RESOLUTION OF IRON CHLOROSIS

S. C. MEHROTRA, N. K. MEHROTRA, S. S. BISHT AND C. P. SHARMA

Department of Botany, Lucknow University, Lucknow

ABSTRACT

The causative factors, diagnostic indices and ameliorative methods of iron chlorosis are discussed. Available information suggests that tissue analysis, even after removal of surface contaminants by suitable detergents, dilute acids and/or complexing agents, is a poor indicator of iron chlorosis. Chemical and bio-chemical indices like increase in P/Fe, K/Ca, Mn/Fe and Peroxidase/Catalase ratios may also not help in the resolution of the cause of chlorosis.

Controlled culture trials and field experiments indicate that increase in Cu/Fe and Zn/Fe ratio resulting from increased accumulation of Cu and Zn in chlorotic tissues responding to iron amendment, as also bio-chemical parameters like decrease in protein and catalase activity in chlorotic tissues and their restoration to near normalcy consequent to suitable iron application, can give an indication of physiological unavailability of iron and can be taken as suitable indices for quick identification of the disorder. Study of varietal reaction under iron stress indicates that iron chlorosis can be overcome by planting chlorosis resistant varieties.

THE PROBLEM OF IRON CHLOROSIS

Chlorosis of plants that can be cured by supplying iron to the chlorotic plants and hence referred to 'iron chlorosis' is not only the oldest known deficiency disease of plants (Gris, 1843, 1844), but is also the most widespread mineral nutritional disorder in the world, especially on calcareous soils which cover more than one-third area of the world (Brown, 1956, 1961; Wallace & Lunt, 1960). The disorder has been reported from different agroclimatic soil regions of India (Agarwala & Mehrotra, 1963; Kanwar & Randhawa, 1974).

As iron is abundantly present in the lithosphere, 0.07% to 4.2% or more (Jackson, 1964), lack of iron in the rooting medium is rarely a cause of iron deficiency (Wallace & Hewitt, 1946). Iron chlorosis is an outcome of soil factors that reduce its availability to plants or is caused by poor efficiency of the plant for absorption, translocation or utilization of iron. The better known factors conducive to iron chlorosis in plants are listed in Table 1. Depending upon the soil factors, iron chlorosis may be qualified as carbonate induced, bicarbonate induced, lime induced, phosphate induced or heavy metal (Mn, Cu, Zn, Mo, V, Co, Ni, etc.) induced.

Since iron chlorosis results in serious economic losses to agricultural and horticultural crops, the problem has been investigated from various angles, and different facets of the disorder have been discussed by several workers (Thorne, Wann & Robinson, 1950; Brown, 1956, 1960, 1961; Brown, Holmes & Tiffin, 1959; Wallace & Lunt, 1960; Wallace, 1962, 1971; Hewitt, 1963; Little, 1971).

Table 1—Factors conducive to Iron Chlorosis (*Adapted from* WALLACE & LUNT, 1960)

1. Low iron supply.
2. High $CaCO_3$ in soil.
3. High bicarbonate in soil or irrigation water.
4. High Phosphate.
5. High levels of nitrate nitrogen.
6. Over-irrigation or high moisture condition.
7. High levels of heavy metals—manganese, copper, zinc, etc.
8. Unbalanced cation ratios.
9. Poor aeration.
10. Low or high temperature.
11. High light intensities.
12. Organic matter addition to soils.
13. Virus infections.
14. Root damage by nematodes or other organisms.
15. Genetical.

DIAGNOSTIC INDICES OF IRON CHLOROSIS

IRON IN TISSUE AND THE ROOTING MEDIUM

Evaluation of availability of iron to plants has been quite problematic as neither analysis of 'available' iron content of soils (THORNE, 1941; THORNE & WALLACE, 1944; OLSEN & CARLSON, 1949; GALLEGO & LABORDA, 1958; AGARWALA & MEHROTRA, 1963) nor the tissue content of iron in plants (Table 2) provide any reliable index of iron availability.

Table 2—Changes in tissue concentration of iron in plants showing iron chlorosis

Tissue iron decreased	Tissue iron increased	Effect on tissue iron inconsistent
	Iron deficiency	
Somers & Shive (1942), Weinstein & Robbins (1955), Fujiwara & Tsutsumi (1957), DeKock, Hall & McDonald (1960), Oertli & Jacobson (1960), Patnaik & Bhadrachalam (1965), Djendov (1971).	Agarwala & Sharma (1961).	Jones & Hewitt (1949), Twyman (1951), Agarwala, Sharma & Farooq (1965).
	Induced Iron deficiency	
Fliche & Grandeau (1873), Vidal (1937), Jacobson (1945), Wallihan (1955), Kuykendall (1956), Harkness & Malcolm (1957), Mayagoitia, Hactor & Uzeanga (1959), Altares (1959), Kartashova (1968), Georgobiani (1969).	Smith, Reuther & Specht (1954), Ahmad & Tewfik (1956), Ivanov (1964), Kenezek & Maier (1966), Shpota (1967).	Gile & Carrero (1920), Milad (1924, 1939), Wallace & Mann (1926), Allyn (1927), Wallace (1928), Oserkovsky (1933), Lindner & Harley (1944), Iljin (1952), Leeper (1952), Sims & Gabelman (1956), Agarwala & Mehrotra (1963), Ostrovskaya & Zaiko (1967).

Several workers (JACOBSON, 1945; THORNE, WANN & ROBINSON, 1950; TAYLOR, 1956; SMITH, 1962) have ascribed high tissue iron in chlorotic leaves to surface contamination which can be removed by suitable detergent or dilute acid (HCl) treatments. It has, however, been observed by the authors that, irrespective of the cleansing procedure used, the chlorotic foliage of *Justicia grandis* always contained higher tissue iron than the comparable green leaves (Table 3).

Table 3—Effect of certain cleansing treatments on the iron content of green and chlorotic *Justicia grandis* leaves (AGARWALA *et al.*, unpublished)

Cleansing Treatments	Tissue	
	Green	Chlorotic
	/μg Fe/g dry matter	
Unwashed	720	823
Washed with deionised water	207	233
Washed with detergent (Lidet)	160	167
Washed with 0.3 N HCl	167	235
Washed with (10^{-2}M) EDTA	153	227

Table 4a—Nutrient imbalance associated with iron chlorosis (Induced iron deficiency)

Nutrient element	Tissue concentration		
	Increased	Decreased	Effect inconsistent
Potassium ..	Church (1886), Colin & Grandsire (1925), Wallace & Mann (1926), Wallace (1928), Lindner & Harley (1944), Thorne & Wallace (1944), Chapman & Brown (1950), Smith, Reuther & Specht (1950), Thorne, Wann & Robinson (1950), Iljin (1952), Higdon (1957), Carpena, Sanchez & Guillen (1957), Shpota, (1967), Agarwala *et al.* (unpublished).		
Calcium ..	Shpota (1967) ..	Church (1886), Colin & Grandsire (1925), Wallace & Mann (1926), Menchikowsky & Puffeles (1935), Iljin (1942), Lindner & Harley (1944), Thorne & Wallace (1944), McGeorge (1948), Chapman & Brown (1950), Higdon (1957), Carpena, Sanchez & Guillen (1957).	Iljin (1952) Agarwala *et al.* (unpublished)
Magnesium	Iljin (1952)	Lindner & Harley (1944), Smith, Reuther & Specht (1950), Higdon (1957).	Agarwala *et al.* (unpublished)
Sulphur ..	Shpota (1967).. ..		Agarwala *et al.* (unpublished)
Phosphorus ..	Smith, Reuther & Specht (1950), Shpota (1967).		Lindner & Harley (1944), Thorne, Wann & Robinson (1950), Iljin (1952), Agarwala *et al.* (unpublished).
Manganese ..		Smith, Reuther & Specht (1950), Ahmad & Tewfik (1956).	Lindner & Harley (1944), Agarwala *et al.* (unpublished).

Concentration of Other Nutrient Elements

Iron deficiency, whether *true* or induced, results in marked changes in the concentration of other nutrient elements (Tables 4a and b) and suggestions have been made to use tissue concentration of certain elements (other than iron) as an index of availability of iron to plants. A perusal of Tables 4a and b would indicate that iron chlorosis is almost always associated with increase in the concentration of potassium, nitrogen, copper, molybdenum and boron, In most cases the chlorotic leaves also contain higher concentration of zinc, sodium, phosphorus and sulphur than normal green leaves.

Table 4b—Nutrient imbalance associated with Iron deficiency

Plant	Nutrient elements			References
	Increased	Decreased	Not affected	
Rice	P			Ohira (1955)
Rice & Barley	K, P, Cu		Ca, Mg, Zn	Fujiwara & Tsutsumi (1957)
Barley	K, Mg, P, Mn, Cu, Zn, Mo		Ca	Agarwala & Sharma (1961)
Tobacco	Na, K, Ca, P, Cu, Mn			Steinberg, Specht & Roller (1955)
Tomato	Na, Mg, Ca, P, Mn			Twyman (1959)
Mustard	K, P	Ca		DeKock, Hall & McDonald (1960), Chaudhary (1971)
Potato	Ca, Mn, P			Jones & Hewitt (1949)
Citrus	K			Wallihan (1955)
Pineapple	Mg, P, Mn		Ca	Sideris, Young & Krauss (1943)
Cocoa	P, K, Ca, Mn, B, Mo	Na		Lockard & Asomaning (1964)
Sugarbeet	Mn, Cu, Zn, Mg, Ca			Nagarajah & Ulrich (1966)
Grasses	Cu, Mn, Ca, P, K, N			O'Sullivan (1969)

Nutrient Ratios

Several workers have suggested the use of certain nutrient ratios for identifying iron chlorosis. DeKock and his associates suggest that high K/Ca and P/Fe ratios in plant tissues are suggestive of iron deficiency. Somers and Shive (1942) and O'Sullivan (1969) identified a high Mn/Fe ratio as characteristic of iron chlorosis irrespective of its cause. The universal applicability of these suggestions have, however, been questioned (Olsen, 1935; Lindner & Harley, 1944; Wallace & Hewitt, 1946; Agarwala & Kumar, 1962 & Agarwala *et al.*, unpublished; O'Sullivan, 1969). Work done at the authors' laboratory under controlled culture conditions with plant species like cauliflower, cabbage, wheat, and chickpea (Table 5b) also does not lend support to the use of the above ratios as indices of iron deficiency. This work (Table 5b), however, suggests that since iron deficient plants are always associated with high Cu/Fe ratio and generally a high Zn/Fe ratio these may be used as indices of iron deficiency.

Table 5a—Changes in certain nutrient ratios in plants suffering from iron chlorosis

Ratio	Type of Fe-chlorosis	Change in ratio	
		Increase	Inconsistent
K/Ca	Lime-induced	Church (1886), Colin & Grandsire (1925), Wallace & Mann (1926), Wallace (1928), Thorne & Wallace (1944), Chapman & Brown (1950), Smith, Reuther & Specht (1950), DeKock (1955), Carpena, Sanchez, Guillen (1957), Higdon (1957)	Agarwala *et al.* (unpublished)
	True deficiency	Hewitt & Bolle-Jones (1953), Bolle-Jones (1955), DeKock & Strmecki (1954), DeKock, Hall & McDonald (1960), Palmer, DeKock & Bacon (1963), O'Sullivan (1969)	Olsen (1935), Lindner & Harley (1944)
P/Fe	Lime-induced	DeKock (1955)	Wallace & Hewitt (1946), Agarwala *et al.* (unpublished)
	Heavy metal induced	DeKock (1956), DeKock & Inkson (1962)	Agarwala & Kumar (1962)
	Genetical or viral	DeKock & Hall (1956)	
	True deficiency	Hewitt & Bolle-Jones (1953), Bolle-Jones (1955), DeKock, Hall & McDonald (1960), Palmer, DeKock & Bacon (1963)	O'Sullivan (1969)
Mn/Fe	Lime-induced		Agarwala *et al.* (unpublished)
	True deficiency	Somers & Shive (1942), O'Sullivan (1969)	
N/Fe	True deficiency	North & Wallace (1952), Wallace & Lunt (1969)	

Table 5b—Changes in plant nutrient ratios in iron deficient plants grown in sand culture (+ = increase; − = decrease, o=No appreciable effect, x = estimation not made)—AGARWALA *et al.*, unpublished.

Ratio	Plant			
	Cauliflower	Cabbage	Wheat	Chickpea
Na/Fe ..	0	0	0	+
K/Fe ..	+	0	+	+
Ca/Fe ..	+	0	+	+
Mg/Fe ..	+	0	+	+
P/Fe ..	+	0	+	+
S/Fe ..	×	×	×	+
Mn/Fe ..	+	0	+	+
Cu/Fe ..	+	+	+	+
Zn/Fe ..	+	0	+	+
Mo/Fe ..	+	0	+	0
K/Ca ..	+	+	+	−

Organic acids—Plants exhibiting iron chlorosis are reported to show a disturbance in organic acid metabolism which is generally associated with increase in the concentration of citric acid (McGeorge, 1949; Iljin, 1951b; Rhoades, Wallace & Romney, 1959; DeKock & Morrison, 1958b). DeKock and Morrison (1956, 1958b) have suggested a high tricarboxylic to dicarboxylic acid ratio in the chlorotic leaves as suggestive of iron deficiency.

Nitrogen metabolism—Several workers have observed that the chlorotic tissue undergoes a major disturbance in nitrogen metabolism (Bennett, 1945; Steinberg, 1949; Iljin, 1951a; Schwarze, 1952, 1954; Holley & Cain, 1955; Sideris & Young, 1956; Demetriades, 1955, 1956; Possingham, 1956; DeKock & Morrison, 1956; 1958a; Rhoades, Wallace & Romney, 1959; Marsh & Evans, 1960), the disorder being generally associated with increased accumulation of amino-acids in the plants showing iron chlorosis.

Enzymes—Under controlled culture conditions, iron deficiency is known to decrease the activity of iron enzymes-catalase (Appleman, 1952; Brown & Steinberg, 1953; Weinstein & Robbins, 1955; Banerjee, 1957; Perur, Smith & Wiebe, 1963; Agarwala & Sharma, 1961; Machold, 1968; Nicholas & Goodman, 1958; DeKock, Commisiong, Framer & Inkson, 1960; Marsh, Evans & Matrone, 1963; Agarwala, Sharma & Kumar, 1964; Agarwala, Sharma & Farooq, 1965; Iyengar & Sheshagiri Rao, 1971), peroxidase (Bar-Akiva, 1961; Bar-Akiva & Lavon, 1968; Nicholas & Goodman, 1958; DeKock, Commisiong, Framer & Inkson, 1960; Marsh, Evans & Matrone, 1963; Agarwala, Sharma & Kumar 1964; Agarwala, Sharma & Farooq, 1965; Iyengar & Sheshagiri Rao, 1971) and cytochrome oxidase (Marsh & Evans, 1960). In a few instances iron deficiency did not depress the activity of catalase (Demetriades, 1955) or peroxidase (Demetriades, 1955; Elmstrom & Howard, 1969).

Plants susceptible to lime induced iron chlorosis are reported to have a predominant iron—requiring metabolic system (Brown, 1953). Their chlorotic leaves show a low activity of catalase (Brown & Hendricks, 1952; Brown, 1953; Brown & Steinberg, 1953; Brown, Holmes, Shapiro & Specht, 1955; Abadia, 1956; Shpota, 1958; Altares, 1959) and often that of peroxidase (Brown & Hendricks, 1952; Brown, 1953; Brown & Steinberg, 1953; Dimza, 1959). Such plants are also reported to exhibit a high activity of a copper requiring enzyme like ascorbic acid oxidase (Brown & Hendricks, 1952; Brown, 1953).

Comparative biochemical studies on normal and chlorotic plants grown under natural conditions have revealed (Table 6) basic similarities in the metabolic derangements in plants suffering from true and induced iron-chlorosis (Agarwala, Sharma & Farooq, 1965; Bar-akiva, Sagir & Hasdai, 1971). As in case of true iron deficiency, plants showing iron chlorosis showed a decrease in the specific activity of aldolase and starch phosphorylase, and a stimulation in the specific activity of ribonuclease; the effect on each of the enzymes being nullified during recovery from iron chlorosis consequent to application of iron chelate (2 mM Fe-EDTA).

We observed that out of the seven plant species showing iron chlorosis under natural conditions, near Lucknow (Table 6) two, *Hibiscus rosa-sinensis* and *Cassia fistula*, showed a decrease in peroxidase/catalase ratio. Application of Fe-EDTA restored peroxidase/catalase ratio to near normalcy in only three out of five plant species that showed a high peroxidase/catalase ratio in chlorotic leaves. These observations do not lend support to the observations of DeKock, Commisiong, Framer and Inkson (1960) that a high peroxidase/catalase ratio can be used as an index of iron deficiency. DeKock's contention

Table 6—Biochemical changes associated with induced iron-chlorosis and recovery therefrom (Agarwala *et al.*, unpublished)

Plant species	Biochemical changes in chlorotic leaves									
	Control (unsprayed)					2mM (Fe-EDTA sprayed)				
	Chlorophyll	Protein	Catalase	Peroxidase	Per./Cat. ratio	Chlorophyll	Protein	Catalase	Peroxidase	Per./Cat. ratio
	% increase (+) or decrease (—) over normal green leaves.					% increase (+) or decrease (—) over unsprayed chlorotic leaves.				
Bougainvillaea spectabilis ..	—55	—50	—49	—6	+86	+68	+53	+63	0	—39
Quisqualis indica	—86	—33	—72	—48	+80	+138	+24	+188	+194	+5
Tabernaemontana coronaria ..	—61	—57	—67	+3	+43	+70	+100	—150	+150	+24
Callistemon citrinus	—75	—33	×	+16	×	+215	+40	×	+114	×
Anthocephalus cadamba ..	—82	—31	—93	—50	+617	+300	+30	+511	+130	—64
Hibiscus rosasinensis	—83	—15	—63	—70	—20	+120	+22	+150	+56	—36
Cassia fistula ..	—69	—48	—77	—79	—9	+75	+77	+118	+131	+6
Justicia grandis ..	—79	—32	—92	—69	+301	+400	+20	+1100	+240	—72

	Aldolase	Starch phosphorylase	Inorganic pyrophosphatase	R-Nase	Aldolase	Starch phosphorylase	Inorganic pyrophosphatase	Ribonuclease
Justicia grandis ..	—46	—43	+123	+104	+67	+48	—62	—47

seems to be applicable only to such plant species (like *A. cadamba*, *B. spectabilis* and *J. grandis* in the present study) wherein iron deficiency influences the activity of catalase more than peroxidase.

From what has been observed above, it appears that decrease in chlorophyll, protein nitrogen and the activity of catalase, aldolase and starch phosphorylase and stimulation in the activity of ribonuclease and inorganic pyrophosphatase in chlorotic leaves may serve as a quick biochemical index of iron deficiency.

Simple inorganic salts of iron like ferrous sulphate may, to some extent, ameliorate iron chlorosis when applied as foliar spray (KANWAR & DHINGRA, 1962; KRANTZ, BROWN, FISCHER, PANDEY & BROWN, 1962) but because of interaction with various physico-chemical properties of soil, iron in these compounds is rendered unavailable to the plant and they are not effective in correcting iron chlorosis as soil amendments. STEWART & LEONARD (1952) suggested the use of Fe-EDTA (Ferric-ethylene diamine tetra acetic acid) to overcome such a situation, and during the last two decades, a number of synthetic iron chelates have been used for correcting iron chlorosis under field conditions—both as soil amendment and as foliar application (SESHAGIRI & MARIAKULANDAI, 1956; BEAR, 1957; WALLACE,

1962, 1971; GAUCH, 1972). Among others, iron chelates of diethylenetriamine penta acetic acid (DTPA), Ethylenediamedi-O-hydroxyphenylacetic acid (EDDHA), Hydroxyethyl ethylene-diamine tetra acetic acid (HEEDTA), Aminopolycarboxylic acid (APCA), Cyclohexanetrans-1, 2-diaminotetra acetic acid (CDTA) have been used with great success. EDTA and HEEDTA are particularly effective in acid and slightly alkaline soils, DTPA and EDDHA in calcareous soils (SAUCHELLI, 1969), and Fe-APCA in alkaline-calcareous soils. During the recent years, successful use has also been made of iron-fritts, in which iron is impregnated in soda glass, for correcting iron chlorosis in acid soils.

Another way to overcome iron chlorosis is to plant chlorosis resistant varieties. Considerable information has accumulated on varietal differences to iron chlorosis (WEISS, 1943; BROWN & HOLMES, 1955; BROWN, WEBBER & CALDWELL, 1967; BROWN, CHANEY & AMBLER, 1971; BELL, BOGORAD & MCILRATH, 1958, 1962; CLARK, TIFFIN & BROWN, 1973; ESTES & BRUTESCH, 1973; AGARWALA, SHARMA, SHARMA & NAUTIYAL, 1971; AGARWALA & SHARMA, 1974; MIKESELL, PAULSEN, ELLIS & CASACY, 1973; O'TOOLE, 1966; O'SULLIVAN, 1969; THORNE & WANN, 1950). Table 7a lists some plant species, varieties and mutants which are resistant to iron deficiency. Some varietal differences in susceptibility to iron chlorosis observed in controlled culture experiments—conducted at the Botany Department, Lucknow University, are indicated in Table 7b. Such information is of great economic value and practical utility for resolving iron chlorosis where it is a problem of continuing nature.

Table 7a—Differential susceptibility of varieties and mutants to iron chlorosis

Plant sp.	Genotypes		References
	Susceptible	Non-susceptible	
Soybean ..	PI-54619-5-1 (PI) ..	Hawkeye (HA) ..	Weiss, 1943; Brown & Holmes, 1955
	A 62-10 (I-10) ..	E-62-9 (E9) ..	Brown, Webber & Caldwell, 1967
Corn ..	YSI/YSI ..	± YSI ..	Bell, Bogorad & McIlrath, 1958, 1962
		W F9 ..	Clark, Tiffin & Brown, 1973
	Sene Ca XX 155 ..	Wisconsin 335A ..	Estes & Brutesch, 1973
Tomato ..	T3238 fe ..	T3238 Fe ..	Brown, Chaney & Ambler, 1971
Sorghum ..	Inefficient Iso-lines ..	Efficient Isolines ..	Mikesell, Paulsen, Ellis & Casacy, 1973
Wheat ..	Choti Lerma ..	Lal Bahadur	Agarwala, Sharma, Sharma & Nautiyal, 1971.
	Safed Lerma ..	U. P. 301 ..	
Grasses ..	Timothy ..	Yorkshire FO9 ..	O'Toole, 1966; O'Sullivan, 1969
Grapevine..	Labrusca ..	Vinefera ..	Thorne & Wann, 1950

Table 7b—Relative susceptibility of varieties of some economically important crops to iron stress (After Agarwala & Sharma, 1974; Agarwala *et al.* unpublished)

Plant species	Varieties	
	Susceptible	Non-susceptible (Resistant)
Pea	T. 61 T. 56..	T. 163
Gram	BG. 1 G. 130 Pusa 53 H. 208 T. 3	C. 235 T. 1 GWL. 2 850-3/27 N. 59
Green Gram	BG. 1 T. 51 T.1 T. 2	T.44 T. 305
Black gram	BG. 369 T. 9	T. 65 K. 3
Wheat	K. 64 K. 68 K. 65 Sonora 63 ..	Sonora 64 NP. 862 NP. 830
Groundnut	T. 28	T. 32

ACKNOWLEDGEMENTS

The authors are grateful to Professor S. C. Agarwala, Head of the Department of Botany, Lucknow University for his guidance during the investigations reported here and for providing facilities for the work.

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