EFFECT OF IRON SUPPLY ON GROWTH AND PHOTOSYSTEM II EFFICIENCY OF PEA PLANTS

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Summary. Pea plants, grown hydroponically, were supplied with different amounts of iron (Fe) ranging from complete deficiency to toxicity. Plant growth, chlorophyll and carotenoid content and chlorophyll fluorescence parameters were recorded at 7-day intervals from day 20 to day 41. Growth decrease, caused by Fe deficiency or excess as a rule accrued with strengthening and prolongation of the imposed stress. The observed decrease in relative growth rate (RGR') was mainly due to the decrease of its physiological component, the net assimilation rate. Being only slightly affected, its morphological component, the leaf area ratio (LAR′) was considered as a dynamic component, too. Opposite trends in the changes of is components, leaf weight ratio (LWR) and specific leaf area (SLA), were observed under Fe deficiency. Under partial Fe deficiency RGR′ was high during the last week of the experiment, suggesting that plants had been adapted to insufficient Fe supply. Stresses differed in regard to their effects on SLA, which reflects leaf morphological peculiarities. SLA tended to increase under Fe deficiency and to decrease under excess Fe supply. Fe deficiency was characterized by low pigment content and high chlorophyll a/chlorophyll b and carotenoids/chlorophylls ratios when chlorosis was well manifested. Significant changes in chlorophyll fluorescence were observed only in strongly chlorotic plants. Plants deprived from iron were marked by low actual PS II efficiency (Φ_{PSII}) at days 34 and 41, which was due to lower proportion of open PS II centers (qP) and to their lower excitation capture efficiency (Φ_{exc}). The maximum quantum yield of PS II (F_v/F_m) decreased, too. Excess Fe caused rise in pigments content and only slight disturbance in their ratio.

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Chlorophyll fluorescence parameters suggested increased thermal dissipation lacking any other significant changes in PS II activity.

Key words: chlorophyll fluorescence, chlorosis, deficiency, excess, Fe, growth analysis, iron, pea

Abbreviations: Chl - chlorophyll, DM - dry biomass; Fe - iron; LA- leaf area; PS II-photosystem II

INTRODUCTION

Due to iron redox properties and its ability to form complexes with diverse ligands, this element is constituent of many electron carriers and enzymes, thus playing an important role in plant metabolism. On the other hand, low solubility of inorganic iron at physiological pH and its high reactivity in presence of oxygen, which brings to generation of toxic hydroxyl radicals, represent severe difficulty (Hell and Stephan, 2003). Soil conditions, causing insufficient (for instance - calcareous soils) or excess (acid soils, water logging) Fe uptake, are widespread in nature. Growth alteration under Fe deficiency or toxicity, characterized by different biometric parameters such as fresh and dry biomass accumulation, shoot and root length, number and area of leaves, has been reported for various plant species grown under natural or laboratory conditions. In a number of studies the relative growth rate (RGR) has been applied to evaluate Fe efficiency and tolerance of plants at suboptimal and supra-optimal Fe concentration (Snowden and Wheeler, 1993; Schmidt and Fühner, 1998; de la Guardia and Alcántara, 2002a).

Chlorosis of young leaves is often the first visual sign of iron deficiency. It is associated not only with loss of chlorophyll, as several steps of its biosynthesis depend on Fe, but also with changes in the expression and assembly of other components of the photosynthetic apparatus (Terry and Abadía, 1986). On the other hand, evidence exists that Fe excess increases cytochrome b6/f complex in thylakoids (Suh et al., 2002). In recent years chlorophyll fluorescence analysis has been applied as a rapid non-destructive tool to obtain information about the state of photosynthetic apparatus, and especially of photosystem II (PSII), under Fe deficiency or toxicity. It has been demonstrated that photoprotection through excessive light dissipation as well as reversible photosynthesis down-regulation and sustained photoinhibitory damage might occur (Kampfenkel et al., 1995; Abadía et al., 1999; Morales et al., 2000; Gogorcena et al., 2001; Donnini et al., 2003).

Plant reaction towards inadequate Fe supply is a complex phenomenon. Growth reduction due to excess Fe takes place above a threshold Fe concentration in the medium, but does not depend directly on Fe concentration in aerial plant tissues (Batty and Younger, 2003). Sometimes under Fe deficiency severe chlorosis may develop without producing any effect on growth (Gogorcena et al., 2001). On the

other hand, growth depression can occur before the start of the leaf yellowing or even without yellowing (Kosegarten et al., 1998; Gruber and Kosegarten, 2002).

The present work reports results on growth analysis, photosynthetic pigment concentration measurement and chlorophyll fluorescence analysis, which were applied in order to track dynamic changes in growth and PS II activity in pea plants developing under different iron supply, ranging from complete deficiency to toxicity.

MATERIALS AND METHODS

Growth conditions

Pea plants (*Pisum sativum L.* cv. Manuela) were grown hydroponically in a growth chamber (air temperature 22-25^{0}C, relative air humidity about 60%, irradiance 110 μmol photon.m^{-2}.s^{-1}, 14/10 h day/night period). After germination on wet filter paper, seedlings were transferred into containers with half strength Hoagland-Arnon nutrient solution I with micronutrient supply according to modified Hoagland's "A-Z" solution (Hoagland and Arnon, 1938). At day 10 after seed soaking, plants were supplied with different amounts of iron in the form of Fe (III) ethylenediamine tetraacetate and cotyledons were removed. The control plants were supplied with 2.0 mg.l^{-1} Fe. Nutrient solution has been changed every 3-4 days. The analyses were made at day 20, 27, 34 and 41 after seed soaking.

Pigment concentration and chlorophyll fluorescence

The youngest fully expanded leaves were used for these analyses. Concentrations of chlorophyll a (Chl.a), chlorophyll b (Chl.b) and carotenoids were measured in three replicates after acetone extraction according to Arnon (1949) and calculated according to McKinney (1941). Chlorophyll fluorescence was measured in four replicates in leaf discs by a pulse modulation chlorophyll fluorometer (PAM 101, H. Walz, Effeltrich, Germany) after 5 min of dark adaptation, using actinic light at 100 μmol.m^{-2}.s^{1} and saturating light at 3500 μmol.m^{-2}.s^{-1} photon flux density. Minimal chlorophyll fluorescence in the dark (F_0), maximum fluorescence in dark and in light (F_m and F_m', respectively), fluorescence at steady-state photosynthesis (F_t) and fluorescence after switching off the actinic illumination (F_0') were recorded. Variable fluorescence $F_v=F_m-F_0$; maximum quantum yield of PS II = F_v/F_m; actual quantum yield of PS II $\Phi_{PS\ II}=(F_m'-F_t)/F_m'$; photochemical quenching $qP=(F_m'F_t)/(F_m'-F_0')$; non-photochemical quenching $NPQ=(F_m-F_m')/F_m'$ were calculated according to Maxwell and Johnson (2000). The intrinsic PS II efficiency $\Phi_{exc}=(F_m'-F_0')/F_m'$ and the relative amount of light absorbed by PS II and dissipated thermally $D=F_o'/F_m'$ were calculated according to Abadía et al. (1999).

Growth analysis

Shoot length, fresh biomass of leaves, stems and roots, as well as leaf area (LA) from six plants were measured at each harvest. The content of dry biomass in a unit of fresh biomass was determined after drying the plant material at 105 ^{o}C to a constant weight and the values were used to calculate the dry biomass (DM) of different organs per plant. Parameters of classical growth analysis were computed on a dry biomass basis according to Kerin et al. (1997). Some of them were average values for the 7-day periods between harvests: mean growth rate (GR′) - total DM increase per day; mean relative growth rate (RGR′) - total DM increase per total DM per day; mean net assimilation rate (NAR′) - total DM increase per LA per day; mean leaf area ratio (LAR′) - LA increase per total DM. Other parameters were indicative of the date of harvest: leaf area ratio (LAR) - LA per total DM; specific leaf are (SLA) - LA per leaf DM; leaf weight ratio (LWR), stem weight ratio (SWR) and root weight ratio (RWR) representing the DM of leaves, stems and roots per unit of total DM, respectively.

Two independent experiments were conducted. Data are means of a representative experiment. Values followed by the same letter are not significantly different at P=0.05 according to the Student's t-test.

RESULTS AND DISCUSSION

Growth depression under Fe deficiency developed gradually and depended on Fe concentration. At day 20 under complete deficiency the shoot length and dry biomass of all vegetative organs decreased by no more than 20% (Table 1). Reduction of shoot growth became more evident with time and for leaf DM it was about 30, 50, and 60% at day 27, 34 and 41, respectively. Root biomass reduction was less manifested and because of this RWR increased by up to 48% (Table 2). Biomass allocation towards the roots is not specific for Fe deficiency stress, and might be a mechanism for increasing soil volume exploration. It might be explained by increased phosphoenolpyruvate carboxylase activity and higher dark CO_2 fixation in roots, as well as by assimilates partitioning to roots from lower leaves in conditions of limited shoot growth (de la Guardia and Alcántara, 2002 b). Growth analysis revealed decreasing GR′ for the three examined periods and RGR′ decreased by 55% after day 27. Inhibition of RGR′ was basically due to a decrease in its physiological component, NAR′, while the morphological component of RGR′, LAR′, did not change by more than ±6%. Lower photosynthetic rate and higher dark respiration rate might explain the inhibited NAR′.

As compared to plants deprived of Fe, growth inhibition of plants supplied with 0.1 mg.l^{-1} Fe appeared later and was less pronounced (Tables 1 and 2). It was best exhibited at day 34 when shoot length, DM of leaves and stems were de-

Table 1. Effect of Fe supply on growth, chlorophyll and carotenoid concentrations (mg.g^{-1} fresh biomass) in pea plants. At each harvest date values with same letters are not significantly different at P = 0.95 according to Student's t-test.

Fe (mg.l^{-1})	Shoot length (cm)	Dry biomass (mg.plant^{-1})			Pigment content (mg.g^{-1} FW)		Chl.a / Chl.b	Chl.(a+b) / Caroten.
		Leaves	Stems	Roots	Chl.(a+b)	Caroten.		
Day 20								
0.0	6.5bc	31.5 b	17.8 a	18.7 b	2.063ab	0.151 b	1.94 a	13.65 a
0.1	7.3ab	42.8 a	20.7 a	28.0 a	2.161ab	0.170 a	2.02 a	12.72ab
2.0	7.5 a	37.9ab	21.0 a	23.3ab	2.079 b	0.165ab	2.06 a	12.63ab
10.0	6.5abc	39.5ab	20.7 a	24.2ab	1.957 b	0.176 a	2.02 a	11.13 b
40.0	5.8 c	32.2ab	16.8 a	18.7 b	2.355 a	0.189 a	2.04 a	12.45 b
Day 27								
0.0	9.7 b	70.9 b	36.3 a	47.4 a	1.600 b	0.152 d	2.08 c	10.55 b
0.1	10.8ab	86.5ab	43.6 a	54.7 a	2.161 a	0.170cd	2.02 c	12.72 a
2.0	11.5 a	99.0 a	45.0 a	49.5 a	2.137 a	0.186bc	2.12 bc	11.51ab
10.0	11.5ab	95.9ab	46.8 a	47.5 a	2.257 a	0.207ab	2.17 b	10.95ab
40.0	10.7ab	84.0ab	41.8 a	43.0 a	2.219 a	0.225 a	2.30 a	9.90 b
Day 34								
0.0	13.3 d	94.7 b	54.2 c	52.4 a	0.446 d	0.066 c	2.49 a	6.72 b
0.1	15.3bc	121.1 b	74.1 b	65.5 a	1.885 c	0.162 b	2.17 bc	11.79 a
2.0	18.5 a	191.2 a	95.8 a	60.9 a	2.348ab	0.175ab	1.98 c	13.59 a
10.0	17.3ab	182.5 a	85.9ab	61.8 a	2.223 b	0.181ab	2.10 b	12.25 a
40.0	13.7cd	145.1 b	67.6bc	50.2 a	2.616 a	0.208 a	2.11 b	12.58 a
Day 41								
0.0	15.2 d	120.6 c	73.4 c	62.0 b	0.180 c	0.038 c	3.66 a	4.80 c
0.1	23.2bc	244.9 b	165.2ab	77.3ab	1.693 d	0.168 b	2.30 b	10.1 b
2.0	27.2 a	325.0 a	173.1 a	97.0 a	2.211 b	0.194ab	2.04 c	11.40ab
10.0	24.5 b	262.9 b	129.2b	101.0a	2.422 b	0.223 a	2.11bc	10.80 b
40.0	20.7 c	203.0 b	110.1b	86.4ab	2.825 a	0.215 a	2.05 c	13.13 a

creased by 17, 36 and 23%, respectively, and RWR was increased by 43%. Between days 27-34 RGR′ diminished by 42% due to a drop in NAR′ by 31% and a drop in LAR′ by 15%. At the end of the experiment the growth of these plants was

Table 2. Growth analysis of pea plants grown under different iron supply. At each harvest date values with same letters are not significantly different at P = 0.95 according to Student's t-test.

Fe ($mg.l^{-1}$)	Parameters for given period				Parameters at a given day				
	GR′ $mg.d^{-1}$	RGR′ $mg.g^{-1}d^{-1}$	NAR′ $mg.cm^{-2}.d^{-1}$	LAR′ $cm^2.g^{-1}$	LAR $cm^2.g^{-1}$	SLA $cm^2.g^{-1}$	LWR $g.g^{-1}$	SWR $g.g^{-1}$	RWR $g.g^{-1}$
	day 20 - day 27				day 20				
0.0	12.3	117	0.401	292	299 a	650 a	0.460 a	0.262 a	0.278 a
0.1	13.3	100	0.347	289	291 a	626 ab	0.466 a	0.228 b	0.306 a
2.0	15.9	122	0.402	304	288ab	624 ab	0.461 a	0.253ab	0.286 a
10.0	15.1	116	0.386	301	274ab	593 bc	0.464 a	0.251ab	0.285 a
40.0	14.5	131	0.468	280	257 b	556 c	0.464 a	0.257ab	0.280 a
	day-27 - day 34				day 27				
0.0	6.7	38	0.117	322	287 b	631 a	0.456 b	0.237 a	0.307 a
0.1	10.8	49	0.168	293	291 b	620 a	0.469 b	0.238 a	0.293ab
2.0	22.1	84	0.244	344	320 a	625 a	0.512 a	0.232 a	0.256bc
10.0	20.0	79	0.242	326	321 a	637 a	0.504 a	0.249 a	0.248bc
40.0	13.4	63	0.201	315	299ab	602 a	0.497 a	0.262 a	0.241c
	day 34 - day 41				day 34				
0.0	7.8	34	0.094	366	358 a	760 a	0.471 b	0.270 a	0.259 a
0.1	32.4	89	0.271	330	297 c	641bc	0.464 b	0.285 a	0.251 a
2.0	35.3	77	0.211	363	367 a	668 b	0.549 a	0.276 a	0.175 b
10.0	19.0	48	0.139	347	330 b	598 d	0.552 a	0.260 a	0.188 b
40.0	10.9	37	0.107	343	342ab	618cd	0.554 a	0.262 a	0.185 b
					day 41				
0.0					375ab	793 a	0.473 b	0.287 a	0.240 a
0.1					360ab	723abc	0.510ab	0.332ab	0.159 b
2.0					362 a	667 b	0.544 a	0.289 a	0.167 b
10.0					343 b	644 b	0.534 a	0.261 b	0.206ab
40.0					298 c	589 c	0.505ab	0.277ab	0.217 a

less affected. Obviously, plants had been adapted to low Fe supply by switching to some mechanisms of better Fe mobilization from solution (Hell and Stephan, 2003) and/or for its more efficient utilization. For the period between days 34 and 41

RGR′ was increased by 16%, and the increase in NAR′ was even greater - by 28%, while LAR′ dropped by 9%.

More detailed look at LAR components revealed that although its values were often close to those of control plants under complete and partial deficiency, it was a dynamic component, too. At day 20 LAR, SLA and LWR remained unchanged. Since day 27 the portion of leaf DM in the total biomass had decreased and LWR under both degrees of deficiency had dropped by 10-15%. "Adapted" plants, grown under partial deficiency at day 41, were the only exception. At day 27 low LWR was the cause for the LAR decrease by about 10%. With aggravation of Fe deficiency in plants not supplied with Fe SLA started to increase by 14% and 19% at days 34 and 41, respectively. According to Kerin and co-workers (1997) decreased thickness of the leaves is due to morphological changes and reflects the altered proportions between assimilatory, mechanical and vascular tissues. Opposite trends of LWR and SLA changes resulted in almost unaffected values of LAR at days 34 and 41 under complete deficiency.

Excess Fe also caused growth reduction and depended on Fe concentration in the solution. Shoot length and biomass under 40 mg. l^{-1} Fe lessened by 25-40% at day 34 and 41, the inhibition being stronger at the end of the experiment. At day 41 significant inhibition of growth was registered in plants grown under 10 mg. l^{-1} Fe as well, but it was less pronounced as compared to that found under the highest Fe concentration. Excess Fe did affected less the root growth so RWR grew up, which did not support the findings of Snowden and Wheeler (1993) that root growth of most investigated dicotyledonous fen species was more susceptible than shoot growth, but coincided with the observed changes in common reed (Batty and Younger, 2003). Under 40 mg. l^{-1} Fe during the second and the third period RGR′ decreased by 25% and 52%, respectively, while under 10 mg. l^{-1} Fe it dropped by 38% between days 34-41. Low NAR′ was again the main reason for the changes observed in RGR′. Iron toxicity, involving formation of reactive oxygen species, might be the reason for growth inhibition. On the other hand, growth inhibition may take place before toxic Fe concentration in plant tissues is reached, thus suggesting an impeded uptake of other nutrients (Batty and Younger, 2003). The morphological component of RGR′, LAR, decreased by not more than 10%, except for plants supplied with 40 mg.l^{-1} Fe at the end of the experiment. In the case of toxicity, its decrease was mainly attributed to lower SLA i.e. to morphological changes, driving to formation of small thick leaves.

A decrease of photosynthetic pigments under Fe deficiency as a rule followed the same trend as growth depression (Table 1). Under complete deficiency at days 27, 34 and 41 total chlorophyll concentration decreased by 25, 81 and 92 %, respectively. Under partial deficiency the decrease was about 20% at days 34 and 41. The drop in carotenoid concentration was less pronounced, by not more than 80%. So the chlorophylls/carotenoids ratio diminished by more than 50% in plants supplied with

no Fe at days 34 and 41. In these plants the chl. a/chl. b ratio increased by 26% and 79% at days 34 and 41, respectively. The same, but less pronounced variation of pigments proportions were found with partial Fe deficiency.

A high chl. a/chl. b ratio, observed usually in strongly chlorotic plants, indicates a reduced antenna size relative to reaction centers (Terry and Abadía, 1986; Gogorcena et al., 2001). More detailed analysis of carotenoids revealed that the higher carotenoid/chlorophylls ratio was due to higher lutein/chl and xanthophylls/chl ratios. Violaxanthin cycle was fully functional and was supposed to be involved in photoprotection by rising the amount of light, dissipated within the PSII antenna (Abadía et al., 1999; Gogorcena et al., 2001; Donnini et al., 2003). In our experiments, higher thermal dissipation in chlorotic leaves at day 41 by up to 59% was found when estimated by D (Table. 3). An increase in NPQ was also anticipated, but not found. This parameter detects changes in efficiency of heat dissipation relative to the dark-adapted state and according to Maxwell and Johnson (2000) might give ambiguous information when used for comparison between leaves with different history. Activated photoprotective mechanisms were not sufficient to prevent photoinhibition. At steady-state photosynthesis the actual efficiency of PS II under

Table 3. Effect of Fe supply on chlorophyll fluorescence parameters of pea plants. At each harvest date values with same letters are not significantly different at P = 0.95 according to Student's t-test.

Fe ($mg.l^{-1}$)	$\Phi_{PS\ II}$	qP	Φ_{exc}	F_v/F_m	F_v/F_0	NPQ	D
Day 34							
0.0	0.411 b	0.622 b	0.654 a	0.692 b	2.369 b	0.320 b	0.347 a
0.1	0.544 a	0.760 a	0.719 a	0.788 a	3.737 a	0.425 b	0.281 a
2.0	0.539 a	0.776 a	0.698 a	0.796 a	3.921 a	0.504ab	0.302 a
10.0	0.481ab	0.765 a	0.630 a	0.778ab	3.528 a	0.774 a	0.370 a
40.0	0.533ab	0.795 a	0.673 a	0.776ab	3.471 a	0.419 b	0.327 a
Day 41							
0.0	0.332 b	0.588 b	0.563 c	0.643 b	1.862 b	0.443ac	0.437 a
0.1	0.523 a	0.795 a	0.658 b	0.778 a	3.511 a	0.476bc	0.342 b
2.0	0.547 a	0.755 a	0.725 a	0.789 a	3.768 a	0.462 c	0.275 c
10.0	0.518 a	0.775 a	0.669ab	0.779 a	3.559 a	0.611 a	0.331bc
40.0	0.510 a	0.777 a	0.656 b	0.780 a	3.543 a	0.641ab	0.344 b

complete Fe deficiency decreased by 24 and 39% at days 34 and 41, respectively. Low $\Phi_{PS\ II}$ was due to both low proportion of open PS II centers (qP) and low excitation capture efficiency (Φ_{exc}). Under complete deficiency qP dropped by about 20% at days 34 and 41, but Φ_{exc} was inhibited by 22% only at day 41. In plants supplied with 0.1 mg. l^{-1} Fe only a 9% decrease in Φ_{exc} at the end of the experiment was found. The decrease of the maximum quantum yield of PS II, as pointed by the 14-18% drop in F_v/F_m, suggested also development of sustained photoinhibitory damages. The F_v/F_0 ratio, which is also sensitive to changes in efficiency in dark-adapted state, decreased by 40-50%, as well.

Excess Fe resulted in risen pigment concentrations by up to 28%. Some changes in the pigment ratios were also observed, suggesting that their increased content could not be attributed only to "concentration" effect due to inhibited growth. At the end of the experiment NPQ and D increased by 20-40% under both 10 and 40 mg. l^{-1} Fe. Kampfenkel and co-workers (1995) observed similar changes when inducing strong Fe excess upon root cutting of *Nicotiana plumbaginifolia* plants. They also found proofs for photoinhibition, as the F_m/F_0 ratio (hence the F_v/F_m ratio) diminished. Suh and co-workers (2002) found also proofs for photoinhibition in pea. In our experiments Φ_{exc} decreased by 9% at day 41 in plants supplied with 40 mg. l^{-1} Fe, but no other significant changes in photochemical quenching parameters were found. One might suppose that the stress was not strong enough and/or not prolonged enough in order to provoke strong inhibition of PS II activity.

To summarize, when plants are not supplied with an optimum amount of Fe, growth inhibition and physiological changes develop gradually, depending on the strength and duration of the imposed stress. Growth analysis, with all its components, is a very useful tool to bring forward subtle deviations in growth. Chlorophyll concentration is also very sensitive to Fe supply. Chlorophyll fluorescence analysis can give appreciable data under more severe stress.

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