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Research article

The memory of iron stress in strawberry plants

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ABSTRACT

To provide information towards optimization of strategies to treat Fe deficiency, experiments were conducted to study the responses of Fe-deficient plants to the resupply of Fe. Strawberry (*Fragaria* × *ananassa* Duch.) was used as model plant. Bare-root transplants of strawberry (cv. 'Diamante') were grown for 42 days in Hoagland's nutrient solutions without Fe (Fe0) and containing 10 μM of Fe as Fe-EDDHA (control, Fe10). For plants under Fe0 the total chlorophyll concentration of young leaves decreased progressively on time, showing the typical symptoms of iron chlorosis. After 35 days the Fe concentration was 6% of that observed for plants growing under Fe10. Half of plants growing under Fe0 were then Fe-resupplied by adding 10 μM of Fe to the Fe0 nutrient solution (FeR). Full Chlorophyll recovery of young leaves took place within 12 days. Root ferric chelate-reductase activity (FCR) and succinic and citric acid concentrations increased in FeR plants. Fe partition revealed that FeR plants expressively accumulated this nutrient in the crown and flowers. This observation can be due to a passive deactivation mechanism of the FCR activity, associated with continuous synthesis of succinic and citric acids at root level, and consequent greater uptake of Fe.

1. Introduction

Plants are an important source of iron (Fe) in human diet. Although Fe is quite abundant in soils, the incidence of Fe deficiency is common in plants grown in calcareous soils due to reduced bioavailability of the metal (Hindt and Guerinot, 2012). The low mobility of Fe in calcareous soils reduces the photosynthetic rate and alters chloroplast structure, leading to symptoms in young leaves generally described as chlorosis. Under these conditions plants show limited growth and yields, and the quality of fruit is poor (Domenico Rombolà et al., 2003; Larbi et al., 2006; Pestana et al., 2003) Under Fe deficiency, sensitive crops often present a nutrient imbalance of Fe, P, Mn and Zn resulting in low availability of such nutrients for metabolic needs (López-Millán et al., 2001a; Ortas et al., 2015; Venturas et al., 2014).

Iron acquisition and transport by plants follow two general strategies (Marschner et al., 1986; Muneer et al., 2014). Strategy I, also known as the reduction strategy, occurs in non-grass species which require a reduction of Fe(III) to Fe(II) before uptake through a membrane bound ferric-chelate reductase (FCR, belonging to the FRO/Ferric Reduction Oxidase family genes). Fe(II) is then transported to the symplast by Iron Regulated Transporters (IRTs), which belong to the ZIP family (ZIP: zinc and iron protein; a metal transporter family which are able to transport a selection of cations, such as cadmium, iron, manganese and zinc) and show affinity to other transition metals. Plants following this strategy also release organic acids through their roots, resulting in an acidification of the rhizosphere, accentuated by the H^+ excretion. Organic acids also play an important role in Fe bioavailability in the rhizosphere and in Fe translocation within the plant (Abadía et al., 2002; López-Millán et al., 2001a). Citric acid transports Fe(III) in the xylem sap and usually shows higher concentrations in the xylem and roots of Fe-chlorotic plants. Concentration increases of citric acid as well as in malic have been related with Fe uptake into various plant organs (Gayomba et al., 2015; López-Millán et al., 2000; Rellán-Álvarez et al., 2010; Zocchi et al., 2007). A second Strategy II, also referred

Abbreviations: BPDS, Fe(II)-bathophenantrolinedisulfonate; Chl, Chlorophyll; DW, dry weight; EC, electrical conductivity; EDDHA, ethylenediamine-N-N'bis(*o*-hydroxyphenylacetic) acid; EDTA, ethylenediamine-tetraacetic acid; FCR, ferric chelate reductase; FW, fresh weight; MES, 2-(N-morpholino)ethanesulfonic acid; SPAD, soil and plant analyser development.

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as the chelation strategy, is confined to gramineous plants. Grasses release Fe chelator compounds, for example phytosiderophores from the mugineic acid (MAs) family, to solubilize Fe in the rhizosphere prior to uptake (Jeong and Guerinot, 2009).

To sustain metal homoeostasis, plants developed signalling mechanisms that control internal and external metal concentrations, and evaluate interactions between different nutrients (Rubio et al., 2009). These regulation mechanisms determine a balanced nutritional status and lead to a continuous management of uptake, utilization and storage of metals in plants.

The dynamics of nutrient partition in plants under Fe-deficiency have been documented in several crops. For example, in chlorotic citrus rootstocks, macronutrients and micronutrients decreased in the shoots (Pestana et al., 2005). In rice, changes in the partition of nutrients in response to Fe stress depend on the organ: macronutrients accumulate in leaves (Ca, Mg and K) while micronutrients accumulate in roots (Zn, Mn and Cu). Fe deficiency can lead to decreases of Ca, Mn and Fe in roots, and of Zn and Ni in leaves (Sperotto et al., 2012). Another study conducted in kiwi showed increases in leaf N and P and a decrease in Fe concentration (Domenico Rombolà et al., 2003).

Strawberries are popular worldwide due to their antioxidant capacity and richness in essential nutrients (Giampieri et al., 2014, 2015; Manganaris et al., 2014). However, in strawberry (Fragaria × ananassa Duch.) the knowledge about the effects of Fe homoeostasis is scarce. As described for other crops, Fe deficiency induces higher activity of the FCR enzyme as part of the standard reduction strategy (López-Millán et al., 2001b). It was reported by Pestana et al. (2012b) that the deactivation of the root FCR enzyme in chlorotic strawberry plants (cv. 'Selva') was slower if a pulse of Fe, in this case as ferrous sulphate, was added to the root system compared to foliar application. It was concluded that this "delay" was an opportunity for plants to take up greater amounts of Fe from the solution.

Under Fe deficiency a complex set of mechanism is activated and we expect that during this window of time an opportunity is created to potentiate Fe uptake and translocation. Moreover, we expect that this translocation will be directed towards selective and physiological important sinks. The aim of this work is to decipher the responses of Fe deficient strawberry plants upon Fe resupply, relating the profile of organic acids and the concentration of nutrients in different organs.

2. Material and methods

2.1. Plant material and growth conditions

Strawberry bare-root plants, obtained from a nursery, were grown in a glasshouse under natural photoperiod conditions and air temperature ≤25 °C. Plants were disinfected by soaking them in a 2 g l^{-1} fosetyl-aluminium solution for 2 h. They were then transferred to 20-l plastic containers filled with a complete and full-strength Hoagland solution with (in mM): 5 $Ca(NO_3)_2$, 5 KNO_3, 1 KH_2PO_4, 2 $MgSO_4$ and (in μM): 46 H_3BO_3, 0.8 $ZnSO_4$, 0.4 $CuSO_4$, 9 $MnCl_2$, and 0.02 MoO_3. Iron was supplied as Fe(III)-EDDHA (Basafer® from Compo, with 6% of Fe; 5.0% of Fe chelated by ortho–ortho EDDHA) at two concentrations: 0 (Fe0; n = 119 plants) or 10 μM of Fe (Fe10; n = 77 plants). Plants in Fe10 (positive control plants) were grown with an adequate Fe level. Fe0 plants were used as a negative control. The initial pH of nutrient solutions was adjusted to 6.0 ± 0.1 using a NaOH 0.1 M aqueous solution and their electrical conductivity (EC) was 2.2 ± 0.1 dS m^{-1} in all cases. The pH and EC of solutions were monitored daily. When the EC value reached 2.0 ± 0.1 dS m^{-1} the solutions were renewed.

As expected, control plants grown with Fe (Fe10) in the nutrient solution did not develop symptoms throughout the entire experiment and Chlorophyll values were always above 720 μmol m^{-2}. After 35 days plants under Fe0 conditions developed chlorotic strawberry leaves showing SPAD values between 0 and 21 (Chl < 376 μmol m^{-2}). At this stage, the chlorotic plants were divided into two groups: (i) one set remained without Fe (Fe0), and (ii) for the other set (FeR) the nutrient solution was supplemented with 10 μM of Fe in order to evaluate the effects of recovery from the stress. Plants growing under the three treatment conditions were maintained for additional two weeks: Fe0 (always grown without Fe; n > 36); Fe10 (control plants, always grown with Fe; n > 42), and FeR (chlorotic plants treated by adding Fe to nutrient solution; n > 20). The containers used in the experiment were placed in a randomized design, each one with six plants.

2.2. Leaf chlorophyll assessment

During the experimental period, leaf Chl concentrations were estimated non-destructively using the Chlorophyll Metre SPAD-502 device (Minolta Camera Co., Osaka, Japan), at least thrice a week. SPAD readings began 12 days after the development of the first/second new leaves. For comparison purposes one mature-basal leaf and one fully-expanded new leaf (apical leaf) were measured. To account for leaf heterogeneity, SPAD values were taken in all three leaflets of each leaf. SPAD readings were converted into total Chl concentration (μmol m^{-2}) using the calibration curve for 'Diamante' strawberry:

$$\mathrm{Chl} = 0.38 \times \mathrm{SPAD}^2 + 6.63 \times \mathrm{SPAD} + 71.55 \left(r^2 = 0.97;\ n = 38;\ P < 0.001\right).$$

This calibration curve was established by extracting pigments from leaves with different degrees of chlorosis from the same area where SPAD readings were carried out. Pigment extraction was done with pure acetone and then Chl was measured spectrophotometrically in the presence of Na ascorbate (Abadía and Abadía, 1993).

2.3. Biomass

Strawberry plants (at least 5 per treatment) were harvested at four different stages: (i) (***day 1***) - before the beginning of the experiment plants without leaves were divided into roots and crown; (ii) ***(day 15)*** two weeks after the imposition of Fe treatments; plants were divided into roots, crown, young leaves and flowers; (iii) ***(day 35)*** – when Fe0 plants developed Fe deficiency symptoms; plants from each treatment (Fe0 and Fe10) were separated into roots, crowns, mature leaves, young leaves and flowers; (iv) ***(day 47***) at the end of the recovery period plants from each treatment (Fe0, Fe10 and FeR) were separated into roots, crown, mature leaves, young leaves, flowers and runners. Samples were washed with a non-ionic detergent (0.1%) to remove surface contamination, then with tap water, and finally rinsed three times with deionised water. Fresh weight (FW) was determined for each collected sample and dry weight (DW) estimated after drying at 60 °C until constant weight.

2.4. Mineral composition

Dried samples were ground to powder, ashed at 450 °C and digested with HCl (1 M) according to standard laboratory procedures (A.O.A.C., 1990). Total N and P concentrations were determined by the Kjeldahl and molybdo-vanadate methods, respectively. Potassium, Ca, Mg, Cu, Mn, Zn and Fe concentrations