Plant and Soil **165:** 275–283, 1994.

Iron availability in plant tissues – iron chlorosis on calcareous soils

Konrad Mengel
Institute of Plant Nutrition of the Justus Liebig University, Südanlage 6, D–35390 Giessen, Germany

Key words: apoplast, Fe reductase, iron chlorosis, pH

Abstract

The article describes factors and processes which lead to Fe chlorosis (lime chlorosis) in plants grown on calcareous soils. Such soils may contain high HCO_3^- concentrations in their soil solution, they are characterized by a high pH, and they rather tend to accumulate nitrate than ammonium because due to the high pH level ammonium nitrogen is rapidly nitrified and/or even may escape in form of volatile NH_3. Hence in these soils plant roots may be exposed to high nitrate and high bicarbonate concentrations. Both anion species are involved in the induction of Fe chlorosis.

Physiological processes involved in Fe chlorosis occur in the roots and in the leaves. Even on calcareous soils and even in plants with chlorosis the Fe concentration in the roots is several times higher than the Fe concentration in the leaves. This shows that the Fe availability in the soil is not the critical process leading to chlorosis but rather the Fe uptake from the root apoplast into the cytosol of root cells. This situation applies to dicots as well as to monocots. Iron transport across the plasmamembrane is initiated by Fe^{III} reduction brought about by a plasmalemma located Fe^{III} reductase. Its activity is pH dependent and at alkaline pH supposed to be much depressed. Bicarbonate present in the root apoplast will neutralize the protons pumped out of the cytosol and together with nitrate which is taken up by a H^+/nitrate cotransport high pH levels are provided which hamper or even block the Fe^{III} reduction.

Frequently chlorotic leaves have higher Fe concentrations than green ones which phenomenon shows that chlorosis on calcareous soils is not only related to Fe uptake by roots and Fe translocation from the roots to the upper plant parts but also dependent on the efficiency of Fe in the leaves. It is hypothesized that also in the leaves Fe^{III} reduction and Fe uptake from the apoplast into the cytosol is affected by nitrate and bicarbonate in an analogous way as this is the case in the roots. This assumption was confirmed by the highly significant negative correlation between the leaf apoplast pH and the degree of iron chlorosis measured as leaf chlorophyll concentration. Depressing leaf apoplast pH by simply spraying chlorotic leaves with an acid led to a regreening of the leaves.

Introduction

Iron chlorosis of crop plants and fruit trees on calcareous soils is an old problem and was already precisely described at the beginning of this century by Molz (1907). In recent years a lot of work has been done on iron availability in soils, on iron acquisition by plant roots, and iron uptake by plants. Nevertheless the old problem of iron chlorosis or lime chlorosis, as it is also called, is not completely understood and until now the means available for curing iron chlorosis in the field are not yet satisfying. In the following it is shown that the problem is a physiological one closely related to metabolic processes in roots and in leaves.

Iron mobility in the root tissue

Experimental results of various authors have shown that plants grown in artificial root media show high iron concentrations in their roots (Bienfait et al., 1985; Longnecker und Welch, 1990; Mengel and Geurtzen, 1988). This obviously is also the case for plants cultivated in soils as was shown by Schaumberger (1990). He grew an iron efficient cultivar (Mutin) and a non-iron efficient cultivar (Anjou) of maize in pot experiments with 6 soils differing widely in soil characteristics, particularly in pH and the concentration of $CaCO_3$ as is shown in Table 1. The most important result is that the Fe concentrations in roots were several times higher than the Fe concentrations in the leaves, although the roots were thoroughly washed with distilled water

Table 1. Fe concentration in young leaves and roots of the maize cultivars Mutin and Anjou as related to soil pH and $CaCO_3$ concentration (Schaumberger, 1990)

pH	$CaCO_3$	Leaves		Roots	
	%	Mutin	Anjou	Mutin	Anjou
		$\mu g\ Fe\ g^{-1}FW$			
4.75	–	9.8	9.9	70	100
6.05	–	12.5	10.5	500	580
6.98	–	14.5	10.5	420	480
7.51	5	10.5	10.5	380	380
7.61	37	9.2	11.1	220	340
7.70	14	8.8	7.1	360	290

Table 2. Iron concentration in young roots and leaves of green and chlorotic plants

	Roots	Leaves
	$\mu g\ Fe^{-1}DM$	
Peaches		
Chlorotic	1441	34
Green	1682	54
Vine		
Chlorotic	1027	84
Green	1364	109
Chlorotic	1059	–
Green	420	–

Analysis in vine were carried out in our laboratory, analysis in peaches in the laboratory of the Botany Dep. Barcelona according to our prescription.

Table 3. Effect of $Ca(NO_3)_2$, $(NH_4)_2)SO_4$, and diluted HCl (pH 3.5) on the chlorophyll concentration in the youngest leaves, on the Fe concentration in leaves, stems, and roots, and on the Fe concentration in the outer medium 72 h after transfer of the plants from the NO_3^- containing medium. *, **, *** significant from the nitrate medium at 5%, 1%, and 0.1% level, respectively

Plant part/medium	NO_3^-	NH_4^+	pH 3.5
	Chlorophyll, $mg\ g^{-1}FW$		
Youngest leaves	0.58	1.03***	0.72
	Fe concentration, $\mu g\ g^{-1}DW$		
Leaves	100	163*	157*
Stems	86	105*	134
Roots	588	414*	398*
	Fe concentration, μM		
Medium	0.00	5.15**	3.72*

and 0.5 *M* HCl in order to get rid of all adhering soil particles. The Fe concentrations in the roots reflected somehow the Fe availability in the soil, because the Fe found in the roots must have been moved from the soil medium towards the roots. Availability was poorest in the organic soil (soil with pH 4.75) and declined in the soils with higher concentrations of $CaCO_3$. Nevertheless the Fe concentrations in the roots are considered as sufficient, also in the roots of the soil which was absolutely low in Fe (pH 4.75). In this experiment no iron chlorosis was found in the leaves but there was a slight tendency that the chlorophyll concentration in the leaves declined with an increase of the $CaCO_3$ concentration in the soils ($r = -0.732$). There was no correlation between the Fe concentration in the youngest leaves and the $CaCO_3$ concentration in the soils. From this result one may already conclude that also on calcareous soils the Fe concentration in roots is high and the transport of iron from the soil medium towards the roots is not a limiting factor in Fe supply of crops on calcareous soils. This conclusion is confirmed by the results shown in Table 2. Peaches and vine of this investigation were from farmers' fields, the peaches from Spain, the vine from Franconia in Germany. Here again it is clearly evident that even plants showing severe chlorosis were rich in root Fe.

The Fe accumulation in the roots may be explained by microbial processes in the soil producing siderophores which mobilize insoluble soil Fe (Neilands and Leong, 1986) and transport it to plant roots (Crowley et al., 1991). Obviously the chelator is decomposed by microbes at the root surface so that the iron released probably is strongly adsorbed to cell wall adsorption sites or precipitated as $Fe^{III}(OH_3)$ (Crowley and Gries, 1993). Since washing roots with diluted HCl did only remove a small proportion of root Fe (Schaumberger, 1990) Fe must be strongly fixed in whatsoever a form. It is assumed that most of this iron is in the root apoplast according to Bienfait et al. (1985) and Longnecker and Welch (1990).

Since also chlorotic plants had high Fe concentrations in roots, chlorosis could be related to the mobilization of root Fe and its translocation to upper plant parts. Mengel and Geurtzen (1988) reported that Fe chlorotic maize plants became green within three days

if their roots were exposed to an ammonium containing solution or to distilled water with pH 3.5. Most important data of this experiment are shown in Table 3. It is evident that the Fe concentrations in the roots declined and in the upper plant parts increased in plants exposed to an ammonium containing solution or to the water with pH. 3.5. Also the outer solution contained some soluble Fe in the treatment with ammonium nutrition or with the pH 3.5 solution. Since ammonium nutrition decreases rhizosphere soil pH, low pH leads to a mobilization of root Fe. It is hypothized that this mobilization is not only a simple dissolution process but that it is also related to plant metabolism.

As can be seen from the figures in Table 3, exposition of the roots to an ammonium containing medium or to water with pH 3.5 initiated the translocation of Fe from the roots to upper plant parts. This process implicates the uptake of Fe by root cells which means the translocation of Fe across the plasmamembrane. There is now sufficient evidence that this uptake process is initiated by a reduction of Fe^{III} at the plasmamembrane (Brüggemann and Moog, 1989; Buckhout et al., 1989; Holden et al., 1991). According to Holden et al. (1991) the Fe^{III} reductase is located in the plasmamembrane and its activity is pH dependent. Maximum activity was found at pH 6.5 while at higher pH the activity rapidly declined. Nitrate nutrition in combination with ample bicarbonate should lead to rather high pH levels at the root surface and in the root apoplast because the uptake of nitrate is brought about by a proton/nitrate cotransport across the plasmamembrane (Ullrich, 1991). Nitrate nutrition and high HCO_3^- concentration in the soil solution are typical for calcareous soils since in these soils ammonium accumulation rather occurs because of ammonia volatilization. Bicarbonate can be permanently produced particularly at the root surface where root respiration provides CO_2 for the dissolution of $CaCO_3$. Bicarbonate is a strong buffer and may neutralize the H^+ released by proton pumps of the root plasmalemma and hence it is justified to assume that the pH in the apoplast of roots under such conditions is raised to a high level so that the plasmalemma located Fe^{III} reductase is more or less inhibited (Romera et al., 1991; Toulon et al., 1992). Bicarbonate as a factor inducing Fe chlorosis is well documented in literature (Kolesch et al., 1984; Mengel and Bübl, 1983; Mengel and Malissiovas, 1981; Mengel et al., 1984; Rutland and Bukovac, 1971). It must be emphasized that the bicarbonate concentration depends also on the partial pressure of CO_2 which may be rather high at microsites in soils especially under condition of high soil compaction. According to experimental results of Loeppert and Hallmark (1985) and Mengel and Geurtzen (1988) high root medium pH induced Fe chlorosis with respectively sorghum and maize although these species belong to the Gramineae. Obviously in these particular cases the Fe supply by phytosiderophores was insufficient and an ample Fe provision required also an Fe^{III} reduction at the root plasmamembrane.

Long distance translocation of Fe and its mobility in leaves

The mechanism described above does well explain why iron chlorosis occurs on calcareous soils. According to the relationships presented here the critical process appears to be the Fe^{III} reduction at the root plasmamembrane followed by Fe^{2+} uptake. Still in the symplasm of the root system Fe^{II} is oxidized (Brown and Jolley, 1989) and complexed by citrate and the Fe^{III} dicitrate is translocated *via* the xylem to the upper plant parts (Chaney, 1989). If the reduction of Fe^{III} at the root plasmamembrane is the only critical process in the phenomenon of Fe chlorosis then the degree of Fe chlorosis, generally measured as chlorophyll concentration in leaves, should be closely reflected by the Fe concentration in the leaves. This, however, is not the case. From all plant nutrients, to my knowledge, Fe is the only one which in many cases does not show a correlation between the concentration in the plant tissue and the degree of deficiency. This enigma has intrigued plant physiologists since decades. Numerous examples in literature show that there is no correlation between the Fe concentration in leaves and the chlorophyll concentration as an indicator for the degree of Fe deficiency (Loeppert and Hallmark, 1985; Mengel and Malissiovas, 1981; Plänker, 1991; Rashid et al., 1990). Aktas and van Egmond (1979) cite an example of soybean of which the cultivar Hawkeye had green leaves and the cultivar T–203 had yellow leaves. Although the Fe concentrations in leaves of both cultivars were virtually the same. According to this result Fe deficiency is not only a question of Fe translocation from the roots to the upper plant parts but is in some way inherent in the leaves.

Inefficiency of Fe may even occur in the same cultivar of one species caused by nutritional conditions as was shown by Mengel and Malissiovas (1981). The most important results of their investigations are shown in Table 4. Vine plants were grown during a

certain period with three different nutrient solutions: control solution with Fe, solution without Fe, solution with Fe^{+} bicarbonate. Eleven days after the application of bicarbonate first Fe chlorotic symptoms were observed in the youngest leaves in form of yellowing spots between the veins. In this treatment plant growth was strongly reduced as can be seen from the yield of young leaves in the bicarbonate treatment. Lowest Fe concentration in the young leaves was found in the treatment without Fe. But these leaves were not chlorotic in contrast to young leaves of the treatment with bicarbonate which were chlorotic and had a Fe concentration which was more than twice as high (184 $\mu g\ g^{-1}$ dry matter) as the Fe concentration in leaves of the treatment without Fe. One may explain the high Fe concentration in the bicarbonate treatment with a concentration effect because the leaf growth was affected. This, however, does not explain why leaves with such a high Fe concentration are chlorotic.

Until now it is not yet completely understood why bicarbonate has such an effect. Saglio (1969) experimented with decapitated vine plants and found that an addition of bicarbonate to the nutrient solution raised the pH of the exudation sap from pH 4.8 to 5.0. In this experiment the bicarbonate concentration in the nutrient solution was 3 m*M* and therefore much lower than the bicarbonate concentration we applied in our nutrient solution (*see* Table 4) which was almost 16 m*M*. This high concentration of HCO_3 is not exceptional but may well occur under water saturating soil conditions (Mengel et al., 1984). Plänker (1991) found that an application of bicarbonate significantly increased the pH in the leaf apoplast from pH 6.2 to 7.0. From these results one may conclude that bicarbonate in the root system may raise the pH of the xylem sap. That bicarbonate affects the Fe translocation in plants and the distribution of Fe in leaves was already reported by Rutland (1971) and Rutland and and Bukovac (1971). The effect of bicarbonate on the distribution of labelled Fe was clearly shown by Mengel and Bübl (1983) with vine plants in which one treatment was supplied with bicarbonate by stem infusion. As is shown in Plate 1 in the control treatment (no bicarbonate) the labelled Fe was distributed evenly while in the bicarbonate treatment the labelled iron mainly remained in the veins.

From this observation one may conclude that the uptake of Fe from the apoplastic leaf system into the cytosol across the plasmalemma is affected by bicarbonate. Young tissues and particularly the growing shoot apex are mainly supplied with Fe *via* the ploem because in growing tissues phloem elements differentiate earlier than xylem vessels (Pate, 1975). Xylem sap transporting the Fe dicitrate from the roots to the leaves may exchange along the vascular path with the phloem (Stephan and Scholz, 1993). The crucial step in this exchange is the transport of Fe across the plasmamembrane. This process presumably is associated with a Fe^{III} reduction. According to Stephan and Scholz (1993) the Fe^{2+} thus entering the cytoplasmatic pathway is then complexed by nicotianamine and in this form Fe is evenly distributed in the symplasm and is supposed to provide Fe to the Fe requiring processes. All multicellular plants possess nicotianamine as it is presumably required for the symplastic distribution of heavy metals (Stephan and Scholze, 1993). As is discussed below more in detail it is assumed that the transport of Fe across the leaf plasmamembrane is initiated by the reduction of Fe^{III}. Reduction may occur along the vascular path at the interfaces apoplast/symplast. If this loading process is affected the Fe supply of young tissues will become insufficient. In such a case the Fe will be only supplied by the xylem and the distribution of Fe in the intercostal areas of young leaves is hampered. This is exactly the pattern of chlorophyll distribution in chlorotic leaves which frequently are still green along the vascular veins (see also Plate 1). The picture resembles much the chlorophyll distribution in the tomato mutant "Chloronerva" which lacks the nicotianamine (Stephan and Scholz, 1993).

According to Brown (1978) green plants require a continous supply of Fe as they grow. As soon as this supply is interrupted growth is hampered (see Table 4) and iron deficiency occurs as yellowing of leaves. The

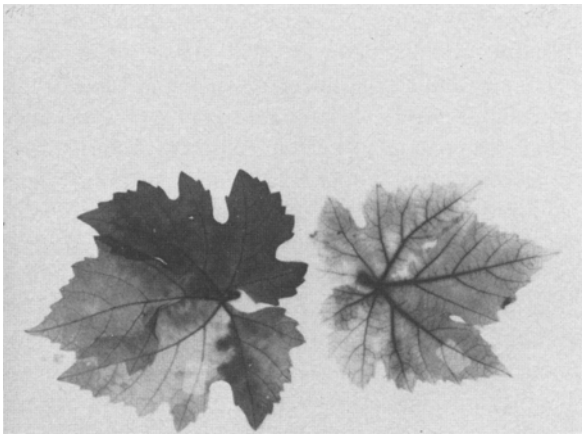

Plate 1. Effect of bicarbonate perfusion on the distribution of labeled Fe in young vine leaves (after Mengel and Bübl, 1983). Left: control (without bicarbonate perfusion. Right: with bicarbonate perfusion.

Table 4. Effect of Fe and bicarbonate on the yield of leaves, the Fe concentration in younger and older leaves, and the Fe and HCO_3^- concentration in the nutrient medium. Quartz sand solution culture (Mengel and Malissiovas, 1981)

Treatment	Young leaves		Older leaves	Nutrient medium	
	DM	Fe conc.	Fe conc.	pH	HCO_3
	g plant^{-1}	μg g^{-1} DM	μg g^{-1} DM		mM
1. Control (+Fe) without HCO_3^-)	6.6	106	223	5.2	–
2. without Fe without HCO_3^-	6.0	82	196	5.0	–
3. with Fe with HCO_3^-	3.3**	215 184[a]	250	8.5	15.7

[a] Chlorotic leaves.

** Significantly different from the other two treatments at the 1% level.

primary process which needs Fe and which induces growth reduction is not yet known. According to recent results the reduction of ribonucleotide to deoxyribonucleotide is brought about by a reductase in which Fe plays an essential role (Reichard, 1993). This reduction is a fundamental process since it is the prerequisite for the synthesis of DNA which is required for cell growth and cell division.

pH in leaf apoplast and chlorophyll concentration

It is not only the application of bicarbonate which reduces the efficiency of Fe in leaves but also nitrate because both raise the leaf apoplast pH. Mengel et al. (1994) carrying out experiments with young sunflower plants (*Helianthus annuus*) which were grown in a nutrient solution without Fe so that plants were only fed with Fe from the seed. In one treatment plants were supplied with ammonium nitrate and in one with Ca-nitrate. Plants grown without ammonium developed typical symptoms of Fe chlorosis, while the plants grown with ammonium nitrate had green leaves. After 12 days of growth in the different nutrient media the nitrate grown plants had a leaf chlorophyll concentration of 0.2 mg g^{-1} fresh weight while the plants grown with ammonium nitrate had a chlorophyll concentration of 1.18 mg g^{-1} fresh weight. The difference was highly significant. In this particular case Fe uptake from the outer medium was not involved; therefore it was simply the efficiency of Fe in the leaves which was affected by the type of nitrogen nutrition. The Fe concentration in the leaves was the same for both treatments, namely 51 μg g^{-1} dry weight. From these results it was hypothesized that also nitrate may have an influence on the pH in the apoplast and analogous to the root also the pH in the apoplast of leaves is of importance for the uptake of Fe from the apoplast into the symplasm. It is supposed that also this process is initiated by a reduction of Fe^{III} dicitrate brought about by a Fe^{III} reductase located in the plasmamembrane of leaves (Moog, 1993). Mengel et al. (1994) measured leaf apoplast pH by fluorescence according to the technique described by Hoffmann et al. (1992). In the experiments of Mengel et al. (1994) young sunflower plants were grown with and without Fe and with Ca-nitrate and ammonium nitrate in the various treatments. Chlorophyll concentration was measured in the opposite leaf in which leaf apoplast pH was measured. In the latter case leaves were excised and put with their petioles in a solution containing the fluorescence dye (5-carboxyfluorescein) and nitrogen and Fe according to the nutrient solution of the corresponding treatments (concerning the technique of this experimentation see Mengel et al. (1994). In Figure 1 the results of this experiment are shown. There was a very close correlation (r = - 0.97) between the degree of iron chlorosis, indicated as leaf chlorophyll concentration, and the pH in the apoplast. Lowest pH values were obtained in the ammonium nitrate treatment and highest pH in the treatment with Ca-nitrate. The experiment was repeated three times at different dates with almost identical results which may show the high reproducibility of the technique. It is of interest that in this experiment also the Fe supply had an impact on leaf apoplast pH. Probably plants not sup-

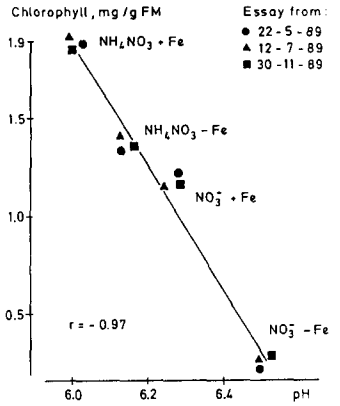

Fig. 1. Relationship between leaf apoplast pH, leaf chlorophyll concentration and source of nitrogen supply and Fe supply. Each symbol is the mean of three replicates. The different symbols indicate the date at which the experiments were carried out (after Plänker, 1991).

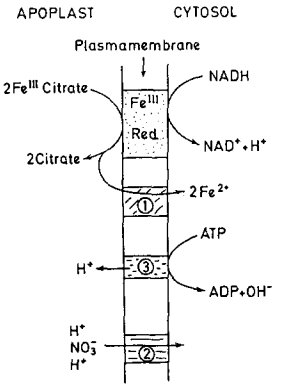

Fig. 2. Scheme of Fe^{III} reduction at the apoplast/symplast interface (plasmalemma) associated with the channel for Fe^{2+} uptake (1), the proton/nitrate cotransport (2) and the proton pump (3).

plied with Fe were affected in their plasmamembrane ATPase activity. Englisch (1993) found similar close correlations between the leaf apoplast pH and chlorophyll concentrations in soya (r = - 0.97) and maize (r = - 0.99). Tagliavini et al. (1993) came to analogous results. These researchers worked with leaves of Kiwifruit vines (*Actinidia deliciosa*) grown in the field and showing different degrees of iron chlorosis. They measured the chlorophyll concentration in the leaves and the pH in the leaf extract. Tagliavini et al. (1993) found also a very close negative correlation between the leaf sap pH and the chlorophyll concentration in leaves. This result, obtained with another technique, confirms our hypothesis that the leaf apoplast pH is of crucial importance for the efficiency of Fe in leaves.

As can be seen from Figure 1 the pH between the treatments varied within only 0.4 pH units. Nevertheless this pH range had a remarkable influence on the chlorophyll concentration which finding may demonstrate that the process of Fe^{III} reduction is highly pH sensitive. This conclusion is in line with experimental data of Brüggemann et al. (1989), who found with membrane vesicles obtained from barley roots a pH optimum of 6.8 for the Fe^{III}-EDTA reduction and with results of Holden et al. (1991) who reported a pH optimum of 6.5 for the reduction of Fe^{III} citrate of vesicles obtained from tomato roots. These authors found pH optima for the Fe^{III} reduction. Tagliavini et al. (1993) and we found a linear relationship between the chlorophyll concentration and the pH in the apoplast indirectly providing evidence that the Fe^{III} reduction is the better the lower the pH. This finding agrees well with research data of Toulon et al. (1992) who found with excised rape roots that the rate of Fe^{III} reduction was the higher the lower the pH in the root apoplast. At pH 4 the rate of Fe^{III} reduction was about five times higher than at pH 8. Obviously vesicle production may lead to some disturbance of the membranes and the pH optima obtained by Brüggemann et al. (1989) and by Holden et al. (1991) may therefore rather reflect an artefact than the real in vivo situation. The assumption hence is justified that also in leaf apoplast a low pH in particular favours the Fe^{III} reduction.

Iron is especially required in meristematic tissues where new Fe containing prostetic groups such as FeS clusters, ferredoxins and hem groups are synthesized. These tissues demand also much nitrogen which may be supplied in form of amino acids mainly over the phloem and in form of nitrate mainly *via* the apoplastic pathway. High uptake rates of nitrate at the interfaces apoplast/symplast may result in high pH levels due to the nitrate H^+ cotransport. This should be especially the case if plants are only supplied by nitrate as the sole nitrogen form (Fig. 2). Nitrate concentrations in the xylem sap of decapitated tomato and sunflower plants were in a range of 20 to 30 m*M* as was reported by Kirkby et al. (1981) and Mengel and Simic (1973), respectively. The concentration of nitrate still may be higher at microsites of the apoplast/symplast interfaces where nitrate is taken up into the symplasm with high rates. This should raise the pH considerably. This speculation is in line with our observation that nitrate as the sole nitrogen source raised the apoplast pH and induced chlorosis.

In a preliminary experiment Mengel et al. (1994) could show that under the conditions of nitrate supply

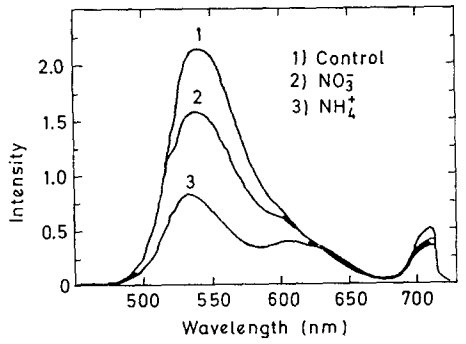

Fig. 3. Light transmission spectrum. 1. Leaf blade without ferrocene perfusion. 2. Leaf blade with ferocin perfusion and nitrate supply. 3. Leaf blade with ferocin perfusion and ammonium supply.

Table 5. Effect of sprayings with various chemicals on the chlorophyll concentration in leaves and pot yield of peas grown on a calcareous soil (Sahu et al., 1987)

Treatment	Chlorophyll mg g^{-1} FM	Yield t ha^{-1}
Control	1.37	1.79
Mixtalol (SF)	1.85*	3.38
Giberellic acid (SF)	1.32	2.36
Naphtalene acetic acid (SF)	1.84*	3.32
H_2SO_4 (F)	1.83*	3.36
Fe-EDDHA (F)	1.78	3.15

* Significantly different from the control at the 5% level.
(SF) = Seed and foliar application.
(F) = Foliar application.

the reduction of Fe^{III} was depressed as compared with ammonium supply. In this experiment the leaves were perfused with ferrocene which forms a precipitation with Fe^{2+}. As shown in Figure 3, light transmission was much higher in the nitrate treatment than in the ammonium treatment. The difference is explained by the Fe^{II} ferrocene precipitation which was obviously much stronger in the ammonium treatment.

If our concept of the leave apoplast pH as the crucial factor of Fe chlorosis on calcareous soils is correct than a simple treatment of leaves with a diluted acid should cure the chlorosis provided that this is not caused by an absolute Fe deficiency. Plänker (1991) treated chlorotic sunflower leaves with an acid mist (pH 3.2) which resulted in a regreening of the leaves. Tagliavini et al. (1993) sprayed citric acid which treatment cured the chlorosis of Kiwi leaves grown in the field. The spectacular results of Sahu et al. (1987) are shown in Table 5. In this experiment the spray with sulphuric acid yielded the best results. The authors speculate whether this could be a sulphur effect and they did not consider that this could have been a simple pH influence. It is of interest that in the experiment of Sahu et al. (1987) also the application of "Mixtalol", a mixture of long-chain aliphatic alcohols, yielded a remarkable effect. One may speculate whether the alcohol had an influence on the plasmamembrane and even on the Fe^{III} reductase. According to the concept outlined above also measurements which stimulate the plasmamembrane proton pumps should alleviate Fe chorosis. This was actually found by Mengel and Geurtzen (1986) who reported that chlorotic maize leaves after having been sprayed with fusicoccin as well as with indole acetic acid regreened.

Concluding remarks

Iron chlorosis in practical farming is still a severe problem. Most of the remedies applied and recommended are not always successful and in many cases expensive. The cure of the chlorosis should come from the biological side and for developing biological means to overcome Fe deficiency the sequence of reactions should be known which cause the chlorosis on calcareous soils. As was shown in this article it is a physiological problem which is related to processes both in the roots and in the leaves. Even on calcareous soils the Fe availability in the soil is sufficient due to numerous siderophores produced by microbes. The critical problem is the reduction of Fe^{III} in the root apoplast; hence the activity of the enzyme Fe^{III} reductase and the conditions that influence the activity of this enzyme are of importance. In this respect the pH in the apoplast and therefore the plasmalemma located proton pumps are of paramount importance. This applies not only for dicots but probably also for monocots. Hence further research and the development of new types should have a look on these proton pumps.

In many cases sufficient Fe is translocated from the roots to the leaves and it is the efficiency of the leaf Fe which plays the critical role. There is now enough evidence that this efficiency is related to the pH in the leaf apoplast and to the activity of the plasmamembrane located Fe^{III} reductase. It is contended that this activity also is pH dependent and is severely depressed at high pH levels. Hence also in this case further research should have a closer look at the proton pumps in the leaves. In addition also the nitrate situation in leaves

should be investigated. There are species which reduce much of the absorbed nitrate in the roots. These species should be less prone to Fe chlorosis and *vice versa*.

References

Aktas M and van Egmond F 1979 Effect of nitrate nutrition on iron utilization by an Fe-efficient and an Fe-inefficient soybean cultivar. Plant and Soil 51, 257–274.

Bienfait H F, van den Briel W and Mesland-Mul N T 1985 Free space iron pools in roots, generation and mobilization. Plant Physiol. 78, 596–600.

Brown J C 1978 Mechanism of iron uptake by plants. Plant, Cell, Environ. 1, 249–257.

Brown J C and Jolley V D 1989 Plant metabolic responses to iron-deficiency stress. Biol. Sci. 39, 546–551.

Brüggemann W and Moog P R 1989 NADH-dependent Fe^{3+} EDTA and oxygen reduction by plasma membrane vesicles from barley roots. Physiol. Plant. 75, 245–254.

Buckhout T J, Bell P F, Luster D G and Chaney R L 1989 Iron-stress induced redox activity in tomato (*Lycopersicum esculentum* Mill.) is localized on the plasma membrane. Plant Physiol. 90, 151–156.

Chaney R L 1989 Translocation of iron from plant roots to shoots. 5. International Symposium on Iron Nutrition, Jerusalem, Israel.

Crowley D E and Gries D 1993 Modeling of iron competition in the plant rhizosphere. 7th International Symposium on Iron Nutrition and Interactions in Plants, Zaragoza, Spain.

Crowley D E, Wang Y C, Reid C P P and Szaniszlo P J 1991 Mechanisms of iron acquisition from siderophores by microorganisms and plants. *In* Iron Nutrition and Interactions in Plants. Ed. Y Chen and Y Hadar. pp 213–217. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Englisch G 1993 Der Einfluß unterschiedlicher Stickstoff-Ernährungsformen auf den pH-Wert im Apoplasten von Blattmesophyllzellen sowie auf die Ausbildung einer Eisenmangelchlorose bei *Glycine max* L. und *Zea mays* L. 1993. Master-Thesis, Fac. Agriculture, Justus Liebig University Giessen.

Hoffmann B, Plänker R and Mengel K 1992 Measurements of pH in the apoplast of sunflower leaves by means of fluorescence. Physiol. Plant. 84, 146–153.

Holden M J, Luster D G, Chaney R L, Buckhout T J and Robinson C 1991 Fe-chelate reductase activity of plasma membranes isolated from tomato (*Lycopersicum esculentum* Mill.) roots. Plant Physiol. 97, 537–544.

Kirkby E A, Armstrong M J and Leggett J E 1981 Potassium recirculation in tomato relation to potassium supply. J. Plant Nutr. 3, 955–966.

Kolesch H, Oktay M and Höfner W 1984 Effect of iron chlorosis-inducing factors on the pH of the cytoplasm of sunflower (*Helianthus annuus*). Plant and Soil 82, 215–221.

Loeppert R H and Hallmark C T 1985 Indigenous soil properties influencing the availability of iron in calcareous soils. Soil Sci. Soc. Am. J. 49, 597–603.

Longnecker N and Welch R M 1990 Accumulation of apoplastic iron in plant roots. Plant Physiol. 92, 17–22.

Mengel K, Breininger M and Bübl W 1984 Bicarbonate, the most important factor inducing iron chlorosis in vine grapes on calcareous soils. Plant and Soil 81, 333–344.

Mengel K and Bübl W 1983 Verteilung von Eisen in Blättern von Weinreben mit HCO_3 induzierter Chlorose. Z. Pflanzenernähr. Bodenkd. 146, 560–571.

Mengel K and Geurtzen G 1986 Iron chlorosis on calcareous soils. Alkaline nutritional condition as the cause for the chlorosis. J. Plant Nutr. 9, 161–173.

Mengel K and Geurtzen G 1988 Relationship between iron chlorosis and alkalinity in *Zea mays*. Physiol. Plant. 72, 460–465.

Mengel K and Malissiovas N 1981 Bicarbonat als auslösender Faktor der Eisenchlorose bei der Weinrebe (*Vitis vinifera*). Vitis 20, 235–243.

Mengel K, Plänker R and Hoffmann B 1994 Relationship between leaf apoplast pH and Fe chlorosis of sunflowers (*Helianthus annuus L.*). J. Plant Nutr. 17, 1053–1064.

Mengel K and Simic R 1973 Effect of potassium supply on the acropetal transport of water, inorganic ions, and amino acids in young decapitated sunflower plants. Physiol. Plant. 28, 232–236.

Molz E 1907 Untersuchungen über die Chlorose von Reben. Ph.D. Thesis, University Jena.

Moog P R and W Brüggemann 1994 Iron reductase systems on the plant plasma membrane. Plant and Soil 165, 241–260.

Neilands J B and Leong S A 1986 Siderophores in relation to plant growth and disease. Annu. Rev. Plant Physiol. 37, 187–208.

Pate J S 1975 Exchange of solutes between phloem and xylem circulation in the whole plant. *In* Transport in Plants, I. Phloem Transport. Eds. M H Zimmermann and J A Milburn. pp 451–473. Springer Verlag Berlin.

Plänker R 1991 Bedeutung des Apoplasten pH-Wertes für die Eisenchlorose. Untersuchungen an *Helianthus annuus*. Ph.D. Thesis, Fac. Biol., Justus Liebig University, Giessen.

Rashid A, Couvillon G A and Benton Jones J 1990 Assessment of Fe status of peach rootstocks by techniques used to distinguish chlorotic and non-chlorotic leaves. J. Plant Nutr. 13, 285–307.

Reichard P 1993 From RNA to DNA, why so many ribonucleotide reductases? Science 260, 1773–1777.

Romera F J, Alcantara E and de la Guardia M D 1991 Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution. II Iron-stress response mechanisms. *In* Iron Nutrition and Interactions in Plants. Eds. Y Chen and Y Hadar. pp 151–155. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rutland R B 1971 Radioisotope evidence of immobilization of iron in Azalea by excess calcium bicarbonate. J. Am. Hort. Sci. 96, 653–655.

Rutland R B and Bukovac M J 1971 The effect of calium bicarbonate on iron absorption and distribution by *Chrysanthemum morifolium* (Ram.). Plant and Soil 35, 225–236.

Saglio P 1969 Nutrition en fer de la vigne. I Essai d'induction d'une chlorose ferrique par l'action combinée du bicarbonate et de l'orthophosphate sur deux variétés: L'une sensible et l'autre resistante. Ann. Physiol. Veg. 11, 27–35.

Sahu M P, Sharma D D, Jain G L and Singh H G 1987 Effects of growth substances, sesquestrene 138-Fe and sulphuric acid on iron chlorosis of garden peas (*Pisum sativum L.*). J. Hortic. Sci. 62, 391–394.

Schaumberger P 1990 Einfluß von pH-Wert und Kalkgehalt des Bodens auf die Eisenaufnahme und Eisenverlagerung in den Sproß bei einer Fe-ineffizienten und einer Fe-effizienten Sorte von *Zea mays*. Master-Thesis, Fac. Agriculture, Justus Liebig University Giessen.

Stephan U W and Scholz G 1993 Nicotianamine: mediator of transport of iron and heavy metals in the phloem? Physiol. Plant. 88, 522–529.

Tagliavini M, Scudellari D, Marangoni B and Toselli M 1994 Acid spray regreening of kiwifruit leaves affected by lime induced Fe chlorosis. Proc. 7th International Symposium on Iron Nutrition and Interactions in Plants. Ed. J Abadia.

Toulon V, Sentenac H, Thibaud J B, Davidian C, Moulineau C and Grignon C 1992 Role of apoplast acidification by the H^+ pump. Effect on the sensitivity to pH and CO_2 of iron reduction. Planta 186, 212–218.

Ullrich W R 1991 Transport of nitrate and ammonium through plant membranes. *In* Nitrogen Metabolisms in Plants. Eds. K Mengel and D J Pilbeam. pp 121–137. Oxford University Press, Oxford, Great Britain.

Section editor: H Lambers