Journal of Plant Physiology **166** (2009) 375—384

Available online at www.sciencedirect.com

ScienceDirect

JOURNAL OF PLANT PHYSIOLOGY

www.elsevier.de/jplph

Metabolic responses in iron deficient tomato plants

Ana Flor López-Millán[a], Fermín Morales[a], Yolanda Gogorcena[b], Anunciación Abadía[a], Javier Abadía[a,*,1]

[a]*Department of Plant Nutrition, Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (CSIC), P.O. Box 13034, E-50080 Zaragoza, Spain*
[b]*Pomology Department, Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (CSIC), P.O. Box 13034, E-50080 Zaragoza, Spain*

Received 18 April 2008; received in revised form 13 June 2008; accepted 13 June 2008

KEYWORDS
Iron;
Organic anions;
Phospho*enol*pyruvate carboxylase;
Strategy I;
Tomato

Summary

The effects of Fe deficiency on different metabolic processes were characterized in roots, xylem sap and leaves of tomato. The total organic acid pool increased significantly with Fe deficiency in xylem sap and leaves of tomato plants, whereas it did not change in roots. However, the composition of the pool changed with Fe deficiency, with major increases in citrate concentrations in roots (20-fold), leaves (2-fold) and xylem sap (17-fold). The activity of phospho*enol*pyruvate carboxylase, an enzyme leading to anaplerotic C fixation, increased 10-fold in root tip extracts with Fe deficiency, whereas no change was observed in leaf extracts. The activities of the organic acid synthesis-related enzymes malate dehydrogenase, citrate synthase, isocitrate dehydrogenase, fumarase and aconitase, as well as those of the enzymes lactate dehydrogenase and pyruvate carboxylase, increased with Fe deficiency in root extracts, whereas only citrate synthase increased significantly with Fe deficiency in leaf extracts. These results suggest that the enhanced C fixation capacity in Fe-deficient tomato roots may result in producing citrate that could be used for Fe xylem transport. Total pyridine nucleotide pools did not change significantly with Fe deficiency in roots or leaves, although NAD(P)H/NAD(P) ratios were lower in Fe-deficient roots than in controls. Rates of O_2 consumption were similar in Fe-deficient and Fe-sufficient roots, but the capacity of the alternative oxidase pathway was decreased by Fe deficiency. Also, increases in Fe reductase activity with Fe deficiency were only 2-fold higher when measured in tomato root tips. These values are significantly lower than those found in other plant species, where Fe deficiency leads to larger increases in organic acid synthesis-related enzyme activities and flavin accumulation. These data support the hypothesis that

Abbreviations: AOX, alternative oxidase; CS, citrate synthase; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; PDC, pyruvate decarboxylase; PEPC, phospho*enol*pyruvate carboxylase; SHAM, hydroxy-salicylic acid.

*Corresponding author. Tel.: 34 976 716056; fax: 34 976 716145.
E-mail address: jabadia@eead.csic.es (J. Abadía).
[1]Courier address: Av. Montañana 1005, Campus de Aula Dei, E-50059 Zaragoza, Spain.

0176-1617/$ - see front matter
doi:10.1016/j.jplph.2008.06.011

the extent of activation of different metabolic pathways, including carbon fixation via PEPC, organic acid synthesis-related enzymes and oxygen consumption is different among species, and this could modulate the different levels of efficiency in Strategy I plants.

Introduction

Iron is an essential microelement for plant growth and development. Soils normally contain high amounts of Fe, but in well aerated and alkaline soils the availability of Fe for plant uptake is very limited. Plants have developed two different strategies in response to Fe shortage: Strategy II, which occurs in *Poaceae* species, and Strategy I, which occurs in dicotyledonous and non-grass monocotyledonous species (Marschner et al., 1986). In both strategies, Fe deficiency induces several mechanisms aimed to increase Fe uptake from the soil. In Strategy II species, there is an increase in the synthesis and secretion of phytosiderophores to the rhizosphere, parallel to an induction of an Fe(III)–phytosiderophore complex transport system (Kobayashi et al., 2006). Strategy I plants induce a two-step mechanism for root Fe uptake that includes the induction of an Fe(III) reductase (Chaney et al., 1972) and an Fe(II) transporter (Eide et al., 1996). In addition to these changes, Strategy I plants have developed several physiological responses that aid Fe uptake from the soil by lowering soil pH and increase Fe(III) solubility. These responses can include, depending on the species, enhanced excretion of protons to the rhizosphere mediated by a plasma membrane-bound H^+-ATPase (Schmidt, 1999; Zocchi et al., 2007), excretion of phenolics, and accumulation and/or excretion of flavin compounds (Susín et al., 1994) and organic acids (Abadía et al., 2002).

In the last decade, many of the key components for increased Fe uptake have been identified at the molecular level. Plasma membrane-bound Fe reductases belonging to the FRO family have been cloned in *Arabidopsis thaliana*, *Pisum sativum and Lycopersicon esculentum* (see references in Kim and Guerinot, 2007), and it has been demonstrated that Fe transporter genes involved in root uptake belong to the ZIP family of metal transporters. Members of this family have been cloned from several plant species; these include *IRT1* and *IRT2* from *Arabidopsis thaliana*, *RIT1* from *Pisum sativum*, *LeIRT1* and *LeIRT2* from *Lycopersicon esculentum* and *MtZIP6* from *Medicago truncatula* (Kim and Guerinot, 2007).

In xylem sap, Fe is transported as Fe(III), likely chelated by citrate (Tiffin, 1966; López-Millán et al., 2000a; Rellán-Álvarez et al., 2008). Several authors have reported an increase in xylem sap organic acid concentrations with Fe deficiency (reviewed in Abadía et al., 2002). Although the precise role of these organic acids in Fe transport and uptake by mesophyll cells is still unclear, recent advances have indicated that a citrate transporter, FRD3, is necessary for efficient Fe translocation to the xylem sap (Durrett et al., 2007), supporting a role for citrate in long-distance Fe transport. Once in the leaf apoplast, Fe(III) is reduced before uptake by the leaf cell, and this process may be mediated by a plasma membrane-bound ferric chelate reductase similar to that present in roots (González-Vallejo et al., 1999). In agreement with the biochemical data, expression of several members of the FRO family, *AtFRO6*, *AtFRO7* and *ATFRO8*, has been observed in leaves (Wu et al., 2005; Mukherjee et al., 2006).

Several changes occur at the metabolic level in order to sustain the increased Fe uptake capacity of Fe-deficient plants (Zocchi, 2006). These changes include an accumulation of organic acids throughout the plant, primarily malate and citrate (reviewed in Abadía et al., 2002), shifts in the redox state of the cytoplasm (Schmidt, 1999; López-Millán et al., 2000b), increases in the activity of PEPC and in several enzymes of the Krebs cycle such as citrate synthase (CS), isocitrate dehydrogenase (ICDH), fumarase and aconitase and of the glycolytic pathway such as glyceraldehyde 3-phosphate dehydrogenase (reviewed in Zocchi, 2006). Some of these increased activities are associated with enhanced expression of the corresponding genes (Thimm et al., 2001). The increase in PEPC activity correlates with the organic acid accumulation in Fe-deficient roots (López-Millán et al., 2000b) and is localized mainly in the external layers of the cortical cells of Fe-deprived root apical sections, which are very active in proton extrusion (Zocchi, 2006). An up-regulation of the glycolytic pathway, as well as increases found in the activities of several NAD(P)H-producing enzymes, can produce reducing equivalents to keep the Fe reductase working at the necessary rate (Sijmons