Occurrence and correction of chlorosis in young petunia plants

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ABSTRACT: A glasshouse pot experiment tested the effects of 14 different combinations of substrate type, pH and nutrient treatments on the occurrence and severity of leaf chlorosis in a susceptible variety of petunia. Plants grown at optimal pH level (4.7) in peat substrate with low limestone dose were symptom-free even without added micronutrients. Severe chlorosis occurred in plants grown at high pH in peat substrate with high limestone (pH 6.7) and in peat-bark-compost (pH 6.2); it was associated with decreased Fe and Mn content in leaves. Regular application of nutrient solution with low concentration of Fe, Mn, and other micronutrients as EDTA chelates greatly reduced chlorosis in plants grown in peat-bark substrate and in peat-bark-compost, and it improved Fe uptake. An exception was peat substrate at high pH level and high limestone where chlorosis was only partially reduced by this treatment. Regular application of Fe, Mn, and other micronutrients as sulphates or citrates had no substantial effect; only application of three additional substrate drenches of 30 mg/l Mn from Mn-EDTA and 90 mg/l Fe from either Fe-EDTA or Fe-EDDHA substantially corrected chlorosis and increased foliar Fe and Mn in cases where plants were grown in high limestone peat substrate.

Keywords: *Petunia* × *atkinsiana*; chlorosis; iron; manganese; iron chelates; Fe-EDTA; Fe-DTPA; Fe-EDDHA; Mn-EDTA

Abbreviations: Fe-EDTA – ferric salt of ethylenediaminetetraacetic acid; Fe-DTPA – ferric salt of diethylenetriaminepentaacetic acid; Fe-EDDHA – ferric salt of N,N'-ethylenediamine-di-(o-hydroxyphenylacetic acid); Mn-EDTA – manganese salt of ethylenediaminetetraacetic acid

Petunia and closely relative *Calibrachoa* belong among the Fe-inefficient plants that are very susceptible to neutral and above pH, and react by decreasing Fe uptake followed by chorosis and growth depression (Fisher et al. 2003; Smith et al. 2004a,b; Wik et al. 2006). In neutral and well-aerated soils iron occurs in a ferric form and most of it is bound in insoluble oxyhydroxide polymers with very low concentration of ferric ions in the soil solution. The situation gets much worse in soils of high pH (Guerinot, Yi 1994; Marschner 1995). Crop plants uptake iron mainly from natural or synthetic chelates which are therefore preferred as a Fe source in most of nutritional systems.

Several Fe chelates are characterized by different stability at high pH. Ferric salt of ethylenediaminetetraacetic acid (Fe-EDTA) contains 6% Fe and is stable below pH 6 but very unstable above pH 6.5 and only 5% Fe remains chelated at pH 7.5. Its effectiveness is very low in soils where the iron is displaced by other cations (Ca^{2+}, Zn^{2+}, Cu^{2+}) and is precipitated (Tills 1987; Reed 1996; De Kreij 1998; Pestana et al. 2003). It is valuable mostly as a relatively cheap foliar spray that does not tend to cause leaf damage (Tills 1987). Foliar spray of chlorotic *Calibrachoa* plants with Fe-EDTA at 60 mg/l Fe is more effective than foliar spray with ferrous sulphate. Higher concentrations increase chlorophyll content even more efficiently but can necrotize leaves (Fisher et al. 2003).

Ferric salt of diethylenetriaminepentaacetic acid (Fe-DTPA) contains 11% Fe and is stable at pH below 7 but above pH 7.5 only 60% Fe remains chelated (Tills 1987; Reed 1996). Ferric salt of N,N'-ethylenediamine-di-(o-hydroxyphenylacetic acid) (Fe-EDDHA) with 6% Fe was a very efficient source

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of Fe even in calcareous soils because of its stability at pH above 9 (Tills 1987; Reed 1996). Fe-EDDHA applied in nutrient solution at 1–4 mg/l Fe to severely chlorotic *Calibrachoa* plants grown in substrate with high pH was more efficient than Fe-EDTA which only worked at the highest concentration (Wik et al. 2006). A single drench with Fe-EDDHA at high concentrations from 20 to 80 mg/l Fe was more effective than a single drench of Fe-DTPA or a foliar application of ferrous sulphate, Fe-EDTA or Fe-DTPA (Fisher et al. 2003). Three soil drenches of 30 mg/l Mn from $MnSO_4$ and 90 mg/l Fe either from Fe-EDTA, Fe-DTPA, or Fe-EDDHA stimulated Fe and Mn uptake and nearly eliminated lime-induced chlorosis in petunia mother plants (Šrámek, Dubský 2008).

High substrate pH can also limit the uptake of manganese and its deficiency symptoms are similar to Fe deficiency symptoms. The two ions are antagonistic and several authors found a negative effect of iron chelates application on Mn uptake in the case of Fe-EDDHA in soybean plants (Roomizadeh, Karimian 1996; Ghasemi-Fasaei et al. 2003) and Fe-EDDHA and Fe-DTPA in petunia plants (Smith et al. 2004b).

The objective of this work was to compare the treatments consisting of different substrate composition, pH and concentrations and forms of Fe and Mn on mineral uptake, chlorosis and growth of petunia plants.

MATERIAL AND METHODS

The variety *Petunia* × *atkinsiana* Karma was chosen as the experimental plant because of its known enhanced susceptibility to the iron deficiency. Cuttings were rooted in peat-perlite substrate (ratio 70:30 by volume) amended with dolomitic limestone (2 g/l) and later transplanted into 10 cm plastic pots (volume 400 ml) containing one of four substrates. Altogether 14 different treatment combinations of substrate, lime and nutrients were applied (Table 1) with three replications with 8 plants per replicate arranged in a fully randomized design.

Treatments P3 and P12 were peat substrates with 1 g/l PG Mix fertilizer (14% N, 16% P_2O_5, 18% K_2O, 0.7% MgO) amended with dolomite limestone (85% $CaCO_3$, 5% $MgCO_3$) at the rates of 3 g/l (P3) and 12 g/l (P12). Treatment B was peat-bark substrate (ratio 60:40 by volume) amended with 2 g/l dolomite limestone, 6 g/l ammonium nitrate (27.5% N), 0.8 g/l Fosmag MK (25% P_2O_5), and 0.25 g/l potassium sulphate (50% K_2O). Treatment G was peat-bark-compost substrate (ratio 60:30:10 by volume) amended with 1.3 g/l dolomite limestone and 0.4 g/l ammonium nitrate.

All plants were pinched, cultivated in a greenhouse at the day/night temperature of 17/15°C and were irrigated manually, which, from April 2 to April 25, included nutrient solution in six-day intervals. All nutrient solutions contained 380 mg/l N

Table 1. Treatment combinations

Treatment	Substrate	Limestone (g/l)	Form of Fe and Mn in nutrient solution	Form of additional Fe and Mn
P3-W	P3	3	–	–
P3-S	P3	3	sulphates	–
P3-C	P3	3	citrates	–
P3-E	P3	3	EDTA chelates	–
P12-W	P12	12	–	–
P12-W-E	P12	12	–	Fe-EDTA, Mn-EDTA
P12-W-D	P12	12	–	Fe-DTPA, Mn-EDTA
P12-S	P12	12	sulphates	–
P12-C	P12	12	citrates	–
P12-E	P12	12	EDTA chelates	–
B-W	B	2	–	–
B-E	B	2	EDTA chelates	–
G-W	G	1.3	–	–
G-E	G	1.3	EDTA chelates	–

Fig. 1. A symptom-free plant evaluated on the scale (1–8) with the degree of chlorosis 1

(144 mg/l N–NH_4), 35 mg/l P, 330 mg/l K, and 35 mg/l Mg. Experimental treatments consisted of four different forms of micronutrients. Treatment W (combinations P3-W, P12-W, B-W, G-W) contained no micronutrients while all the other treatments included 1.4 mg/l Fe, 0.8 mg/l Mn, 0.5 mg/l Zn, 0.2 mg/l Cu, 0.5 mg/l B and 0.08 mg/l Mo. Treatment S (combinations P3-S, P12-S) contained Fe, Mn, Zn, and Cu in sulphate form. Treatment C (combinations P3-C, P12-C) contained Fe, Mn, and Zn in citrate form and Cu as sulphate. Treatment E (combinations P3-E, P12-E, B-E, G-E) contained Fe, Mn, Zn, and Cu as EDTA chelates. Boron and molybdenum were always applied as H_3BO_3 and Na_2MoO_4. Treatment combinations P12-W-E and P12-W-D were regularly fertilized without micronutrients and got additional three drenches of 30 mg/l Mn from Mn-EDTA and 90 mg/l Fe from either Fe-EDTA or Fe-DTPA (combinations P12-W-E, P12-W-D). High Fe doses were applied together with Mn, since previous works, mentioned above, showed that Fe chelates (Fe-DTPA, Fe-EDDHA) can decrease Mn uptake causing the deficiency (Roomizadeh, Karimian 1996; Ghasemi-Fasaei et al. 2003; Smith et al. 2004b).

The components and the substrates were analyzed for chemical properties according to the European Standards. Electric conductivity (EN 13 038), pH value (EN 13 037), and the content of available calcium (EN 13 652) were estimated in water extract 1:5 (v:v); content of other available nutrients (EN 13 651) was estimated in CAT extraction (0.01 mol/l $CaCl_2$ and 0.002 mol/l DTPA) with the extract ratio 1:5 (v:v).

Fig. 2. A severely chlorotic plant evaluated on the scale (1–8) with the degree of chlorosis 8

Table 2. Chemical properties of substrates at the beginning of the experiment, before planting of petunia plants

Substrate	pH	EC*	N**	P**	K**	Mg**	Ca**	Fe**	Mn**	Zn**	Cu**	B**	Mo**
P3	4.7	0.34	225	67	158	90	50	20.5	3.4	4.0	1.8	0.21	0.17
P12	6.6	0.30	164	24	141	146	69	15.7	4.2	3.0	1.6	0.17	0.08
B	5.1	0.31	190	28	191	97	78	34.5	25.6	7.0	1.3	0.48	0.02
G	5.3	0.38	159	43	577	130	58	38.8	19.9	6.6	1.6	0.78	0.03

*Electric conductivity (mS/cm), **available nutrients (mg/l)

At the end of the experiment the incidence and severity of leaf chlorosis was estimated visually. To set the degrees of chlorosis, a scale from 1 to 8 was used; symptom-free plants were evaluated 1, most severely chlorotic plants 8 (Figs. 1 and 2). Moreover, fresh and dry weight of the plants was evaluated. All the data sets were tested for normality and analyzed with one-way ANOVA (Unistat 5.2). The significance level $P = 0.05$ was used and significant differences between means were evaluated with the Duncan's multiple range test.

The content of macronutrients and micronutrients in the leaves was determined from milled samples mineralized in a microwave digestion appliance (Mileston model MLS 1200) according to the manufacturer's recommended procedure. Concentrations of P, K, Ca, Mg, and micronutrients were determined using inductively coupled plasma spectrometer (Thermo Jarrell Ash Trace Scan model ICP-OES). For determination of total nitrogen, leaf samples were mineralized according to Kjeldahl in H_2SO_4 with selenium, and were spectrometrically analyzed according to the recommended procedure (Skalar model SAN plus System).

RESULTS AND DISCUSSION

The substrate components differed in pH value, EC and available macronutrients and micronutrients. Compost and bark had sufficient natural content of all micronutrients with exception of molybdenum. Bark was very high in Mn, compost in Zn and B.

All substrates were within the acceptable range for available nutrients at the start of the experiment with exception of substrate G which was very high in available potassium (Table 2). Substrate composition and limestone doses in peat substrates affected their initial pH values as well as available nutrients content. The lowest pH was determined in the low-limestone substrate P3, the highest in the high-limestone substrate P12 (Table 2). This was consistent with our goal to set conditions unfavourable for micronutrients (especially Fe) uptake in this substrate. Substrate P12 contained more Ca and Mg and less Fe than P3. Substrate G was high in Mg and both peat-reduced substrates (B, G) were higher in nearly all micronutrients than peat substrates, only Mo and Cu were exceptions (Table 2).

By the end of the experiment there were no significant differences among substrates in available nutrients except for substrate G which remained very high in potassium. Substrates B and G had higher content of available Fe, Mn, Zn and B (50.3, 20.4, 7.38 and 0.62 in B; 55.1, 18.2, 8.19 and 0.80 in G,

Table 3. Degree of chlorosis (1–8) at the experimental treatments

Treatment	Chlorosis evaluation*
P3-W	1.0^{d}
P3-S	1.0^{d}
P3-C	1.0^{d}
P3-E	1.0^{d}
P12-W	6.7^{b}
P12-W-E	1.7^{d}
P12-W-D	1.7^{d}
P12-S	5.3^{c}
P12-C	7.7^{a}
P12-E	5.0^{c}
B-W	1.7^{d}
B-E	1.0^{d}
G-W	5.0^{c}
G-E	1.5^{d}

*1 – non-chlorotic leaves, 8 – fully chlorotic leaves, mean values with the same letter were not significantly different at the level $P = 0.05$

Table 4. Content of macronutrients (%) and micronutrients (ppm) in the leaves

Treatment	Ca	Mg	K	Fe	Mn	Cu	Zn	B
P3-W	0.94^{bcd}	0.35^{ab}	3.24^{bc}	128^{bc}	111^{bcd}	7^{bc}	39^{a}	20^{cd}
P3-S	0.87^{cd}	0.33^{ab}	2.86^{c}	116^{bcd}	108^{bcd}	7^{bc}	38^{a}	48^{ab}
P3-C	0.90^{cd}	0.34^{ab}	2.96^{c}	111^{bcde}	114^{bcd}	8^{b}	40^{a}	50^{a}
P3-E	0.79^{d}	0.32^{ab}	2.89^{c}	147^{b}	93^{cde}	8^{b}	38^{a}	50^{a}
P12-W	1.46^{a}	0.43^{a}	4.28^{abc}	71^{e}	61^{ef}	3^{c}	46^{a}	14^{d}
P12-W-E	1.08^{abcd}	0.31^{ab}	3.44^{abc}	145^{b}	80^{def}	15^{a}	58^{a}	15^{d}
P12-W-D	1.06^{abcd}	0.31^{ab}	3.28^{abc}	230^{a}	130^{bc}	5^{bc}	54^{a}	13^{d}
P12-S	1.24^{abc}	0.36^{ab}	3.61^{abc}	74^{de}	50^{f}	4^{bc}	42^{a}	32^{abcd}
P12-C	1.33^{ab}	0.35^{ab}	3.92^{abc}	105^{bcde}	65^{ef}	3^{c}	46^{a}	39^{abc}
P12-E	1.20^{abc}	0.34^{ab}	3.34^{abc}	73^{e}	42^{f}	3^{c}	44^{a}	28^{bcd}
B-W	1.10^{abcd}	0.32^{ab}	3.98^{abc}	85^{de}	177^{a}	5^{bc}	53^{a}	36^{abc}
B-E	0.96^{bcd}	0.28^{ab}	3.63^{abc}	94^{cde}	118^{bcd}	5^{bc}	41^{a}	37^{abc}
G-W	0.76^{d}	0.23^{ab}	5.35^{a}	77^{de}	138^{ab}	7^{bc}	47^{a}	33^{abcd}
G-E	0.71^{d}	0.21^{b}	5.07^{ab}	90^{cde}	92^{cde}	7^{bc}	49^{a}	30^{abcd}

Mean values with the same letter were not significantly different at the level $P = 0.05$

respectively) than peat substrates (37.2, 2.7, 4.0 and 0.28, respectively). Initial pH values did not change during cultivation in peat substrates whereas an increase from 5.3 to 6.2 was recorded in substrate G and from 5.1 to 5.4 in substrate B; the differences were significant ($P = 0.05$) between P3 on one side and P12, B and G on the other side, as well as between P12 and both B and G.

Composition of substrates and limestone doses had a great effect on the degree of chlorosis (Table 3) and on Fe and Mn content in the leaves (Table 4). Among treatment combinations without micronutrient application, only plants in P3-W had healthy dark green leaves. The rest of plants were considered slightly chlorotic (B-W plants), chlorotic (G-W plants) or severely chlorotic (P12-W plants). The leaves of P3-W plants had the highest Fe content and were higher in Mn than the leaves of P12-W plants. The highest amount of foliar Mn was found in the combinations B-W and G-W (Table 4), clearly due to the effect of high available Mn in these substrates (Table 2).

Application of micronutrient in sulphate form (P12-S) only slightly corrected chlorosis (Table 3) and had no effect on foliar Fe and Mn (Table 4). Micronutrients in citrate form (P12-C) had more severe chlorosis (Table 3) despite a slight positive effect on foliar Fe. In all cases, Fe and Mn content in chlorotic leaves was below 80 and 65 ppm, respectively (Table 4).

Nutrient solution E improved the colour of plants grown in substrate B and, to some extent, also in substrate G (treatment combinations B-E, G-E). In P12-E the effect was weak and the plants remained chlorotic (Table 3).

In the experiments carried out by Smith et al. (2004a) regular fertilization with solution containing 2 mg/l Fe from Fe-EDTA similarly did not prevent a decrease in chlorophyll and carotenoids content when substrate pH was above 6. On the other hand regular fertilization with 4 g/l Fe from Fe-EDDHA had a positive effect in *Calibrachoa* plants grown at high substrate pH. Lower concentrations of Fe-EDDHA (2 mg/l Fe) or the maximal used concentration of Fe-EDTA (4 mg/l Fe) gave worse but acceptable results (Wik at al. 2006).

Solution E increased Fe content in the leaves in P3-E and, to a lesser extent, in B-E and G-W (compared with P3-W, B-W, and G-W, respectively). On the other hand, leaf Mn decreased in P12-E and, to a lesser extent, in B-E and G-E (Table 4), where substrates with very high content of available Mn were used (Table 2).

Mills and Jones (1996) reported 84–168 ppm Fe and 44–177 ppm Mn in dry matter of healthy petunia leaves. In our experiment foliar Fe and Mn

in P3-W as well as in several other treatments were within this range (Table 4).

Smith et al. (2004b) measured 159 ppm Fe in the leaves of *Petunia* grown at pH 4.6 and 91 ppm Fe at pH 7 with a decrease of chlorophyll content when foliar Fe content was below 100 ppm. Other findings showed that correlation between Fe content and chlorophyll content does not always occur in the case of lime-induced chlorosis. The Fe concentration (on dry weight basis) could be greater in chlorotic leaves than in non-chlorotic ones because Fe can be immobilized in leaf free space due to too high pH (Mills, Jones 1996; Pestana et al. 2003). Smith et al. (2004b) found that foliar Mn was affected to a lesser extent than Fe by substrate pH in the range of 4.6 to 6.1 and that substantial increase in foliar Mn occurred at pH 7, which was inconsistent with our results.

Three additional drenches of 90 mg/l Fe from Fe-EDTA and 30 mg/l Mn from Mn-EDTA (P12-W-E) substantially corrected chlorosis with increased foliar Fe and Mn. Effect of Fe-DTPA and Mn-EDTA (P12-W-D) on chlorosis was similar, with an even greater increase in foliar Fe and Mn (Tables 3 and 4).

Fisher et al. (2003) reported similar results with *Calibrachoa* plants cultivated at high pH and treated with additional single drench of Fe. The most efficient source of Fe was Fe-EDDHA regardless of concentration (20–80 mg/l Fe). The efficiency of Fe-DTPA was always worse and depended on concentration.

Despite severe chlorosis in some plants, none of the treatment combinations significantly affected plant fresh weight (87.9 ± 8.2 g) or dry weight (11.6 ± 1.0 g). The market quality of chlorotic plants was very poor and it was supposed that growth of these plants would worsen during subsequent cultivation.

Substrate composition and different application of micronutrients did not show a great effect on the leaf content of macronutrients and other micronutrients. Only the plants in P12-W treatment had higher foliar Ca and K than P3-W plants. G-W plants were lower in foliar N and higher in K. Application of micronutrients increased boron in the leaves of plants grown in peat substrates (Table 4).

CONCLUSIONS

Severe chlorosis of petunia plants grown at pH 6.6 in peat substrate with high limestone dose was substantially corrected by substrate drenches of 30 mg/l Mn from Mn-EDTA and 90 mg/l Fe from either Fe-EDTA or Fe-EDDHA. This treatment also increased foliar Fe and Mn.

Regular application of nutrient solution with low concentration of Fe (1.4 mg/l), Mn (0.8 mg/l), and of other micronutrients as EDTA chelates was not sufficiently effective for plants grown in the high-limestone peat substrates but increased foliar Fe and eliminated chlorosis of plants grown in peat-bark substrate (pH 5.4) as well as in peat-bark-compost (pH 6.2).

Regular application of Fe, Mn, and other micronutrients as sulphates or citrates had no positive effect.

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Výskyt a korekce chloróz u petúnií

ABSTRAKT: Cílem pokusu s petúniemi bylo zjistit, jak složení substrátu, vysoké hodnoty pH substrátu a aplikace Fe a Mn v různé formě a koncentraci ovlivní příjem Fe a Mn, chlorózu a růst. Zdravé rostliny byly vypěstovány při optimálních hodnotách pH v rašelinovém substrátu s nízkou dávkou vápence bez ohledu na to, zda roztok použitý pro přihnojování obsahoval stopové živiny nebo ne. Vysoké hodnoty pH substrátu složeného z rašeliny, kůry a kompostu a hlavně rašelinového substrátu s vysokou dávkou vápence způsobily silnou chlorózu a snižovaly obsah Fe a Mn v listech. Pravidelné přihnojování roztokem obsahujícím Fe, Mn a další stopové živiny ve formě chelátů EDTA v nízké koncentraci mělo pozitivní vliv na rostliny pěstované v rašelinokůrovém substrátu a v substrátu z rašeliny, kůry a kompostu, kde zlepšovalo příjem Fe a (téměř) eliminovalo chlorózu. Toto ošetření mělo pouze slabý účinek na rostliny v rašelinovém substrátu s vysokou dávkou vápence a v důsledku toho s vysokými hodnotami pH. Pravidelná aplikace Fe, Mn a dalších stopových živin ve formě síranů a citrátů se téměř neprojevila. Pouze trojnásobná zálivka roztokem s 30 mg/l Mn ve formě Mn-EDTA a 90 mg/l Fe ve formě Fe-EDTA nebo Fe-EDDHA výrazně snižovala stupeň chlorózy a zvyšovala obsah Fe a Mn v listech rostlin pěstovaných v rašelinovém substrátu s vysokou dávkou vápence.

Klíčová slova: *Petunia* × *atkinsiana*; chloróza; železo; mangan; cheláty železa; Fe-EDTA; Fe-DTPA; Fe-EDDHA; Mn-EDTA

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