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A NEW TECHNIQUE OF PLANT ANALYSIS TO RESOLVE IRON CHLOROSIS

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KEY WORDS

Fe^{2+}-iron Iron chlorosis o-Phenanthroline

SUMMARY

Iron though indispensable for the biosynthesis of chlorophyll, its total content in the plant was not associated with the occurrence of chlorosis. In order to overcome this inconsistency a new technique of plant iron analysis has been developed. It consists of the determination of Fe^{2+}, the fraction of iron involved in the synthesis of chlorophyll.

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 could preferentially chelate Fe^{2+}. The highly specific orange colour of the Fe^{2+}-phenanthroline complex made possible the determination of Fe^{2+} by reading the transmittancy at 510 nm.

The procedure involves extraction of 2 g of thoroughly washed, chopped, fresh plant by 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 sharp contrast to total iron the green plants always contained more Fe^{2+} than chlorotic plants. The technique has been developed for rice but is expected to be successful for other crops also.

INTRODUCTION

Plant analysis is one of the accepted tools for diagnosing deficiency disorders. Deficient plants, if analysed at the right stage, usually contain lower amount of the deficient element than the corresponding healthy checks. Perhaps, iron is the only essential element which often evades this most simple definition of deficiency, since instance are not uncommon when the total content of iron in the chlorotic plants was higher than in the green plants[7,15,19,20,22,26,32,35,36]. In order to overcome this elusive behaviour of iron, it has been suggested: *i*) That in place of total iron the plant should be analysed for active iron[14] or dilute acid-extractable iron[17]. In some instances, this fraction of iron correlated well with the chlorotic plants[3] while in others it did not[17]. Possibly, this inconsistency coupled with unspecific form of iron being extracted has not widened the popularity of this technique. *ii*) Since iron deficiency leads to imbalance in other plant nutrients, several workers[2,5,15,18,26] have suggested the use of certain nutrient ratios for diagnosing iron chlorosis. Their universal applicability has however, been contested[1,13,18,33]. *iii*) Iron is linked with a number of enzyme systems[21]. Thus, the change in activities of enzymes has been used as a yardstick to distinguish iron deficiency in plants[8].

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Nevertheless, a close scrutiny of these techniques does not lend support to the common belief that the deficiency of iron is associated with low amount of iron. Thus, Wallace[32] and Patel *et al.*[19] have suggested to analyse the plants for that fraction of iron which correlates well with the occurrence of chlorosis. In this context, we assume that Fe^{2+} fraction of iron is more important for synthesis of chlorophyll and consequently occurrence of chlorosis. The procedure described in this paper has been developed to analyse plants for their Fe^{2+} content.

THEORY

The new technique of plant iron analysis to resolve iron deficiency chlorosis is based upon the following considerations: Iron in the plant is present in the form of different complexes. These complexes vary in their stability constants. When the deficiency occurs, according to chelate model, the iron compounds of low stability may decompose and are possibly transformed into compounds of high stability[21]. Irrespective of the size of the molecule of the iron compounds, most of the Fe^{3+} complexes are more stable than those of Fe^{2+}. Therefore, when there is iron deficiency, perhaps, there is lack of Fe^{2+} form of iron.

On this basis, we assume that it is the Fe^{2+} fraction of the total iron which is responsible for green or chlorotic plants and is, thus, indispensable for chlorophyll synthesis. This assumption is apparently true and it is in line with the evidence[6] that the Fe^{2+} is involved in the condensation of succinic acid and glycine to form δ-amino leavulinic acid (ALA). The ALA then condenses to form pyrrole groups and they condense to give rise to protoporphyrin IX which by insertion of Mg^{2+} produces chlorophyll. Additionally, the enzyme ferrochelatase which catalyses the insertion of Fe^{2+} into protoporphyrin may apparently have low stability constant (<7.0)[21], thus, it will be an early victim of ligand competition. The deficiency of Fe^{2+}, accordingly may restrict chlorophyll synthesis and consequently may lead to chlorosis. Hence for the diagnosis of iron deficiency chlorosis Fe^{2+} should be determined. Although this hypothesis involving Fe^{2+} estimation alone has been contested by Price[21].

Our technique is based upon the direct determination of Fe^{2+} in the fresh plant material. In our search for an appropriate extracting reagent for Fe^{2+}-iron, we selected 1–10 o-phenanthroline ($C_{12}H_8N_2$). The choice of o-phenanthroline was based upon the following considerations:

1. The Fe^{2+}-phenanthroline complex $[Fe(C_{12}H_8N_2)_3]^{2+}$, ferroin, has significantly higher stability constant (Table 1) than Fe^{3+}-phenanthroline complex, ferriin. Therefore, when o-phenanthroline is added to plant material which contains Fe^{2+} and Fe^{3+}-complexes it is expected to preferentially chelate Fe^{2+}. Additionally, Fe^{3+}-complexes are known to have higher stability constants and thus o-phenanthroline will be less effective in extracting Fe^{3+}. On this basis, Fe^{2+} of the plant material in contact with o-phenanthroline extractant will be mobi-

lized into the solution. It will form a stable, raddish orange colour complex ferroin. The ferroin so formed can be determined directly by reading the transmittancy at 510 nm.

2. The iron in the plant is transported as relatively large negatively charged molecules of stability constants varying between 17.0–20.7[23]. However, recent findings indicated that low molecular weight organic molecules may function in cation transport[27,28,29,30,31]. The stability constant of o-phenanthroline-Fe^{2+} complex is neither too low to render it incapable to extract most of the plant Fe^{2+}, nor it is unusually high to remove its non-reactive forms.

3. The orange colour of ferroin is highly specific for Fe^{2+}. Thus, by reading the transmittancy at 510 nm the interference from Fe^{3+} can be avoided. The low levels of Cu present in the plants are not expected to be an impediment to the Fe^{2+}-o-phenanthroline colour.

4. The ferroin is stable and it prevents the oxidation of Fe^{2+}.

5. Other commonly available chelating agents have remarkably higher stability constants for Fe^{3+} (Table 1). These prefer Fe^{3+} for chelation. Thus, in contrast to o-phenanthroline their success for extracting Fe^{2+} in plants is doubtful.

Table 1. Stability constants of some Fe^{2+} and Fe^{3+} iron complexes[24]

	DTPA	EDDHA	EDTA	o-Ph
Fe^{2+}	16.5	14.3	14.3	21.3
Fe^{3+}	28.6	33.9	25.1	14.1

Values for stability constants are the overall stability constant values.

MATERIALS AND METHODS

Plant samples used

During the wet season of 1978, the rice cultivation in parts of Ludhiana district of Punjab (India) was initiated for the first time on low organic matter ($<0.5\%$), alkaline (pH > 8.0) loamy sand to loamy soils. The farmers could not maintain continuously standing water uniformly in their fields. By and large, rice within 10–12 days after transplanting suffered from chlorosis which could be alleviated by repeated sprays of 1 per cent iron sulfate. So severe was the chlorosis that in some fields the crop failed. While in others, where the soil texture was relatively heavier, and the surface submergence could be achieved for a day or so the chlorosis was moderate and it was distributed unevenly. Strikingly, the green and chlorotic hills were growing side by side.

For our analysis, if not specified, we collected green and chlorotic rice plant samples after 30–40 days of transplanting. The mother tillers, the tallest tiller in a hill, were collected from 20 different green and chlorotic hills, respectively. Each tiller was cut about 5 cm above the ground level. The plant

samples were transported immediately to the laboratory in close polyethylene bags. These were washed copiously with running tap water, followed by 0.1 *N* HCl and distilled water. The samples were freed off the sticking water drops by sandwiching them between sheets of clean blotting papers. They were cut into small pieces of approximately 1–2 mm with the help of stainless steel scissors. Each sample was analysed in duplicate and only the mean results are presented in this paper.

In order to remove discrepancies arising due to varying moisture contents of the wet samples the fresh chopped plant samples were dried to a constant weight at 50°C in an oven and moisture per cents were computed. The results for the iron contents were then calculated and are presented on oven dry weight basis.

o-phenanthroline extracting solution

In the initial experiments 1.5 g 1–10 o-phenanthroline (o-Ph) was dissolved in alcohol and the volume made to 100 ml. This extractant imposed serious limitations in the colorimetric determination of iron because of simultaneous extraction of chlorophyll. Additionally, the filtrate could only be analysed for total iron by atomic absorption spectrophotometer. Therefore, in the subsequent experiments the o-Ph was dissolved in water.

o-Ph ($C_{12}H_8N_2$) is a monoacid base and it was soluble to the extent of 0.1 per cent in the hot boiling water only. Therefore, the acidification of the suspension was essential to dissolve desired quantities of o-Ph. The procedure used to prepare 1 l extractant was to add exactly 15 g of o-Ph to about 850 ml of distilled water. To this continously stirring solution approximately 1 *N* HCl was added dropwise. When the last traces of the salt were soluble the pH was determined. It was found to be around 5.5. The volume of the solution was made to 1 l. Since pH of o-Ph (or of any complexing agent) in solution influences its capability to chelate and thus extract iron the o-Ph extracting solutions of varying pH were prepared (Table 6). The fresh green and chlorotic rice plant samples were extracted.

Extraction procedure

Two g of the fresh-chopped samples were weighed immediately and transferred to 100 ml capacity glass bottles. Twenty ml of o-Ph solutions were added and the contents of the bottles were stirred gently in order to embathe the plant samples with the extractant. The bottles were stoppered and allowed to stand for about 16 hours at room temperature. The contents were filtered through Whatman No. 1 filter paper. Fe^{2+} and total iron were estimated directly in the filtrate by measuring the transmittancy at 510 nm and by atomic absorption spectrophotometer, respectively.

Total iron

The oven-dried, ground plant samples were digested in a triacid mixture[11]. The extracts so obtained were analysed for their total iron by atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

By and large it is accepted, that iron, though not part of chlorophyll, is indispensable for its bio-synthesis[14]. Despite this the total plant iron contents have usually failed to regulate greenness or chlorosis in plants. This was reflected in our study also when the total iron contents of the green plants were strikingly lower than

Table 2. Iron content (ppm) of plants by 2 methods of determination to separate iron-deficient from non-deficient plants

Site No.	Total		o-Phenanthroline	
	Green	Chlorotic	Green	Chlorotic
1	135	260	51	27
2	170	200	55	29
3	140	220	53	29
4	130	270	50	34
5	160	260	52	30

Table 3. Effect of washing on the total iron content (ppm) of green and chlorotic plants

Plant	Green		Chlorotic	
	Washed	Unwashed	Washed	Unwashed
Leaf	115	250	231	375
Tiller	121	260	250	375

the chlorotic rice plants (Table 2). Careful washing in dilute acid as suggested by Jacobson[12], Smith, Reuther and Spacht[25] and Wallihan[34] for proper evaluation of iron status of pear and citrus trees was of no avail for rice in our study. The chlorotic plants despite proper cleaning like the results obtained by Mehrotra *et al.*[15], contained more iron than green plants (Table 3). On the contrary, the o-Ph (1.5% o-phenanthroline in alcohol) extractable iron though only fraction of the total iron was about 1.5 times more in green than in the chlorotic plants (Table 2). These results clearly demonstrated the effectiveness of the o-Ph extractant for separating iron-deficient chlorotic plants from non-deficient green plants.

Due to simultaneous extraction of chlorophyll, we were restricted to analyse iron in the filtrate by atomic absorption spectrophotometer. Similar to dilute acid-extractable iron, determined by earlier workers[2,3,10,15], we also could not distinguish the fraction of iron being extracted. Therefore, to avoid interference of chlorophyll and to measure the transmittance of the orange-coloured complex of Fe^{2+}-phenanthroline in the following experiments we used 1.5 per cent o-Ph solution of pH 3.0 prepared in distilled water (The basis for selecting this concentration and pH of the extractant is discussed subsequently).

Table 4. Total and o-Phenanthroline (1.5%, pH 3.0) extractable iron in rice plants at different stages of growth

Growth stage	Total Fe (ppm)		o-Ph-extractable Fe (ppm)	
	Green	Chlorotic	Green	Chlorotic
Tillering	115	135	55	26
Pre-panicle initiation	115	154	51	36
50% flowering	120	140	46	30

Unlike with alcohol, when the rice plant samples were extracted with o-Ph extractant prepared in distilled water adjusted to pH 3.0 the filtrates were free from green-colouring matter. On the contrary, the colour of the filtrates was orange red which is the characteristic colour of Fe^{2+}-phenanthroline complex. The non-interference of the chlorophyll and/or other plant pigments was confirmed by the 100 per cent transmittance readings of the filtrate obtained by treatment of the fresh plant material with distilled water adjusted to pH 3.0.

The data on iron content, total and o-Ph extractable, are presented in Table 4. Similar to the results of the previous experiment the total iron was higher in the chlorotic plants. In sharp contrast to total iron, irrespective of the stage of growth, the o-Ph extractable iron was more in the green than in the chlorotic plants.

Form of iron being extracted

1–10 o-phenanthroline forms a raddish orange complex with Fe^{2+} which has an absorbance maximum at 510 nm. It also forms a yellow complex with Fe^{3+} which exhibits little absorbance at 510 nm. However, the yellow colour of the Fe^{3+}-complex can be transformed into orange red by reducing Fe^{3+} to Fe^{2+}. Hydroxylamine hydrochloride is the most widely used reducing agent for this purpose.

In order to determine the state of iron being extracted the o-Ph extracts of the green and chlorotic plants obtained after filtration were divided into 2 parts. One part was analysed for Fe^{2+} by reading the transmittancy of the orange-coloured ferroin at 510 nm. To bring about the reduction of the Fe^{3+}, 4 ml of the second part of the filtrate was treated with 1 ml of 2 per cent hydroxylamine hydrochloride and the contents were mixed thoroughly and allowed to stand for about 10 minutes. Total Fe^{2+} was estimated by reading again the transmittancy at 510 nm. The correction for the dilution brought about the hydroxylamine

Table 5. Iron content in the o-Phenanthroline extract of fresh rice plants as influenced by treatment of the extract with reducing agent and method of determination

Plants	Spectrophotometer, Fe^{2+} ppm		AAS*, Fe ppm
	o-Ph	o-Ph + hydroxylamine hydrochloride	o-Ph
Green	53.0	53.0	54.5
Chlorotic	29.1	29.0	29.1

* AAS: Atomic absorption spectrophotometer.

hydrochloride solution was applied to calculate Fe^{2+} content. Additionally, the original filtrates were also analysed for their iron contents by atomic absorption spectrophotometer.

The data for Fe^{2+} contents with and wihout treatment of the filtrate with reducing agent determined by reading the transmittancy at 510 nm and for total iron estimated by atomic absorption spectrophotometer are presented in Table 5. A comparison of these data clearly elucidate that irrespective of the treatment of the filtrate with the reducing agent and method of analysis the iron contents were more or less the same. Failure of the hydroxylamine hydrochloride to alter the iron content of the filtrate was an evidence that the o-Ph was largely extracting Fe^{2+} only. Additionally, the atomic absorption spectrophotometer estimates the total iron. The lack of difference between iron contents by reading the transmittancy of the ferrous complex with the help of a spectrophotometer and total iron estimated by atomic absorption spectrophotometer was supportive of the fact that the o-Ph was mostly extracting Fe^{2+} fraction of the iron only.

EXPERIMENTAL VARIABLES

A number of variables were examined to determine their effect on the o-Ph extractable iron. For this purpose, green and chlorotic rice plants were collected from the same field and were extracted depending upon the variable under investigation.

pH of o-Ph extractant

The influence of pH on the o-Ph-extractable iron was studied by altering the pH of the extractant from 5.0 to 1.0. The samples were collected from two different sites (Table 6).

Table 6. Influence of pH of o-Phenanthroline on the extractability of iron (Fe^{2+} ppm) from green and chlorotic rice plants

pH	Green	Chlorotic
Site 1		
5.5	42.3	26.5
5.0	52.8	36.0
4.0	54.4	35.0
3.0	64.8	42.3
Site 2		
3.0	50.5	42.9
2.0	61.8	55.5
1.0	72.3	75.4

The original pH of the extractant had a significant influence on the extractable iron (Table 6). The amount of iron being extracted increased sharply with the fall in pH. So intense was the influence of low pH on the extractable iron that the chlorotic plants which had lower Fe^{2+} than the green plants at pH 2.0 or above contained a slightly higher Fe^{2+} than green plants at pH 1.0.

The rise in extractable iron with the fall in pH was brought about by increase in instability of the organo-metallic complexes. Further, at low pH H^+ ions and metal ions compete for the ligands. Thus, more iron will potentially be extracted.

Lack of difference in the Fe^{2+} contents between green and chlorotic plants might have arisen due to reduction of Fe^{3+} to Fe^{2+} by excess of H^+ at low pH ($2Fe^{3+} + 2H^+ = 2Fe^{2+} + H_2$). A slight increase in Fe^{2+} content in the chlorotic plants was in conformity with this hypothesis. Thus, at low pH relatively more iron was extracted into the solution. This might have contributed to increased Fe^{2+} pool.

Hence the pH of the extractant should be such that:

1. A maximum difference in Fe^{2+} content between green and chlorotic plants is obtained.
2. At the same time sufficient iron is extracted to be measured conveniently.

This pH was found to be around 3.0.

Concentration of o-Ph

The amount of metal being chelated will depend upon the availability of ligands, consequently the concentration of the chelating agent. Thus, the higher is the

Table 7. Effect of different concentrations of o-Ph (pH 3.0) on the extractability of iron (Fe^{2+} ppm) from green and chlorotic plants

o-Ph (%)	Green	Chlorotic
0.1	3.1	nd*
0.5	36.8	31.3
1.0	47.0	38.6
1.5	50.5	42.9
2.0	54.2	45.2

* nd: not determined.

chelate concentration the more stress it is expected to exert to dissolve labile metals from the plant.

Theoretically, to complex each mole of Fe^{2+} 3 moles of o-Ph are required. Hence, while extracting plant samples the extractant must contain excess o-Ph. In order to find out the optimum concentration of o-Ph the extractants were prepared by altering its concentration from 0.1–2.00 per cent.

The data on the influence of concentration of chelating agent on the extractable Fe^{2+} are presented in Table 7. The increase in concentration of the chelating agent, by and large, extracted more iron. The improvement in Fe^{2+} with the initial rise in the o-Ph concentration was dramatic. However, the increase in Fe^{2+} beyond 1.0 per cent o-Ph was only marginal.

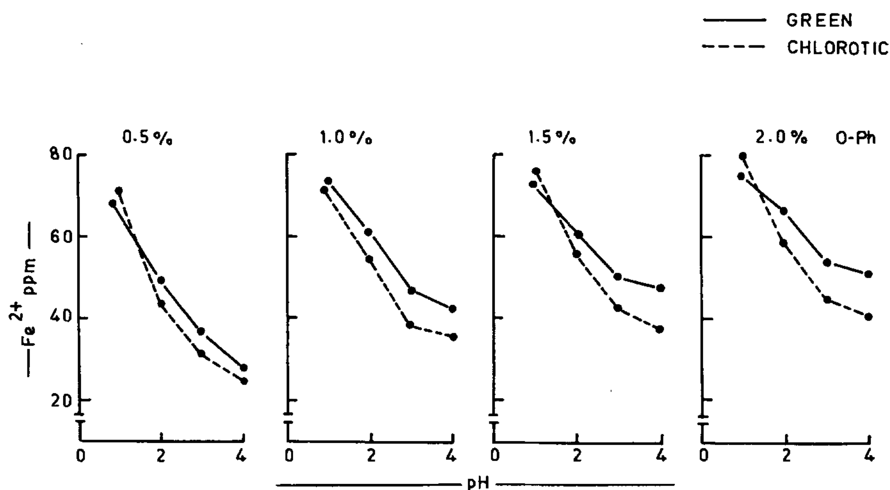

Fig. 1. Influence of pH and concentration of o-Ph on the extractability of Fe^{2+}.

The results discussed thus far indicated the need to find out the optimum combination of pH and o-Ph concentration of the extractant to establish the maximum difference in the Fe^{2+} content between green and chlorotic plants. For this purpose, the same plant material was extracted with different extractants prepared by varying their pH and content of o-phenanthroline (Fig. 1). As in the previous experiments, the extractable Fe^{2+} both in green and chlorotic plants increased with the fall in pH and increase in o-Ph content of the extractant.

The optimum combination of pH and o-Ph which could distinguish green from chlorotic plants on the basis of iron was worked out by fitting the regression of difference in Fe^{2+} contents between green and chlorotic plants (Y) against pH (x_1) and per cent o-Ph (x_2) as independent variables. The functional relationship between Y and x_1 and x_2 was as follows:

$$Y = -13.1562 + 11.8012x_1 + 5.6700x_2 - 2.3937x_1^2 - 4.5000x_2^2 + 2.5520x_1x_2 \quad (R^2 = 69\%).$$

Maximum difference in Fe^{2+} content between green and chlorotic plant occurs for the curve fitted (Fig. 2) for the two values of x_1 and x_2.

$$\left[\frac{d^2Y}{dx_1^2} < 0 \text{ and } \frac{d^2Y}{dx_2^2} < 0\right]$$

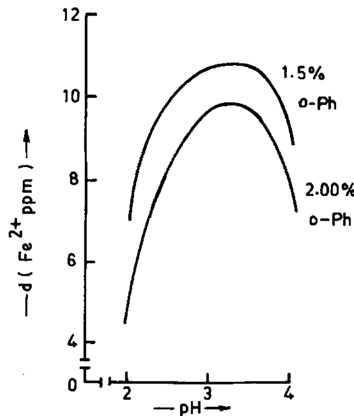

Fig. 2. Difference (d) in Fe^{2+} between green and chlorotic rice at varying pH.

The values of x_1 and x_2 for which iron content difference is maximum are:

x_1 (pH) = 3.30
x_2 (o-Ph%) = 1.56

On this basis we suggest that to get the maximum difference in Fe^{2+} contents between green and chlorotic plants the pH of the extractant should be around 3.0 and it should contain 1.5 per cent o-Ph.

Time after sampling and extraction of Fe^{2+}

The interval between sampling and analysis is expected to influence the status of iron in the plant. The longer is this time, the more of Fe^{2+} may get oxidized to Fe^{3+}. In order to investigate the influence of time after sampling on the Fe^{2+} content the green and chlorotic plant samples were collected and processed for analysis then and there in the field. Two g of the each sample was weighed and treated with 20 ml of the extractant. A part of each of the unprocessed samples was saved, placed in polyethylene bags and brought to the laboratory for preparation and analysis. The time gap between 2 extractions was about 4 hours.

The data presented in Table 8 revealed that there was no appreciable change in Fe^{2+} content whether the samples were analysed in the field, that is immediately after collection or in the laboratory, that is about 4 hours after sampling. However, when the same samples were stored for 5 days in a refrigerator the decline in Fe^{2+} content was significant.

Table 8. Influence of time between sampling and analysis on the o-Ph-extractable Fe^{2+} (ppm)

Time (hours)	Green	Chlorotic
0	52	40
4	52	39
120	41	33

We have not investigated the influence of time intervals between 4 hours and 5 days. However, a decrease in Fe^{2+} content by increasing the time interval beyond 4 hours is expected. On the basis of these results, the samples should be analysed immediately after being brought to the laboratory and their storage for longer intervals should be avoided.

Table 9. Influence of time of extraction on the extractability of Fe^{2+} (ppm) from green and chlorotic plants (1.5% o-Ph of pH 3.0)

Time (hours)	Green	Chlorotic
Experiment 1		
1	12.5	14.6
4	18.5	22.6
8	33.3	24.5
12	39.4	27.3
24	41.1	32.8
36	41.9	31.2
Experiment 2		
12	38.7	28.1
16	45.9	28.1
20	45.6	29.8

Time of extraction

The time of contact between the plant sample and chelating agent will influence the amount of Fe^{2+} being extracted. In order to determine the optimum time of extraction the samples after treatment with o-Ph were filtered after various intervals of time (Table 9) in two separate experiments.

The results presented in Table 9 show that the extractable Fe^{2+} both in green and chlorotic plants increased by extending the period of extraction upto 12 hours. Beyond this period, the increase was marginal and after 24 hours the content of Fe^{2+} being extracted levelled off. These data revealed that the optimum time of extraction should be more than 12 hours. In a subsequent experiment the plant samples were extracted for 12, 16 and 20 hours. The results thus obtained suggest that for extracting the maximum amount of Fe^{2+} from green and chlorotic plants the contact time between sample and the extractant should not be less than 16 hours. Longer periods than this do not offer any distinct advantage.

Influence of ratio of plant sample to extractant

For finding out the optimum ratio of plant sample and extractant, 2 g of the chopped plant samples were treated with varying volumes of extractant and Fe^{2+} was analysed (Table 10).

Table 10. Influence of plant to extractant ratio on the extractability of Fe^{2+} (ppm) from green and chlorotic plants

Extractant plant ratio	Green	Chlorotic
2.5	No filtrate could be obtained	
5.0	31.8	20.0
10.0	41.2	32.8
15.0	43.2	32.7

When the extractant to plant ratio was 2.5 no filtrate was obtained (Table 10). As the extractant to plant ratio increased the amount of iron being extracted also increased. However, the increase was only slight when this ratio was 10 or higher. The low amount of iron being extracted when the extractant to plant ratios were lower than 10 was apparently due to a portion of plant sample not being bathed by the extractant completely and thus it was partially extracted.

We suggest a sample to extractant ratio of 10 for removing the maximum amount of Fe^{2+}.

CONCLUSIONS

Attempts to relate total iron content of the foliage with the occurrence of chlorosis have often failed. Similar to the results of this study a number of earlier workers also obtained strikingly more iron in the chlorotic than in the green plants. While Oserkowsky[17], Bolle-Jones[3] and Machold and Stephan[14] could discern chlorotic plants from green plants on the basis of active iron content, Patel *et al.*[19] were unable to confirm these results for rice. The basis for denoting the dilute acid extractable iron as 'active iron' has not been elaborated. The technique discussed in this paper measures Fe^{2+}, the fraction of iron involved in the synthesis of protoporophyrin IX, and thus indispensable for the manufacture of chlorophyll.

The procedure essentially consists of extracting the fresh plant material with o-phenanthroline (pH 3.0, concentration 1.5%) and measuring the transmittancy of the orange coloured ferroin (Fe^{2+}-o-phenanthroline complex) of the filtrate at 510 nm. The Fe^{2+} content of green plants was always higher than chlorotic plants. The technique has been used for rice. But it is expected to be successful for other crops also. Additionally, the critical level of Fe^{2+} below which rice plants develop chlorosis needs to be worked out.

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