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Effect of iron chlorosis on mineral nutrition and lipid composition of thylakoid biomembrane in *Prunus persica* (L.) Bastch.

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Abstract

The effect of iron chlorosis on mineral, thylakoid lipids and fatty acids composition of field grown peach tree leaves was studied. Significant differences were found in iron extracted by using α,α'-dipyridyl (active iron), total iron, P, K, Cu and the P/Fe and Fe/Mn ratios. The levels of total chlorophyll, total glycolipids and phospholipids were reduced under iron chlorosis. A slight iron deficiency does not modify the fatty acid composition of thylakoid membranes, while a strong deficiency changes the proportion of some fatty acids.

Abbreviations: Chl – chlorophyll, DGDG – digalactosyldiglycerol, MGDG – mono-galactosyldiglycerol, PC – phosphatidycholine, PE – phophatidylethanolamine, PG – phophatidylglycerol, TLC – thin layer chromatography, 16:0 – palmitic acid, 16:1 – palmitoleic acid, 16:1t – trans-hexadecenoic, 18:0 – steric acid, 18:1 – oleic acid, 18:2 – linoleic acid, 18:3 – linolenic acid

Introduction

Iron-chlorosis is a world-wide problem, particularly in arid and semi-arid regions. The soils of these naturally dry areas are of lime-induced chlorosis and frequently contain high concentrations (more than 20%) of calcium carbonate. Although, iron is abundant in these soils, it is not readily available for uptake to many types of plants including peach trees.

Iron stressed plants shown visible symptoms in the youngest leaves, which become yellow (chlorotic) due to a decline of the Chl content and therefore have lower net photosynthesis (Terry, 1980). Leaves suffering from lime-induced chlorosis often have a content of total iron similar to, or ever higher than that of green leaves, indicating a physiological inactivation of iron. Studies on Fe-chlorosis are often controversial on the significance of the some nutrient ratios, mainly P/Fe, K/Ca and Fe/Mn on the development of lime-induced chlorosis.

Iron deficiency preferentially inhibits the development of the photosynthetic membranes, and its effects are particularly confined to the chloroplasts, while other cell organelles seem to be unaffected (Platt-Aloia et al., 1983). The membrane lipid composition of chloroplasts depends on plant nutritional status and in general, on all factors affecting the membrane staking degree (Horvath et al., 1987; Murata et al., 1990; Murphy and Woodrow, 1983).

Lipids are recognized as major chemical components of biomembranes. Thylakoid membranes contain lipids arranged in a double-layered structure. Most of the chloroplast acyl lipids are located in the lamellae (70%) and osmiophilic globuli. 70% of these lipids correspond to unsaturated chargeless galactolipids (MGDG and DGDG) and all the others (PG, PC and SQDG) are anionic lipids (Leech and Murphy, 1976).

Most of the knowledge about iron-deficient plants has been obtained with annual plants

(bean, sugar beet, barley and sunflower) grown in nutritive media (with and without iron) and then subjected to iron stress. These plants are usually grown in greenhouse or culture chamber, under controlled conditions of light, photoperiod, temperature and humidity (Newman, 1964; Nishio and Terry, 1983). However, cultures developed in field conditions affected by environmental stresses whose physiological effects over the thylakoid lipid membranes have received little attention. The purpose of the present study is to show the influence of induced iron deficiency on nutrient elements, their ratios and chloroplast membrane lipid composition of healthy, moderate and strong chlorotic peach trees grown in field conditions.

Materials and methods

About 40–60 leaves (5th–8th fully developed in the year growth) of each tree were harvested on adult peach trees in July (*Prunus persica* L. cv. Miraflores) grown on calcareous soils (pH = 7.9) in Zaragoza (Spain).

Extractable iron

The determinations were carried out by using α,α'-dipyridyl in order to extract the active iron in leaves, according to Abadía et al. (1984). The extracts were purified by a Sep-Pak, C_{18} cartridge (Millipore) and its absorbance measured at 522 nm.

Mineral elements determination

Leaves were carefully washed with a soft brush and liquid soap (1%) and rinsed with tap water and deionized water to eliminate surface contamination. Dry ashing was carried out following the methods of 'Comité Inter-Institutos' (C.I.I., 1969) and Pinta and DeWele (1975). Ca, Mg, Fe, Mn, Cu and Zn were determined by atomic absorption spectroscopy, K and Na by flame emission and P by the vanodomolibdophosphoric method.

Pigment determination

Pigments were extracted from leaf disks cut with a cork borer (0.358 cm^2) which were ground with 5 mL of 100% acetone and a few mg of sodium ascorbate to prevent the formation of phaeophytins. The quantitative determination was made by reverse phase HPLC according to the method of Val and Monge (1990).

Polar lipids determination

Thylakoid polar lipids were extracted according to the method described by Bligh and Dyer (1959). Later they were resolved by TLC following the method of Trémolières and Lepage (1971). Methyl-esters of each lipid were obtained by trans-esterification carried out by the methanol-sulphuric method (Calvo et al., 1988). Quantification was performed by using gas chromatography (HP 5840A GC), equipped with a flame ionised detector and a 2 m column (15% DEGS in 80/100 Chromosorb). Methyl pentadecanoate (15:0) was used as internal standard. The identification of each fatty acid was made by comparing their retention times with commercially available standards.

Results

The levels of total Chl were significantly decreased under iron chlorosis (Tables 1a and b). We classified into three groups according to their contents in chlorophyll per leaf area. Leaves under severe iron deficiency (**S**) consisted of samples with a chlorophyll concentration below 10 $nmol.cm^{-2}$ (average 8.04 $nmol.cm^{-2}$); (**L**), between 15–35 $nmol.cm^{-2}$ (mild iron deficiency) with an average level of 20.75 $nmol.cm^{-2}$ and (**C**) above 35 $nmol.cm^{-2}$ control.

The levels of several macro (N, Ca, Mg and Na) and micronutrients (Mn and Zn) were not significantly affected by iron chlorosis, however P, K, Fe and Cu concentrations were significantly different under iron chlorosis (Tables 1a and 1b). The iron extracted with α,α'-dipyridyl decreased significantly in (**S**) and (**L**) cases and was also positively correlated with chlorophyll (Table 2). Phosphorus and P/Fe ratio were significantly

Table 1a. Averages and standard deviations (St) of chlorophyll concentrations (nmol/cm^2) and macronutrient levels (mg/100 mg dry matter) of peach trees (cv. Miraflores) under several iron deficiency degrees ($n = 10–12$)

Sample		Chl-t	N	P	Ca	Mg	K	Na	P/Fe	K/Ca
S	Average	8.04	3.73	0.35	1.39	0.42	2.73	0.07	47.43	2.05
S	St	1.75	0.47	0.04	0.31	0.03	0.20	0.01	6.16	0.44
L	Average	20.75	3.69	0.30	1.31	0.46	2.37	0.08	33.51	2.44
L	St	2.38	0.50	0.03	0.42	0.05	0.27	0.01	5.56	2.24
C	Average	35.21	4.01	0.25	1.58	0.45	2.27	0.07	23.63	1.45
C	St	3.39	0.31	0.02	0.21	0.05	0.32	0.01	2.07	0.24

Chl-t, total chlorophyll.
Sample: C, control leaves; L, leaves under mild iron deficiency; S, severe deficiency.

Table 1b. Averages and standard deviations (St) of chlorophyll concentrations (nmol/cm^2), micronutrients levels (mg kg^{-1}) and active iron (μg/cm^2) of peach trees (cv. Miraflores) under several iron deficiency degrees ($n = 10–12$)

Sample		Chl-t	Fe-a	Fe	Mn	Cu	Zn	Fe/Mn
S	Average	8.04	1.72	74.41	40.00	9.98	28.42	1.86
S	St	1.75	0.31	5.78	0.88	0.67	1.80	0.18
L	Average	20.75	2.54	88.93	41.05	10.83	30.19	2.17
L	St	2.38	0.37	6.58	2.16	0.52	1.82	0.20
C	Average	35.21	3.67	105.37	39.66	11.30	29.60	2.66
C	St	3.39	0.41	4.40	1.67	0.54	1.17	0.19

Chl-t, total chlorophyll; Fe-a, active iron.
Sample: C, control leaves; L, leaves under mild iron deficiency; S, severe deficiency.

Table 2. Regression equations and variance analysis among total chlorophyll and the significant nutrients

Elements	Regression	Coefficient	Significant
Fe-a	$Y = 0.12 + 5.35X$	0.94	***
P	$Y = 0.38 - 3.77X$	0.85	***
K	$Y = 2.82 - 0.02X$	−0.59	**
Fe	$Y = 65.87 + 1.11X$	0.91	***
Cu	$Y = 9.78 + 0.04X$	0.64	**
P/Fe	$Y = 5.34 - 8.69X$	−0.90	***
Fe/Mn	$Y = 1.60 - 0.03X$	0.89	***

** and *** mean significant at the $p < 0.01$ and $p < 0.001$ levels respectively.
Y = elements; X = total chlorophyll.

higher in (**S**) and (**L**) cases under iron chlorosis, nevertheless, the Fe/Mn ratio was significantly different under the three treatments, but increased as the total chlorophyll decreased.

The analysis of lipid extracts performed by TLC revealed the existence of MGDG, DGDG, PC and PG, but no SQDG was detected. Table 3 shows the average values (percent) as well as the standard deviations of different lipids and the total glycolipid (DGDt) and phospholipid (PLt) concentrations. These lipids were differentiated in their major lipids: MGDG, DGDG and PG, PC, respectively.

Differences in total galactolipids (DGDt) amongst the treatments (**C**, **L** and **S**) were due to a diminution of MGDG in **L** samples, while under severe deficiency (**S**) the decrease in MGDG was also accompanied by a reduction in the DGDG concentration. Iron deficiency acts by diminishing galactolipids.

Table 3. Percent composition of galactolipids and phospholipids from peach tree thylakoid membranes (cv. Miraflores), under several degrees of iron chlorosis

Sample	GDG(t)	MGDG	DGDG	PL(t)	PG	PC
C	60.42	22.44 ± 1.49	37.98 ± 2.32	39.58	28.91 ± 2.70	10.67 ± 1.06
L	56.23	19.69 ± 1.72	36.54 ± 2.16	43.76	28.71 ± 1.63	15.05 ± 2.09
S	51.68	20.26 ± 2.31	31.43 ± 2.12	48.32	22.26 ± 2.15	26.06 ± 2.74

The results are the average of six determinations ± standard error.
Sample: C, control leaves; L, leaves under mild iron deficiency; S, severe deficiency.

Iron deficiency also alters the phospholipid levels. The percent of total phospholipids (PLt) increased with the degree of iron deficiency. Of the phospholipids, the iron deficiency affected mainly PC. In **L** cases, PG suffered no modification, while PC was affected. However, **S** thylakoids have shown a slight diminution of PG corresponding with a strong relative increment of PC.

The fatty acid analysis reveal that the light iron deficiency (**L**) does not disturb the fatty acid composition of lipid thylakoid membranes, while a strong deficiency (**S**) changes the proportion of some fatty acids (Table 4).

The analysis of these results demonstrates that the 18:3 proportion of PG and PC fractions, from **S** samples, decreases practically to half, while the 18:0 proportion is increased consequently. The same variation seems to occur with the 16:1t and 16:0 fatty acids, while 16:lt is reduced from 40% to 23%, the 16:0 is increased in the same proportion. The acid trans-hexadecenoic (16:lt) only could be detected in the PG, while cis is the usual form, amongst the other lipids.

Discussion

When yellowing of the leaves from plants grown in calcareous soils appears, it could be due to several causes. In most cases, it is caused by iron-deficiency which is determined by analysis of physiological active iron in leaves (Abadía, et al., 1984). The high correlation between extractable iron and Chl (Table 2) demonstrated that

Table 4. Percent composition of fatty acids from galactolipids and phospholipids from peach tree thylakoid membranes (cv. Miraflores), under strong iron deficiency

Sample	Fatty cid	MGDG	DGDG	PG	PC
C	16:0	6.88 ± 0.70	20.80 ± 1.60	14.84 ± 1.80	38.20 ± 0.26
S		8.10 ± 0.60	19.60 ± 1.50	39.93 ± 2.20	44.40 ± 3.60
C	16:1	4.21 ± 0.60	3.61 ± 0.60	40.70 ± 3.30[a]	2.66 ± 0.30
S		1.23 ± 0.15	0.63 ± 0.20	23.48 ± 1.60[a]	traces
C	18:0	1.06 ± 0.18	3.11 ± 0.24	0.70 ± 0.20	8.04 ± 0.80
S		2.76 ± 1.80	12.65 ± 1.00	4.56 ± 0.50	19.90 ± 1.40
C	18:1	2.00 ± 0.24	1.82 ± 0.20	2.96 ± 0.20	7.40 ± 0.60
S		2.68 ± 0.20	1.92 ± 0.22	6.38 ± 0.82	6.75 ± 0.64
C	18:2	4.34 ± 0.60	1.91 ± 0.24	6.80 ± 0.80	24.77 ± 2.10
S		5.58 ± 0.70	1.80 ± 0.24	8.60 ± 0.80	18.30 ± 1.40
C	18:3	81.51 ± 6.20	68.75 ± 4.60	34.00 ± 2.60	19.00 ± 1.40
S		79.64 ± 6.60	63.40 ± 5.80	17.06 ± 1.80	10.64 ± 1.20

[a] Trans-hexadecenoic acid. The results are the average of six determinations ± standard error.
Sample: C, control leaves; S, leaves under severe deficiency.

the lack of Chl in the peach tree leaves studied was induced by iron-deficiency.

In our study, phosphorus and total iron contents were affected by iron chlorosis. The P is probably involved in the interaction with iron nutrition (Bindra, 1980; DeKock, 1981). In chlorotic leaves, the P levels are higher than in the healthy ones, while the iron contents were lower in chlorotic than in control leaves. The P/Fe ratio was significantly affected in both chlorotic samples (**S** and **L**). Although Abadía et al. (1985) and Heras et al. (1976) pointed out the significance of the K/Ca ratio in chlorotic samples, in our experiment, the K/Ca ratio was significantly affected, nevertheless the Fe/Mn ratio was affected. This is in agreement with Bindra (1980), which has shown that there is a close relationship between Fe/Mn proportions and iron chlorosis.

Total polar lipid concentrations in thylakoid membranes from peach tree leaves diminished in relation to iron deficiency, but not all in the same proportion. A peculiarity of iron deficiency is the lack of thylakoid membrane staking organization (grana) in the chloroplasts of leaf cells (Platt-Aloia et al., 1983). In our study, this iron deficiency probably induced the lack of staking organization, diminutions of galactolipids and concomitantly an increase in phospholoids, being the PC the more increased. These results are in agreement with those found by Abadía et al. (1988), which could indicate that lipid levels depend on the species and growth conditions. It is necessary to bear in mind that peach tree chloroplasts, in our particular field conditions, were exposed to very high light intensities.

The lack of iron also modified the fatty acid composition. A lower unsaturated degree of galacto and phospholipids were due to an increase in saturated fatty acids. Similar results were found by Abadía et al. (1988) and Newman (1964).

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