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Diagnosis of iron deficiency in groundnut, *Arachis hypogaea* L.

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Summary Investigations into iron deficiency have been hindered by the lack of a satisfactory diagnostic tissue test, which in turn results from the total iron content of plant tissue commonly being an unreliable index of the iron status. Our measurements of chlorotic and normal leaves of field grown groundnut (*Arachis hypogaea* L.) showed that total iron was unsatisfactory as the measure of iron status of plant tissue. It was found that iron status was better assessed from an estimate of the ferrous iron content of fresh leaf materials obtained by extraction with o-phenanthroline. Extractable iron content increased with leaf age. Chlorotic buds or the first fully opened leaf always contained less than 6 μg extractable-Fe/g fresh tissue.

Introduction

Chlorosis is widespread in groundnut (*Arachis hypogaea* L.) grown on calcareous and alkaline soils in India. It is suspected that alkalinity-induced iron deficiency is the primary cause because the visual symptoms are very consistent with those caused by iron deficiency, yet the response to iron applications has been very variable. It is possible that other nutrient deficiencies may have been present[7]; diagnostic tests are therefore needed to aid in interpreting the reasons for success or otherwise of the amelioration techniques. There is not a widely-accepted satisfactory diagnostic tissue test for iron, because total iron concentrations of plant tissue do not provide a suitable index of the iron status of plants[4,8,9,13].

Several techniques based on plant tissue analysis have been proposed for diagnosis of iron deficiency in plants (for review see[8,11]). Various extractants have been proposed to extract the fraction of total iron, which is metabolically active and is related to occurrence of iron chlorosis. These extractants include water, dilute acids (hydrochloric acid, acetic acid, oxalic acid and citric acid), dilute NaOH, chelating agents such as EDTA, DTPA, tartaric acid and some organic solvents including 2,2′ dipyridyl and its derivatives, o-phenanthroline and several other compounds.

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Table 3. Mean extractable and total iron contents of groundnut leaves of different age (cv TMV 2)*

Leaf sample	Concentration of iron (μg/g)		
	Extractable		Total
	Fresh weight basis	Dry weight basis	Dry weight basis
Main bud	4.6	34.5	267
Lateral bud	5.1	38.2	408
Leaf 1	5.9	37.3	217
Leaf 2	6.6	36.2	206
Leaf 3	7.2	37.2	229
Leaf 4	7.6	39.0	315
Leaf 5	7.9	40.5	313
SE ±	0.38	2.08	34.6

* Sampled on 29 and 31 July, and 3 August, 1981. Main and lateral buds were healthy on 29 July and chlorotic on 31 July and 3 August.

The development of chlorosis in the youngest leaves indicated that the extractable-iron content of these should provide a better index of the iron status of a plant than total iron content. The first three samplings of the monitoring program in 1981 were therefore used to determine the iron content of all leaves on plants of cv TMV 2. The extractable-iron content of the fresh tissue consistently increased as a leaf aged (Table 3), but there was no consistent relationship between leaf age and total iron, or the extractable iron content expressed on a dry weight basis. Extractable iron content decreased in each of the three samplings, reflecting the onset of chlorosis, but similar decreases were observed in leaves of all ages.

Table 4. Extractable and total iron in lateral buds (Lb), main bud (Mb) and first fully opened leaf (L-1) of groundnut (cv TMV 2) in the rainy season, 1981

Date 1981	O-phenanthroline-extractable iron (μg/g)						Total iron (μg/g)		
	Fresh wt. basis			Dry wt. basis			Dry wt. basis		
	Lb.	Mb	L-1	Lb	Mb	L-1	Lb	Mb	L-1
Jul 29	6.0	5.4	7.2	35.6	34.4	40.5	236	233	246
Jul 31	5.0*	4.3*	5.5	45.2*	35.6*	39.3	456*	230*	188
Aug 3	4.2*	4.1*	4.9	33.2*	33.2*	32.0	531*	340*	217
Aug 5	6.7	6.3	8.3	47.8	42.8	42.5	279	317	212
Aug 7	4.7	5.1	5.5	26.2	26.8	26.5	253	264	239
Aug 10	5.4	6.0	6.0	27.8	29.1	21.4	181	242	132
Aug 12	6.2	6.2	7.7	34.7	33.3	31.8	232	215	139
Aug 14	5.6	6.0	8.2	35.7	35.3	30.9	260	175	206
Aug 17	5.2	5.0	6.3	32.7	28.9	28.2	154	157	160
Aug 19	8.3	8.2	14.0	49.0	44.3	49.3	147	177	106
Aug 26	7.0	7.9	8.3	37.1	36.2	31.3	159	177	128
Sep 7	–	4.7*	10.1	–	29.1	39.5	–	77*	124
Sep 11	–	5.6	5.3*	–	32.5*	20.8*	–	55*	52*
Sep 25	–	–	6.7	–	–	26.5	–	–	81
SE ±	0.28	0.43	0.42	2.49	2.36	1.93	24.9	27.8	14.7

* Marked chlorosis.

The monitoring program in 1981 was restricted to the youngest leaf material (the main bud, lateral buds, and the first opened leaf). Chlorosis developed less frequently and less severely in 1981 than in previous seasons. However, the extractable-iron content of buds and the first opened leaf was usually lowest during or shortly after the few occasions that chlorosis did develop (Table 4). Again, there was no consistent relationship between total iron content and the incidence of chlorosis.

Severe chlorosis developed during the 1981 rainy season in one experiment that contained a large number of breeding lines. The growth of individual plants, and the severity of chlorosis, was consistent within a breeding line, but there was very wide variation across the lines. The most diverse lines for growth and chlorosis were selected for sampling and analysis. The results again showed that the youngest leaves of chlorotic plants contained less extractable iron than those from healthy plants (Table 5).

Analysis of plant samples for other elements such as Mn showed that their contents were above the deficiency levels both in green and chlorotic tissue (unpublished data).

The consistently lower extractable iron and higher total iron in chlorotic than in normal leaves (Tables 1, 4 and 5) showed that the iron deficiency was due to poorer utilization of iron within a leaf rather than to decreased absorption of iron by the root or translocation from the

Table 5. Content of extractable and total iron (μg/g) in main bud (Mb) and first fully opened leaf (L-1) of different groundnut breeding lines*

Extent of chlorosis†	Plant growth†	Breeding entry	Extractable Fe**		Total Fe***	
			Mb	L-1	Mb	L-1
Severe	Poor	FESR 12-P5	4.0	4.4	413	302
		FESR 12-P6	4.1	5.0	438	225
Severe	Good	NCAC 664	4.8	4.5	416	325
		U-1-2-1	4.1	4.4	429	371
Nil	Poor	TMV-2	5.4	9.0	286	196
		Krapovikas 16	6.5	11.4	267	174
Nil	Good	C. No. 501	5.8	9.9	231	202
		T. Runner	6.0	10.3	252	263
SE ±			0.36	0.58	15.1	7.2

* Leaves sampled on 1 September 1981, 72 days after sowing.
** Fresh weight basis.
*** Dry weight basis.
† Extent of chlorosis and plant growth scores made on a scale of 0–10; the highest value was given for maximum growth or maximum chlorosis.

root to the leaf. This pattern is consistent with the common association of chlorosis in our fields with high soil moisture content, resulting from either rainfall or irrigation. High soil moisture content, by reducing aeration would cause an increase in the ferrous iron in the soil solution, thus promoting iron uptake and an increase in the total iron content of the leaf tissue. Reduced aeration would also restrict carbon dioxide escape from the soil thus causing, in these alkaline soils, increased bicarbonate concentration in the soil solution, which is known to reduce the availability of iron in the plant[12].

Although the extractable iron content of fresh leaf tissue increased with leaf age, the first fully-opened leaf appears to exhibit a wider range in extractable iron content (Tables 4 and 5), than the younger unopened leaves (main bud or lateral buds). More detailed studies are needed to determine whether the first fully opened leaf or a bud is the most reliable test organ for diagnostic work. From the data in both Tables 4 and 5, chlorosis was observed in the present work only when the extractable-iron content of the buds of first opened leaf was less than 6 μg/g fresh tissue.

Conclusions

The results with groundnut are consistent with those from recent studies on rice, which indicate that the o-phenanthroline extractable iron content of fresh leaf tissue appears to be a much better index of the iron status of the plant than total iron content. Extractable-iron in chlorotic buds or the first fully opened leaf was always less than 6 μg/g of fresh tissue.

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