelled pods increased from 0.94 t/ha in untreated controls to 1.47 to 4.3 t/ha by the iron applications. Yields of haulms were also increased from 2.2 to 4.35 t/ha. Increases in yield were obtained by the application of iron compounds to plants.

Even though chelates have been usually much more efficient than inorganic compounds, soil applications have commonly not been economical, as their cost remains high (Murphy and Walsh,1972).

Hartzook (1975) also reported that favourable response was obtained to iron chelates. The optimal date of spraying onto plants was found to be between 40 and 50 days after emergence, and the recommended rate was 10 to 15 kg/ha, applied as suspension of 1 to 5 percent concentration. The iron chelate treatments corrected the chlorosis within seven to ten days after application, and they increased the number of pods per plant, the average pod and kernel weights, and consequently the yield per unit area.

Patil (1978) reported that foliar spray of 0.5 percent ferrous sulphate in combination with 2 percent urea at 90 and 100 days after sowing helped to correct the chlorotic symptoms in groundnut and enhanced

the pod yield by 8.2 percent compared to unsprayed control. The higher pod yield obtained was attributed to greater 100 kernel weight (2.1 percent) and pod weight per plant (12.2 percent) compared to unsprayed control. The foliar spray of ferrous sulphate and urea corrected the chlorotic symptoms and increased the leaf dry matter and total dry matter production. Improvement in the oil and protein content to the extent of 0.5 percent and 1.7 percent by foliar spray of ferrous sulphate and urea was observed as compared to unsprayed control.

Recently Chandrasekhar Reddy (1979) reported that 'lime induced chlorosis' in groundnut can be corrected by spraying 0.5 percent ferrous sulphate, four times at fortnightly intervals starting from 15th day after sowing.

III. MATERIALS AND METHODS

III.1 EXPERIMENTAL SITES

III.1.1 Location

All experiments were conducted at ICRISAT Center, Patancheru, which is located 26 km North-West of Hyderabad, and is the headquarters of the International Crops Research Institute for the Semi-Arid Tropics

III.1.2 Weather

Long-term monthly means of the meteorological observations of rainfall, temperature, and relative humidity are presented in Table 2.

III.1.3 Soils and Water

The soils used for the experimentation were alfisols and an entisol. The physical and chemical characteristics of the surface soils (0-15cm) used in the experiments are presented in Table 3. Some properties of the water used in the pot experiment are given in Table 4; single-distilled (glass) water was used for all laboratory analyses.

III.2 FIELD EXPERIMENTATION

III.2.1 Monitoring of Iron Chlorosis and Iron Content of Leaves of Groundnut (cv TMV 2) grown on an Alfisol during Rainy Season

Groundnut (cv TMV 2) leaves were sampled at intervals of 2-14 days during the rainy season in 1981 from the 4 replicate plots of an existing

Table 2: Rainfall, temperature and relative humidity at ICRISAT Center, Patancheru in 1981, and long-term means.

Month	Rainfall (mm)		Temperature (°C)				Relative Humidity (%)			
			Maximum		Minimum		Mean$^{\delta}$		1981$^{\alpha}$	
	Mean*	1981$^{\alpha}$	Mean$^{\sigma}$	1981$^{\alpha}$	Mean$^{\sigma}$	1981$^{\alpha}$	am	pm	am	pm
Jan	6	16	29	27	15	14	79	36	81	38
Feb	11	0	31	32	17	16	64	35	61	19
Mar	13	77	35	34	20	20	54	30	70	27
Apr	24	3	37	38	24	23	51	31	53	19
May	27	2	39	39	26	26	50	33	51	22
Jun	115	202	34	35	24	24	71	54	79	45
Jul	171	209	30	31	22	23	83	69	84	58
Aug	156	218	29	28	22	22	82	70	89	70
Sep	181	287	30	29	22	22	82	71	91	72
Oct	67	154	30	30	20	20	73	58	88	53
Nov	23	2	29	28	16	15	68	48	82	40
Dec	6	0	28	27	13	14	71	42	84	41
Total	800	1170								

* Based on 1901-70 rainfall data at Hyderabad

$^{\delta}$ Based on 1931-60 relative humidity data at Hyderabad

$^{\alpha}$ Recorded at ICRISAT Center, Patancheru, A.P. India.

$^{\sigma}$ Based on 1931-60 temperature data at Hyderabad

Table 3: Characteristics of surface soils (0-15 cm) used for experimentation

Characteristic	Field experiment		Pot experiment
	*RM 5	*RP 7C	*RP 3C
Particle size distribution (%)			
Sand (2-0.02 mm)	72	62	64
Silt (0.02-0.002 mm)	14	9	8
Clay (<0.002 mm)	14	29	28
Organic matter (%)			
Organic carbon	0.69	0.37	0.49
Total nitrogen	0.066	0.034	0.054
Available nutrients (µg/g)			
Nitrogen	94	101	105
Phosphorus	4	17	12
Exchangeable potassium	-	110	100
pH	8.12	7.98	8.29
Electrical conductivity (millimho/cm)	0.28	0.23	0.23
Cation exchange capacity (meq/100 g)		13.1	12.2
DTPA extractable micronutrients (µg/g)			
Iron	9.2	7.4	6.5
Zinc	4.4	4.3	1.3
Manganese	14.2	18.0	11.6
Copper	2.1	1.3	1.2
Calcium carbonate (%)	0.50	0.28	0.25
Moisture content at field capacity (%,w/w)	17	21	20

[CRISAT Field which was the site, or source of soil, for experiments.

Table 4: Properties of water used in pot experiment.

Property	*Source of water	
	Deionized	Borewell
pH	5.40	7.90
EC (millimhos/cm)	< 0.15	0.75
Carbonate (µg/ml)	Nil	15
Bicarbonate (µg/ml)	61	366
Total iron (µg/ml)	0.09	0.10
Dissolved nutrients (µg/ml)		
Sodium	12	65
Calcium	1	5
Magnesium	1	3
Boron	Nil	0.30
Sodium adsorption ratio	2.0	5.7

* Central supplies at ICRISAT Center.

experiment in RP7C-North, which is one of ICRISAT Center's Alfisol Precision Fields. The first sampling was made on 29 July, and the last on 25 September. A row of 5 plants was harvested from each of two areas within a plot. The plants were placed in plastic bags to minimize moisture loss during their transit from the field to laboratory. Leaves of the same age from the plants within a plot were bulked together prior to preparation for chemical analysis. For most samplings only the main bud, lateral bud and first fully opened leaf were taken.

For the last 7 samplings soil samples were collected at the same time as the plant samples, and from the vicinity of the plants sampled. Five cores were taken from each of the two sampling areas within each plot, and the cores from each plot were bulked together. A sub-sample of the moist soil was retained for analyses for DTPA extractable iron and moisture content; the remainder of the soil was air dried.

III.2.2 Sampling procedure

To provide information on the most suitable leaves for analysis, the first three samplings on the alfisol (in section III.2.1) were more detailed than at the subsequent eleven sampling occasions. For these initial samplings, the 2nd, 3rd, 4th and 5th fully opened leaves (abbreviated to L-2 to L-5) were sampled in addition to the main bud (Mb)

lateral buds (Lb) and the first fully opened leaf (L-1); the latter three plant parts only viz., main bud, lateral bud and first fully opened leaf (FOL or L-1) were sampled as part of the regular monitoring program.

III.2.3 Field observations of Chlorosis in Groundnut Breeding entries on an Entisol

Widespread marked chlorosis developed in August 1981 on one field (RM5) which was used regularly for screening of groundnut breeding entries. Samples were collected from 8 cultivars, which had been selected to provide 4 pairs of cultivars to represent extremes of growth and susceptibility to chlorosis. The selection method involved scoring each of the 64 entries in the field for:

a) total growth
b) proportion of leaves with mild chlorosis
c) proportion of leaves with severe chlorosis

On 1st September 1981, 20 plants were taken from each plot of each of the 8 chosen cultivars. There were three replicate plots for each entry, arranged in a randomized block design. The main buds (Mb) and the first fully opened leaves (L-1) were taken and bulked together within a plot, as described earlier.

Soil samples were collected on 1st September with a core sampler from the vicinity of the plants sampled. The cores within a plot were

bulked together, and separated into a small subsample (100 g) for analyses on the moist soil (on the same day) and a remaining large sample which was air-dried and ground prior to analysis. The moist sample was used for the estimation of DTPA extractable Fe and moisture content. All other analyses were made on the air-dried ground sample.

III.2.4 Correction of iron deficiency in groundnut on an entisol

Iron chelate treatments were applied to a number of ICRISAT groundnut breeding entries within an existing experiment on an entisol; this experiment was located within the same field (RM5) as the cultivars examined in the previous section. The 7 breeding entries examined in this work were:

i) Var. 27
ii) U-1-2-1
iii) EC 76446
iv) Gangapuri
v) PI 337394
vi) J 11
vii) TMV 2

There were 6 rows (4 m long) in each plot of the above cultivars. The plot size was 18 m^2 (4x4.5 m) and the area of each row was 3 m^2. The

two border rows were eliminated and the following four treatments were imposed on the middle four rows.

a) control

b) spray : iron chelate Sequestrene 138 (1%, w/v) sprayed onto the foliage

c) soil application : iron chelate applied at 1 g/m^2 by drenching the soil in rows of groundnut plants with iron chelate solution

d) spray + soil application.

III.3 POT EXPERIMENT

A sample of an alfisol surface soil (0-15 cm) was collected from the RP3C Precision Field. About 5 kg soil was collected from each of 20 different locations in this field. This bulk sample was air-dried, then lightly ground using a wooden mallet to pass a brass sieve with 2 mm mesh. Plastic pots of 1 liter capacity were filled with 1 kg of soil. The dimensions of the pots were: height 12 cm, diameter 12.5 cm (top) and 8.3 cm (bottom). There were 6 holes in the base for drainage, which was collected in a saucer.

The soil was moistened with water to 70% of field capacity prior to filling the pots. Groundnut (cv TMV 2) was sown at 5 seeds/pot on

4th July; they emerged on 11th July 1981. The populations were then thinned to 3 plants per pot on 20th July 1981. Deionised or borewell water was applied daily to compensate for loss of moisture by evapotranspiration.

The following treatments were examined, using a randomized block design with 6 replications:

Treatment No.	Iron	Sodium carbonate	Water	
			Borewell	Deionised
A	-	-	-	+
B	-	+	-	+
C	-	-	+	-
D	-	+	+	-
E	+	-	-	+
F	+	+	-	+
G	+	-	+	-
H	+	+	+	-

Iron was applied as Sequestrene 138-Fe (Fe-EDDHA) in aqueous solution to the soil surface of the pots. It was applied 7, 16 and 44 days after sowing. The rate of application was 3, 2 and 3 μg chelate per g of soil on the three separate occasions. Sodium carbonate was applied as an aqueous solution to the soil surface in 5 split applications each consisting of 325 μg/g of soil 9, 16, 27, 36 and 44 days after seeding.

Zinc was applied as 10 μg $ZnSO_4 \cdot 7H_2O$ per g of soil on the 23rd day after seeding. On the same day all plants were sprayed with 0.4% manganous chloride. Potassium was supplied as K_2SO_4; 100 μg of the salt was added per g of soil 44 days after seeding. Phosphorus was not added because the soil contained adequate amounts of available phosphorus as shown by soil test value of 12 μg/g (Table 3).

A few of the youngest leaves were sampled for chemical analysis at 105 days after seeding. The crop was harvested 30 days later, that is 135 days after sowing. The plants were separated into haulms, pods and roots. All the plant parts were thoroughly washed with dilute (0.3N) hydrochloric acid and distilled water, dried and ground.

III.4 METHODS OF PLANT AND SOIL ANALYSIS

III.4.1 Plant analyses

Orthophenanthroline extractable iron was determined on samples of fresh tissue by the method described by Katyal and Sharma (1980). The procedure involves extraction of 2 g of thoroughly washed, chopped, fresh plant tissue with 20 ml of o-phenanthroline extractant (pH 3.0; conc 1.5%) The plant samples treated with the extractant are allowed to stand for 16 hours and Fe^{2+} is determined in the filtrate by reading the transmittancy at 510 nm in a spectrophotometer. All other analyses were made on oven-dried material; the samples were dried for 48 hours at $60^{o}C$ prior to

grinding to pass a 40 mesh sieve. For nitrogen and phosphorus, 100-150 mg sample was weighed into "Tecator" digestion tubes (75 ml capacity); 4 ml of concentrated sulfuric acid containing 0.5% selenium powder was added to each tube. Digestion was continued at a temperature of 360°C until 30 minutes after clearing; the total time of digestion was about 1 hour and 30 minutes. The tubes were then allowed to cool, and the contents made to volume (75 ml) with distilled water. Nitrogen and phosphorus content in the digests were estimated colorimetrically by the indophenol blue method in alkaline medium (for nitrogen), and for phosphorus, the vanado molybdo-phosphoric yellow colour method in acid medium; the "Technicon" Autoanalyzer II was used for the colorimetry (Technicon, 1972).

Total calcium, magnesium, potassium, iron, copper and manganese contents of the air-dried ground sample were estimated by the atomic absorption spectrophotometer using the tri-acid digestion method (Jackson, 1967), which involved the digestion of 200-250 mg of oven-dried, ground plant tissue with 6 ml of tri-acid; nitric acid, sulfuric acid, perchloric acid in the ratio of 10:0.5:2 for 2 or 3 hours on a sandhbath. Sulfur was analysed by the modified colorimetric method as described by Palaskar et al. (1981).

III.4.2 Soil analyses

Soil pH was measured using a glass electrode, a calomel reference electrode, and pH meter (Mocel LI-10) all supplied by ELICO (Hyderabad,A.P).

Salt content was measured by using an electrical conductivity bridge. Both pH and EC measurements were made on 1:2 soil:water suspension (Jackson 1967).

Organic carbon was determined by Walkley-Black method as described by Allison (1965). Cation exchange capacity was determined by the sodium acetate (pH 8.2) method as outlined by Jackson (1967). Exchangeable potassium was determined using an atomic absorption spectrophotometer, after extraction of the soil with neutral 1N ammonium acetate (Jackson 1967).

Available nitrogen was determined by the alkaline permanganate method outlined by Subbiah and Asija (1956), and available phosphorus by the sodium bicarbonate method as described by Olsen and Dean (1965).

Total nitrogen was determined by the modified Kjeldahl method described by Jackson (1967). The particle size distribution of the soil (w/w) was measured using the hydrometer method (Day 1965). Available iron, copper, manganese, zinc were determined by extracting the soil with DTPA (Diethylenetriamine penta acetic acid) as suggested by Lindsay and Norwell (1969).

The carbonates and bicarbonates in deionised and borewell water were estimated by the method of neutralization with 0.05N H_2SO_4 using phenolpthalein and methylorange as indicators (Chapman and Pratt 1961).

All chemicals used were of AR grade. Distilled water was used for all laboratory work.

IV. RESULTS AND DISCUSSION

IV.1. SAMPLING PROCEDURE

IV.1.1 Results

Examination of the leaves of different ages (Table 5) showed that extractable iron content increased with age of leaf. This pattern was consistent over all 3 samplings, even though the extractable iron content decreased consistently in all leaves over the 3 successive samplings during the period July 30 - August 3 when chlorosis became increasingly obvious in this field (see Section IV.2.1). The chlorosis was confined to the youngest leaf tissue (the mainbud, lateral bud and first fully-opened leaf); that is, the tissue which had the lowest concentration of extractable iron.

The relationship between the concentration of extractable iron in leaf tissue and the age of leaves was less clear when the concentration measured in the fresh tissue was expressed on an oven dry tissue basis (Table 6). The occurrence of chlorosis was not associated with an immediate decrease in extractable iron (on a DW basis) but all of the opened leaves (Leaf 1 to Leaf 5) showed a decrease in extractable iron between July 29 and August 3.

Total iron concentrations were not consistently related to age of leaf, nor was there a decrease in total iron content with the onset of chlorosis (Table 7). In fact, the total iron content of the buds (but

Table 5' Concentration of o-phenanthroline extractable iron (µg/g) in groundnut (cv TMV 2) leaves of different age : Results expressed on fresh weight basis; alfisol, 1981.

Sampling date	Leaf age $^{\phi}$						
	Mb	Lb	L-1	L-2	L-3	L-4	L-5
July 29	5.4	6.0	7.2	7.5	8.5	8.7	9.6
July 31	4.3*	5.0**	5.6*	6.5	7.0	8.1	8.0
Aug 3	4.0**	4.2**	4.9*	5.8	6.0	6.1	6.2
SE ±				0.39			
Mean	4.6	5.1	5.9	6.6	7.2	7.6	7.9
SE ±				0.22			

$^{\phi}$ Leaf age : Mb is main bud; Lb is lateral bud; L-1 is youngest unfolded leaf, and L-5 is the oldest unfolded leaf.

* Slight chlorosis

** Marked chlorosis

Table 6 Content of o-phenanthroline extractable iron (µg/g) in groundnut (cv TMV 2) leaves of different age : Results expressed on dry weight basis; alfisol 1981.

Sampling date	Leaf age $^{\phi}$						
	Mb	Lb	L-1	L-2	L-3	L-4	L-5
July 29	34.2	35.5	40.7	38.0	43.5	42.5	43.7
July 31	35.7*	45.5**	39.5*	38.7	35.5	42.5	45.7
Aug 3	33.5**	33.5**	31.7*	32.0	32.7	32.0	32.2
SE ±				2.08			
Mean	34.5	38.2	37.3	36.2	37.2	39.0	40.5
SE ±				1.20			

$^{\phi}$ Leaf age : Mb is main bud; Lb is lateral bud; L-1 is youngest unfolded leaf, and L-5 is the oldest unfolded leaf.

* Slight chlorosis

** Marked chlorosis

Table 7 Content of total iron (µg/g of O.D. tissue) in groundnut (cv TMV 2) leaves of different age ; alfisol 1981.

Sampling date	Leaf age ϕ						
	Mb	Lb	L-1	L-2	L-3	L-4	L-5
July 29	233	237	246	180	213	293	310
July 31	230*	456**	188*	231	272	305	289
Aug 3	340**	531**	217*	207	203	347	341
SE ±				34.6			
Mean	267	408	217	206	229	315	313
SE ±				20.0			

ϕ Leaf age : Mb is main bud, Lb is lateral bud, L-1 is youngest unfolded leaf and L-5 is the oldest unfolded leaf.

* Slight chlorosis

** Marked chlorosis

not the older leaves) increased significantly between July 29 and August 3.

The fraction of total iron that could be extracted with o-phenanthroline decreased markedly during the period July 31 - August 3 (Table 8), but the very low values of 0.06-0.09 were not confined only to leaves that were chlorotic. Some older leaves also gave low values. There was no consistent relationship with age of leaf.

Table 9 shows the content of other nutrients in the leaves of different age. Nutrient contents of leaves differed between leaf ages and between samplings, although a number of results are not consistent.

The most marked relationships observed were those between leaf age and phosphorus, potassium and calcium; the change in concentrations were highly significant ($P < 0.01$); with increasing age of leaf, the phosphorus concentration decreased from 0.70% in the buds to 0.30% in the older leaves, and potassium concentration decreased from 3.3 - 3.6% in buds to 1.0% in the older leaves. Calcium concentration increased with leaf age from less than 1% in buds to over 2% in leaf-5.

Magnesium contents also increased with age, but to much lesser extent than calcium. Nitrogen concentration decreased markedly with leaf age to L-2 but older leaves showed little change with age.

Table 8. Fraction of total iron (µg/g of O.D. tissue) extractable with o-phenanthroline in groundnut (cv TMV 2) leaves of different age: alfisol 1981.

Sampling date	Leaf age ϕ						
	Mb	Lb	L-1	L-2	L-3	L-4	L-5
July 29	0.15	0.15	0.17	0.21	0.20	0.14	0.14
July 31	0.16*	0.09**	0.20*	0.16	0.13	0.14	0.16
Aug 3	0.09**	0.06**	0.14*	0.15	0.16	0.09	0.09
		SE ± 0.016					
Mean	0.13	0.09	0.17	0.17	0.16	0.12	0.13
		SE + 0.009					

ϕ Leaf age: Mb is main bud, Lb is lateral bud, L-1 is youngest unfolded leaf and L-5 is the oldest unfolded leaf.

*Slight chlorosis

** Marked chlorosis

Table 9. Total nutrient contents of groundnut leaves of different age (cv TMV 2); alfisol 1981.

Sampling date	Leaf age $^{\phi}$						
	Mb	Lb	L-1	L-2	L-3	L-4	L-5
(a) Total nitrogen (%)							
July 29	5.73	5.32	4.34	3.90	3.90	3.94	3.61
July 31	4.37	6.16	5.34	3.90	4.08	4.26	3.98
Aug 3	6.34	6.16	5.35	4.57	4.61	4.26	4.23
SE ±				0.145			
Mean	5.48	5.88	5.01	4.13	4.20	4.16	3.94
SE ±				0.083			
(b) Total phosphorus (%)							
July 29	0.74	0.68	0.43	0.29	0.25	0.28	0.28
July 31	0.70	0.70	0.51	0.41	0.35	0.38	0.33
Aug 3	0.70	0.70	0.45	0.38	0.34	0.29	0.28
SE ±				0.025			
Mean	0.71	0.69	0.46	0.36	0.32	0.32	0.30
SE ±				0.015			
(c) Total potassium (%)							
July 29	3.83	3.28	2.92	1.53	1.55	1.06	1.00
July 31	2.52	3.94	2.71	1.85	1.39	1.34	0.95
Aug 3	3.45	3.77	2.76	2.02	1.57	1.25	1.12
SE ±				0.152			
Mean	3.27	3.66	2.80	1.80	1.37	1.21	1.02
SE ±				0.088			
(d) Total calcium (%)							
July 29	0.90	0.78	1.12	1.47	1.76	1.92	2.20
July 31	0.81	0.77	0.92	1.35	1.77	1.96	2.25
Aug 3	0.59	1.00	1.08	1.40	1.53	2.09	2.10
SE ±				0.096			
Mean	0.77	0.85	1.04	1.40	1.68	1.99	2.18
SE ±				0.056			

Cont.....

Table 9 (cont'd)

Sampling date	Leaf age ϕ						
	Mb	Lb	L-1	L-2	L-3	L-4	L-5
(e) Total magnesium (%)							
July 29	0.55	0.45	0.39	0.38	0.48	0.48	0.63
July 31	0.38	0.41	0.31	0.37	0.38	0.53	0.65
Aug 3	0.47	0.52	0.55	0.56	0.57	0.65	0.71
SE ±				0.043			
Mean	0.47	0.46	0.41	0.44	0.48	0.55	0.66
SE ±				0.025			
(f) Total zinc (µg/g)							
July 29	67	66	49	46	47	43	33
July 31	55	64	54	52	49	54	54
Aug 3	70	68	48	55	50	48	52
SE ±				3.0			
Mean	64	66	50	51	48	48	46
SE ±				1.7			
(g) Total manganese (µg/g)							
July 29	38	39	40	31	44	44	38
July 31	36	35	32	43	46	46	53
Aug 3	23	27	31	36	46	40	51
SE ±				5.12			
Mean	32	33	34	37	45	43	47
SE ±				2.95			
(h) Total copper (µg/g)							
July 29	9.1	9.4	9.2	6.7	10.5	12.3	8.4
July 31	13.1	11.6	11.1	11.5	13.2	10.3	10.7
Aug 3	18.2	18.0	15.8	15.2	7.1	7.3	8.6
SE ±				1.57			
Mean	13.5	13.0	12.0	11.1	10.2	10.0	9.2
SE ±				0.85			

ϕ Leaf age : Mb is main bud; Lb is lateral bud; L-1 is youngest unfolded leaf and L-5 is the oldest unfolded leaf.

Note : Values on dry weight basis

Zinc and manganese concentrations changed significantly with leaf age, but the major difference was between buds and the older leaves. The zinc concentration was significantly higher in the buds (65 ppm) than in all opened leaves (47-51 ppm); manganese concentration of buds and first two opened leaves (32-37 ppm) was less than that of the three older leaves (43-47 ppm). Copper concentration usually decreased with leaf age.

IV.1.2 Discussion

Because iron chlorosis is invariably confined to the younger leaves of groundnut, it was expected that a successful diagnostic tissue test would indicate lower levels of 'active' iron in these younger leaves. Examination of iron contents of leaves of different ages showed that the o-phenanthroline extractable iron was lower in the younger leaves than in the older leaves, not only during the periods when chlorosis developed but also prior to the onset of chlorosis. On the three sampling occasions when all leaves were examined, extractable iron increased consistently with leaf age, regardless of chlorosis, and the extractable iron content of leaves decreased during the onset of chlorosis. Because extractable iron was lowest in the youngest leaves and these were the first to be affected by iron deficiency, the youngest leaves (buds and L-1) were selected as the plant parts most suitable for analysis in the subsequent field monitoring program.

There was not a close relationship between the age of leaves and the concentration of extractable iron in leaf tissue when the concentration

measured in the fresh tissue was expressed on an oven dry weight basis (Table 6). Further, the onset of chlorosis was not associated with an immediate decrease in extractable iron (on a dry weight basis) such as observed for extractable iron on a fresh weight basis between July 29 and August 3 (Table 5), but all of the opened leaves (L-1 to L-5) showed a decrease in extractable iron on a dry weight basis when the duration of chlorosis was prolonged, e.g. from July 31 to August 3 (Table 6). Even though the extractable iron content on a dry weight basis decreased in all opened leaves between July 31 and August 3, there was still no clear relationship between the extractable iron content and leaf age.

Total iron was higher in the younger tissue, especially during the development of chlorosis. The lack of a consistent relationship between chlorosis and total iron content was in agreement with the results of other workers (Singh 1970; Patel et al. 1977); these and other workers showed that total iron content of leaves was not satisfactory as an index of iron deficiency (Singh 1970; Patel et al. 1977).

With the onset of chlorosis, total iron contents increased in the buds, but not in the opened leaves. During this time the main bud also accumulated iron to much greater levels than the lateral bud. Some examination of factors causing these very high and diverse levels between lateral and main buds is merited, because this lies at the very basis of the cause of iron deficiency. Obviously, the onset of chlorosis was not due to a total shortage of iron in tissue, but rather it was due to a factor or

factors that caused precipitation or conversion of iron from the ferrous form.

Calcium and phosphorus have been implicated by other workers (Brown, 1961; DeKock and Stremecki, 1954) as nutrients involved in causing precipitation of iron in plant tissue. But the phosphorus concentration in the buds was consistently about 0.70%, and decreased with leaf age, and calcium concentration increased from 0.59-1.00% in the buds to over 2% in the older leaves. The higher phosphorus concentration in the young tissue could therefore be a contributing factor to the lower iron concentration in this tissue.

However, these detailed samplings were only preliminary and therefore could not reveal the cause of chlorosis. The extractable iron contents of the younger leaves and buds show that it was this tissue that was the most sensitive to changes when chlorosis occurs, as well as being the parts in which chlorosis occurs. In the subsequent monitoring program, it was not possible to continue to analyse all leaves of the plants; it was, therefore, clear that the younger tissues would be those most suitable for sampling and analysis.

IV.2 MONITORING OF IRON CHLOROSIS AND IRON CONTENT OF LEAVES OF GROUNDNUT (cv TMV 2) GROWN ON AN ALFISOL DURING RAINY SEASON 1981.

IV.2.1 Results

IV.2.1.1 Occurrence of chlorosis: The groundnut plants in the field available for repeated sampling during the 1981-82 season (TMV 2) developed only

mild chlorosis and on only a few occasions during this rainy season. The chlorosis in this field was not as severe as that observed in many other fields in other years, both in terms of the area of the field affected and the severity with which individual plants were affected.

Plants became mildly chlorotic in this field only during two periods: between about July 30 - August 2nd and between about September 6th and 13th. During these periods, the buds and first opened leaf of cv TMV 2 developed mild to marked chlorosis. Older leaves were not obviously affected. The development of iron chlorosis in groundnut at ICRISAT Center has been related in the past to rainfall or irrigation. The first occurrence of chlorosis in RP7C in 1981 (July 29 - August 3rd) developed shortly after a period of heavy rainfall (83 mm during 26-27 July); the second occurrence (September 7 to 11th) also developed after a succession of days giving a total of 91 mm between 1-6 September. Daily rainfall and temperature data over the period of monitoring are shown in Table 10. Although chlorosis developed after rain or wet periods, the occurrence of chlorosis was not closely related to prior weather conditions in 1981, because some wet periods did not cause chlorosis.

IV.2.1.2 Relationship between occurrence of chlorosis and iron contents of leaves: The results of the monitoring of iron content of the younger leaves (Table 11) showed that the occurrence of chlorosis was usually associated with low extractable iron in the fresh tissue. For the first

Table 10: Relationship between sampling date and occurrence of chlorosis; alfisol 1981.

Date	Severity of chlorosis				Rainfall (mm)	Temperature °C		Relative humidity(%)	
		Mb	Lb	L-1		Max	Min.	am	pm
July 15					0.0	32.5	23.3	79	47
July 16					0.0	33.6	23.9	76	40
July 17					0.0	32.9	24.0	73	46
July 18					0.0	32.9	24.0	75	44
July 19					0.0	32.0	24.5	74	44
July 20					0.0	33.2	24.5	73	45
July 21					0.0	33.0	23.3	82	45
July 22					19.0	33.0	23.0	87	47
July 23					31.8	28.7	22.3	91	69
July 24					0.9	26.5	22.7	87	84
July 25					0.8	30.1	21.8	88	67
July 26					0.0	31.2	23.3	84	58
July 27					83.2	31.7	22.6	95	54
July 28					0.0	26.5	23.0	90	81
July 29	S	-	-	-	0.0	29.6	23.2	91	66
July 30					0.5	29.0	23.2	90	83
July 31	S	+	++	+	4.8	30.7	22.8	91	63
Aug 1					7.8	26.0	21.5	96	88
Aug 2					41.5	26.2	21.0	98	94
Aug 3	S	++	++	+	18.0	27.4	21.0	94	76
Aug 4					31.8	25.1	21.4	95	89
Aug 5		-	-	-	7.9	22.9	21.5	89	94
Aug 6					0.0	27.0	21.3	88	72
Aug 7	S	-	-	-	0.0	27.9	21.6	89	68
Aug 8					2.6	27.4	21.9	88	75
Aug 9					0.0	25.8	22.0	85	77
Aug 10	S	-	-	-	0.0	26.6	22.1	82	75
Aug 11					0.0	30.0	19.7	88	58
Aug 12	S	-	-	-	0.0	30.3	21.5	87	58
Aug 13					28.4	30.0	21.8	88	58
Aug 14	S	-	-	-	6.0	28.0	22.0	87	69
Aug 15					1.2	27.0	21.8	93	73
Aug 16					6.8	25.2	22.6	83	92
Aug 17	S	-	-	-	0.0	29.2	21.5	88	69
Aug 18					0.0	30.2	21.6	88	60
Aug 19	S	-	-	-	4.6	30.2	21.5	90	59
Aug 20					0.0	30.8	21.7	85	60
Aug 21					0.0	30.9	22.0	85	57
Aug 22					0.0	30.1	22.4	86	59
Aug 23					0.0	28.6	22.5	82	62
Aug 24					0.4	30.2	21.5	83	57
Aug 25					0.0	30.8	22.4	87	54
Aug 26	S	-	-	-	8.4	30.9	22.5	90	86

Contd..

Contd.. Table 10.

Date 1981	Severity of chlorosis			Rainfall (mm)	Temperature °C		Relative humidity (%)	
	Mb	Lb	L-1		Max	Min	am	pm
Aug 27				0.0	29.6	21.8	86	62
Aug 28				0.0	30.2	21.5	86	55
Aug 29				0.0	29.0	21.8	83	59
Aug 30				0.0	30.2	21.0	88	54
Aug 31				0.0	30.5	22.5	85	52
Sep 1				0.0	30.0	22.8	84	77
Sep 2				34.3	29.3	21.9	96	68
Sep 3				24.2	26.8	21.7	90	75
Sep 4				5.1	24.0	21.5	88	87
Sep 5				0.3	25.4	22.0	90	86
Sep 6				27.6	28.7	21.8	90	71
Sep 7	S ++	NS	-	0.0	28.2	21.2	87	72
Sep 8				0.0	29.8	20.0	88	56
Sep 9				0.0	30.0	21.5	80	58
Sep 10				0.0	29.9	21.0	85	60
Sep 11	S +	NS	++	0.0	30.9	23.0	80	53
Sep 12				0.0	30.5	22.8	79	65
Sep 13				8.2	29.2	21.7	85	67
Sep 14				0.0	28.6	20.8	81	61
Sep 15				0.0	31.1	20.9	87	47
Sep 16				6.8	28.1	21.5	94	71
Sep 17				8.5	29.0	21.5	95	66
Sep 18				15.4	30.2	20.6	95	63
Sep 19				0.0	31.1	22.8	91	63
Sep 20				2.0	30.5	22.5	95	64
Sep 21				0.0	30.5	23.2	89	66
Sep 22				12.8	30.6	20.7	95	62
Sep 23				0.0	31.0	21.9	93	57
Sep 24				7.6	29.0	22.0	91	70
Sep 25	S NS	NS	-	2.2	28.2	22.2	91	71

* Sampling denoted by: S

** Severity of chlorosis indicated by:

- No chlorosis
+ Slight chlorosis
++ Marked chlorosis

NS Not sampled

Table 11 Extractable and total Fe contents (µg/g) of main buds (Mb), lateral buds (Lb) and first opened leaf (L-1) of groundnut (cv TMV 2), alfisol 1981

Date	Fresh wt. basis			Dry wt. basis			Total			Fraction of active iron		
	Mb	Lb	L-1	Mb	Lb	L-1	Mb	Lb	L-1	Mb	Lb	L-1
July 29	5.4	6.0	7.2	34.2	35.5	40.7	233	237	246	0.15	0.15	0.17
July 31	4.3*	5.0**	5.6*	35.7*	45.5**	39.5*	230*	456**	188*	0.16*	0.09**	0.20*
Aug 3	4.0**	4.2**	4.9*	33.5**	33.5**	31.7*	340**	531**	217*	0.09**	0.06**	0.14*
Aug 5	6.3	6.7	8.3	42.8	47.8	42.5	317	279	212	0.13	0.18	0.20
Aug 7	5.1	4.7	5.5	26.8	26.2	26.5	264	253	239	0.10	0.10	0.11
Aug 10	6.0	5.4	6.0	29.1	27.8	21.4	242	181	132	0.12	0.15	0.17
Aug 12	6.2	6.2	7.7	33.3	34.7	31.8	215	232	139	0.15	0.14	0.22
Aug 14	6.0	6.6	8.2	35.3	35.7	30.9	175	260	206	0.20	0.14	0.15
Aug 17	5.0	5.2	6.3	28.9	32.7	28.2	157	154	160	0.18	0.21	0.17
Aug 19	8.2	8.3	14.0	44.3	49.0	49.5	177	147	106	0.23	0.33	0.46
Aug 26	7.9	7.0	8.3	36.2	37.1	31.3	177	159	128	0.20	0.23	0.23
Sept 7	4.7**	-	10.1	29.1**	-	39.5	77**	-	124	0.36**	-	0.31
Sept 11	5.6*	-	5.3**	32.5*	-	20.8**	55*	-	52**	0.59*	-	0.40**
Sept 25	-	-	6.7	-	-	26.5	-	-	81	-	-	0.33
SE ±	0.43	0.28	0.42	2.36	2.49	1.93	27.8	24.8	14.7	0.025	0.017	0.019
Means												
July 29-Aug 3	4.6	5.0	5.8	34.4	38.0	37.2	267	407	217	0.13	0.10	0.17
S.E ±	0.31	0.28	0.30	2.83	2.43	2.19	39.1	30.3	27.4	0.026	0.013	0.023
July 29 to Aug 26	5.8	5.9	7.4	34.5	36.8	34.0	230	262	179	0.15	0.16	0.20
SE ±	0.44	0.28	0.39	2.42	2.49	1.82	29.9	24.8	16.6	0.021	0.017	0.017

* Slight chlorosis
** Marked chlorosis

leaf, the lowest concentrations coincide with the 3 samplings on which these leaves were chlorotic; for the mainbud, 3 of the four samplings coincided with the lowest extractable iron contents; and, the two samplings in which the lateral buds were chlorotic fall within the three lowest extractable iron contents of the lateral buds.

From the limited data in this table, chlorosis occurred in the particular plant part only when extractable iron content of the lateral bud was less than 5.1 μg/g, that of the main bud less than 4.8 μg/g, and that of the first opened leaf less than 5.4 μg/g (on a fresh wt. basis).

The first leaf, on average, contained more extractable iron 7.4 μg/g fresh tissue) than the buds (Mb 5.8 μg/g fresh wt. Lb 5.9 μg/g fresh wt.) and these differences in concentrations were significant at the 5% level of significance. The average concentrations of the mainbud and lateralbud were not significantly different (at the 5% level of probability). On a dry weight basis, the average concentration in the mainbud is 34.5 μg/g; lateralbud 36.8 μg/g and Leaf-1 34.0 μg/g (Table 11).

Total iron contents of the buds and first leaf did not show any clear relationship with the occurrence of chlorosis. The outstanding feature was the marked decrease in concentration during the season, with the highest concentration of 531 μg/g occurring early in the season when the lateral bud involved was chlorotic. This pattern contrasts strongly with the extractable iron contents, which remained at about the same level during the season, and additionally some of the highest values for

extractable iron occurred late in the season. The highest values for total iron contents of the buds appeared to be associated with the onset of chlorosis. This increase of iron content with onset of chlorosis is similar to that observed by other workers (Singh 1970; Patel et al. 1977) and is the reason why total iron content of tissue is not satisfactory as a diagnostic test.

IV.2.1.3 Other nutrients: In general there was a gradual decline during the season in the N, P and K contents of the first opened leaf (Appendix A); although the values missing from this table (due to an insufficient amount of sample) prevents a full interpretation of the changes in nutrients with time. However, the decrease in concentration (of N, P and K) in the main buds and lateral buds was less pronounced than in L-1. There were no pronounced associations between manganese contents of mainbud, lateral bud with age of the plant and there is no consistent relationship between calcium, magnesium, copper and zinc contents with plant age.

IV.2.1.4 Soil analyses: Analyses of soils, which were sampled during the last 7 plant samplings (Table 12), showed that soil moisture content and DTPA extractable iron changed significantly with time,at the 5% level of significance. The changes in soil pH and salt content (EC) were not statistically significant. The plants showed chlorotic symptoms on September 7, 1981 when the soil moisture content was highest;the chlorosis was still present later (11 September) when the soil moisture content

was not particularly high. The DTPA-extractable iron contents of soil show an increasing trend with time; the DTPA-extractable iron increased from about 6.0 µg/g in mid-August to about 7.5 µg/g in mid-September. However, there is no clear relationship between these values and soil moisture content, which has been implicated as one factor causing chlorosis. The DTPA-extractable iron levels were much higher than the critical level of 2 ppm obtained by Sankara Reddi and Adivi Reddy (1979). Thus the occurrence of chlorosis could not be related to the previous criterion defining soil iron status. The DTPA-extractable iron levels and changes in pH did not indicate any causal relationship between these and the occurrence of chlorosis.

Table 12: Results of analysis of soil samples (0-15 cm) for DTPA-extractable iron, pH, EC and moisture content. RP7C, alfisol (1981).

Date of Sampling	Occurrence of chlorosis	DTPA-extractable Fe (µg/g)	pH	E.C. (millimhos/cm)	Moisture content (%)
14 Aug	-	6.0	7.98	< 0.15	14.5
17 Aug	-	5.0	8.00	< 0.15	12.9
19 Aug	-	6.4	7.98	< 0.15	9.9
26 Aug	-	6.0	8.02	< 0.15	14.1
7 Sep	+	7.5	7.96	< 0.15	18.0
11 Sep	+	7.3	7.94	< 0.15	11.4
25 Sep	-	7.4	7.99	< 0.15	15.4
S.E. +		0.30	0.057		0.76

IV.2.2 Discussion

Although chlorosis was mild, and occurred in this field (RP7C) during only two periods (July 30 - August 3 and September 7-11), the relationship between occurrence and other variables has yielded valuable data. Chlorosis occurred shortly after heavy rainfall, although not always. This observation was in agreement with observations by other workers (Wallace et al. 1976a) and by staff at ICRISAT Center in previous years (Burford and Sahrawat, 1981). The reason why high soil moisture content favours the occurrence of chlorosis was not obviously clear. The availability of iron in the soil increased during the period of onset of chlorosis in late July, because the total iron contents of leaves increased (Table 7). This increase in iron uptake with increase in soil moisture content is logical, because the reduced aeration would have promoted reduction of Fe^{3+} to Fe^{2+}

The higher total iron and lower extractable iron in plants at the time of chlorosis, therefore, reflect an increased availability of iron in the soil and poorer solubility within the plant. The factors that would cause such diminished solubility within the plant are high phosphorus, high bicarbonates and high calcium. Contents of phosphorus and calcium in the leaves did not change significantly during the onset of chlorosis. Although no definite proof is available, it can be speculated that bicarbonates were the causative factor from a consideration of the change in

soil condition when the soil becomes very wet. The reduced aeration that promotes iron solubility also promotes bicarbonate accumulation because the escape of carbon dioxide from the soil is reduced; the higher levels of carbon dioxide and its participation in carbonate-bicarbonate equilibria would increase bicarbonate levels in the soil; these in turn would increased bicarbonate levels in the plants, causing preciptation of iron within the plant tissues (Porter and Thorne, 1955).

Bicarbonate has been suggested as the prime factor causing iron deficiency in a number of calcareous soils. The effect of bicarbonate and carbonate on reducing the availability of iron inside the plant system has been highlighted by many other workers (Harley & Linder, 1945; Wadleigh and Brown, 1952; Saglio,1969; Boxma, 1972). The higher carbon dioxide increases the concentration of bicarbonate and carbonate in solution, and it appears that these are absorbed by plant and cause precipitation of iron at least in leaves.

However, despite the uncertainty over the cause of the induced deficiency, the extractable iron content of young leaves appears to reflect satisfactorily the iron status of the groundnut plant. Certainly it appears to be a much better guide than total iron content. However, the above observations were for one cultivar (cv TMV 2) during one season. The test of this index will be its applicability across a range of cultivars.

IV.3 FIELD OBSERVATIONS OF CHLOROSIS IN GROUNDNUT BREEDING ENTRIES ON AN ENTISOL

IV.3.1 Results

IV.3.1.1 Iron contents: The wide variations in growth and chlorosis among 64 breeding entries were shown by the visual scores of total growth and proportion of leaves with mild and severe chlorosis (Appendix C). Duplicate breeding entries were selected to provide a 2 x 2 factorial of the most diverse entries for the two characteristics; total growth, and severity of chlorosis. Scores for these eight entries are presented in Table 13a.

Table 13a: Scores of relative growth and incidence of chlorosis in 8 groundnut breeding entries from an entisol, 1981.

Breeding entry	Taxonomic group	Score		
		Total* growth	Proportion of* leaves with mild chlorosis	Proportion of* leaves with severe chlorosis
FESR 12-P5	Virginia bunch	5.0	4.0	2.6
FESR 12-P6	Virginia bunch	4.0	5.0	2.8
NCAC 664	Valencia	9.0	6.0	5.0
U-1-2-1	Virginia bunch	8.0	5.6	4.6
TMV 2	Spanish	3.3	2.5	0.7
Krapovikas	Valencia	6.3	1.0	Nil
C.No.501	Virginia runner	9.3	0.8	0.6
E.runner	Virginia runner	10.0	Nil	Nil

* Scores made on a scale of 0-10; the highest value was given for maximum growth or maximum chlorosis.

From this table, it appeared that the virginia bunch taxonomic group may be relatively susceptible to iron chlorosis and the virginia runner group relatively tolerant, because these groups provided cultivars that were only in the most chlorotic or least chlorotic group (Table 13a). Examination of the data for the 64 breeding entries (Appendix C) on the basis of grouping the cultivars with their taxonomic group confirms this suggestion (Table 13b); the virginia bunch and virginia runner groups had high and low mean scores for chlorosis. The valencia group appeared to be quite diverse with respect to susceptibility to iron chlorosis; it had the highest mean score for chlorosis (Table 13b). The valencia group contained an entry which was among the most chlorotic as well as one which was among the least chlorotic of the cultivars (Table 13a).

Table 13b: Scores of relative growth and incidence of chlorosis in 64 groundnut breeding entries from an entisol, 1981.

Botanical name	Taxonomic group	Number of entries scored	Total* growth	Proportion of* leaves with mild chlorosis	Proportion of* leaves with severe chlorosis
Arachis hypogaea	Virginia bunch	8	7.1	2.7	1.6
Arachis fastigiata	Valencia	12	8.3	3.0	1.9
Arachis vulgaris	Spanish	36	8.5	1.9	0.7
Arachis hypogaea	Virginia runner	8	9.7	0.7	0.3
Weighted mean		64	8.4	2.1	1.0

* Values - Mean of 3 replications.

Extractable iron in youngest leaves of plants collected on 1st September reflected the severity of chlorosis (Table 14). Buds or leaves from chlorotic plants contained significantly lower ($P < 0.05$) extractable iron (≤ 5.0 µg/g fresh weight) than healthy buds or leaves (≥ 5.4 µg/g fresh weight). There was a much wider range in the extractable iron contents of the first fully opened leaves than those of the main buds, viz.

Main bud	- 4 chlorotic lines contained	≤ 4.8 µg/g fresh weight
	4 healthy lines contained	≥ 5.4 µg/g fresh weight
Leaf-1	4 chlorotic lines contained	≤ 5.0 µg/g fresh weight
	4 healthy lines contained	≥ 9.0 µg/g fresh weight

In contrast to the strong association between chlorosis and extractable iron contents, there was no obvious association between total growth and extractable iron contents.

The expression of extractable iron on a dry matter basis gave fairly similar results to the fresh weight basis results. Chlorotic cultivars contained less than 24 µg/g dry weight iron, and healthy cultivars contained more than 27 µg/g dry weight (Table 14).

Total iron contents again did not directly reflect iron status. The young leaves of chlorotic cultivars contained significantly higher total iron, whereas those in healthy cultivars contained less of total

Table 14: Content of extractable and total iron (µg/g) in main bud (Mb) and first fully opened leaf (L-1) of different groundnut breeding entries*

Extent of chlorosis	Plant growth	Breeding entry	Extractable Fe (F.W.B)**		Extractable Fe (D.W.B)***		Total Fe (D.W.B)***		Fraction of active iron	
			Mb	L-1	Mb	L-1	Mb	L-1	Mb	L-1
Severe	Poor	FESR 12-P5	4.0	4.4	19.3	19.7	413	302	0.05	0.07
		FESR 12-P6	4.1	5.0	18.7	20.8	438	225	0.04	0.09
Severe	Good	NCAC 664	4.8	4.5	23.2	22.0	416	325	0.06	0.07
		U-1-2-1	4.1	4.4	19.0	22.4	429	371	0.04	0.06
Nil	Poor	TMV 2	5.4	9.0	27.7	29.9	286	196	0.10	0.15
		Krapovikas	6.5	11.4	31.4	33.6	267	174	0.12	0.19
Nil	Good	C.No. 501	5.8	9.9	29.8	35.3	231	202	0.13	0.18
		E.runner	6.0	10.3	29.0	37.3	252	263	0.11	0.14
SE ±			0.36	0.58	1.62	1.54	15.1	7.2	0.006	0.009

* Leaves sampled on 1-9-1981, 72 days after sowing

** F.W.B. fresh weight basis

*** D.W.B. dry weight basis

iron. (Table 14). Total iron concentrations tended to be higher in the mainbud than in first fully unfolded leaf. Growth did not influence total iron in buds, but concentrations were higher in Leaf-1 for good growth than poor growth (Table 14).

The fraction of total iron extractable with o-phenanthroline was very much higher (over 2 fold) in healthy cultivars than chlorotic cultivars; these effects were highly significant ($P < 0.01$) (Table 14).

IV.3.1.2 Other nutrients in chlorotic and healthy cultivars: The chlorosis caused very few changes in the elemental contents of leaves that were consistent in both main bud and the first unfolded leaf, and in the plants of contrasting vigour. Magnesium concentrations were higher in chlorotic than in healthy tissue of both buds and leaf tissue of the vigorous cultivars. Calcium concentration were also consistently higher in the chlorotic than healthy buds, but not L-1, whereas phosphorus concentrations were higher in L-1 but not the Mb. Manganese contents were variable but chlorosis decreased concentration in L-1 (Table 15). The healthy cultivars (except Krapovikas strain) contained significantly lower amounts of sulfur than chlorotic cultivars (Appendex B).

IV.3.1.3 Soil analyses from chlorotic and helathy areas: Analyses of soils from the plots (Table 16) confirmed that the observable differences in chlorosis amongst the strains were not associated with soil properties; DTPA-extractable iron, pH, and EC did not differ significantly with the severity of chlorosis. However, the moisture content was significantly

Table 15. Content of N, P, K, Ca, Mg (%) in main bud (Mb) and first fully opened leaf (L-1) of different groundnut breeding entries*.

Extent of chlorosis	Plant growth	Breeding entry	N		P		K		Ca		Mg	
			Mb	L-1	Mb	L-1	Mb	L-1	Mb	L-1	Mb	L-1
Severe	Poor	FESR 12-P5	4.65	3.86	0.61	0.40	3.33	3.27	1.61	1.51	0.66	0.57
		FESR 12-P6	4.66	3.86	0.57	0.40	3.34	3.08	1.23	1.49	0.56	0.68
Severe	Good	NCAC 664	5.25	4.23	0.52	0.41	3.10	3.04	1.47	2.08	0.65	0.68
		U-1-2-1	5.47	4.29	0.55	0.40	3.10	2.94	1.66	2.46	0.69	0.67
Nil	Poor	TMV 2	4.78	3.28	0.59	0.27	3.36	2.97	1.06	1.72	0.47	0.33
		Krapovikas	5.12	4.42	0.58	0.23	3.53	3.30	0.88	1.75	0.41	0.37
Nil	Good	C.No. 501	4.91	4.16	0.56	0.27	3.58	2.08	0.89	1.62	0.42	0.31
		E. runner	5.35	4.00	0.61	0.25	3.83	2.00	1.16	1.73	0.55	0.19
SE ±			0.090	0.143	0.017	0.013	0.125	0.054	0.059	0.079	0.018	0.029

*Leaves sampled on 1-9-1981, 72 days after sowing.

Table 15. Content of Mn, Zn, Cu (µg/g) in mainbud (Mb) and first fully opened leaf (L-1) of different groundnut breeding entries.*

Extent of chlorosis	Plant growth	Breeding entry	Mn		Zn		Cu	
			Mb	L-1	Mb	L-1	Mb	L-1
Severe	Poor	FESR 12-P5	54.3	51.0	72.6	57.3	10.6	9.0
		FESR 12-P6	44.0	60.0	72.6	47.6	8.0	8.0
Severe	Good	NCAC 664	73.0	88.0	69.3	51.6	11.3	8.3
		U-1-2-1	58.3	56.6	65.6	49.6	12.6	9.3
Nil	Poor	TMV 2	54.0	105.0	73.0	50.6	10.3	9.3
		Krapovikas	44.0	75.0	56.0	62.0	8.0	11.0
Nil	Good	C.No. 501	47.3	122.0	58.0	40.0	8.6	6.0
		E. runner	47.0	90.0	63.3	38.6	12.3	9.0
SE ±			2.73	7.15	2.01	3.47	0.86	1.34

* Leaves sampled on 1-9-1981, 72 days after sowing

Table 16 Results of analysis of soil samples for DTPA extractable iron, pH, moisture content, and EC

Extent of chlorosis	Plant growth	Breeding entry	DTPA extractable Fe(µg/g)	pH	Moisture content(%)	EC (millimho/cm)
Severe	Poor	FESR 12-P5	3.4	8.75	18.5	< 0.15
		FESR 12-P6	3.2	8.78	16.8	< 0.15
Severe	Good	NCAC 664	2.8	8.69	18.1	< 0.15
		U-1-2-1	3.3	8.70	15.8	< 0.15
Nil	Poor	TMV 2	2.7	8.68	15.8	< 0.15
		Krapovikas	3.9	8.60	12.1	< 0.15
Nil	Good	C.No. 501	2.9	8.85	16.2	< 0.15
		E. runner	3.6	8.75	16.2	< 0.15
SE +			0.056	0.037	0.85	

higher on average in the chlorotic areas (FESR 12-P5; NCAC 664). Further studies will be needed to test whether the apparent relationship between severity of chlorosis and soil moisture content represented a causal or a fortuitous relationship. This point is important because it would establish whether the chlorosis is due to genetic or environmental factors. But chlorosis amongst cultivars in field appeared to be related to cultivar rather than to soil conditions.

IV.3.2 Discussion

Previous research on iron deficiency has emphasized the genotypic variation in absorption and utilization of iron among cultivars of maize (Brown and Bell, 1969) and soybean (Weiss, 1943) and more recently in groundnut (Hartzook et al. 1974). Appearance of obvious genotypic variations in 64 breeding entries from the RM5 field provided an excellent opportunity to test the effectiveness of the o-phenanthroline assay for estimating ferrous iron across the range of genotypic material. The demonstration that the o-phenanthroline extractable iron in young leaves was inversely related to the severity of chlorosis across a range of genetic material provided more confirmation of earlier suggestions that the ferrous iron content of fresh tissue was the physiologically active fraction; and that this correctly reflected the iron status of a plant. The fact that concordant results were obtained across the 8 cultivars examined indicated that, perhaps a similar critical level may apply to most groundnut cultivars.

Extractable iron contents were closely related to chlorosis, and for L-1, the range in concentration was much larger than for the buds.

The four genotypes showing marked chlorosis had extractable iron contents less than 5.1 µg/g (fresh weight basis), where as the four healthy cultivar always contained much more than this concentration i.e. more than 8.9 µg/g fresh weight (Table 14). In contrast, all elements other than active iron were either the same in chlorotic than healthy tissue or significantly higher. Total iron was again not a satisfactory index of iron nutritional status of the plant. The apparent applicability of this test across a range of cultivars had been tested and it appears to be useful in diagnosing iron chlorosis in groundnut.

IV.4 CORRECTION OF IRON DEFICIENCY IN GROUNDNUT ON AN ENTISOL

IV.4.1 Results

The chlorotic young leaves rapidly changed color (from yellow and yellowish green to green) within 3 days of application of the iron chelate in the spray and spray + soil application treatments. The leaves remained chlorotic in the control and the soil application treatments.

IV.4.2 Discussion

Variable results have been obtained at ICRISAT when attempts have been made to correct iron chlorosis by either foliar sprays or soil applications (Burford and Sahrawat, 1981). This led us to question whether other nutritional disorders were also involved in causing the chlorosis

(see also, Section IV.3.3.1). However, the results obtained in this particular field (RM5) showed quite clearly that the chlorosis in this field was due primarily to iron-deficiency, and that the deficiency could be readily corrected by a spray of 1% iron chelate (Sequestrene 138) to the groundnut foliage.

IV.5 POT EXPERIMENT

IV.5.1 Results

IV.5.1.1 Effect of alkalinity in inducing chlorosis: The pot experiment was conducted to determine whether the chlorosis was indeed due to iron deficiency, and whether this could be initiated by use of the center's borewell water (rich in carbonates and bicarbonates) or increasing the alkalinity of the soil artificially by addition of sodium carbonate.

Only partial success was achieved by the addition of sodium carbonate, as judged by the development of chlorosis in the young leaves of the groundnut plants. The carbonate was added in 5 successive increments each separated by intervals of a few days, because it was not known how much alkali would be required to cause chlorosis. The addition of 5th increment (325 µg/g) of sodium carbonate to the soil 44 days after sowing resulted in the fairly general development of mild interveinal yellowing on the sodium carbonate treatments. Chlorosis also developed to a slight extent where borewell water was used without the addition of sodium carbonate;

it was most pronounced where both sodium carbonate was applied and bore-well water was used as the source of water. The chlorosis in all cases was only mild, and consisted of an interveinal lightening of color only to a pale yellowish green of the youngest leaves; there was no intense bleaching.

However, the chlorosis was not obviously prevented by the first addition of iron chelate (7 days after sowing) i.e. 10th July 1981. Therefore, an additional application of iron chelate (2 μg/g soil) was made on 20th July 1981, and another again of 3 μg/g soil on 18th August 1981 (44 days after sowing). No clearcut effect of the chelate addition could be detected at anytime. Because it was then suspected that additional nutrients might be preventing the utilization of iron, manganese was added as a spray to the canopy; also, zinc, potassium and sulfur were added to the soil. These amendments did not satisfactorily correct the chlorosis. The reason for this failure was not clear.

It had been intended to harvest 3 replicates about 10 days after iron deficiency had developed. But the lack of a clear demonstration of iron deficiency, as indicated by the lack of responses to chelate additions, it was decided to allow all replicates to continue until the final harvest and to allow a more accurate determination of the effect of the iron additions on final yield.

But, pod development was very poor, and variable in most of the replicates. Almost all pods failed to mature properly. Senescence

occurred unexpectedly at 135 days after sowing, apparently because the pots were too small for plants grown through to maturity (Williams, 1981). Further, senescence occurred earlier in the treatments which had not received sodium carbonate.

IV.5.1.2 Nutrient content of young leaves sampled at 105 days after sowing: The extractable iron content of the youngest leaves varied from 5.8-11.1 µg/g of fresh weight across the different treatments (Table 17). Unexpectedly, addition of chelate caused a significant decrease in extractable iron. The phosphorus and copper contents were very low in all the treatments. These concentrations, and those of manganese and zinc in some treatments were lower than the critical levels reported by Sankara Reddi and Adivi Reddy (1979); these critical levels are given in Table 18. The potassium content (Table 17) was about the same as the critical limit given in Table 18. Total iron content was also lower in the treatments which were watered with borewell water. Magnesium concentrations were increased significantly by both iron and carbonate applications. Of particular relevance to the nutrient interactions usually involved with the development of iron chlorosis is the effect of sodium carbonate; zinc, copper and phosphorus concentrations increased where carbonate was applied. Carbonate caused a decrease in total iron contents, and borewell water caused a decrease in total iron where iron was not applied.

IV.5.1.3 Nutrient content of haulms at maturity: Application of iron caused a significant ($p < 0.05$) reduction in iron content of haulms and

Table 17: Analyses of youngest leaves from pot experiment for extractable iron and other nutrients at 105 days after sowing.

	Treatment			Extractable*	Macronutrients (%)**					Micronutrients (µg/g)**			
	Iron	Alkali	Water+	Fe (µg/g)	N	P	K	Ca	Mg	Total Fe	Mn	Zn	Cu
A	-	-	D	8.8	1.90	0.080	0.58	2.15	0.48	130	68	16	2.7
E	+	-	D	7.3	2.11	0.095	0.55	2.64	0.44	96	35	17	2.6
B	-	+	D	11.1	2.34	0.105	0.58	2.41	0.52	105	35	19	3.9
F	+	+	D	7.1	2.59	0.150	0.75	2.22	0.61	99	33	18	3.6
C	-	-	B	8.4	2.03	0.100	0.56	2.44	0.61	50	16	13	2.7
G	+	-	B	7.1	2.05	0.085	0.48	2.46	0.65	53	13	17	2.2
D	-	+	B	6.0	2.31	0.135	0.52	2.31	0.69	62	27	18	4.0
H	+	+	B	5.8	2.29	0.135	0.50	2.09	0.85	56	22	23	3.7
	SE ±			0.52	0.109	0.0071	0.018	0.094	0.017	8.4	3.5	1.5	0.54

* Fresh weight basis

** oven dry weight basis

\+ D is deionised water

B is borewell water.

Table 18: Critical limits for concentrations of nutrients in the groundnut plant*

Nutrient	Plant status	
	Deficient	Sufficient
Maeronutrient (%)**		
Phosphorus	< 0.20	> 0.20
Potassium	< 0.50	> 0.50
Calcium	< 0.75	> 0.75
Magnesium	< 0.30	> 0.30
Micronutrients (µg/g)**		
Iron	< 68	> 68
Zinc	< 20	> 20
Manganese	< 25	> 25
Copper	< 6	> 6

* Source: Sankara Reddi and Adivi Reddy (1979).

** All nutrient concentrations expressed on an OD basis.

Table 19: Critical concentration (µg/g) of available nutrients in soils for groundnut culture*

Nutrient	Availability test	Soil status	
		Deficient	Sufficient
P	Olsen	< 9.0	> 9.0
K	Exchangeable	< 68.0	> 68.0
Fe	DTPA	< 2.0	≥ 2.0
Zn	DTPA	< 0.75	> 0.75
Mn	DTPA	< 1.0	> 1.0
Cu	DTPA	< 0.50	> 0.50

* Source: Sankara Reddi, G.H. and A. Adivi Reddy (1979).

significantly higher concentrations of potassium, manganese, zinc and copper. Addition of sodium carbonate significantly increased the concentrations of nitrogen, phosphorus, zinc and copper (Table 20). The use of borewell water decreased the potassium content.

IV.5.1.4 Nutrient content of roots in relation to haulms (at maturity): The concentration of total iron in the haulms was significantly lower than that of roots. Calcium, magnesium and manganese contents were also significantly higher in haulms than that of roots. Copper contents were significantly lower in haulms than that of roots. There were no significant differences in contents of N, P, K of both haulms and roots (Tables 20 & 21).

IV.5.1.5 Soil reaction, salt content and DTPA-extractable micro nutrients in soil from different treatments: Addition of sodium carbonate and borewell water caused a significant increase in soil pH and salt content (Table 22). The treatments which received iron contained significantly higher amounts of iron. None of the treatments caused significant changes in contents of manganese, zinc and copper.

IV.5.2 Discussion

Tissue and soil analyses were undertaken because the development of chlorosis in the young groundnut leaves was not prevented by the addition of iron chelate to the soil. It was hoped to obtain indications of other factors involved in causing the chlorosis, and also the reason for the lack of response to iron.

Table 20: Analyses of haulms for total nutrients at final harvest; pot experiment.

	Treatment			Macronutrients (%)*					Micronutrients (µg/g)*			
	Iron	Alkali	Water**	N	P	K	Ca	Mg	Total Fe	Mn	Zn	Cu
A	-	-	D	1.37	0.061	0.67	1.93	0.52	1195	186	23	4.5
E	+	-	D	1.22	0.053	0.72	2.16	0.51	515	359	30	5.3
B	-	+	D	1.73	0.070	0.62	1.79	0.55	861	277	27	5.3
F	+	+	D	2.15	0.120	0.81	1.04	0.60	302	340	38	6.2
C	-	-	B	1.21	0.065	0.28	1.98	0.70	309	304	27	4.0
G	+	-	B	1.07	0.041	0.31	1.69	0.76	563	416	37	4.8
D	-	+	B	2.10	0.116	0.45	1.58	0.61	239	183	34	5.7
H	+	+	B	2.22	0.142	0.48	1.35	0.69	360	232	39	6.2
	SE ±			0.070	0.0070	0.015	0.114	0.016	26.0	14.0	1.0	0.29

* Oven dry weight basis; ** D is deionised water

B is borewell water

Table 21: Analyses of roots for total nutrients at final harvest, pot experiment.

Treatment				Macronutrients (%)					Micronutrients (µg/g)*			
	Iron	Alkali	Water	N	P	K	Ca	Mg	Total Fe	Mn	Zn	Cu
A	-	-	D	1.46	0.053	0.58	0.71	0.25	2076	30	30	7.8
E	+	-	D	1.38	0.046	0.48	0.63	0.21	2906	44	31	11.2
B	-	+	D	2.51	0.063	0.40	0.38	0.17	2171	42	24	6.2
F	+	+	D	2.87	0.083	0.33	0.48	0.30	2001	49	32	10.0
C	-	-	B	1.45	0.073	0.31	0.62	0.37	2798	85	46	14.0
G	+	-	B	1.51	0.053	0.33	0.74	0.38	2339	68	40	14.2
D	-	+	B	2.66	0.085	0.30	0.38	0.40	1402	49	31	8.5
H	+	+	B	2.33	0.075	0.28	0.47	0.35	2405	42	41	11.5
	SE ±			0.154	0.0122	0.016	0.042	0.010	94.7	3.3	1.7	0.51

* Oven dry weight basis

Table 22: Post harvest soil analyses for pH, EC and DTPA extractable Fe, Mn, Zn, Cu; pot experiment.

	Treatment			DTPA-extractable micronutrients (µg/g)				pH	EC (milli-mho /cm)
	Iron	Alkali	Water*	Fe	Mn	Zn	Cu		
A	-	-	D	8.7	16.6	2.3	2.0	8.28	0.28
E	+	-	D	10.4	18.6	2.5	1.7	8.25	0.31
B	-	+	D	9.3	20.6	2.3	1.8	8.99	0.89
F	+	+	D	10.1	17.4	2.4	1.7	9.09	0.88
C	-	-	B	8.2	10.3	2.1	1.5	9.11	0.83
G	+	-	B	10.3	12.4	1.9	1.8	9.15	0.85
D	-	+	B	8.6	19.4	2.9	1.7	9.49	1.36
H	+	+	B	13.4	18.5	2.4	2.3	9.49	1.44
	SE ±			0.90	0.65	0.06	0.09	0.004	0.039

* D is deionised water

B is borewell water

Extractable iron in youngest leaves was decreased by the application of iron chelate. Also, extractable iron in leaves from all treatments was generally higher than the concentration associated with the occurrence of chlorosis in the field (Table 17). Available iron in the soil (as estimated by DTPA-extractable iron) was higher where iron had applied, therefore the iron applied as chelate was not inactivated in the soil. Further, the extractable iron content of the youngest leaves did not usually decrease as a result of the addition of carbonate or borewell water. Thus, although the symptoms were similar to those of iron deficiency, it would seems that some other nutritional disorder was either also involved in causing the chlorosis or it was the main cause of chlorosis in the pot experiment.

Copper and phosphorus concentrations in the youngest leaves (sampled at 105 days after sowing) were much lower than those given as critical limits by Sankara Reddi and Adivi Reddy (1979). Using the criteria established by the same authors, zinc concentrations were usually less than the critical limits, and manganese concentrations were marginal being less than the critical limit in three treatments. However, the addition of carbonate did not cause consistent decrease in concentration for any of these nutrients, although the chlorosis was clearly related to the carbonate additions. Therefore, it is not possible at this stage to identify a specific nutrient as the cause of the chlorosis observed, although the low concentration of Mn, Zn and especially P and Cu indicate the need for further studies on these nutrients in alfisols.

Of course, the sodium carbonate and borewell water treatment caused significant increases in pH and salinity (as measured by EC) of the soil. But there is no published description of the visual symptoms of groundnut plants affected by high pH or salinity.

The critical concentrations given by Sankara Reddi and Adivi Reddy (1979) have been used as a guide, but caution is indicated in using these for interpretations, because the authors do not state:

(i) the plant part sampled
(ii) the stage of plant development
(iii) whether there are cultivar differences.

It could perhaps be assumed that the critical concentrations quoted were for whole plants at flowering.

Total iron concentrations were significantly higher in roots than haulms (Tables 20 and 21). The cause could be immobilization of iron on the surface of roots, or within the roots. Previous workers (Rogers and Shive, 1932) have shown these effects. But the importance for this study is that it was earlier shown that iron was also immobilized within leaf tissue during the onset of chlorosis (Section IV.1.2). Thus immobilization of iron in both leaves and roots has been demonstrated in this study.

However, apart from these effects noted above, it must be concluded that the treatment of the soil with borewell water and sodium carbonate was not successful in creating uniform conditions for studying iron

chlorosis, primarily because iron deficiency was not induced even though the pH and salinity levels increased to undesirable high levels. It was not clear why the plants did not develop an iron deficiency. Therefore, there is a need to characterize the nutritional disorders connected with iron chlorosis in the soils at ICRISAT Center, and also in soils where iron deficiency is likely to occur.

V GENERAL DISCUSSION

The results obtained in this research work have provided new information about the occurrence and diagnosis of "iron-chlorosis". The results can be summarized under the following headings:

1. Causes of chlorosis: The occurrence of chlorosis in fields at ICRISAT has been erratic. Not only is the occurrence of chlorosis during the season unpredictable but it is confined to irregular patches within fields; so far the only consistent feature of the disorder has been that it commonly, but not always, develops after heavy or prolonged rainfall (Sahrawat and Burford, 1981). However, such development of iron deficiency under transient high soil moisture content is contradictory to the classical view of the factors affecting the availability of iron *in soil*. As soil dries, more ferrous iron would be oxidised to ferric and thus deficiency should be initiated. When soil becomes wetter than field capacity, the reduced aeration would be expected to cause an increase in ferrous iron content of the soil, an increase in uptake of iron, and therefore alleviation of iron deficiency.

The plant data showed that the onset of chlorosis was in fact accompanied by an increase in total iron content, which indicated an improved availability of iron in the soil (Table 7); however, o-phenanthroline extractable iron in the plant decreased (Table 5). This increase in total iron and concurrent decrease in extractable ('ferrous') iron in the plant during the onset of chlorosis indicated that chlorosis was

not caused by an unavailability of iron within the soil but instead by some factor that decreased solubility within the plant. Phosphorus or calcium could not be clearly implicated as causing poor iron solubility within the plant (Table 9). Speculatively it was therefore suggested that increased bicarbonate levels in the plant induced an iron deficiency within the plant.

This mechanism appeared quite feasible because the reduced aeration would have caused decreased oxygen levels in soil, increased ferrous iron concentration in the soil, and therefore increased iron uptake (Tables 11 and 12), would also have caused an increase in carbon dioxide concentration and an increase in bicarbonate and carbonate concentrations (Boxma, 1972). Absorption of bicarbonate by the plant could therefore have reduced the ferrous iron content within leaves (Porter and Thorne, 1955). The higher concentrations of iron in roots than in haulms indicated that transfer of iron from the roots to the leaves was restricted. There was no evidence to indicate whether the higher concentrations in the roots were due to poor transport of iron per se, or whether precipitation had occurred. Neverthless the results are in agreement with those of previous authors (Biddulph, 1951) which showed that both accumulation in roots, and poor solubility in leaves, were factors involved in the development of iron chlorosis.

If the chlorosis was primarily due to plant factors such as decreased transport or solubility of iron within the plant, rather than to

unavailability of iron in the soil solution, then this raises an implication for the management of the soils at ICRISAT Center. High moisture contents were considered to cause chlorosis, primarily because of its effect on aeration. However, the structure of the surface soil of these alfisols will deteriorate under continuous intensive cultivation. Such structure deterioration would also cause poorer aeration, higher carbon dioxide and bicarbonate levels in the soil, and higher bicarbonate uptake by crops for a 'standard' input of water (by irrigation or natural rainfall). While these aspects are speculative, they do indicate the approaches that should be considered in future research.

2. Diagnostic test: The results provided good preliminary evidence to indicate that the o-phenanthroline extractable iron content of fresh young leaf tissue may be a good index of the iron status of groundnut. The two series of field examinations gave compatible results:

	Extractable-Fe contents (µg/g fresh weight)					
	Chlorotic			Healthy		
	Mb	Lb	L-1	Mb	Lb	L-1
1. Monitoring of cv TMV 2 during the season	<4.8	<5.1	<5.4	>4.8	>5.1	>5.4
2. Breeding entries from an entisol	<4.9	*-	<5.1	>5.3	*-	>8.9

*- Not sampled

These results are in agreement with the earlier findings of Katyal and Sharma (1980) for rice who, however, gave their results on an ovendried, whole plant basis. A summary of the results in Tables 2, 4 and 6 of their paper were:

	Chlorotic Plants	Healthy Plants
Total iron (μg/g)	135-270	115-170
o-Ph extractable iron (μg/g)	<43	>46

The only comparative results from the present work are those for the breeding entries (Table 14), in which o-Ph extractable iron (on a dry weight basis) was less than 24 μg/g in chlorotic tissue and more than 27 μg/g in healthy tissue.

The youngest leaves were chosen as the plant part which was most suitable for analysis for two reasons:

i) This youngest tissue was that which was most severely affected by the onset of chlorosis;

ii) this tissue also contained the lowest concentration of extractable iron.

The data were insufficient to clearly show whether the buds or the first opened leaves were the best plant parts for diagnostic testing (Table 11). Data from different cultivars indicated that perhaps Leaf-1 may exhibit a much wider range of concentrations and may be more sensitive

than buds. It is perhaps pertinent to mention that the relationship between concentration of total iron in the leaves of groundnut does not appear to have been examined previously in relation to leaf age. Although there was little consistent change with leaf age for total iron (Table 7), the changes in extractable iron with age of leaf were marked and consistent (Table 5); they indicated the usefulness of sampling leaves of the same age.

Previous authors had indicated clearly that total iron was quite unsuitable as a diagnostic test, because the total iron contents were not related to the occurrence of deficiency symptoms. Similar results were obtained in this study, that is, total iron contents were usually not lower in chlorotic tissue; in fact, they were commonly higher (Table 14). Additionally, total iron contents tended to decrease from very high concentrations in the early stages of growth to low concentrations during later stages (Tables 7 and 11). In contrast, the extractable iron concentrations in the fresh tissue of the same age remained relatively constant over the life of the plant.

Many workers have suggested that total iron was unsatisfactory for diagnosis of the iron status of plants, because only a small fraction of the total iron was actively involved in metabolism. Measurement of the active fraction of iron in leaves was desired to give a better indication of the iron status in plants. The o-phenanthroline extractable

iron estimates ferrous iron (Gupta, 1968); the results obtained here and in the work of Katyal and Sharma (1980), who used o-phenanthroline, and earlier workers who attempted to measure Fe^{2+} directly (Gupta, 1968), all support the hypothesis that an estimate of the physiologically active iron, viz. ferrous iron, is a better index than total iron.

However, the above results provided evidence over only one season. Future work will need to test the applicability of o-phenanthroline extractable iron as a diagnostic test over a range of seasonal conditions, soils and cultivars. At the same time, some further investigation into the most suitable plant part for analysis is merited. The main bud and leaf-1 were selected after only a limited investigation; because the results indicated that leaf-1 may be more sensitive than the main bud, this aspect should be examined further.

3. Predictive soil tests: Various extractants have been proposed for estimating the iron status of a soil. Within India, DTPA (Diethylene triaminepentaacetic acid) is the usual recommended extractant (Katyal and Agarwala, 1982). However, from a number of considerations, the usefulness of this extractant can be questioned:

i) Chlorosis occurred in groundnut grown on soil in which DTPA extractable iron levels were well above the critical limits of 2 µg/g of soil given by Sankara Reddi and Adivi Reddy (1979).

ii) The factors affecting iron chlorosis in groundnut in our soils appear to be related to factors other than only the availability of iron in the soil.

It would seem that plant characteristics, and the concentration of bicarbonate in the soil are more important. These aspects indicate that a re-evaluation should be made of the present policy within India of placing reliance on the DTPA-extractable iron in soil for predicting the iron-status of a soil. Analysis of plant tissue would appear to be preferable to analysis of the soil for available iron.

4. Genotypic variations: Studies in maize (Brown and Bell, 1969) and soybean (Weiss, 1943) by other workers have indicated that there was considerable genotypic variation in the absorption of iron from the soil and also its efficient utilization with in the plant. Such results led to strong pleas for the breeding of iron-efficient cultivars (e.g. Early runner, C.No. 501). The results obtained here (Table 14) indicated that the iron-efficient cultivars maintain a higher level of iron in their tissue. Apart from indicating that the ferrous iron or extractable iron assay will be effective as a diagnostic test over a range of cultivars, the results also reinforce the previous pleas that the best means of alleviating iron deficiency is not by amelioration with iron applications, but by the breeding of iron-efficient cultivars.

VI SUMMARY AND CONCLUSIONS

The major objective of this study was to investigate the factors causing chlorosis in groundnut at ICRISAT Center. This involved much preliminary work to investigate the suitability of using orthophenanthroline extractable iron as a diagnostic test for iron deficiency in groundnut; The main findings from the experiments conducted are:

1. The results were in agreement with those of Katyal and Sharma (1980) for rice, in that, the concentration of extractable iron appeared to be a suitable index of the iron status of the groundnut plant. Some of the detailed conclusions are:

   i) Extractable iron contents of groundnut leaves decreased with decreasing leaf age; thus the youngest leaves, which were those most severely affected by chlorosis, also contained the lowest concentration of extractable iron.

   ii) Chlorosis developed in cv TMV 2 (twice during the growing season), after heavy rainfall, in the field under observation; the onset of chlorosis was accompanied by a decrease in extractable iron content of the youngest leaves, i.e. the buds or first opened leaf (L-1).

   iii) Chlorosis occurred only when the youngest leaves (buds or L-1) contained less than 6 µg extractable - Fe/g fresh weight.

iv) Examination of young leaves of cultivars showing a diverse susceptibility to iron deficiency showed that the extractable iron contents of the youngest leaf tissue were closely related to the development of chlorosis. Plants which exhibited marked chlorosis contained less than 4.9 and 5.1 μg/g fresh weight in the main bud and leaf-1 repsectively; those which developed little or no chlorosis contained greater than 5.3 μg/g fresh weight and 8.9 μg/g fresh weight in main bud and leaf-1 respectively.

v) More detailed testing is required to establish the accuracy and reliability of extractable iron in fresh tissue as a diagnostic test and also to test the suitability of buds or youngest opened leaves as the plant part to be sampled for analysis; preliminary evidence indicate that the range in concentration in the leaf-1 may be larger than the main bud.

2. Expression of extractable iron in green tissue on a dry matter basis did not correlate well with the occurrence of chlorosis.

3. Total iron contents were not reliable indicators of the iron status of groundnut; these results were in agreement with findings of other workers.

4. Soil analysis for available iron (DTPA - extractable iron) did not appear to provide a suitable predictive test. The levels in all soils tested were significantly higher than the critical levels reported in India.

5. Attempts to induce iron deficiency in pot experiment, by using borewell water or adding sodium carbonate to an alfisol, causing the development of a mild chlorosis, but this could not be corrected by the use of an iron chelate (Sequestrene 138). The herbage had particularly low concentration of potassium and copper, and further studies are needed on the nutrition of groundnuts in these soils.

6. Although the increasing occurrence of chlorosis in the fields at ICRISAT has been attributed to increasing soil pH due to irrigation with borewell water containing carbonates and bicarbonates, the pot experiment indicated the pH per se was not the sole factor causing iron deficiency through lack of available iron in the soil. It is suggested that the deficiency arises due to the combination of high pH and high soil moisture content, and, additionally, the use of iron - inefficient cultivars. The major cause of intermittent iron deficiency appears to be unavailability of iron within the plant; the results obtained indicate that this is due to bicarbonates causing precipitation of iron with in roots and leaf cells.

7. The best approach to minimizing the effect of iron deficiency is the use of iron-efficient cultivars in areas where iron chlorosis is a major problem.

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Appendix-A.1 Contents of nitrogen, phosphorus, potassium (%) of Main buds (Mb), Lateral buds (Lb) and first opened leaf (L-1) of groundnut (cv TMV 2); alfisol 1981

Date	Nitrogen (%)			Phosphorus (%)			Potassium (%)		
	Mb	Lb	L-1	Mb	Lb	L-1	Mb	Lb	L-1
Jul 29	5.73	5.32	4.34	0.74	0.68	0.43	3.83	3.28	2.92
Jul 31	4.37*	6.16**	5.34*	0.70*	0.70**	0.51*	2.52*	3.94**	2.71*
Aug 3	6.34**	6.16**	5.35*	0.70**	0.70**	0.45*	3.45**	3.77**	2.76*
Aug 5	-	6.24	4.47	-	0.70	0.36	4.22	4.20	2.43
Aug 7	-	-	5.00	-	-	0.32	3.92	4.00	2.44
Aug 10	-	5.71	3.94	-	0.63	0.28	3.87	3.80	1.69
Aug 12	5.85	7.27	3.84	0.63	0.78	0.24	3.66	3.76	1.91
Aug 14	-	5.76	3.64	-	0.62	0.24	3.42	3.63	1.69
Aug 17	5.77	5.92	3.90	0.55	0.68	0.28	3.84	3.53	1.72
Aug 19	5.71	5.82	3.82	0.59	0.63	0.26	2.94	3.13	1.58
Aug 16	5.37	5.34	3.70	0.54	0.67	0.25	3.03	3.00	1.60
Sep 7	5.79**	-	3.83	0.64**	-	0.25	3.34**	-	1.11
Sep 11	5.52*	-	3.07**	0.58*	-	0.27**	2.81*	-	1.36**
Sep 25	-	-	3.09	-	-	0.22	-	-	0.73

- Not analysed, quantity of sample insufficient

* Slight chlorosis

** Marked chlorosis

Appendix-A.2 Contents of calcium (%), Magnesium (%), Copper (µg/g) of Main buds (Mb), Lateral buds (Lb) and first opened leaf (L-1) of groundnut (cv TMV 2); alfisol 1981

Date		Calcium (%)			Magnesium (%)			Copper (µg/g)		
		Mb	Lb	L-1	Mb	Lb	L-1	Mb	Lb	L-1
Jul	29	0.90	0.78	1.12	0.55	0.45	0.39	9.1	9.4	9.2
Jul	31	0.81*	0.77**	0.92*	0.38*	0.41**	0.31*	13.1*	11.6**	11.1*
Aug	3	0.59**	1.00**	1.08*	0.47**	0.52**	0.55**	18.2**	18.0**	15.8*
Aug	5	0.22	0.26	0.71	0.85	0.91	0.60	10.0	7.0	7.0
Aug	7	0.49	0.44	0.59	0.97	0.96	0.62	18.0	10.0	8.0
Aug	10	0.65	0.81	0.62	0.90	0.94	0.45	8.0	9.0	6.0
Aug	12	0.68	0.85	0.70	0.86	0.82	0.40	15.0	7.0	4.0
Aug	14	0.56	0.52	0.72	0.68	0.71	0.35	5.0	5.0	4.0
Aug	17	0.59	0.59	1.00	0.69	0.63	0.44	5.0	5.0	4.0
Aug	19	0.81	0.91	0.95	0.66	0.90	0.57	4.0	6.0	5.0
Aug	26	1.20	1.24	1.04	1.01	1.01	0.71	9.0	8.0	7.0
Sep	7	0.89**	-	2.11	0.91**	-	0.69	9.0**	-	8.0
Sep	11	1.08*	-	1.62**	0.53*	-	0.43*	6.0*	-	3.0**
Sep	25	-	-	2.54	-	-	0.44	-	-	5.0

* Slight chlorosis

** Marked chlorosis

Appendix-A.3 Contents of total manganese and zinc (µg/g) of Main buds (Mb), Lateral buds (Lb) and first opened leaf (L-1) of groundnut (cv TMV 2); alfisol 1981

Date	Manganese (µg/g)			Zinc (µg/g)		
	Mb	Lb	L-1	Mb	Lb	L-1
July 29	38	39	40	67	66	49
July 31	36*	35**	32*	55*	64**	54*
Aug 3	23**	27**	31*	70**	68**	48*
Aug 5	26	25	25	67	56	35
Aug 7	35	36	24	60	52	32
Aug 10	37	39	18	52	43	23
Aug 12	32	37	23	39	44	26
Aug 14	30	30	23	47	47	27
Aug 17	25	28.0	26	51	52	28
Aug 19	28	25	22	46	54	38
Aug 26	29	29	24	57	58	40
Sep 7	21**	-	19	73**	-	43
Sep 11	24*	-	17**	48*	-	21**
Sep 25	-	-	15	-	-	23

* Slight chlorosis

** Marked chlorosis

Appendix B: Sulphate sulfur (%) in main bud (Mb) and first fully opened leaf (L-1) of different groundnut breeding entries.

Extent of chlorosis	Plant growth	Breeding entry	Sulfate sulfur (%)	
			Mb	L-1
Severe	Poor	FESR 12-P_5	-*	0.66
		FESR 12-P_6	0.59	0.52
	Good	NCAC 664	-*	0.54
		U-1-2-1	-*	0.49
Nil	Poor	TMV-2	0.67	0.40
		Krapovikas	-*	0.56
Nil	Good	C.No. 501	0.42	0.39
		E.runner	-*	0.26
SE +				0.029

* Not analysed; insufficient sample.

Appendix C: Scores of 64 groundnut breeding entries for growth and severity of chlorosis; Entisol 1981.

ICG No.	Entry Name	Taxonomic group (Type)	Growth				Proportion of leaves with mild chlorosis				Proportion of leaves with severe chlorosis			
			Replicate				Replicate				Replicate			
			I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
2031	Ah 3533	S	10.0	9.0	7.0	8.7	1.0	5.0	1.0	2.3	0.0	4.0	0.0	1.3
5655	Ah 6715	Vr	10.0	10.0	10.0	10.0	1.0	0.0	0.0	0.3	0.5	0.0	0.0	0.2
4015	Ah 6738	V	10.0	10.0	6.0	8.7	3.0	7.0	2.0	4.0	2.0	5.0	0.5	2.5
4866	Ah 7013	Vr	10.0	9.0	9.0	9.3	1.0	1.0	0.5	0.8	0.5	0.0	0.0	0.2
4799	Ah 7202	Vb	9.0	10.0	8.0	9.0	2.0	2.0	1.0	1.7	1.0	1.0	0.0	0.7
3700	Ah 7223	S	10.0	10.0	6.0	8.7	1.0	1.0	0.5	0.8	0.5	0.5	0.0	0.3
2051	Ah 7299	V	8.0	9.0	7.0	8.0	4.0	3.0	1.0	2.7	3.0	2.0	0.0	1.7
1277	Ah 7319	S	10.0	9.0	7.0	8.7	3.0	4.0	3.0	3.3	1.5	2.0	1.0	1.5
2056	Ah 7336	S	10.0	9.0	7.0	8.7	6.0	0.0	7.0	4.3	3.0	0.0	6.0	3.0
1289	Ah 7984	S	10.0	10.0	10.0	10.0	1.0	1.0	2.0	1.3	0.0	0.0	1.0	0.3
4551	Ah 8068	S	10.0	8.0	7.0	8.3	3.0	0.5	0.5	1.3	1.0	0.0	0.0	0.3
846	C 100	Vr	10.0	10.0	10.0	10.0	1.0	1.0	0.0	0.7	0.0	0.0	0.0	0.0
4562	C.No.55-437	S	9.0	7.0	8.0	8.0	5.0	6.0	2.0	4.3	4.0	5.0	0.5	3.2
1904	C.No.677	S	10.0	10.0	10.0	10.0	2.0	1.0	0.0	1.0	0.0	0.0	0.0	0.0
2601	C.No.501	Vr	8.0	10.0	10.0	9.3	2.0	0.5	0.0	0.8	2.0	0.0	0.0	0.6
4589	Exotic-2	S	10.0	10.0	9.0	9.7	3.0	1.0	0.0	1.3	1.0	0.0	0.0	0.3

Contd.. Appendix C.

ICG No.	Entry Name	Taxonomic group (Type)	Growth				Proportion of leaves with mild chlorosis				Proportion of leaves with severe chlorosis			
			Replicate				Replicate				Replicate			
			I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
3551	Exotic 3-5	S	10.0	9.0	10.0	9.7	1.0	3.0	0.0	1.3	0.0	0.0	0.0	0.0
3556	Exotic 6	S	10.0	9.0	9.0	9.3	1.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
3621	EC 206965	S	9.0	6.0	7.0	7.3	2.0	8.0	3.0	4.3	0.5	6.0	1.0	2.5
1849	EC 24419	S	9.0	8.0	8.0	8.3	4.0	6.0	2.0	4.0	1.0	5.0	0.0	2.0
3316	EC 27446	V	10.0	8.0	7.0	8.3	4.0	4.0	6.0	4.7	3.0	3.0	6.0	4.0
2716	EC 76446	V	9.0	8.0	8.0	8.3	1.0	2.0	0.5	1.2	0.0	0.0	0.0	0.0
1949	EC 264743	S	9.0	9.0	6.0	7.3	2.0	0.5	1.0	1.2	0.5	0.0	0.0	0.2
3008	Early runner	Vr	10.0	10.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2224	Faizpur	S	10.0	10.0	8.0	9.3	1.0	1.0	0.0	0.7	0.0	0.0	0.0	0.0
4590	Florigiant	S	10.0	10.0	9.0	9.7	1.0	0.5	0.0	0.5	0.0	0.0	0.0	0.0
4593	GFA Spanish	S	8.0	6.0	8.0	7.3	2.0	6.0	0.0	2.7	1.0	4.0	0.0	1.7
1326	J 11	S	10.0	10.0	8.0	9.3	1.0	2.0	4.0	2.3	0.0	1.0	3.0	1.3
4790	Krapovikas	V	6.0	6.0	7.0	6.3	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0
3388	KG 61-240	S	9.0	10.0	9.0	9.3	1.0	1.0	4.0	2.0	0.0	0.0	3.0	1.0
3391	Khandesh-2	S	8.0	8.0	5.0	7.0	2.0	2.0	1.0	1.7	0.5	0.0	0.0	0.2
3400	Local-3	S	8.0	8.0	7.0	7.6	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0
156	M 13	Vr	9.0	9.0	10.0	9.3	0.5	3.0	0.5	1.3	0.0	2.0	0.0	0.7
2800	Monir 240-30	Vr	10.0	10.0	10.0	10.0	1.0	1.0	0.0	0.7	0.0	0.5	0.0	0.2
3424	NG 387	V	9.0	10.0	10.0	9.7	1.0	1.0	0.5	0.8	0.0	0.0	0.0	0.0

Contd.. Appendix C.

ICG No.	Entry Name	Taxonomic group (Type)	Growth				Proportion of leaves with mild chlorosis				Proportion of leaves with severe chlorosis			
			Replicate				Replicate				Replicate			
			I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
6090	NCAC 664	V	9.0	10.0	8.0	9.0	5.0	7.0	6.0	6.0	4.0	5.0	6.0	5.0
316	NCAC 688	S	8.0	5.0	10.0	7.7	5.0	7.0	0.0	4.0	3.5	6.0	0.0	3.2
2288	NCAC 841	Vb	8.0	8.0	6.0	7.3	1.0	0.5	0.0	0.5	0.0	0.0	0.0	0.0
6812	NCAC 2592	S	10.0	8.0	10.0	9.3	0.8	1.0	0.5	0.8	0.0	0.0	0.0	0.0
1881	Pircom	S	9.0	9.0	9.0	9.0	0.7	1.0	0.5	0.7	0.0	0.0	0.0	0.0
4748	PI 337394	S	10.0	10.0	7.0	9.0	3.0	4.0	1.5	2.8	1.0	2.0	1.0	1.3
4750	PI 337409	S	10.0	9.0	10.0	9.7	2.0	2.0	0.5	1.5	0.0	1.0	0.0	0.3
-	RMP-12	Vb	10.0	8.0	10.0	9.3	0.5	1.5	0.0	0.7	0.0	0.0	0.0	0.0
799	Robut 33-1	Vb	9.0	9.0	8.0	8.7	2.0	1.0	0.0	1.0	1.0	0.0	0.0	0.3
5469	Sir of Bijapur	S	8.0	10.0	9.0	9.0	3.0	1.0	1.0	1.7	1.0	0.0	0.0	0.3
4770	Shantungku No.203	Vb	5.0	5.0	7.0	5.7	2.0	6.0	0.5	2.8	0.0	4.0	0.0	1.3
4660	Tiftan 1134	S	10.0	9.0	10.0	9.7	1.5	3.0	2.0	2.2	0.0	1.0	0.0	0.3
221	TMV 2	S	4.0	3.0	3.0	3.3	1.0	4.0	2.0	2.5	0.0	2.0	0.0	0.7
200	S 196	S	9.0	10.0	8.0	9.0	2.0	0.0	2.0	1.3	0.0	0.0	0.5	0.2
4528	U-1-2-1	Vb	8.0	10.0	6.0	8.0	4.0	6.0	7.0	5.6	3.0	5.0	6.0	4.6
1435	U-4-4-23	S	9.0	9.0	9.0	9.0	1.0	0.0	1.0	0.7	0.0	0.0	0.0	0.0
4699	U-4-3-25	S	9.0	10.0	10.0	9.7	1.0	1.5	1.0	1.2	0.0	0.0	0.0	0.0
1452	U-4-12-3	S	6.0	7.0	5.0	6.0	2.0	4.0	0.5	2.3	1.0	2.0	0.0	1.0
4672	U-4-4-1	V	8.0	8.0	8.0	8.0	1.0	6.0	0.0	2.3	0.5	4.0	0.0	1.5
-	Gangapuri	V	10.0	6.0	9.0	8.3	4.0	6.0	1.5	3.8	3.0	5.0	0.0	2.7
1393	U-2-1-26	V	9.0	9.0	9.0	9.0	2.0	5.0	1.0	2.7	1.0	3.0	0.0	1.3

Contd.. Appendix C

ICG No.	Entry Name	Taxonomic group (Type)	Growth Replicate I	II	III	Mean	Proportion of leaves with mild chlorosis Replicate I	II	III	Mean	Proportion of leaves with severe chlorosis Replicate I	II	III	Mean
3263	U-4-47-7	S	10.0	9.0	8.0	9.0	2.0	2.0	0.5	1.5	0.0	0.0	0.0	0.0
4601	Var-27	V	6.0	8.0	8.0	7.3	5.0	5.0	1.0	3.7	4.0	4.0	0.0	2.7
2902	Var-42-9	Vr	10.0	10.0	10.0	10.0	0.0	1.0	1.0	0.7	0.0	0.0	0.5	0.2
3556	Var-26-5-2	S	10.0	10.0	6.0	8.7	1.0	0.5	0.5	0.7	0.0	0.0	0.0	0.0
-	FESR 12-P5	Vb	5.0	5.0	5.0	5.0	4.0	4.0	4.0	4.0	3.0	2.0	3.0	2.6
-	FESR 12-P6	Vb	5.0	2.0	5.0	4.0	4.0	8.0	3.0	5.0	2.5	6.0	0.0	2.8
3604	319 of Russia	V	9.0	9.0	7.0	8.3	4.0	2.0	3.0	3.0	2.0	0.5	2.0	1.5
1740	AK 10-24	S	10.0	10.0	7.0	9.0	0.0	2.0	3.0	1.7	0.0	0.5	1.5	0.7

* S - Spanish

V - Valencia

Vb - Virginia bunch

Vr - Virginia runner

V I T A

I, J. Koteshwar Rao, was born on February 2, 1958 to Kamala and Premchander Mohan, in Miryalaguda, Nalgonda Dist, Andhra Pradesh, India. I obtained my B.Sc., degree in Agriculture from Andhra Pradesh Agricultural University, Rajendranagar, Hyderabad (A.P.) in 1979. I married Miss Revathi of Miryalguda on February 15, 1981. During 1981 I was sponsored by the International Mineral Corporation for M.Sc. research work at the International Crops Research Institute for the Semi-Arid Tropics. I worked for my M.Sc. degree in Chlorosis in groundnut under the guidance of Drs. J.R. Burford and T.M. Vithal Rao.