International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (12), pp. 001-010, December, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

Full Length Research Paper

The response to iron deficiency of two sensitive grapevine cultivars grafted on a tolerant rootstock

M. A. Russo*, F. Sambuco and A. Belligno

Department of Agrochemistry, University of Catania, Italy.

Accepted 22 April, 2018

Two sensitive cultivars were examined, *VITTORIA* and *ITALIA,* grafted on a tolerant rootstock (140 Ruggeri). Two levels of iron chlorosis in scions were selected, initial and evident, and compared to the healthy rootstock (control). The fractions of extracellular and cytoplasmatic cations, chloroplastic mobile, loosely linked, strongly linked and residual cations as well as the active fraction were extracted from the fresh matter. In the chlorotic the plant inability to use Fe^{2+} uptaken by the rootstock was highlighted, with a different response from *VITTORIA* and *ITALIA*, as evidenced by the decrease in the available fraction of Fe^{2+}, particularly in the case of evident chlorosis. The increase in leaf content of the active forms of K^{+} and Ca^{2+} resulted directly correlated to the intensity of iron deficiency, since they bring about a higher pH and a destabilization of membranes, respectively, both hindering iron utilization. The modified response in terms of reduced photosynthetic activity in chlorotic scions was evidenced through the decrease in the active form of Mg^{2+} and consequently in chlorophyll content.

Key words: Fe and nutrition unbalance, active Fe fraction, iron chlorosis.

INTRODUCTION

The vast variability of soil properties requires that appropriate grapevine rootstocks be selected able to adapt to specific soil conditions; developing rootstocks able to uptake iron ions under conditions of deficient availability is one of the present challenges to genetic improvement (Gupton and Spiers, 1992).

However, since iron uptake must be followed by its translocation to leaves, the tolerance of rootstock to iron deficiency is not sufficient to prevent leaf chlorosis, which can be brought about by iron inactivation in leaves (Mengel and Malissovas, 1981).

Until now, research has focused mainly on the soil-root interface but not enough is known on the relationships between rootstock and scion: consequently the present research addressed the response to iron deficiency of two different cultivars of *Vitis vinifera* grafted on a tolerant rootstock (*Vitis labrusca*).

Chlorosis in fact can be evidenced even in some scions grafted on tolerant rootstocks: even if the rootstock is able to mobilize iron at root level, the reduction in mobility which can be formed at the grafting level can impair plant ability to satisfy its metabolic requirements.

Many pedological factors as well as anthropic interventions can impair plant iron uptake: example it has been highlighted that high levels of potassium or cultivation practices can reduce iron availability by raising soil pH reaction thus bringing about conditions unfavourable to maintain iron in its reduced form (Lucena et al., 1990; Pal et al., 1990; Szlek et al., 1990). Furthermore, even concentrations are at an optimal level and it is present in an available form, an unbalance due to excess in Mn^{2+} and Cu^{2+} can cause iron deficiency (Lucena et al., 1990; Mench and Fargues, 1994; Pich et al., 1994; Welch et al., 1993).

Some rootstock cultivars are able to reduce Fe^{3+} to Fe^{2+}, making the ions mobile in the soil and enhancing their uptake (Brown and Draper 1980; Brancadoro et al., 1995; Tagliavini et al., 1995). Also roots of some cultivars can reduce Fe^{3+} to Fe^{2+} encouraging its migration from roots to leaves (Cinelli, 1995).

It can be presumed that such plants have an enzymatic redox equipment depending on the Fe^{2+}/Fe^{3+} ratio (Nenova and Stoyanov, 1995), able to make the microelement in its active form available to the plant. Some varieties of grapevine, particularly rootstocks of *V. labrusca* and scions grafted on them, achieve a higher

*Corresponding author. E-mail: marcoanton.russo@tiscali.it. Tel: +39 095 7580202. Fax: +39 095 7141581.

Table 1. Selected properties of the soils.

Farm n.	1	2	3	4
Sand (%)	68.10	67.30	67.90	66.90
Silt (%)	13.00	13.50	13.10	13.60
Clay (%)	18.90	19.20	19.00	19.50
pH	7.89	7.98	7.79	7.86
Total ca (%)	47.30	48.20	49.00	46.90
Active ca (%)	11.80	12.60	13.20	11.40
Organic C (%)	1.34	1.36	1.39	1.40
C/N	7.44	8.00	7.85	7.61
Total N (%)	1.80	1.70	1.77	1.84
Available P (kg ha^{-1})	1361.00	1257.00	1385.00	1360.00
Available K (kg ha^{-1})	997.00	986.00	1005.00	1097.00

ability in uptaking iron, even in markedly alkaline soils. Such tolerant varieties can mobilize iron by reducing soil pH at root level, thanks to their ability to emit H^+ and/or organic acids; in the latter case, iron is absorbed and transferred as a complex (Brancadoro et al., 1995).

In this research the trade-offs between mobile and non-mobile forms of iron during plant development were evaluated, considering that the fraction of chloroplastic mobile iron is largely represented, at least in those leaves where no chlorosis is evidenced, by the iron-protein-chlorophyll complex at basically constant levels, whereas it seems that iron in chlorotic leaves is stored as a non-active state, possibly such as ferritin, a protein which captures iron as Fe-phosphate in a non-readily usable form (Grossman et al., 1992).

Since all nutrients concur to the development of plants throughout all their life cycle, this research was aimed at assessing the evolution during a full season of the impact of different Fe levels on the mineral nutrition of two iron deficiency-sensitive grapevine cultivars grafted on a tolerant rootstock. The results were compared to the response of the tolerant rootstock.

While this paper deals with the evolution of mineral components, total chlorophyll and proteins throughout a vegetative cycle, in a companion paper the impact of iron deficiency on organic components at harvesting time will be described.

MATERIALS AND METHODS

The field investigation was conducted in four farms, representative of the typical conditions for table grape production, with uniform pedologic conditions; soils are loamy sands and their averaged main characteristics are reported in Table 1. Two iron deficiency-sensitive cultivars of table grape *V. vinifera*, "Italia" and "Vittoria" grafted on a tolerant rootstock, *V. labrusca* ("140 Ruggeri"), were examined.

Six plants per cultivar in each of the four farms were labelled on healthy rootstocks (2.27 - 3.84 mg total chlorophyll g^{-1} fresh matter (f.m.): chlorosis absent) and two levels of chlorosis were identified in the scions, namely incipient (1.47 to 1.82 total chlorophyll g^{-1} f.m.) and evident (0.77 - 1.30 total chlorophyll g^{-1} f.m.).

Plant development was followed from May to August, prior to fruit ripening time, with four monthly leaf samplings from apical shoots of rootstocks and scions, in three replications.

The sampled leaves were dried and the dry matter (d.m.) was mineralized at 500 - 550°C; after that, the content in Fe, K, Ca, Mg, Mn, Cu was determined by a atomic absorption spectrophotometry (AA Perkin Elmer mod, 4000).

The nitrogen (Kjeldahl), after wet-ashing in conc. HNO_3, was determined also by means of atomic absorption spectroscopy (Perkin Elmer, 4000).

In fresh plant matter, total protein content was determined (Bradford, 1976) as well as cations in their fractions a) available, on the extract obtained with N HCl (Köseoglu and Açikgöz, 1995); and as components: 1- extra-cellular and cytoplasmic; 2- mobile and loosely linked chloroplastic; b) unavailable (strongly linked and residual).

The three fractions were obtained by applying an exhaustive extraction, using in succession solvents with growing extracting strength, namely NaCl 0.35 M; NaEDTA 3 x 10^{-3} M for the available fraction and Triton-X 1.5% in water for the unavailable fraction (Machold, 1968). Additionally total chlorophyll content (Arnon, 1964) was determined.

The extracts from fractions a) and b) as well as the residues were subdivided in two parts: one part was dried and mineralized and the cations were determined as described above; the second part was used to determine the protein content. Finally, also the cation and protein content in the residues was determined.

RESULTS AND DISCUSSION

The values of dry matter, total proteins and chlorophyll were always higher in rootstock leaves, that showed no chlorosis, and opposite to this, nitrogen content was higher in leaves showing iron chlorosis (Figures 1 - 2).

Total and available iron content (Table 2) was in chlorotic leaves compared to the unstressed leaves, with the iron content of the active form proportional to chlorosis intensity, in accordance to results obtained by Brancadoro, 1995 with the roots of *V. vinifera*.

Throughout the period of plant development, the iron content in its two forms, total and active, increased only in non-chlorotic plants (Table 2). Parallel to this, a considerable decrease in the forms “strongly linked” and “residual” was found in non-chlorotic plant matter.

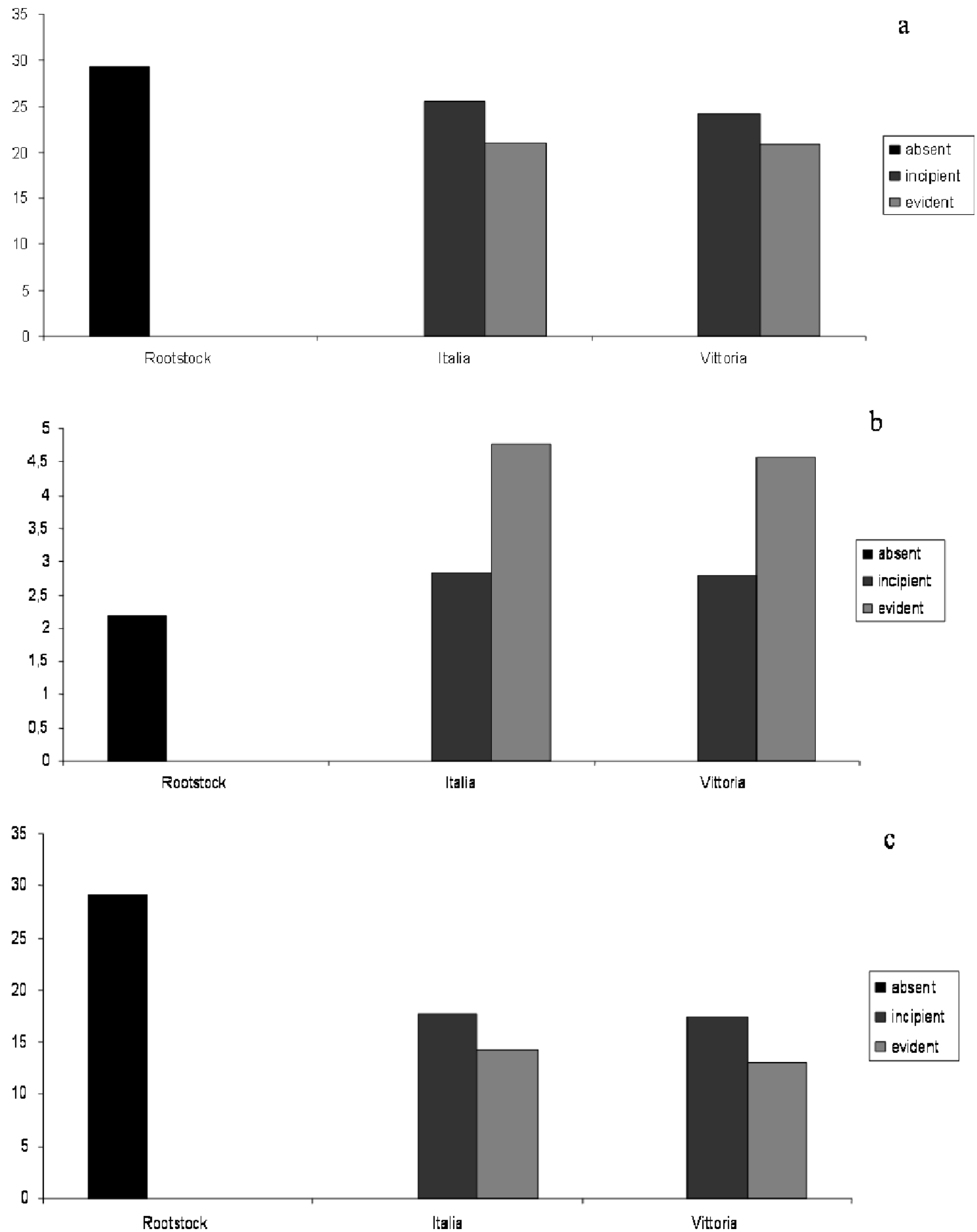

Figure 1. Dry matter (%, a), total nitrogen (% dm, b) and total proteins content (mg g^{-1} dm, c) in unstressed (rootstock) and variously stressed scions.

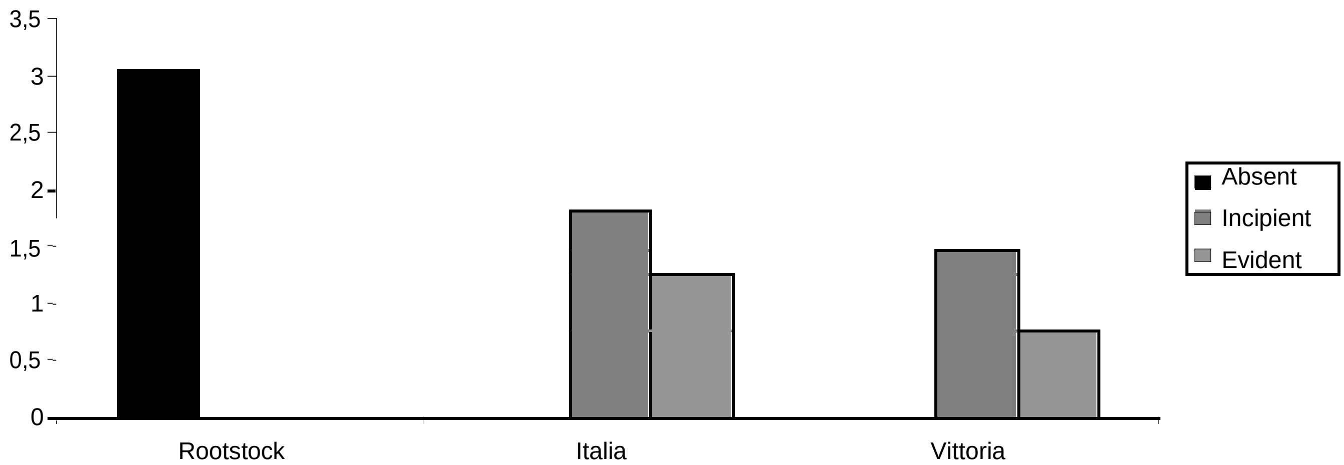

Figure 2. Total chlorophyll content (mg g^{-1} fm) in unstressed (rootstock) and variously stressed scions.

Table 2. Content of the various forms of iron in the rootstock and the variously stressed scions.

Parameters		Iron (mg g^{-1} d.m.)			Iron (% on total)			
					Available		Unavailable	
Cultivars and chlorosis intensity	Sampling date	Total	Available	Unavailable	Extra cell and cytopl.	Chloroplastic mobile	Strongly linked	Residual
	06-May	1.12 d	0.98 e	0.16 f	34.52	39.83	10.93	14.72
	10-Jun	1.27 c	1.13 d	0.15 f	38.20	43.02	8.11	10.69
Rootstock (absent)	13-Jul	1.45 b	1.32 c	0.13 g	41.60	45.14	5.31	7.95
	10-Aug	1.62a	1.53 a	0.12 g	42.40	47.29	3.39	6.92
	Means	1.37 a	1..24 b	0.14 c				
	06-May	1.16 a	0.42 e	0.25e	33.74	35.24	12.27	18.75
	10-Jun	1.14 a	0.80 cd	0.21 f	29.81	32.72	16.63	20.84
Italia (incipient)	13-Jul	1.14 a	0.85 c	0.23 ef	26.34	28.59	19.21	25.86
	10-Aug	1.05 b	0.76 d	0.42 d	25.80	24.97	23.21	26.02
	Means	1.12 a	0.71 b	0.28 c				
	06-May	1.08 a	0.54 c	0.34 e	29.56	26.10	15.43	23.08
	10-Jun	1.12 a	0.55 c	0.34 e	29.42	25.33	18.72	26.53
Vittoria (incipient)	13-Jul	1.12 a	0.63 b	0.34 e	25.71	22.05	20.57	31.67
	10-Aug	1.02 a	0.60 b	0.32 e	22.70	22.76	22.28	32.26
	Means	1.09 a	0.58 b	0.34 c				
	06-May	1.06 a	0.56 d	0.20g	21.20	17.57	20.81	40.42
	10-Jun	1.09 a	0.69 c	0.20g	17.38	16.27	24.53	41.82
Italia (evident)	13-Jul	1.11 a	0.68 c	0.20g	14.10	11.40	30.82	43.68
	10-Aug	1.09 a	0.60 d	0.24f	11.90	9.70	32.19	46.21
	Means	1.09 a	0.63 b	0.21 c				
	06-May	0.97 b	0.53 b	0.23 f	9.21	14.01	26.44	50.34
	10-Jun	1.01 a	0.53 b	0.28 e	8.52	11.14	29.11	51.23
Vittoria (evident)	13-Jul	1.02 a	0.49 b	0.33 d	6.71	8.42	31.74	53.13
	10-Aug	1.00 a	0.38 c	0.30 e	3.43	4.48	35.37	56.72
	Means	1.00 a	0.48 b	0.29 c				

The values of the “total” form may differ from the summation of “available” and “unavailable” forms due to the different analytical methodology.

Table 3. Content of the various forms of potassium in the rootstock and the variously stressed scions.

Parameters		Potassium (mg g^{-1} d.m.)			Potassium (% on total)			
					Available		Unavailable	
Cultivars and chlorosis intensity	Sampling date	Total	Available	Unavailable	Extra cell and cytopl	Chloroplastic mobile	Strongly linked	Residual
Rootstock (absent)	06-May	13.37 a	12.83 ab	0.54 g	93.91	1.80	1.55	2.80
	10-Jun	12.23 b	11.69 bc	0.54 g	92.65	2.55	1.90	2.75
	13-Jul	10.61 c	9.79 c	0.82 f	88.71	3.80	4.45	3.05
	10-Aug	5.46 d	4.56 e	0.91 f	77.49	8.60	7.90	6.00
	Means	10.42 a	9.72 a	0.70 b				
Italia (incipient)	06-May	15.05 a	14.63 ab	0.41 g	95.40	1.50	1.00	2.00
	10-Jun	13.27 bc	12.83 c	0.44 fg	94.30	1.90	1.80	2.00
	13-Jul	11.99 cd	11.57 d	0.42 g	93.90	2.40	2.00	1.80
	10-Aug	9.51 e	9.00 e	0.51 f	87.10	5.80	4.80	2.30
	Means	12.46 a	12.01 a	0.45 b				
Vittoria (incipient)	06-May	13.88 a	13.58 a	0.30 h	96.30	1.20	1.10	1.40
	10-Jun	11.39 b	11.00 bc	0.39 g	93.00	2.70	2.20	2.10
	13-Jul	10.60 c	10.06 c	0.54 f	91.50	3.10	4.10	1.30
	10-Aug	8.57 d	7.97 d	0.60 e	82.90	7.60	7.80	1.70
	Means	11.11 a	10.65 a	0.46 b				
Italia (evident)	06-May	16.46 a	16.04 a	0.42 f	96.10	1.20	1.20	1.50
	10-Jun	14.94 b	14.54 b	0.40 fg	95.30	1.50	1.60	1.60
	13-Jul	13.45 c	13.12 c	0.33 g	94.80	2.40	1.50	1.30
	10-Aug	11.90 d	11.06 d	0.84 e	91.20	4.80	2.90	1.10
	Means	14.19 a	13.69 a	0.50 b				
Vittoria (evident)	06-May	15.08 a	14.72 a	0.36 fg	96.30	1.00	1.20	1.50
	10-Jun	13.06 ab	12.67 b	0.39 f	95.40	1.30	1.78	1.50
	13-Jul	11.78 bc	11.47 c	0.31 g	93.40	3.40	2.10	1.10
	10-Aug	9.44 d	8.62 de	0.82 e	89.30	6.30	3.50	0.90
	Means	12.34 a	11.87 a	0.47 b				

The values of the “total” form may differ from the summation of “available” and “unavailable” forms due to the different analytical methodology.

Total and active potassium percentage decreased during plant development in all the sampled leaves; also the percentage of active form referred to the total was decreasing (Table 3).

The percentages of K increased parallel to chlorosis intensity, and in the cv. Italia were higher than in Vittoria. The higher K content in chlorotic plants could be related to a higher pH in leaf apoplast (Nikolic and Römheld, 1999) which impairs Fe mobilization (Monge et al., 1993; Singh et al., 1995; Szlek et al., 1990). The different levels of K could depend on the unbalance in respiration and photosynthesis typical of sensitive cultivars, where K ions are accumulated to activate stomatal openings(Ward and Schroeder,1994; (solo Blatt), as demonstrated by Lucena et al. (1990) and Pal et al. (1990) for other plant species.

Such assumption is confirmed by the variations in the active form of Ca^{2+} (Table 4) needed to balance ions as required to regulate stomatal openings in response tostress (Lucena et al., 1990; Ward and Schroeder, 1994; McAinsh et al., 1995). In fact under iron stress conditions the ratio K^{+}/Ca^{2+} in their active form decreased about 15% in both sensitive cultivars compared to the tolerant rootstock (Figure 3).

The different percentages of the active Ca^{2+} fraction in chlorotic and non-chlorotic plants (Table 4) indicate a different plant ability in the cation mobilization: as a consequence the active fractions of Fe^{2+} and Ca^{2+} result inversely correlated as found by Pal et al. (1990) in sugarcane.

Variations in Mg^{2+} content as a response to chlorosis (Table 5) are mainly reflected in the photosynthetic process: the lower amounts of its active form in the chlorotic plants demonstrate their lower photosynthetic ability. The active forms of this ion in fact are very significantly correlated to the chlorophyll (Chl) content, and also significantly correlated to Fe^{2+}. Iron in turn, although not present in the chlorophyll molecule, is highly

Table 4. Content of the various forms of calcium in the rootstock and the variously stressed scions.

Parameters		Calcium (mg g^{-1} d.m.)			Calcium (% on total)			
Cultivars and chlorosis intensity	Sampling date	Total	Available	Unavailable	Available		Unavailable	
					Extra cell and cytopl	Chloroplastic mobile	Strongly linked	Residual
	06-May	33.83 c	15.80 g	18.03 f	43.70	2.11	3.20	51.01
	10-Jun	41.81 b	16.86 g	24.96 de	37.95	1.94	3.31	56.82
Rootstock (absent)	13-Jul	47.74 a	21.23 e	26.51 d	42.25	1.99	2.00	53.78
	10-Aug	38.86 bc	19.92 ef	18.94 f	46.45	4.15	9.71	39.70
	Means	40.56 a	18.45 c	22.11 b				
	06-May	28.05 c	13.39 g	14.67 f	44.40	3.06	2.40	50.10
	10-Jun	40.74 a	18.95 e	21.79 d	43.10	3.07	2.00	51.80
Italia (incipient)	13-Jul	40.90 a	22.59 d	18.31 e	52.30	2.88	1.70	43.10
	10-Aug	34.84 b	22.90 d	11.93 h	58.90	6.41	6.20	28.50
	Means	36.16 a	19.46 b	16.68 c				
	06-May	34.89 c	18.92 f	15.97 g	51.50	2.44	2.30	43.80
	10-Jun	41.53 b	22.13 e	19.40 f	49.90	3.130	1.80	45.17
Vittoria (incipient)	13-Jul	49.55 a	27.49 d	22.06 e	52.90	2.24	1.45	43.41
	10-Aug	42.19 b	27.86 d	14.33 h	59.58	4.98	5.64	29.8
	Means	42.04 a	24.1 b	17.94 c				
	06-May	27.10 d	12.52 i	14.58 h	51.00	3.87	2.40	42.74
	10-Jun	41.06 b	21.83 f	19.23 g	48.80	4.03	1.75	45.42
Italia (evident)	13-Jul	49.24 a	35.47 c	13.77 h	67.53	4.47	1.00	27.00
	10-Aug	33.12 c	23.27 e	9.85 l	62.30	7.77	3.80	26.13
	Means	37.63 a	23.27 b	14.36 c				
	06-May	45.29 a	32.99 c	12.30 g	70.30	2.16	1.50	26.04
	10-Jun	37.56 b	27.21 d	10.35 h	65.76	6.39	2.00	25.85
Vittoria (evident)	13-Jul	49.08 a	30.54 c	18.54 f	59.16	2.73	1.30	36.81
	10-Aug	32.97 c	20.01 e	12.96 g	54.60	4.76	4.94	35.70
	Means	41.23 a	27.69 b	13.54 c				

The values of the "total" form may differ from the summation of "available" and "unavailable" forms due to the different analytical methodology.

correlated to Chl (Monge et al., 1993; Van Dijk and Bienfait, 1993; Zhang et al., 1995): in this its active fraction was significantly correlated to chlorophyll in both cultivars.

Manganese and copper percentage, both in their total and active form, exhibits a consistent trend to decrease throughout the season and is lower in non stressed plant (Tables 6 and 7). The higher percentage of such cations in sensitive plants can be explained as a plant defence strategy to balance the insufficient availability of Fe^{2+}, as recorded also in pea (Yi and Guerinot, 1996). This brought to a decrease of about 90% in the ratio Fe^{2+}/Cu^{2+} and about 85% in the ratio Fe^{2+}/Mn^{2+} in both sensitive cultivars (Figure 3): such a decrease can be taken as an indicator of iron availability (Lucena et al., 1990; Monge et al., 1993; Zhang, 1993).

Variations in Cu^{2+} and Mn^{2+} can depend on the need to contrast the unbalance in nutrients due to a reduction in active Fe^{2+} fraction, as reported for peach by Köseoglu (1995) and Monge et al 1993. It has been recently reported that in the same substrate the uptaking of nutrients is different among the cultivars in dependence of their genetic characteristics, since plant ability to use available nutrients is conditioned by specific proteins managing the transport of bivalent elements (Welch et al., 1993).

The higher rate of Cu^{2+} mobilization in chlorotic scions may have caused an inhibition of ferrochelatoreductase activity (Yi and Guerinot, 1996) since it is implied in many factors such as competition for electrons, for chelating agents (Welch et al., 1993) or directly on the redox system (Welch et al., 1993). The competition between Cu^{2+} and Fe^{2+} has been confirmed by many studies with Fe-deficient solutions, where chlorosis was less evident if solutions were also Cu-deficient.

The stress to iron deficiency probably impacted the mobilization of all the cations interacting with nutrition (Mengel et al., 1995), with different responses in the two

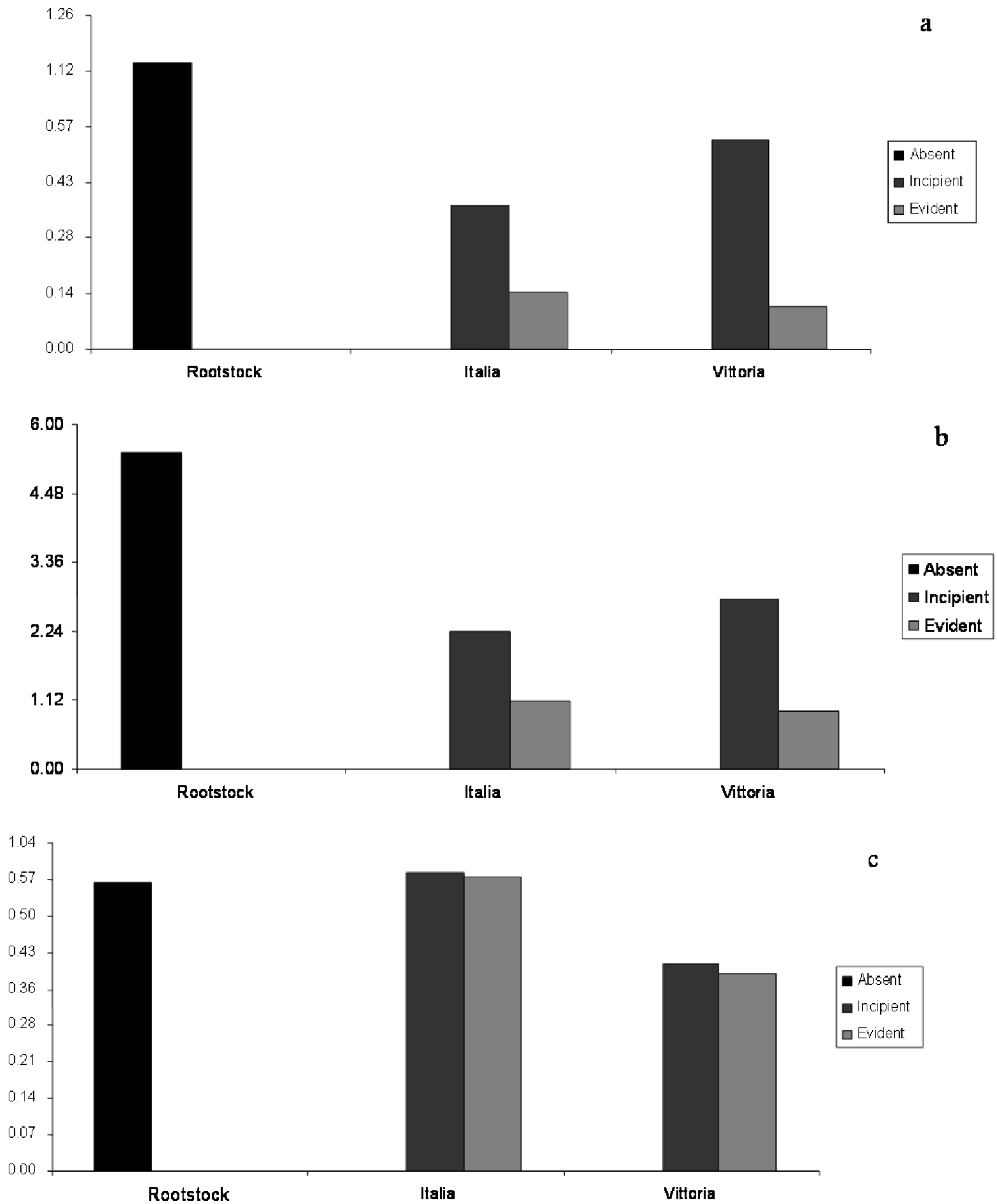

Figure 3. Ratios between the active forms of selected cations, Fe^{2+}/Cu^{2+} (a), Fe^{2+}/Mn^{2+} (b), K^{+}/Ca^{2+} (c) in the rootstock and the variously stressed scions.

Table 5. Content of the various forms of magnesium in the rootstock and the variously stressed scions.

Parameters		Magnesium (mg g^{-1} d.m.)			Magnesium (% on total)			
Cultivars and chlorosis intensity	Sampling date	Total	Available	Unavailable	Available		Unavailable	
					Extra cell and cytopl	Chloroplastic mobile	Strongly linked	Residual
	06-May	9.42 d	5.55 g	3.88 i	48.00	9.81	4.82	37.37
	10-Jun	10.61 c	6.32 f	4.29 i	48.145	10.805	5.10	35.95
Rootstock (absent)	13-Jul	12.28 b	7.37 e	4.97 h	50.27	9.40	4.85	35.48
	10-Aug	14.71 a	9.51 d	5.18 gh	53.38	10.99	5.00	30.64
	Means	11.76 a	7.21 b	4.58 c				
	06-May	6.39de	3.14d	3.25b	44.68	5.63	5.90	43.79
	10-Jun	6.70d	3.50d	3.20b	48.18	5.27	5.61	40.94
Italia (incipient)	13-Jul	7.38cd	3.70cd	3.68b	46.51	5.29	5.73	42.47
	10-Aug	8.64c	4.24c	4.40a	47.40	1.40	5.00	46.20
	Means	7.28 a	3.63 b	3.63 b				
	06-May	7.88 d	4.45 h	3.43 i	49.07	7.11	4.49	39.33
	10-Jun	9.09 c	4.86 gh	4.23 h	45.64	7.53	5.20	41.63
Vittoria (incipient)	13-Jul	10.33 b	5.97 f	4.36 h	50.81	6.68	4.85	37.66
	10-Aug	12.10 a	6.84 e	5.26 g	53.40	2.80	4.23	39.57
	Means	9.82 a	5.53 b	4.32 c				
	06-May	4.46 c	1.86 gh	2.58 e	36.40	4.70	4.75	54.15
	10-Jun	4.96 bc	1.75 h	3.26 d	24.90	4.50	4.71	65.89
Italia (evident)	13-Jul	5.26 b	1.55 i	2.75 e	22.40	5.10	4.78	67.72
	10-Aug	6.85 a	2.01 g	3.59 d	23.00	4.10	4.35	68.55
	Means	5.38 a	1.79 c	3.05 b				
	06-May	4.49 d	1.88 f	2.63 f	37.00	5.00	4.78	53.22
	10-Jun	5.89 b	1.70 f	4.14 de	28.00	6.00	5.06	60.94
Vittoria (evident)	13-Jul	5.58 bc	2.51 e	4.03 e	39.00	8.00	5.39	47.61
	10-Aug	7.27 a	3.25 d	5.26 c	40.00	7.00	4.90	48.1
	Means	5.81 a	2.32 c	4.02 b				

The values of the "total" form may differ from the summation of "available" and "unavailable" forms due to the different analytical methodology.

Table 6. Content of the various forms of manganese in the rootstock and the variously stressed scions.

Parameters		Manganese (mg g^{-1} d.m.)			Manganese (% on total)			
Cultivars and chlorosis intensity	Sampling date	Total	Available	Unavailable	Available		Unavailable	
					Extra cell and cytopl	Chloroplastic mobile	Strongly linked	Residual
Rootstock (absent)	06-May	0.33 a	0.27 c	0.07 f	63.28	17.25	7.45	12.02
	10-Jun	0.30 b	0.23 d	0.06 g	58.91	20.53	4.85	15.71
	13-Jul	0.27 c	0.22 d	0.04 h	66.87	20.02	5.09	8.02
	10-Aug	0.21 de	0.20 e	0.01 i	76.88	18.73	3.07	1.32
	Means	0.28 a	0.23 b	0.06 c				
Italia (incipient)	06-May	0.34 b	0.28 c	0.06 g	67.00	15.36	5.80	11.84
	10-Jun	0.39 a	0.29 c	0.10 f	54.40	20.27	5.20	20.13
	13-Jul	0.28 c	0.23 d	0.05 h	60.50	20.52	6.20	12.78
	10-Aug	0.20 e	0.19 e	0.02 i	68.60	21.77	6.90	2.73
	Means	0.30 a	0.25 b	0.06 c				

Table 6. Contd.

Vittoria (incipient)	06-May	0.52 a	0.38 b	0.14 g	58.03	14.51	9.96	17.50
	10-Jun	0.35 b	0.28 de	0.06 h	61.20	19.88	4.50	14.42
	13-Jul	0.30 c	0.26 e	0.04 i	64.80	21.48	4.37	9.35
	10-Aug	0.22 e	0.21 f	0.01 l	71.40	22.78	3.42	2.40
	Means	0.35 c	0.28 b	0.06 c				
Italia (evident)	06-May	0.41 b	0.32 c	0.09 h	61.80	15.03	5.90	17.27
	10-Jun	0.45 a	0.32 c	0.13 g	50.50	20.21	7.00	22.29
	13-Jul	0.33 c	0.27 d	0.06 i	60.00	20.56	6.60	12.84
	10-Aug	0.24 e	0.22 f	0.02 l	65.12	25.88	5.48	3.52
	Means	0.36 a	0.28 b	0.07 c				
Vittoria (evident)	06-May	0.73 a	0.52 b	0.21 g	54.70	15.75	6.00	23.55
	10-Jun	0.52 b	0.37 c	0.15 h	53.10	18.50	4.12	24.28
	13-Jul	0.33 d	0.28 e	0.05 i	60.10	23.56	4.60	11.74
	10-Aug	0.24 f	0.22 fg	0.01 l	63.60	29.50	3.68	3.22
	Means	0.46 a	0.35 b	0.11 c				

The values of the “total” form may differ from the summation of “available” and “unavailable” forms due to the different analytical methodology.

Table 7. Content of the various forms of copper in the rootstock and the variously stressed scions.

Parameters		**Copper (mg g^{-1} d.m.)**			**Copper (% on total)**			
Cultivars and chlorosis intensity	**Sampling date**	**Total**	**Available**	**Unavailable**	**Available**		**Unavailable**	
					Extra cell and cytopl	**Chloroplastic mobile**	**Strongly linked**	**Residual**
Rootstock (absent)	06-May	1.06 b	0.97 b	0.09 d	66.80	27.55	3.32	2.33
	10-Jun	1.02 b	0.96 b	0.06 e	60.83	33.15	3.01	3.01
	13-Jul	0.96 b	0.91 c	0.06 e	47.875	47.265	2.45	2.41
	10-Aug	1.43 a	1.38 a	0.05 f	50.11	46.93	1.73	1.23
	Means	1.12 a	1.06 a	0.07 b				
Italia (incipient)	06-May	1.47 b	1.35 c	0.12 d	57.60	34.97	5.15	2.28
	10-Jun	1.46 b	1.34 c	0.11d	59.93	33.86	3.20	3.01
	13-Jul	1.43 bc	1.34 c	0.09e	52.54	40.52	3.09	3.85
	10-Aug	2.01 a	1.95 a	0.07f	51.10	44.12	2.21	2.57
	Means	1.59 a	1.50 a	0.10 b				
Vittoria (incipient)	06-May	1.43 b	1.33 b	0.10 e	57.60	34.97	5.15	2.28
	10-Jun	1.33 b	1.00d	0.13 f	59.93	33.86	3.20	3.01
	13-Jul	1.30 b	1.23 b	0.07 g	52.54	40.52	3.09	3.85
	10-Aug	1.84 a	1.78 a	0.05 h	51.10	44.12	2.21	2.57
	Means	1.48 a	1.3 b	0.09 c				
Italia (evident)	06-May	1.93 bc	1.79 c	0.13 e	54.17	38.05	4.80	2.98
	10-Jun	1.86 c	1.69 c	0.17 d	53.14	37.62	6.10	3.14
	13-Jul	2.17 b	2.03 b	0.14 e	54.01	39.07	3.94	2.98
	10-Aug	2.84 a	2.76 a	0.08 f	69.64	27.43	1.17	1.76
	Means	2.20 a	2.07 a	0.13 b				
Vittoria (evident)	06-May	2.14 ab	2.02 b	0.12 f	52.16	40.59	4.45	2.80
	10-Jun	2.31 a	2.15 ab	0.16 d	42.27	50.22	4.61	2.90
	13-Jul	1.67 c	1.53 c	0.14 e	49.3	41.92	4.78	4.00
	10-Aug	2.19 ab	2.08 ab	0.11 f	64.74	29.60	2.16	3.50
	Means	2.08 a	1.95 a	0.13 b				

The values of the “total” form may differ from the summation of “available” and “unavailable” forms due to the different analytical methodology.

cultivars Italia and Vittoria. Accordingly chlorotic scions resulted unable to take advantage of Fe^{2+} uptaken and made available by the tolerant rootstock. This in turn influenced the overall conditions of plants, which could not compensate the unbalances due to Fe^{2+} deficiency, mainly demonstrated by 1) the lower contents in Mg^{2+} and higher contents in K^{+} and Ca^{2+}, depending on the alterations in photosynthetic activity, and 2) the increased percentage of Mn^{2+} and Cu^{2+} in leaves: such elements, active in electron transfer and cofactors of enzymatic activities, can act synergically or antagonistically in Fe^{2+} mobilization in dependence of plant genetic characteristics.

REFERENCES

Arnon DI, Tsujimoto HY, Mcswain BD (1964). Ferredoxin in photosynthetic production of oxygen and phosphorylation by chloroplasts. Proceedings of the National Academy of Sciences of the United States of America 51: 1274-1282.

Bienfait HF, Scheffers MR (1992). Some properties of ferric citrate relevant to the iron nutrition of plants. Plant and Soil 143: 141-144.

Blatt MR (1990). Potassium channel currents in intact stomatal guard cells: rapid enhancement by abscisic acid. Planta. 180: 445-455.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-binding. Anal. Biochem. 72: 248-254.

Brancadoro L, Rabotti G, Scienza A, Zocchi G (1995). Mechanisms of Fe-efficiency in roots of *Vitis* spp. In response to iron deficiency stress. Plant Soil 171: 229-234.

Brown JC, Draper AD (1980). Differential response of “Blueberry ” (Vaccinum) progenies to pH and subsequent use of iron. J. Am. Soc. Hort. Sci. 105: 20-24.

Cinelli F (1995). Physiological responses of clonal quince root-stocks to iron-deficiency induced by addition of bicarbonate to nutrient solution. J. Plant Nutr. 18: 77-89.

Grossman MJ, Hinton SM, Minak-Bernero V, Slaugheter C, Stiefel EI (1992). Unification of ferritin family of proteins. Biochem. 89: 2419-2423.

Gupton CL, Spiers JM (1992). Inheritance of the tolerance to mineral element-induced chlorosis in rabbiteye blueberry. Hort. Sci. 27: 148-151.

Köseoglu AT, Açikgöz V (1995). Determination of iron chlorosis with extractable iron analysis in peach leaves. J. Plant Nutr. 18: 153-161.

Lucena JJ, Garate A, Ramon AM, Manzanares M (1990). Iron nutrition of a hydroponic strawberry culture (*Fragaria vesca* L.) supplied with different Fe chelates. Plant Soil 123: 9-15.

Machold O (1968). Einflub der Ernahrunggsbedingungen auf den Zustand des Eisens in den Blattern, den Chlorophyllgehalt un die Katalase-sowie Peroxydaseaktivitat. Flora Abt. A 159.

McAinsh MR, Webb AAR, Taylor JE, Hetherington AM (1995). Stimulus-induced oscillations in guard cell cytosolic free calcium. The Plant Cell 7: 1207-1219.

Mench MJ, Fargues S (1994). Metal uptake by iron-efficient and inefficient oats. Plant Soil 165: 227-233.

Mengel K, Malissiovas N (1981). Bicarbonate as a factor inducing iron chlorosis in grapevine (*Vitis vinifera*). Vitis 20: 235-244.

Mengel K, Planker R, Hoffmann B (1994). Relationship between leaf apoplast pH and iron chlorosis of sunflower (Helianthus annuus L.). J. Plant. Nutr. 17: 1053-1065.

Monge E, Perez C, Pequerul P, Madero P, Val J (1993). Effect of iron chlorosis on mineral nutrition and lipid composition of thylakoid biomembrane in *Prunus persica* (L.) Bastch. Plant Soil 154: 97-102.

Nenova V, Stoyanov I (1995). Physiological and biochemical changes in young maize plants under iron deficiency: 2. Catalase, peroxidase and nitrate reductase activities in leaves. J. Plant Nutr. 18: 2081-2091.

Nikolic M, Römheld V (1999). Mechanism of Fe uptake by the leaf symplast: Is Fe inactivation in leaf a cause of Fe deficiency chlorosis? Plant Soil 215: 229-237.

Pal AR, Motiramani DP, Gupta SB, Bhargava BS (1990). Chlorosis in sugarcane: associated soil properties, leaf mineral composition and crop response to iron and manganese. Fert. Res. 22: 129-136.

Pich A, Scholz G, Stephan UW (1994). Iron-dependent changes of heavy metals, nicotianamine, and citrate in different plant organs and in the xylem exudate of two tomato genotypes. Nicotianamine as possible copper translocator. Plant Soil 165: 189-196.

Singh AL, Chaudhari V, Koradia VG, Zala PV (1995). Effect of excess irrigation and iron and sulphur fertilizers on the chlorosis, dry matter production, yield and nutrients uptake by groundnut in calcareous soil. Agrochimica 39: 184-198.

Szlek M, Miller GW, Welkie GW (1990). Potassium Effect on iron stress in tomato I. The effect on pH, Fe-reductase and chlorophyll. J. Plant Nutr. 13: 215-229.

Tagliavini M, Rombolà AD, Marangoni B (1995). Response to iron-deficiency stress of pea and quince genotypes. J. Plant Nutr. 18: 2465-2482.

Van Dijk HFG, Bienfait HF (1993). Iron-deficiency chlorosis in Scots pine growing on acid soils. Plant Soil 153: 255-263.

Ward JM, Schroeder JI (1994). Calcium-activated K^{+} channels and calcium-induced calcium release by slow vacuolar ion channels in guard cell vacules implicated in the control of stomatal closure. The Plant Cell 6: 669-683.

Welch RM, Norvell WA, Schaefer SC, Shaff JE, Kochian LV (1993). Induction of iron (III) and copper (II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status: Does the root-cell plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake? Planta 190: 555-561.

Yi Y, Guerinot ML (1996). Genetic evidence that induction of root Fe(III) chelate reductase activity is necessary for iron uptake under iron deficiency. Plant J. 10: 835–844.

Zhang C, Romheld V, Marschner H (1995). Distribution pattern of root-supplied ^{59}iron in iron-sufficient and iron-deficient bean plants. J. Plant Nutr. 18: 2049-2058.

Zhang FS (1993). Mobilisation of iron and manganese by plant-borne and synthetic metal chelators. Plant Soil pp. 155-156.

Zhen HH, Shen T, Korcak RF, Baligar VC (1994). Screening for iron-efficient species in the genus malus. J. Plant Nutr. 17: 579-592.