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“QTL and candidate gene analyses of rootstock-mediated tomato fruit yield and quality traits under low iron stress”

Asins MJ*, Raga MV, Torrent D, Roca D, Carbonell EA

Instituto Valenciano de Investigaciones Agrarias, Carretera de Moncada a Náquera Km 4.5, Apartado Oficial, 46113 Moncada, Valencia, Spain.

* Corresponding author; email: mjasins@ivia.es Telephone: 34 963424067

<https://orcid.org/0000-0002-4330-160X>

Abstract

The use of iron-efficient rootstocks can be a sustainable way to enhance iron (Fe) fruit content in tomato. A hybrid tomato variety was grafted on a genotyped population of recombinant inbred lines derived from *Solanum pimpinellifolium*, and compared with self- and non-grafted controls under low iron (5.2 μ M) growing conditions. Rootstock effects on total fruit yield, fruit [Fe] and yield Fe content (FeUEc) were the target traits; other minerals were also investigated by quantitative trait loci (QTL) and candidate gene analyses.

The rootstock genotype affected fruit concentrations of Fe, Ca, Mg, Mn, Na, P, S, Si and Al. Most rootstocks increased FeUEc. Fruit and leaf [Fe] and FeUEc were genetically complex, involving epistatic interactions. Six and eight QTLs were detected for these traits, respectively, by multiple QTL mapping. Two kinds of relevant genes were found among candidates within QTLs for iron related traits: those coding for secretory proteins specific of the tomato xylem sap under iron deficiency, and others having a role in iron uptake such as TOMLHA1, FRO1, NRAMP2, FER and MYB72. Detected QTLs might reflect differences in the regulatory regions of those relevant genes, more than in their coding sequences.

Key words: Rootstock breeding, *S. lycopersicum*, *S. pimpinellifolium*, Iron deficit, Root transcriptomics, Xylem proteome.

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Introduction

Although undernourishment has decreased in the last decades the prevalence of micronutrient malnutrition is augmenting (Strobe et al. 2018). These micronutrients include iron, zinc, selenium, manganese and a wide range of vitamins (Miller and Welch 2013). Anemia, linked to deficiencies in iron, vitamin B1, B9 and B12 affects one-fourth of human population (McLean et al. 2009) and 32.8% of woman of reproductive age (FAOSTAT 2017). Iron (Fe) enters the food chain mainly through plants. Tomato fruit is a major component of daily meals in many countries and constitutes an important source of minerals, vitamins, and antioxidant compounds. Therefore, biofortification, which consists of enhancing the natural micronutrient content of crops (at edible tissues), can be considered a sustainable and cost-effective means to reduce micronutrient malnutrition (Meenakshi et al. 2010). Iron is abundant in the soil but it is usually present in an oxidized form, difficult to be acquired by plants. Besides, in alkaline soils which affects around 30% of the earth, Fe solubility is low leading to iron deficiency chlorosis (Mengel 1994). Low iron availability affects plant growth and yield of many field crops; thus, Fe is often a component of agricultural fertilizers used to increase crop yields and contributes to increasing cultivation costs; i.e. decreasing farmers income. Since root is the main organ involved in Fe uptake, grafting tomato varieties on improved rootstock for Fe acquisition and translocation might allow the application of less Fe fertilizer, without decreasing

tomato yields and increasing total iron fruit content. However, to our knowledge this approach has not been explored yet. Given that past tomato breeding programs grew plants under optimal (Fe-sufficient) conditions, the genetic variability for Fe uptake and translocation under low Fe input remains largely unexplored. Besides, genetic studies of rootstock effects on Fe assimilation conform a valuable strategy to understand root function since nutrient uptake is shoot-regulated by root-shoot communication (Wang et al. 2006). The identification of rootstock-genomic regions controlling Fe uptake and translocation (Quantitative Trait Loci, QTL) could allow marker-assisted selection in rootstock breeding programs, and the search for new alleles in wild germplasm because, following Price (2006), those rootstock QTLs are expected to contain genes involved in Fe acquisition and distribution.

Genes involved in Fe acquisition and distribution have been previously identified by the reverse genetic strategy through expression studies in mutants and transgenic genotypes in *Arabidopsis* and tomato (Kobayashi and Nishizawa 2012; Connort et al. 2017). For Fe uptake, coumarins, facilitated by the PDR9/ABCG37 transporter (Fourcroy et al. 2014; Tsai and Schmidt 2017) and protons, mediated by a plasmalemma P-Type ATPase (AHA2) (Santi and Schmidt 2009) are pumped to the rhizosphere to increase Fe^{3+} solubility (Kailasam et al. 2019). The secretion of coumarins (derived from precursors of the phenylpropanoid pathway) is dependent on specific β -glucosidases (Clemens and Weber 2015). Other non-grass species secrete flavins instead of coumarins. Besides, the secretion of putrescine (a polyamine) appears to improve mobilization of Fe inside the plant cell wall (Zhu et al. 2016). Before transport across the membrane by Iron-Regulated Transporter 1 (IRT1), Fe^{3+} is reduced by Ferric Reduction Oxidase 2 (FRO2) and this is the main route of iron uptake in *Arabidopsis* (Fourcroy et al. 2016). Importantly, the chelator strategy seems related to iron deficiency chlorosis tolerance in alkaline

77 conditions (Fourcroy et al. 2014; Clemens and Weber 2015; Waters and Amundsen
78 2018). The natural resistance associated macrophage protein NRAMP1 is suggested to
79 cooperate with IRT1 in iron uptake (Castaings et al. 2016). Nutrients can travel through
80 the apoplastic space to reach the endodermis. The main reservoir for iron in the roots is
81 the cell wall of root apoplast. A suberisation reduction of the endodermis (cell wall
82 modifications), induced in Fe deficient plants, has been shown to allow more iron to enter
83 the vasculature (Barberon et al. 2016; Ceballos-Laita et al. 2018). In the symplast, iron is
84 thought to be transported in the form of Fe^{2+} -nicotianamines (NA) complexes. NA, a
85 precursor of mugineic acid, is produced from S-adenosyl methionine. Once iron has
86 passed the endodermis, it can be loaded into the xylem for transport, mainly in the form
87 of Fe^{3+} -citrate, to the shoot (Rellan-Alvarez et al. 2010). Citrate is effluxed into the
88 Arabidopsis xylem by FDR3, a member the multidrug and toxic compound extrusion
89 (MATE) family (Green and Rogers 2004). In the cell, iron can be incorporated into
90 proteins (iron co-factors) or stored in cellular compartments, particularly the vacuole to
91 avoid its toxicity (fenton reaction) and to withdrawal when needed. Vacuolar iron
92 transporters (VIT) catalyze vacuolar Fe transport while its efflux from the vacuole is
93 catalyzed by NRAMP proteins (Gollhafer et al. 2014). In plastids, several thousand Fe
94 atoms are stored per ferritin molecule (Briat et al. 2010). Regulation of iron homeostasis
95 is essential and it occurs through the action of positive and negative regulators (Kailasam
96 et al. 2019). Iron uptake and transport is coordinated by transcription factors including
97 FER in tomato (FIT in Arabidopsis) and many others from the basic Helix-Loop-Helix
98 (bHLH), MYB and WKY families. Phytohormones ethylene, auxin (in the shoot-to-root
99 regulation), as well as molecules such as nitric oxide and sucrose (promoting auxin
100 signaling) positively regulate Fe-deficiency transcription, while others act negatively on

the network (Kailasam et al. 2019; Liu et al. 2016; Li and Lan 2017; Lin et al. 2016; Lucena et al. 2006).

Then, as far as breeding programs is concerned, Fe assimilation is a complex, quantitative trait that involves several biological processes such as biosynthesis of chelators, proton pumps, transporters, root development, suberisation of endodermis, Fe sequestration into vacuoles or into proteins, biosynthesis of Fe cofactors, Sulphur and phosphate uptake and translocation, and Fe sensing and signaling. Therefore, natural variation for Fe uptake and translocation have to involve genes (with unknown position and distribution along the genome) participating in those processes and contributing each in a certain degree to the final phenotype. The present study, including a tomato grafted population of RILs derived from the wild species *S. pimpinellifolium*, focuses on the role of the root (maintaining constant the genotype of the shoot and the level of Fe input) to genetically analyze its effect on total fruit yield, fruit [Fe] and harvested Fe (Fe output, as the main commercial component of Fe use efficiency in tomato) through QTL and candidate gene analyses. QTL peaks shall point out physical positions that will allow the analysis of genes they include, greatly reducing the number of genes belonging to multigene families that could be involved (Price 2006). Further study of candidate genes will consider: (1) their segregation for frameshift mutations in the RIL population, (2) their molecular function as annotated genes, and (3) their relative expression in the root of a reference cultivar (Heinz). Each of these steps, and QTL analysis itself, has limitations but all together defines an alternative strategy to the analysis of mutants or transgenics, that enhances the utilization of wild germplasm (natural variation) for rootstock breeding. Following this strategy, we aim to (1) estimate the heritability of the rootstock effect on leaf chlorophyll content, leaf and fruit mineral concentrations, fruit yield, and total harvested Fe under commercial growing conditions but low iron input, (2)

detect the QTLs involved and study their interactions, (3) infer possible candidate genes for major rootstock QTLs and QTLs governing Fe-related traits of the scion, and (4) explore the origin of such QTLs and the potency (and scope) of this forward genetics strategy.

Materials and Methods

Plant material, growth conditions and trait evaluation

A population of 121 F11 lines (Recombinant Inbred Lines, RILs) derived by single seed descendent from the hybrid between a *Solanum lycopersicum* var. Cerasiforme (formerly *L. esculentum*) genotype (E9) and a *S. pimpinellifolium* L. (formely *L. pimpinellifolium*) line (L5) (Monforte et al. 1997) was used as rootstock for the study reported here.

The commercial tomato hybrid *Solanum lycopersicum* cv. Boludo (Bol) was used as scion. Boludo was also grafted onto roots derived from a different plant of the same genotype (Bol/Bol). Non-grafted Boludo plants (Bol) and Bol/Bol plants were included as controls. Bol/Bol plants were used to evaluate any physiological change that could be induced by the grafting process *per se*.

Grafted plants having approximately 6 leaves were obtained from the company Planters Taches S.L. (Valencia, Spain). Grafting was performed when seedlings had developed 3-4 true leaves, seedlings were cut over the cotyledons, using the shoot as scion and the remaining plant part as rootstock. Grafts were made immediately after cutting the plants and grafting clips were used to adhere the graft union. After grafting, seedlings were grown as described previously (Estañ et al. 2009).

Five grafted plants per RIL, and controls were planted in pots (5L) into a polycarbonate greenhouse, in Valencia, Spain (February 14th, 2017) using cocofiber as a substrate. The greenhouse had automatic roof ventilation and heating system (maintaining

inside air temperature above 8°C). The climate variables, in and out solar global radiation, inside photosynthetic active radiation, temperatures of the air, and air humidity, were recorded by sensors connected to a data acquisition system. A latinised row-column design was proposed with 5 reps along the benches, 22 rows per rep and 6 columns (benches) across the reps. Extra repetitions were used to complete the experimental units. A high frequency fertirrigation system together with 4L/h drippers were used and handled to ensure homogeneity of low [Fe] at the roots of all plants in cultivation at the same time. Plants were grown under standard commercial conditions (**Supplementary Fig. 1**). Initially, during 13 days, the [Fe] in the nutrient solution was standard (15 µM; Asins et al. 2017), and then, it was lowered to one-third (5.2 µM), and maintained so for 141 days, to challenge rootstocks (RILs) for fruit yield. This nutrient solution (pH: 6.4) contained the following concentration of macronutrients (in mM): NO₃⁻ 8.1; H₂PO₄⁻ 4; SO₄²⁻ 1; NH₄⁺ 0.9; K⁺ 4.2; Ca²⁺ 3.5; Mg²⁺ 1; plus, the following concentration of micronutrients (in µM): Fe²⁺ 5.2; Mn²⁺ 8; Zn²⁺ 2.3; B 20, Cu²⁺ 7; Mo⁴⁺ 0.5. The water for the nutrient solution was previously treated with reverse osmosis. The plants were cultivated to only one stem, eliminating all axillary buds.

A total of 43 traits were evaluated, most of them (40) at the scion. Chlorophyll leaf concentration of fully expanded young leaves from each plant was estimated three times during the experiment with the chlorophyll meter SPAD-502 Plus (Konica Minolta, INC., Japan) but only initial and final evaluations were genetically analyzed: SPAD1 (at February 23rd, before starting the Fe deficit treatment) and SPAD3 (at June 28th, the end of the experiment). The fifth Boludo leaves (L) were harvested from 3 (central) out of the 5 reps per both RIL and controls for phenotyping after 123 days of low Fe availability. Three evaluations of scion leaves were carried out: leaf fresh weight (LFW, g); leaf dry weight (LDW, g) measured in samples dried at 80°C for 3 days, and leaf dry matter

(LDM, %) calculated as the percentage of LDW to LFW. At the end of the experiment (141 days of low Fe input), the root system was carefully extracted and washed to determine fresh root weight (FRW, g), root dry weight (RDW, g) and root dry matter (RDM, %).

Ripe fruits from the second or third truss of each plant (3 plants per genotype) were evaluated for soluble-solids content (SSC) and acidity. Fruit juice was obtained by squeezing of tomato through a cloth filter. This filtered juice was used to measure SSC, pH and citric acid content (%Cit). Total soluble solids were measured as °Brix with a digital refractometer (PR-101α; Pallete, Atago). The citric content (%) and pH of fruit juice was measured in a 1:10 mL juice dilution with a digital pH meter (PH-Matic23; Crinon). Dry tissue samples of fruit (raw edible part, F), and leaf (L), were prepared for mineral analysis by digestion in a HNO₃: HClO₄ (2:1, v/v) solution. Inorganic solutes were determined in ppm (mg/Kg) by inductively coupled plasma spectrometry (Varian ICP 720-E, Scientific Instrumentation Service, Estación Experimental del Zaidín, CSIC, Granada, Spain). These traits were named by the element symbol followed by F or L, denoting the tissue. The relative Fe_F to Fe_L was also considered (Fe_F_L) as percentage.

Plants from all reps were evaluated during nine weeks for commercial fruit yield in terms of number of normal, ripe fruits (FNc), their individual weight, (FWm, g) and total fruit weight (TFWc, g). Bronze fruits (**Supplementary Fig. 1C**) and fruits presenting Blossom End Rot (BER), or out of type, were not included to estimate commercial fruit yield. Percentage of fruits per plant showing BER, or fruits out of type from the total were considered (pFNber and pFNnot, respectively) as well as the incidence of plants with bronze fruits (Fe_sym; 1, yes; 0, no). Since this fruit symptom was somehow similar to that caused by Cucumber mosaic virus, its presence in plants showing

bronze fruits was tested (and discarded) by RT-PCR (Lin et al. 2004). Total dry fruit weigh (TDFW) was estimated from TFWc and fruit dry matter (FDM, %). Finally, total harvested Fe (mg of Fe in total fruit yield) was deduced from TDFW and Fe_F. This trait, coded as FeUEc, could be considered as a comparative, agronomic indicator of the rootstock iron uptake and translocation efficiency, under the fixed low Fe input (5.2 μ M).

Statistical analysis

A general factorial mixed model with genotypes (fixed), benches and reps (random) was used to assess the significance of each source of variation following the latinised row-column design. The Bayesian Information Criteria (BIC) was used to select the best model. Given that for most traits the model including genotypes, benches and heterogeneous within-genotype variances, was the most parsimonious with the lowest BIC, it was used to estimate the adjusted mean traits per rootstock genotype for the QTL analysis and to study the grafting effects by comparing Bol vs. Bol/Bol adjusted means.

Pearson correlation coefficients and principal component analysis based on the correlation matrix for the adjusted means was used to study the relations between the different traits.

Molecular markers, QTL and candidate gene analyses

Broad sense heritability (H^2) was calculated for the traits in the RIL population assuming that the individuals from the tenth self-pollinated generation were nearly homozygous for all loci. Heritability was calculated as reported previously (Villalta et al. 2007) by using the formula: $H^2 = V_g / (V_g + V_e)$ where V_g and V_e are the estimates of genotype and environmental variance, respectively, by Restricted Maximum Likelihood (REML). These estimates were obtained by considering genotypes as random effects.

Genotypes from the recombinant population were obtained at F10 for 7720 SNPs from the SolCAP tomato panel (Illumina BeadXhip WG-401-1004) and a linkage map

based on 1899 non-redundant SolCAP SNPs, covering 1326.37 cM of genetic length was used for QTL analysis (Asins et al. 2015).

QTL analysis of traits was carried out using Interval Mapping (IM), Multiple QTL Mapping (MQM), and Kruskal-Wallis (KW) procedures in MapQTL ® 6 (Van Ooijen 2009). A 5% experimentwise significance level controlling for dependent markers was assessed by permutation tests. These LOD critical values ranged from 1.8 to 2.3 depending on the trait and chromosome. Significant QTLs were named as the trait followed by the chromosome where they locate. In the case of mineral elements concentrations, the QTLs were named by the element symbol followed by the tissue (F if fruit, L if leaf) and the chromosome they locate.

A two-way ANOVA was used to study the interaction (epistasis) between markers corresponding to QTLs controlling Fe related traits (and others showing disagreements among QTL mapping procedures), and the cofactors used for their MQM analyses.

Genes covering 2.3-2.7 Mbp around the SNP(s) showing maximum LOD score at each QTL governing Fe related traits, fruit citric acid concentration, BER incidence, and major QTLs controlling leaf or fruit element concentrations were downloaded from the Sol Genomics Network (<https://solgenomics.net/>) and studied for the presence of frameshift InDels in the parental genomes (E9 and L5) (Kevei et al. 2015). Root expression of candidate genes was inferred from the Heinz cultivar using the tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi?dataSource=Rose_Lab_Atlas_Renormalized). Overrepresentation tests (Released 2018-02-03) of all downloaded genes, or only mutated genes, within the QTLs were carried out by means of the PANTHER Classification System (<http://www.pantherdb.org/>) (Mi et al. 2013, 2016) using the Fisher's Exact with FDR correction for multiple tests.

Results

Grafting *per se* (comparing control lines, Bol versus Bol/Bol) significantly affected chlorophyll leaf concentration before reducing [Fe] in nutrient solution (SPAD1) only.

The mean (and standard error) of the phenotypic values observed for the analyzed traits in control lines, the range of variation in the RIL population and the estimated broad sense heritabilities are presented in Table 1. Some fruit elements (Al, Na, Zn and Si) presented high values of heritability while these values were quite low for Fe_F, Fe_L, SPAD3 and FeUEc (0.0988, 0.0150, 0.0422 and 0.0310, respectively). Heritability of the total fruit yield conferred by the rootstock (TFWc) was moderate (0.1583).

Most of the rootstocks (89%), including self-grafted Boludo (Bol/Bol), increased total harvested Fe (FeUEc) as compared to non-grafted Boludo (Bol; **Fig. 1**). Across all graft combinations in the RIL population, fruit ionomic profile differed from that of the leaf (**Supplementary Fig. 2**). Main fruit element was K followed by P, S, Ca, Mg, Na, Al, Fe, Zn, Si and Mn, while main leaf element was Ca. As visualized by principal components analysis (**Supplementary Fig. 3**) fruit yield traits were strongly related. As expected, FeUEc was related to yield traits and Fe_F which was within a group that included the fruit concentrations of P, K, Mg and S. There was no significant correlation between fruit and leaf concentrations of K, S, Fe and Zn (Supplementary Table 4). There were strong negative correlations between K_L, on one hand, and Ca_L, Mg_L and LDW, on the other, while strong positive relations connected fruit and leaf concentrations for Na or Mn.

In total, 103 significant QTLs were detected by the MQM procedure controlling 33 traits (Table 2), only 41 QTLs were also significant by IM, and 27 of them were not supported by IM nor KW procedures. Above 10 % contribution to the total variance was

found for 28 QTLs governing 20 traits. Significant epistasis (**Fig. 2**) were detected for traits such as Al_F, Fe_F and FeUEc whose QTLs were detected only by MQM. The contributions of Na_F_7 and Na_L_7 were particularly large (49.7 and 22.9, respectively) and at similar genetic positions, indicating the same gene (HKT1 in Supplementary Table 5) acting at the root level might be responsible of both fruit and leaf [Na]. Two Inorganic phosphate transporters (Soly09g09007 and Soly09g090080) were found likely candidates for the fruit [P] QTL in chromosome 9 (P_F_9).

From QTLs controlling Fe related traits, four of them were distributed into two clusters, in chromosomes 9 (Fe_L_9 and FeUEc_9) and 12 (Fe_F_12 and Fe_L_12), showing similar genetic positions, and overlapping physical regions with common candidate genes (Table 3; **Supplementary Fig. 6**). Similarly, the total fruit yield QTL TFWc_11 (and FNC_11) had a similar position than FeUEc_11 (**Fig. 3**) and their physical regions containing candidate genes overlapped. The wild allele of *S. pimpinellifolium* at this rootstock QTL improved both yield and harvested Fe, particularly, if the wild allele at the epistatic locus FeUEc_7.1 was also present (“bb” genotype; **Fig. 2**). Several genes mentioned in the introduction as relevant for Fe uptake such as TOMLHA1 (Soly03g113400), FRO1 (Soly01g094910), NRAMP2 (Soly04g078220), FER (Soly06g051550), and MYB72 (Soly07g055000) were found among candidate genes within Fe_L_3, Fe_L_1, FeUEc_4, Fe_F/L_6 and FeUEc_7.2 (Table 3). Additionally, genes coding for secretory proteins whose concentrations in the xylem sap were significantly altered by Fe deficiency in tomato (Ceballos-Laita et al. 2018) were found within Fe_F_1, Fe_L_1, FeUEc_1, Fe_L_6, FeUEc_7.1, Fe_L_10, FeUEc_11, Fe_F_12, and Fe_L_12 (Table 3).

Some biological processes were overrepresented in downloaded genes from QTLs governing Fe related traits, even when only segregating genes were included in the

analysis (Supplementary Table 7). This is the case of secondary metabolic process (GO:0019748) and sulfur compound metabolic process (GO:0006790) for FeUEc, and ion transport (GO:0006811) for Fe_F. Cellular calcium ion homeostasis (GO:0006874) and RNA splicing, via transesterification reactions (GO:0000375) were the most overrepresented biological processes for downloaded genes at Fe_L and Fe_F_L QTLs, respectively.

A QTL for fruit citric acid content (%Cit_12) was detected in chromosome 12 suggesting that the rootstock could affect this quality fruit trait (Table 2). The position of %Cit_12 was close to Fe_L_12 (and Fe_F_12). Noteworthy, the increasing allele for leaf and fruit [Fe] is *pimpinellifolium* while that for increasing citric acid fruit concentration is *lycopersicum*. Candidate genes within QTLs controlling citric acid content and incidence of blossom end rot were also studied. A phosphoenolpyruvate carboxylase 1 coding gene mutated at the *S. pimpinellefolium* allele was found included among candidate genes in %Cit_12 (Supplementary Table 8). Considering genes within QTLs for blossom end rot incidence as a whole (Supplementary Table 9), malate synthase, peptidase inhibitor, and voltage-gated potassium channel activities were significantly overrepresented (**Supplementary Fig. 10**).

Discussion

Rootstock effects on the scion distribution of mineral nutrients under low Fe input

In the case of tomato, the allocation of minerals (fruit versus leaf, **Supplementary Fig. 2**) is important regarding human health and nutrition. Since the root is involved in nutrient acquisition (uptake and translocation), nutrient re-cycling, and perception and transduction of shoot signals (i.e. nutrient status), it is plausible that the rootstock genotype finally affect fruit concentration of minerals. The detection of twenty-one

rootstock QTLs controlling the fruit concentration of mineral elements (Al, Ca, Mg, Mn, Na, P, S, Si and particularly, Fe; Table 2) in present experiment supports this hypothesis.

Correlation and principal component analyses of leaf and fruit traits determined by the rootstock under low Fe nutrition clearly denoted three groups of elements: Na, Mn, and the group formed by K, S, P, Mg and Fe (**Supplementary Fig. 3**). Potassium was the major nutrient of the tomato fruit (**Supplementary Fig. 2**) as reported previously for non-grafted plants (Davies and Hobson 1981; Erba et al. 2013; Capel et al. 2017). Fruit and leaf K concentrations were not correlated but similar (Table 1), like in a similar experiment under moderate salinity and Fe standard conditions (Asins et al. 2015). Capel et al. (2017) detected 3 QTLs for fruit [K] in a similar RIL population of non-grafted plants. In the present experiment, apparently, the rootstock did not affect fruit [K] since its estimated heritability was very low (Table 1) and no rootstock QTL was detected (Table 2).

The concentrations of Na, or Mn, in fruit and leaf were strongly correlated (**Supplementary Fig. 3** and Supplementary Table 4). These correlations could be explained by genetic linkage (or pleiotropic action) of QTLs governing fruit and leaf concentrations of each of these elements in chromosomes 7 (Na) and 3 (Mn). In both cases, the contribution of the fruit concentration QTL is larger than that controlling the leaf concentration (Table 2), although Na and Mn concentrations in the fruit were much smaller than those in the leaf (Table 1). Previous experiments under moderate salinity (Asins et al. 2015; Jaime-Pérez et al. 2017), and present experiment, points out HKT1;2 (Soly07g014680, Supplementary Table 5) at the root vasculature as candidate for both Na_F_7 and Na_L_7 suggesting that Na could go directly to the fruit from the root via xylem, or via phloem, since 31% xylem-borne Na can be laterally transported to the phloem (Peuke 2010; Aubry et al. 2019). In both cases, the expected amount of Na in the

fruit would be smaller than in the leaf causing a reduction of the non-genetic component of variance of Na_F (consequently increasing a little bit its heritability).

A likely candidate for Mn_F_3 (and Mn_L_3) would be the manganese transporter MntH/NRAMP (Soly03g116900 in Supplementary Table 5). Noteworthy, the RIL population is not segregating for frameshift mutations at both HKT1;2 and MntH/NRAMP coding genes, hence, the differences could be at their expression level such as it was observed for HKT1;2 in NILs derived from *S. lycopersicum* and *S. cheesmaniae* (Jaime-Pérez et al. 2017).

Linkage of QTLs also supports the strong negative correlation found between K and Mg concentrations in the leaf (K_L_7 and Mg_L_7 with opposite gene effects in Table 2) and the positive ones in the fruit between Fe and S (Fe_F_12 and S_F_12), and Mg and P (Mg_F_9.2 and P_F_9). In the case of Mg_F_9.2 and P_F_9, their physical regions overlapped and included a calcium-transporting ATPase 1 and two inorganic phosphate transporters as likely candidates, respectively (Supplementary Table 5).

Factors reducing fruit quality and commercial tomato yield

Commercial fruit yield was reduced by the incidence of two physiological disorders, blossom end rot (pFNber) and bronze, deformed fruits (Fe_sym; **Supplementary Fig. 1C**). Besides, 26% rootstocks allowed the accumulation of aluminium above 27 ppm in the fruit (**Fig. 1**). From these, only blossom end rot was also observed in control plants (Bol and Bol/Bol in Table 1), and in a similar experiment previously carried out under moderate salinity and Fe standard conditions (Asins et al. 2015) although no QTL was detected then. Present experiment has allowed the detection of 2 QTLs controlling pFNber in chromosomes 3 and 8. Both of them were linked to FeUEc QTLs (Table 2). As shown in **Supplementary Fig. 10**, malate synthase, peptidase inhibitor, and voltage-gated potassium channel activities were significantly

overrepresented among genes within pFNber QTLs. In the case of malate synthase, it particularly concerns pFNber_3, and could be related to the accumulation of Fe in the apoplast of root meristematic cell (Mora-Macías et al. 2017). K transport might be also involved in BER since pFNber showed the highest correlation with K_F ($p \leq 0.0031$, $r = -0.27$) in our experiment, and physical genomic regions including pFNber_3 and Na_L_3 overlapped (Supplementary Tables 2 and 6). Noteworthy, tolerance to BER has been located in a tomato introgression line involving chromosome 8 (IL8-3) which showed a higher Ca and K fruit content than that of the cultivated parent (Uozumi et al. 2012), and no Ca-movement-related gene was found within the introgressed region (Ikeda et al. 2017).

Given that aluminium has the potential to produce neurotoxicity and to affect the male reproductive system, the panel on food additives, flavourings, processing aids and food contact materials has established a tolerable weekly intake for aluminum of 1mg/Kg of body weight (EFSA 2008). Aluminium is not expected in the tomato fruit (Erba et al. 2013; Capel et al. 2017). In our experiment, fruit [Al] was significantly correlated with the incidence of plants with bronze, deformed fruits ($p < 0.003$, $r = 0.28$; Supplementary Table 4). Al was not detected at all in the fruits of controls plants (Bol and Bol/Bol in Table 1) or plants grafted on 56 RILs; however, 26% of RILs induced an Al fruit concentration between 27 and 338 ppm suggesting this trait has to be evaluate in rootstock breeding programs. Interestingly, three epistatic interactions, all of them involving Al_F_1, significantly increased Al_F above 90 ppm when homozygous for the lycopersicum allele (“a” genotype; **Fig. 2**).

The rootstock can contribute to biofortification and reduction of fertilizer costs in tomato cultivation

Fruit yield QTLs TFWc_11, FNc_11 and FWm_9, were also detected in previous experiments using both the grafted (Estañ et al. 2009) and non-grafted RIL population (Villalta et al. 2007), indicating they are determined by the root system. Interestingly, TFWc_11 and FeUEc_11 were found to overlap in chromosome 11 (**Fig. 3**) suggesting that the rootstock can increase both total fruit yield and harvested Fe under low Fe input.

As previously reported (Estañ et al. 2009), QTL detection by multiple QTL mapping procedure has unveiled epistatic interactions for some traits, particularly for Fe_F and FeUEc (**Fig. 2**). All of them are similar to Mendelian dominant epistasis in which one locus (SNP or QTL) suppresses the allelic effects of a second locus. This kind of epistatic interactions often impede QTL detection by interval mapping procedure, and might explain differences in QTL results between detection procedures.

Close linkage between FeUEc_8 and pFNber_8 agrees the expected opposite direction of gene effects. Besides, the chlorophyll content at the end of the experiment (SPAD3) was highly related to fruit number ($p < 0.0001$, $r = 0.44$; Supplementary Table 4), and two SPAD3 QTLs were closely linked to FeUEc_3 and Fe_F_11 (epistatic over FeUEc_8; **Fig. 2**). Therefore, Fe_F and FeUEc have shown complex genetic architectures but, both traits can be simultaneously improved through rootstock selection at different genomic regions and taking into account the epistatic interactions involved (particularly, FeUEc_11 with FeUEc_7.1, and FeUEc_8 with Fe_F_11). Candidate genes coding for ethylene receptors are within the epistatic loci FeUEc_8 and FeUEc_11 what could provide biological meaning to the interactions.

Ethylene is known to be an important component in low Fe signaling (Liu et al. 2016; Iqbal et al. 2013) and S-adenosyl-methionine, (SAM, a precursor of ethylene, nicotianamine and polyamines) a central metabolite under Fe deficiency in several species (Li et al. 2017). Some gene coding enzymes related to the SAM metabolism were

found within Fe_F_L_6, Fe_L_9/FeUEc_9 (spermidine synthase), Fe_L_9/FeUEc_9 (acireductone dioxygenase) and Fe_F_7 (Enolase-phosphatase E-1). Genes coding for S-adenosylmethionine synthase and 1-aminocyclopropane-1-carboxylate oxidase are among candidates within Fe_L_10 and Fe_F_11, respectively. Also related to ethylene biosynthesis is the 2-oxoglutarate-dependent dioxygenase activity. Besides, it is involved in iron sensing, reutilization or the scavenging of iron from the soil through the biosynthesis of mugineic acid (Farrow et al. 2014). Two polymorphic candidates coding for these enzymes were found within FeUEc_7.2 and Fe_L_10.

Several genes already known to be relevant for Fe uptake and the iron deficiency response (Zamboni et al. 2012; Li and Lan 2017) such as TOMLHA1 (Soly03g113400), FRO1 (Soly01g094910), NRAMP2 (Soly04g078220), FER (Soly06g051550), and MYB72 (Soly07g055000) were found among candidate genes for QTLs governing Fe concentration in the leaf, the fruit, and harvested Fe (Table 3) although only NRAMP2 was segregating for a frameshift mutation in the RIL population.

Noteworthy, Fe_L_1 overlapped with a QTL detected for root colonization by arbuscular mycorrhizal fungi (AMF) in this same RIL population (Plouznikoff et al. 2019). In fact, important functional candidate genes for both traits (ferric reductase oxidase, FRO1, and Calcium-dependent protein kinase I, CCaMK, respectively) are close together (Table 3) supporting a genetic relationship between the Fe deficiency response and AMF colonization in tomato (Romera et al. 2019).

In the xylem sap, Fe is found complexed with carboxylates, mainly citrate, in agreement with the increases in citric acid cycle metabolites here in response to Fe deficiency (Rellan-Alvarez et al. 2011). Thus, genes coding for citrate synthase and key enzymes regulating the citric acid cycle and glycolysis have been noted within Fe_F_1, Fe_F_12, Fe_L_12, FeUEc_7.1, and Fe_L_9/FeUEc_9 (Table 3). Besides, 10 genes

coding for secretory proteins whose concentrations in the xylem sap were significantly altered by Fe deficiency in tomato (Ceballos-Laita et al. 2018) were also found within QTLs governing Fe related traits (Table 3), although no segregation for frameshift mutations was observed at their coding regions.

Therefore, the gene content of rootstock QTLs controlling total harvested Fe and Fe concentration of leaf and fruit of the grafted variety in our experiment, agreed with both the root transcriptional response and the protein profile of the xylem sap reported for tomato under Fe deficiency. Differences in the regulation of expression of parental alleles [from *S. lycopersicum* (E9) or *S. pimpinellifolium* (L5)] of those genes are suspected to be responsible for the detected QTLs. Moreover, since neighbor genes in the genome are often co-expressed forming clusters of expression neighborhoods (Michalak 2008; O'Rourke et al. 2009), quantitative differences in cis-acting regulatory elements between parental haplotypes of neighbor genes could give rise to the detected QTLs for iron related traits rather than differences in the coding sequence of single genes.

In conclusion, the rootstock can improve the total harvested iron of tomato under commercial but iron limited growing conditions; *pimpinellifolium* allele increased this trait and fruit [Fe] at most QTLs, and decreased Al fruit content, valorizing germplasm utilization in tomato rootstock breeding programs. The gene contents of rootstock QTLs detected for Fe related traits seem to reflect, at least in part, the previously reported Fe deficiency root response at both transcriptomic and proteomic levels.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical standards: The authors declare that the experiment comply with the current laws.

References

473 Asins MJ, Albacete A, Martinez-Andujar C, Pérez-Alfocea F, Dodd IC, Carbonell EA, Dieleman
 474 JA (2017) Genetic analysis of rootstock-mediated nitrogen (N) uptake and root-to-shoot
 475 signalling at contrasting N availabilities in tomato. *Plant Science* 263: 94-106.

476 Asins MJ, Raga V, Roca D, Carbonell EA (2015) Genetic dissection of tomato rootstock effects
 477 on scion traits under moderate salinity. *Theor Appl Genet* 128: 667-679.

478 Aubry E, Dinant S, Vilaine F, Bellini C, Le Hir R (2019) Lateral transport of organic and inorganic
 479 solutes. *Plants* 8: 20. <https://doi.org/10.3390/plants8010020>

480 Barberon M, Vermeer JE, De Bellis D, Wang P, Naseer S, Andersen TG, Humbel BM5, Nawrath
 481 C, Takano J, Salt DE, Geldner N (2016) Adaptation of Root Function by Nutrient-Induced
 482 Plasticity of Endodermal Differentiation. *Cell* 164:447-59. [https://doi.org/](https://doi.org/10.1016/j.cell.2015.12.021)
 483 [10.1016/j.cell.2015.12.021](https://doi.org/10.1016/j.cell.2015.12.021).

484 Briat J-F, Duc C, Ravet K, Gaymard F (2010) Ferritins and iron storage in plants. *Biochemistry*
 485 *Biophysics Acta* 1800: 806-8014.

486 Capel C, Yuste-Lisbona FJ, López-Casado, Angost T, Heredia A, Cuarter J, Fernández-Muñoz
 487 R, Lozano R, Capel J (2017) QTL mapping of fruit mineral contents provides new chances for
 488 molecular breeding of tomato nutritional traits. *Theor Appl Genet* 130: 903-913.

489 Castaings L, Caquot A, Loubet S, Curie C (2016) The high-affinity metal transporters NRAMP1
 490 and IRT1 team up to take up iron under sufficient provision. *Scientific Reports* 6: 37222.
 491 [https://doi.org/ 10.1038/srep37222](https://doi.org/10.1038/srep37222)

492 Ceballos-Laita L, Gutierrez-Carbonell E, Takahashi D, Abadia A, Uemura M, Abadia J, Lopez-
 493 Millan AF (2018) Effects of Fe and Mn deficiencies on the protein profiles of tomato (*Solanum*
 494 *lycopersicum*) xylem sap as revealed by shotgun analyses. *Journal of Proteomics* 170: 117-129.
 495 <https://doi.org/10.1016/j.jprot.2017.08.018>

496 Clemens S, Weber M (2015) The essential role of coumarin secretion for Fe acquisition from
 497 alkaline soil. *Plant Signaling and Behavior* 11: 2, e1114197.

498 Connort JM, Balk J, Rodriguez-Celma J (2017) Iron homeostasis in plants – a brief overview.
 499 Metallomics 9: 813-823.

500 Davies JN, Hobson LE (1981) The constituents of tomato fruit, the influence of environment,
 501 nutrition and genotype. Critical Reviews Food Science Nutrition 15: 205-280.

502 EFSA (2008) European Food Safety Agency. Scientific Opinion of the Panel on Food Additives,
 503 Flavourings, Processing Aids and Food Contact Materials (AFC) on a request from the
 504 European Commission on safety of aluminium from dietary intake. The EFSA Journal 754:1-
 505 34. <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.754>

506 Erba D, Casiraghi MC, Ribas-Agustí A, Cáceres R, Marfà O, Castellari M (2013) Nutritional value
 507 of tomatoes (*Solanum lycopersicum* L.) grown in greenhouse by different agronomic
 508 techniques. Journal of Food Composition and Analysis 31: 245-251

509 Estañ MT, Villalta I, Bolarín MC, Carbonell EA, Asins MJ (2009) Identification of fruit yield loci
 510 controlling the salt tolerance conferred by *solanum* rootstocks. Theor Appl Genet 118: 305-312

511 FAOSTAT (2017) FAO Statistical Databases FAOSTAT: Suite of Food Security Indicators.
 512 <http://www.fao.org/faostat/en/#data> (access March, 6th, 2019)

513 Farrow SC, Facchini PJ (2014) Functional diversity of 2-oxoglutarate/Fe(II)-dependent
 514 dioxygenases in plant metabolism. Frontiers in Plant Science 5: 624. [https://doi.org/](https://doi.org/10.3389/fpls.2014.00524)
 515 10.3389/fpls.2014.00524

516 Fourcroy P, Sisó-Terraza P, Sudre D, Savirón M, Reyt G, Gaymard F, et al. (2014) Involvement
 517 of the ABCG37 transporter in secretion of scopoletin and derivatives by *Arabidopsis* roots in
 518 response to iron deficiency. New Phytologist 201: 155-167

519 Fourcroy P, Tissot N, Gaymard F, Briat JF, Dubos C (2016) Facilitated Fe nutrition by phenolic
 520 compounds excreted by the *Arabidopsis* ABCG37/PDR9 transporter requires the IRT1/FRO2
 521 high-affinity root Fe(2+) transport system. Molecular Plant 9: 485–488. [https://doi.org/](https://doi.org/10.1016/j.molp.2015.09.010)
 522 10.1016/j.molp.2015.09.010

523 Gollhafer J, Timofeev R, Lan P, Schmidt W, Buckhout TJ (2014) Vacuolar-iron-transporter1-
524 like proteins mediate iron homeostasis in Arabidopsis. PloS ONE: e110468

525 Green LS, Rogers EE (2004) FRD3 controls iron localization in Arabidopsis. Plant Physiology
526 136:2523-31. <https://doi.org/10.1104/pp.104.045633>

527 Ikeda H, Shibuya T, Nishiyama M, Nakata Y, Kanayama Y (2017) Physiological Mechanisms
528 Accounting for the Lower Incidence of Blossom-end Rot in Tomato Introgression Line IL8-3
529 Fruit. The horticultural Journal 86: 327-333

530 Iqbal N, Trivellini A, Masood A, Ferrante A, Khan NA (2013) Current understanding on ethylene
531 signaling in plants: the influence of nutrient availability. Plant Physiology and Biochemistry
532 73: 128-138

533 Jaime-Pérez N, Pineda B, García-Sogo B, Atares A, Athman A, Byrt CS, Olias R, Asins MJ,
534 Gilliam M, Moreno V, Belver A (2017) The Na⁺ transporter encoded by the HKT1;2 gene
535 modulates Na⁺/K⁺ homeostasis in tomato shoots under salinity. Plant, Cell and Environment.
536 <https://doi.org/10.1111/pce.12883>

537 Kailasam S, Chien W-F, Yeh K-C (2019) Small-Molecules selectively modulate iron-deficiency
538 signaling networks in Arabidopsis. Frontiers in Plant Science 10:8. [https://doi.org/](https://doi.org/10.3389/fpls.2019.00008)
539 10.3389/fpls.2019.00008

540 Kevei Z, King RC, Mohareb F, Sergeant MJ, Awan SZ, Thompson AJ (2015) Resequencing at
541 ≥ 40 -Fold Depth of the Parental Genomes of a *Solanum lycopersicum* x *S. pimpinellifolium*
542 Recombinant Inbred Line Population and Characterization of Frame-Shift InDels That Are
543 Highly Likely to Perturb Protein Function. G3 Genes/Genomes/Genetics 5: 971-981

544 Kobayashi T, Nishizawa NK (2012) Iron uptake, translocation, and regulation in higher plants.
545 Annual Review Plant Biology 63:131-152.

546 Li W, Lan P (2017) The understanding of the plant iron deficiency responses in Strategy I plants
 547 and the role of ethylene in this process by omic approaches. *Frontiers in Plant Science* 8:40.
 548 [https://doi.org/ 10.3389/fpls.2017.00040](https://doi.org/10.3389/fpls.2017.00040)

549 Lin HX, Rubio L, Smythe AB, Falk BW (2004) Molecular population genetics of Cucumber
 550 mosaic virus in California: Evidence for founder effects and reassortment. *Journal Virology* 78:
 551 6666–6675

552 Lin XY, Ye YQ, Fan SK, Jin CW, Zheng SJ (2016). Increased sucrose accumulation regulates
 553 iron-deficiency responses by promoting auxin signaling in *Arabidopsis* plants. *Plant Physiology*
 554 170: 907-920. <https://doi.org/10.1104/pp15.01598>

555 Liu XX, He XL, Jin CW (2016) Roles of chemical signals in regulation of the adaptive responses
 556 to iron deficiency. *Plant Signaling & Behavior* 11: e1179418 [https://doi.org/](https://doi.org/10.1080/15592324.2016.1179418)
 557 [10.1080/15592324.2016.1179418](https://doi.org/10.1080/15592324.2016.1179418)

558 Lucena C, Waters BM, Romera FJ, Garcia MJ, Morales M, Alcántara E, Pérez-Vicente R (2006)
 559 Ethylene could influence ferric reductase, iron transporter, and H⁺-ATPase gene expression by
 560 affecting FER (or FER-like) gene activity. *Journal of Experimental Botany* 57: 4145-4154.

561 McLean E, Cogswell M, Egli I, Woidyla D, De Benoist B (2009) Worldwide prevalence of anaemia.
 562 WHO vitamin and mineral nutrition information system, 1993-2005. *Public Health Nutrition*
 563 12: 444-454. <https://doi.org/10.1017/S1368980008002401>

564 Meenakshi JV, Johnson NL, Manyong VM, Degroote H, Javelosa J, Yaggen DR, et al (2010) Hoe
 565 cost-effective is biofortification in combating micronutrient malnutrition? An ex ante
 566 assessment. *World Development* 38: 64-75

567 Mengel K (1994) Iron availability in plant-tissues-iron chlorosis on calcareous soils. *Plant Soil*
 568 165: 275-283

569 Michalak P (2008) Coexpression, coregulation, and cofunctionality of neighboring genes in
 570 eukaryotic genomes. *Genomics* 9_243-248

571 Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, Thomas PD (2016) PANTHER
572 version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data
573 analysis tool enhancements. *Nucleic Acids Research*. [https://doi.org/ 10.1093/nar/gkw1138](https://doi.org/10.1093/nar/gkw1138)

574 Mi H, Muruganujan A, Casagrande JT, Thomas PD (2013) Large-scale gene function analysis
575 with the PANTHER classification system. *Nature Protocols* 8: 1551-1566.

576 Miller DD, Welch RM (2013) Foods system strategies for preventing micronutrient malnutrition.
577 *Food Policy* 42, 115-128. [https://doi.org/ 10.1016/j.foodpol.2013.06.008](https://doi.org/10.1016/j.foodpol.2013.06.008)

578 Monforte AJ, Asins MJ, Carbonell EA (1997) Salt tolerance in *Lycopersicon* species. 5. Does
579 genetic variability at quantitative trait loci affect their analysis? *Theor Appl Genet* 95: 284-293.

580 Mora-Macías J, Ojeda-Rivers Jo, Gutierrez-Alanis D, Yong-Villalobos L, Oropeza-Aburto A,
581 Raya-González J, et al (2017) Malate-dependent Fe accumulation is a critical checkpoint in the
582 root development response to low phosphate. *Proceedings National Academy of Science USA*
583 114: E3563-E3572

584 O'Rourke JA, Nelson RT, Grant D, Schmutz J, Grimwood J, Cannon S, Vance CP, Shoemaker RC
585 (2009) Integrating microarray analysis and the soybean genome to understand the soybeans iron
586 deficiency response. *BMC Genomics* 10: 376. <https://doi.org/10.1186/1471-2164-10-376>

587 Peuke AD (2010) Correlations in concentrations, xylem and phloem flows, and partitioning of
588 elements and ions in intact plants. A summary and statistical re-evaluation of modelling
589 experiments in *Ricinus communis*. *Journal of Experimental Botany* 61: 635-655

590 Plouznikoff K, Asins MJ, Dupré de Boulois H, Carbonell EA, Declerck S (2019) Genetic analysis
591 of tomato root colonization by arbuscular mycorrhizal fungi. *Annals of Botany*, mcy240.
592 <https://doi.org/10.1093/aob/mcy240>

593 Price AH (2006) Believe it or not, QTLs are accurate! *Trends in Plant Sci* 11:213-216

594 Rellan-Alvarez R, El-Jendoubi H, Wohlgemuth G, Abadia A, Fiehn O, Abadia J, Alvarez-
595 Fernandez A (2011) Metabolite profile changes in xylem sap and leaf extracts of strategy I

596 plants in response to iron deficiency and resupply. *Frontiers in Plant Science* 2: 66.
 597 <https://doi.org/10.3389/fpls.2011.00066>

598 Rellán-Alvarez R, Giner-Martínez-Sierra J, Orduna J, Orera, I, Rodríguez-Castrillón JA, García-
 599 Alonso JJ, Abadía J, Álvarez-Fernández A (2010) Identification of a Tri-Iron (III), Tri-Citrate
 600 Complex in the Xylem Sap of Iron-Deficient Tomato Resupplied with Iron: New Insights into
 601 Plant Iron Long-Distance Transport. *Plant and Cell Physiology* 51: 91-102.
 602 <https://doi.org/10.1093/pcp/pcp170>

603 Romera FJ, García MJ, Lucena C, Martínez-Medina A, Aparicio MA, Ramos J, Alcántara E,
 604 Angulo M, Pérez-Vicente R (2019) Induced systemic resistance (ISR) and Fe deficiency
 605 responses in dicot plants. *Frontiers in Plant Science* 10: 287

606 Santi S, Schmidt W (2009) Dissecting iron deficiency-induced proton extrusion in *Arabidopsis*
 607 roots. *New Phytologist* 183: 1072-1084.

608 Strobbe S, De Lepeleire J, Van Der Straeten D (2018). From in planta Function to Vitamin-Rich
 609 Food Crops: The ACE of Biofortification. *Frontiers in Plant Science* 9:1862.
 610 <https://doi.org/10.3389/fpls.2018.01862>

611 Tsai H, Schmidt W (2017) Mobilization of iron by plant-borne coumarins. *Trends in Plant Science*
 612 22: 538-548

613 Uozumi A, Ikeda H, Hiraga M (2012) Tolerance to salt stress and blossom-end rot in an
 614 introgression line, IL8-3, of tomato. *Scientia Horticulturae* 138: 1-6

615 Van Ooijen JW (2009) MapQTL 6. Software for the mapping of quantitative trait loci in
 616 experimental populations of diploid species. Kyazma BV: Wageningen, Netherlands

617 Villalta I, Bernet GP, Carbonell EA, Asins MJ (2007) Comparative QTL analysis of salinity
 618 tolerance in terms of fruit yield using two *Solanum* populations of F7 lines. *Theor Appl Genet*
 619 114: 1001-1017

620 Wang H, Inukaia Y, Yamauchia A (2006) Root development and nutrient uptake. Critical Reviews
621 in Plant Sciences 25: 279-301

622 Waters BM, Amundsen K, Graef G (2018) Gene expression profiling on iron deficiency chlorosis
623 sensitive and tolerant soybean indicates key roles for phenylpropanoids under alkalinity stress.
624 Frontiers in Plant Science 9:10. [https://doi.org/ 10.3389/fpls.2018.00010](https://doi.org/10.3389/fpls.2018.00010)

625 Zamboni A, Zanin L, Tomasi N, Pezzotti M, Pinton R, Varanin, Z, Cesco S (2012) Genome-wide
626 microarray analysis of tomato roots showed defined responses to iron deficiency. BMC
627 Genomics 13: 101. <https://doi.org/10.1186/1471-2164-13-101>

628 Zhu XF, Wang B, Song WF, Zheng SJ, Shen RF (2016) Putrescine alleviates iron deficiency via
629 NO-dependent reutilization of root cell wall Fe in Arabidopsis. Plant Physiology 170: 558-567
630

631 **Table 1-** The mean and standard error of the phenotypic values observed for the analyzed traits
632 in control lines (Bol and Bol/Bol). Minimum (Min) and maximum (Max) means in RIL population
633 and estimated broad sense heritabilities (H^2) are also included.

Trait	Abbreviation	Bol	Bol/Bol	Min (RIL)	Max (RIL)	H^2
Citric acid fruit content	%Cit	0.85±0.26	0.53±0.03	0.54	1.17	0.0000
[Al] in fruit	Al_F	0±0	0±0	0.00	337.99	0.8209
[Al] in leaf	Al_L	53.87±6.12	45.5±5.19	27.69	169.24	0.3321
[Ca] in fruit	Ca_F	1404.61±46.33	1405.24±202.52	890.06	2011.18	0.0726
[Ca] in leaf	Ca_L	47114.85±1686.49	41030.17±2572.3	30779.56	53305.40	0.2169
[Cu] in leaf	Cu_L	7.34±0.65	9.49±1.87	6.48	35.55	0.1206
Fruit dry matter	FDM	7.3±0.44	6.28±0.36	5.22	8.26	0.0000
[Fe] in fruit	Fe_F	28.17±1.66	38.2±2.12	22.15	42.09	0.0988
100 (Fe_F/Fe_L)	Fe_F/L	28.74±4.56	42.56±2.77	20.80	49.78	0.0451
[Fe] in leaf	Fe_L	104.29±20.02	90.72±9.18	78.05	180.78	0.0150
Bronze-fruits incidence	Fe_Sym	0±0	0±0	0.00	0.43	0.0677
Total harvested Fe	FeUEc	2.31±0.63	3.64±0.41	1.92	5.17	0.0310
Commercial Fruit number	FNc	20.76±1.07	17.85±1.61	12.27	37.06	0.1316
Mature fruit weight	FWm	69.86±2.6	72.52±3.57	45.29	97.11	0.1152
[K] in fruit	K_F	25991.52±1741.38	28655.45±2113.81	23164.05	31479.14	0.0732
[K] in leaf	K_L	24735.97±4696.6	28000.11±2309.65	17582.83	33966.03	0.1811
Leaf dry matter	LDM	13.21±1.64	12.35±0.7	11.12	22.41	0.0737
Leaf dry weight	LDW	4.51±0.73	3.64±0.23	2.25	4.76	0.0000
Leaf fresh weight	LFW	33.98±2.92	29.47±1.07	17.27	32.65	0.0000
[Mg] in fruit	Mg_F	1295.57±11.87	1396.98±46.99	1185.58	1703.18	0.0335
[Mg] in leaf	Mg_L	6949.65±1230.15	6377.59±997.51	3870.30	12070.60	0.2970
[Mn] in fruit	Mn_F	10.54±0.34	13.01±0.12	7.90	21.12	0.1737
[Mn] in leaf	Mn_L	181.42±25.32	194.07±42.27	131.46	356.87	0.1328
[Na] in fruit	Na_F	353.26±50.64	328.39±29.62	234.24	720.36	0.5516
[Na] in leaf	Na_L	1158.71±223.51	1085.57±334.81	800.09	4533.91	0.5025
[P] in fruit	P_F	4972.82±183.43	5390.36±243.63	4054.18	5654.26	0.0938
[P] in leaf	P_L	9765.3±372.02	10291.89±697.3	7045.93	11259.28	0.1784
Blossom end rot incidence	pFNber	1.3±0.72	1.03±0.57	0.00	15.48	0.0000
Out-of-type fruit incidence	pFNnot	0±0	0±0	0.00	9.55	0.0291
Fruit juice pH	pH	4.14±0.14	4.26±0.03	4.03	4.63	0.0000
Root dry matter	RDM	13.2±1.09	11.48±0.81	9.07	19.95	0.1734
Root dry weight	RDW	2.22±0.34	1.83±0.11	1.00	3.49	0.1075
Root fresh weight	RFW	16.91±2.7	16.25±2.1	9.36	33.08	0.1966

[S] in fruit	S_F	1775.4±29.48	2034.68±169.33	1418.28	2360.68	0.1420
[S] in leaf	S_L	15572.02±937.14	16209.07±2388.98	10498.74	20662.51	0.2309
[Si] in fruit	Si_F	21.64±2.07	19.76±1.04	11.26	36.43	0.4287
[Si] in leaf	Si_L	363.99±54.92	249.03±11.99	219.71	621.06	0.2255
SPAD before treatment	SPAD1	38.86±0.71	29.73±0.95	32.40	49.41	0.3308
SPAD at the end	SPAD3	53.27±1.18	50.58±2.17	42.50	63.52	0.0422
Fruit soluble-solids content	SSC	5.9±0.55	5.13±0.35	4.61	6.78	0.0000
Commercial total fruit weight	TFWc	1392.8±63.14	1265.55±102.72	870.76	2243.83	0.1583
[Zn] in fruit	Zn_F	16.17±1.44	19.42±1.2	10.67	97.86	0.5629
[Zn] in leaf	Zn_L	52.68±8.2	57.44±10.13	26.92	97.04	0.2057

634

635 **Table 2-** List of QTLs (named by the trait, the tissue: Fruit or Leaf, and the chromosome)
636 that were detected by using MQM procedure (5% overall significance level) and
637 corresponding SNPs (mostly SolCAP SNPs named by the number) at the LOD peak. The
638 map position (cM) of QTL peaks in the tomato chromosomes (Chr) and the means for both
639 homozygous genotypes, *LL* and *PP*, are indicated. The estimated additive value is **a**, and the
640 percentage of explained variance, PEV. QTLs with an asterisk were not significant by the
641 Kruskal-Wallis procedure. QTLs in bold were detected by IM. SNPs in bold were used to
642 search for candidate genes in the corresponding genomic region.

QTL	Chr.	cM	SNP	LOD	<i>LL</i>	<i>PP</i>	PEV	a
%Cit_12	12	51.970	65953	2.55	0.83	0.75	9.5	0.04
Al_F_1	1	126.672	40303	3.64	74.81	18.81	11.1	28.00
Al_F_12	12	108.310	31405	2.56	69.66	23.96	7.6	22.85
Al_F_5*	5	90.079	37400	3.19	72.56	21.06	9.6	25.75
Al_L_1*	1	117.053	44394	3.31	16.26	105.44	9.8	-44.59
Al_L_9	9	99.662	69633	3.57	71.58	51.57	12.2	10.01
Ca_F_11	11	91.800	SL1_00sc6004_1339647	2.83	1398.82	1536.16	9.5	-68.67
Ca_F_6	6	60.181	42119	2.95	1538.27	1396.70	9.9	70.79
Ca_L_12	12	107.846	31495-31403	3.46	43367.50	40545.30	9.4	1411.07
Ca_L_4	4	19.369	34675	2.32	40801.50	43215.10	6.5	-1206.75
Ca_L_7	7	47.604	CL017821_0277-38888	2.97	40645.20	43325.90	8.3	-1340.35
Cu_L_11	11	33.627	21087	2.52	11.17	13.05	5.8	-0.94
Cu_L_8.1*	8	73.277	64706	8.17	15.35	8.85	21.1	3.25
Cu_L_8.2	8	76.682	64865	4.12	9.46	14.74	9.8	-2.64
Fe_F_1	1	70.833	27286	3.27	30.02	32.11	7.1	-1.05
Fe_F_11*	11	98.543	2733	2.91	32.07	30.06	6.3	1.00
Fe_F_12	12	38.979	1495	4.73	29.35	32.78	10.6	-1.71
Fe_F_4*	4	9.099	63976	3.84	29.83	32.30	8.4	-1.24
Fe_F_7*	7	92.858	70912	4.34	32.28	29.85	9.6	1.21
Fe_F_8*	8	0.292	56416	4.17	29.80	32.33	9.2	-1.27
Fe_F/L_6	6	24.576	44694	2.37	31.23	34.00	8.3	-1.38
Fe_L_1	1	96.007	28057-28168	2.84	95.64	105.63	7.7	-4.99
Fe_L_10*	10	7.447	61108-61089	2.27	104.90	96.06	6.2	4.42
Fe_L_12	12	44.174	1573	3.53	94.96	106.00	9.9	-5.52
Fe_L_3*	3	90.075	62473	5.14	116.64	83.52	12.1	16.56
Fe_L_6	6	14.306	25166-35174	3.35	106.14	95.42	9.2	5.36
Fe_L_9*	9	71.915	CL016636_0429	2.04	104.49	96.18	5.1	4.15
FeUEc_1	1	136.127	31775	4.3	3.04	3.42	9.5	-0.19
FeUEc_11	11	2.586	66491	2.73	3.09	3.38	5.8	-0.15
FeUEc_3	3	54.202	30006	3.03	3.08	3.39	6.5	-0.16
FeUEc_4*	4	42.238	23559	2.62	3.09	3.38	5.6	-0.15
FeUEc_7.1	7	13.491	68417	4.13	3.05	3.42	9.1	-0.18
FeUEc_7.2*	7	79.630	53387	2.75	3.38	3.09	5.9	0.15
FeUEc_8*	8	50.429	48484	3.86	3.05	3.42	8.4	-0.19

FeUEc_9*	9	79.450	58161	3.36	3.40	3.07	7.3	0.17
FNc_10	10	8.942	61127-45992	2.54	28.88	25.74	7.5	1.57
FNc_11	11	3.889	66528	2.7	26.05	28.31	8.1	-1.13
FNc_8	8	96.487	34862	2.46	26.07	28.29	7.4	-1.11
FWm_8.1*	8	57.277	29418-48426	5.96	68.88	47.38	15.7	10.75
FWm_8.2*	8	58.792	48414	3.76	52.56	63.89	11.0	-5.67
FWm_8.3	8	102.803	SGN_U580186	3.83	60.48	55.97	11.2	2.26
FWm_9	9	42.226	39492	3.86	60.43	56.03	11.3	2.20
K_L_12	12	12.183	45816	2.87	25784.00	27598.70	8.4	-907.37
K_L_7	7	84.149	53288	3.01	27625.20	25757.50	8.8	933.82
Mg_F_9.1	9	34.586	39725	2.83	1362.25	1415.65	9.0	-26.70
Mg_F_9.2	9	85.839	58269	5.09	1425.88	1352.02	16.9	36.93
Mg_L_5	5	58.641	50940	3.5	7452.02	6553.31	10.3	449.36
Mg_L_7	7	87.245	55513	2.2	6652.72	7350.03	6.3	-348.65
Mg_L_9	9	96.935	CL017713_0530	2.57	7384.06	6621.28	7.4	381.39
Mn_F_3	3	94.404	CL015369_0414	4.48	13.38	11.99	12.3	0.69
Mn_F_7	7	94.149	37096	2.72	13.23	12.14	7.2	0.54
Mn_L_11	11	12.236	36091	4.17	215.13	241.22	9.4	-13.05
Mn_L_12	12	44.463	1568	3.62	216.11	240.24	8.0	-12.06
Mn_L_2	2	80.025	67389	3.61	240.20	216.15	8.0	12.02
Mn_L_3	3	83.743	69353	3.96	240.66	215.69	8.8	12.48
Mn_L_4*	4	5.136	63865	2.45	238.22	218.29	5.3	9.96
Mn_L_6*	6	32.827	CL016124_0483	2.9	238.97	217.37	6.3	10.80
Mn_L_7	7	43.816	52180	4	241.20	215.15	8.9	13.02
Na_F_11	11	77.484	SL10715_489	2.45	473.80	424.09	4.2	24.86
Na_F_12*	12	99.704	31628	2.56	475.49	423.45	4.5	26.02
Na_F_7	7	39.963	57007	19.79	363.01	535.93	49.7	-86.46
Na_L_1.1	1	33.125	59890	3.06	1758.75	2124.73	5.3	-182.99
Na_L_1.2*	1	102.212	43843	2.14	2092.23	1791.25	3.7	150.49
Na_L_3	3	80.054	20088	7.1	1660.23	2223.25	13.4	-281.51
Na_L_5	5	60.651	37122	2.35	2095.40	1788.08	4.0	153.66
Na_L_7	7	41.461	45589-38698	11.26	1561.51	2313.45	22.9	-375.97
P_F_9	9	93.838	36845-69503	4.57	4868.52	4577.33	16.5	145.59
P_L_12	12	63.238	53955-53918	5.5	8494.61	9302.41	15.9	-403.90
P_L_2	2	57.866	49909	4.1	8494.11	9308.49	11.6	-407.19
P_L_3	3	103.530	20803	4.03	9724.83	8016.67	9.7	854.08
pFNber_3	3	76.686	CL015971-0495	4.47	4.35	2.38	13.8	0.99
pFNber_8	8	50.942	48469	3.93	4.34	2.39	12.1	0.98
RDW_1.1*	1	54.068	SL20127_1427	3.11	1.85	2.21	8.8	-0.18
RDW_1.2	1	84.674	34545	4.45	2.23	1.83	13.0	0.20
RDW_2	2	46.390	SGN_U313245	3.48	1.85	2.21	10.0	-0.18
RDW_6	6	101.466	54244	2.78	2.18	1.88	7.8	0.15
RFW_1	1	84.674	34545	6.32	18.67	14.66	14.3	2.00
RFW_10.1	10	12.031	46056	2.02	17.70	15.64	4.2	1.03
RFW_10.2*	10	43.752	31839	3.5	15.19	18.15	7.5	-1.48
RFW_2	2	61.475	CL017838_0265	3.81	15.26	18.07	8.2	-1.41
S_F_12	12	47.517	32638	2.39	1826.44	1922.28	9.0	-47.92
S_L_2.1	2	0.303	36484	5.98	14548.80	16913.20	15.2	-1182.17

S_L_2.2*	2	14.844	59581	2.59	16484.60	14977.40	6.2	753.60
S_L_5	5	59.492	51111	3.99	16405.20	15056.80	9.8	674.17
S_L_6	6	59.267	SGN_U316705	2.18	16203.10	15258.90	5.1	472.14
S_L_7	7	39.465	56997	4.14	15066.00	16396.00	10.2	-665.03
S_L_9*	9	114.318	63742	2.47	15225.20	16236.80	5.9	-505.81
Si_F_3	3	94.404	CL015369_0414	3.94	17.97	21.85	12.1	-1.94
Si_L_1.1	1	33.125	59890	3.91	341.19	385.15	9.6	-21.98
Si_L_1.2*	1	139.174	54685	2.22	380.56	348.73	4.9	15.92
Si_L_3.1	3	50.562	30031	4.35	387.03	339.31	10.8	23.86
Si_L_3.2	3	80.054	20088	6.39	333.73	392.61	16.5	-29.44
Si_L_8	8	74.850	64736	2.85	382.38	343.96	6.9	19.21
Si_L_9	9	94.890	69503	2.85	382.05	344.29	6.9	18.88
SPAD1_6	6	55.729	17019	2.35	44.60	42.87	8.8	0.86
SPAD3_11	11	99.658	28430	2.82	57.76	55.61	8.0	1.07
SPAD3_3.1	3	52.755	30657	4.72	55.16	58.20	13.8	-1.52
SPAD3_3.2*	3	75.667	35697	2.19	57.70	55.67	6.1	1.01
TFWc_11	11	3.889	66528	2.56	1477.12	1618.62	8.9	-70.75
Zn_L_3	3	92.566	62300	2.37	51.92	58.18	5.3	-3.13
Zn_L_6.1	6	39.866	55921	3.52	50.72	59.38	8.0	-4.33
Zn_L_6.2*	6	59.267	SGN_U316705	2.21	58.47	51.63	4.9	3.42
Zn_L_9	9	101.937	69669	3.67	62.12	47.98	8.4	7.07

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645 **Table 3-** Summary list of candidate genes for QTLs detected for Fe related traits, some
646 segregating for frameshift Indels (Kevei et al. 2015) in parental genomes, E9 or L5, (Mut.). The
647 Locus reference, its relative root expression (Exp.) in Heinz cultivar (Max: maximum, H: high,
648 M: medium, VL: very low, L: low and N: no data), and the number of genes counted from the
649 QTL peak (Ord.) are also shown. In bold, genes coding for reported proteins (in parenthesis) in
650 the tomato xylem sap (Ceballos-Laita et al. 2018).

QTL	Start	Annotated gene	Locus	Ord.	Mut.	Exp.
Fe_F_1	80826712	Auxin-regulated protein	Solyc01g081630	55		Max
Fe_F_1	82088252	Mate efflux family protein	Solyc01g087150	38	L5	Max
Fe_F_1	81107161	Citrate synthase	Solyc01g073740	43		Max
Fe_F_1	80471010	Beta-glucosidase	Solyc01g081170	101	L5	VL
Fe_F_1	82719917	Subtilisin-like protease (O82777)	Solyc01g087850	107		Max
Fe_L_1	86291764	Ferric reductase oxidase	Solyc01g094890	84		Max
Fe_L_1	86303058	Ferric reductase	Solyc01g094900	83		L
Fe_L_1	86314679	Ferric reductase oxidase (FRO1)	Solyc01g094910	82		L
Fe_L_1	87180948	Auxin response factor 9	Solyc01g096070	34		Max
Fe_L_1	87543814	Aspartic proteinase nepenthesin-1 (K4B033)	Solyc01g096450	72		M
Fe_L_1	87817159	Calcium-dependent protein kinase 1 (CCaMK)	Solyc01g096820	109		Max
FeUEc_1	95138462	LRR receptor-like ser/thr-protein kinase (K4B2Y3)	Solyc01g107670	85		M
FeUEc_1	95374230	PI-PLC X domain-containing protein (K4B315)	Solyc01g107990	117		M
FeUEc_3	10614784	Methyl jasmonate esterase	Solyc03g044820.2.1	27		VL
FeUEc_3	11305087	Aromatic L-amino acid decarboxylase	Solyc03g045010.1.1	8	L5	N
FeUEc_3	11305456	Aromatic L-amino acid decarboxylase	Solyc03g045020.2.1	7	L5	N
FeUEc_3	11495587	Ammonium transporter	Solyc03g045070.1.1	2	L5	VL
FeUEc_3	12235129	Leaf senescence protein-like	Solyc03g046300.1.1	20	L5	M
FeUEc_3	12320018	Ribosomal RNA-processing protein 7 homolog A	Solyc03g046350.2.1	25	L5	M
Fe_L_3	62715230	Multidrug and toxin extrusion protein 1	Solyc03g112250	74		Max
Fe_L_3	62719449	MATE efflux family protein	Solyc03g112260	73		Max
Fe_L_3	62756705	Ferric reductase oxidase	Solyc03g112320	67		M
Fe_L_3	63555824	H-ATPase (TOMLHA1)	Solyc03g113400	40		Max
Fe_L_3	63563822	Auxin response factor 9	Solyc03g113410	41		Max
Fe_L_3	64444360	Ethylene-responsive transcription factor 3	Solyc03g114440	144		Max
Fe_F_4	1717764	Vacuolar iron transporter (CCC1)	Solyc04g008060	17		M
Fe_F_4	2859789	Ethylene-responsive transcription factor 4	Solyc04g009450	156	L5	VL
FeUEc_4	61109238	ABC transporter G family member 31	Solyc04g076170	119	E9	M
FeUEc_4	61654640	Oligopeptide transporter 4	Solyc04g076780	58		Max
FeUEc_4	61903881	Sucrose transporter	Solyc04g076960	40		Max
FeUEc_4	62429315	AP2-like ethylene-responsive transcription factor	Solyc04g077490	12		Max
FeUEc_4	63020978	Metal transporter (NRAMP2)	Solyc04g078220	85	L5	N
FeUEc_4	63021305	NRAMP 5	Solyc04g078230	86	L5	Max
FeUEc_4	63021935	NRAMP	Solyc04g078240	87	L5	N
Fe_L_6	2369458	Auxin signaling F-box 2 (Fragment)	Solyc06g008460	33	L5	M
Fe_L_6	2052601	50S ribosomal protein L14 (K4CUW3)	Solyc06g008170	4		M
Fe_L_6	948619	Beta-D-glucosidase	Solyc06g005970	94		L
Fe_F_L_6	35057313	Transcription Factor (FER)	Solyc06g051550	8		Max

Fe_F_L_6	35553671	Ethylene responsive transcription factor 1a	Solyc06g051840	19		Max
Fe_F_L_6	36306186	Spermidine synthase	Solyc06g053510	86		Max
Fe_F_L_6	36312412	Spermidine synthase	Solyc06g053520	87		Max
FeUEc_7.1	261349	Glucan endo-1 3-beta-glucosidase (B5M9E5)	Solyc07g005330	121		M
FeUEc_7.1	1637797	Dihydrolipoyllysine-residue acetyltransferase	Solyc07g006790	25		Max
FeUEc_7.1	1755020	Auxin efflux carrier protein	Solyc07g006900	36		Max
FeUEc_7.1	1836680	Zinc transporter	Solyc07g007060	52		M
FeUEc_7.1	2161684	Iron-sulfur cluster assembly scaffold protein IscU	Solyc07g007450	91	L5	VL
FeUEc_7.1	2181989	Multidrug and toxin extrusion protein 2	Solyc07g007480	94	L5	N
FeUEc_7.1	2183276	MATE efflux family protein expressed	Solyc07g007490	95	L5	N
FeUEc_7.1	2165949	Cytochrome P450	Solyc07g007460	92	L5	Max
FeUEc_7.2	63048971	2-oxoglutarate-iron (II)-dependent oxygenase	Solyc07g054870	97	L5	Max
FeUEc_7.2	63145852	Myb-related transcription factor 72 (MYB72)	Solyc07g055000	110		VL
Fe_F_7	65633175	Enolase-phosphatase E-1 (DEP1)	Solyc07g063040	40		M
Fe_F_7	66113374	Ferredoxin-like	Solyc07g063740	30	L5	M
Fe_F_7	66633462	Chromatin remodeling complex subunit	Solyc07g064540	108	L5	Max
Fe_F_8	331751	CAX-interacting protein 4	Solyc08g005450	4		M
Fe_F_8	1811001	Ethylene responsive transcription factor 1a	Solyc08g007230	171	L5	VL
Fe_F_8	2654348	Cation/H(+) antiporter 18	Solyc08g008190	264	L5	VL
Fe_F_8	2658313	Cation/H(+) antiporter 18	Solyc08g008200	265		
FeUEc_8	57160821	Globin	Solyc08g068070	11	L5	L
FeUEc_8	57170272	Globin	Solyc08g068090	13	L5	M
FeUEc_8	57454951	Ethylene receptor	Solyc08g068360	40	L5	Max
Fe_L_9/FeUEc_9	66885408	Beta-glucosidase	Solyc09g075070	66		Max
Fe_L_9/FeUEc_9	67316194	Gibberellin receptor GID1L2	Solyc09g075670	6		Max
Fe_L_9/FeUEc_9	67318526	Gibberellin receptor GID1L2	Solyc09g075680	5		Max
Fe_L_9/FeUEc_9	67322749	Gibberellin receptor GID1L2	Solyc09g075690	4		Max
Fe_L_9/FeUEc_9	67537185	Spermidine synthase	Solyc09g075900	0		Max
Fe_L_9/FeUEc_9	68101382	Globulin-like protein (Fragment)	Solyc09g082360	4	L5	N
Fe_L_9/FeUEc_9	68348659	Acireductone dioxygenase	Solyc09g082630	31	L5	VL
Fe_L_9/FeUEc_9	68367293	Acireductone dioxygenase	Solyc09g082650	33	L5, E9	M
Fe_L_9/FeUEc_9	68558897	Calcium-transporting ATPase 1	Solyc09g082880	56	L5	Max
Fe_L_9/FeUEc_9	68643060	Pyruvate kinase	Solyc09g082970	66	L5	M
Fe_L_10	63076400	Auxin response factor 1	Solyc10g083210	49	L5	M
Fe_L_10	63665213	S-adenosylmethionine synthase	Solyc10g083970	18	E9	Max
Fe_L_10	63669724	2-oxoglutarate-dependent dioxygenase	Solyc10g083980	19	E9	Max
Fe_L_10	63916061	Subtilisin-like protease (K4D3L8)	Solyc10g084320	53		M
Fe_L_10	64550002	Mate efflux family protein	Solyc10g085340	155		M
FeUEc_11	854682	Ethylene-responsive transcription factor 2	Solyc11g006050	34		Max
FeUEc_11	662989	Pectinesterase inhibitor (K4D4J5)	Solyc11g005820	57		M
FeUEc_11	915958	Ethylene receptor	Solyc11g006160	23	L5	M
FeUEc_11	924556	Ethylene receptor	Solyc11g006180	21		Max
FeUEc_11	1431026	Ferredoxin	Solyc11g006910	50		Max
FeUEc_11	1435246	Iron-sulfur cluster insertion protein erpA	Solyc11g006920	51		M
FeUEc_11	1576541	Fe/S biogenesis protein nfuA	Solyc11g007120	71		M
Fe_F_11	55058013	Beta-D-glucosidase	Solyc11g071640	26	L5	Max
Fe_F_11	55063075	Beta-D-glucosidase	Solyc11g071650	25	L5	H
Fe_F_11	55291627	Nitric oxide reductase FIRd-NAD(+) reductase	Solyc11g071990	9		Max

Fe_F_11	55371935	1-aminocyclopropane-1-carboxylate oxidase	Solyc11g072110	21	L5	VL
Fe_F_11	55379104	1-aminocyclopropane-1-carboxylate oxidase	Solyc11g072130	23	L5	M
Fe_F_11	55424089	1-aminocyclopropane-1-carboxylate oxidase 3	Solyc11g072200	30		Max
Fe_F_11	55742768	Proton-dependent oligopeptide transporter	Solyc11g072570	67		Max
Fe_F_11	55748597	Proton-dependent oligopeptide transporter	Solyc11g072580	68		Max
Fe_F_11	56158392	Dirigent-like protein	Solyc11g073030	113	L5	Max
Fe_F_12/Fe_L_12	2943402	Plant synaptotagmin	Solyc12g009700	42	L5	Max
Fe_F_12/Fe_L_12	3005117	Purple acid phosphatase 3 (K4DC68)	Solyc12g009800	32		L
Fe_F_12/Fe_L_12	3818534	50S ribosomal protein L5P (K4CG62)	Solyc12g010930	0		M
Fe_F_12/Fe_L_12	3858049	Citrate synthase	Solyc12g011000	0		M
Fe_F_12/Fe_L_12	4505048	Ethylene responsive transcription factor 2a	Solyc12g013660	48		Max
Fe_F_12/Fe_L_12	4564215	LRR receptor-like serine/threonine-protein kinase	Solyc12g013740	56	L5	Max
Fe_L_12	5069479	Phosphoenolpyruvate carboxylase 1	Solyc12g014250	107	L5	H

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Figure legends

Fig. 1- Distributions of total harvested Fe (FeUEc), Total commercial fruit weight (TFWc), Fe and Al fruit concentrations (Fe_F and Al_F, respectively), leaf [Fe] (Fe_L) and the relative Fe_F to Fe_L (Fe_F/L). The position of controls Bol and Bol/Bol is indicated.

Fig. 2- Genotypic means and standard errors for significant epistatic interactions between QTLs and/or cofactors (SNP number) governing Ca_L, Fe_L, Fe_F, Al_F, FWm, and FeUEc. Homozygotes for the lycopersicum or the pimpinellifolium allele are coded as **a** or **b**, respectively, at the first locus (X axis), and a square (**a**) or a triangle (**b**) at the second locus (in parenthesis).

Fig. 3- LOD profiles of QTLs for FeUEc and TFWc. Encircled two QTL peaks detected for both traits at the same region in chromosome 11. Genetic positions along the 12 chromosomes are shown under the X axis.

Electronic Supplementary Material

Supplementary Fig. 1- Course of the experiment; (A), general view of grafted tomato plants grown under commercial conditions but limiting Fe nutrition (March 2017); (B), fruit yield (May 2017); (C), a hand is holding a truss with bronze, deformed tomatoes.

Supplementary Fig. 2- Relative (A) and absolute (B) mean concentrations of elements at the fruits (left) and leaves (right) of the commercial tomato variety grafted on the recombinant inbred lines. Y axes in B correspond to element concentration in ppm.

Supplementary Fig. 3 - Graphic representation of principal component analysis of variability found among grafted RILs under commercial, iron limiting conditions for vegetative, mineral, and fruit yield and quality traits. Three groups of elements are noted (encircled).

Supplementary Table 4- Pearson coefficients between significantly correlated traits ($p \leq 0.05$).

Supplementary Table 5- Summary list of candidate genes for major QTLs (See QTLs with SNP in bold and excluding those related to Fe traits at Table 2), some of them are segregating for frameshift Indels (Kevei et al. 2015) in parental genomes, E9 or L5, (Mut.). The Locus reference, its relative root expression (Exp.) in Heinz cultivar (Max: maximum, H: high, M: medium, VL: very low, L: low and N: no data), and the number of genes counted from the QTL peak (Ord.) are also shown.

Supplementary Fig. 6- LOD profiles of QTLs for Fe_L, Fe_F and FeUEc. QTL peaks detected for two traits at the same region are encircled. Genetic positions along the 12 chromosomes are shown under the X axis.

Supplementary Table 7- Overrepresented Biological processes within QTLs detected for iron related traits by means of the PANTHER Classification System (<http://www.pantherdb.org/>) using the Fisher's Exact with FDR multiple test correction. The suffix _All denotes all downloaded genes were considered, while _Seg corresponds to those genes segregating in the RIL population for frameshift mutations only.

Supplementary Table 8- List of genes within %Cit_12, the QTL detected for citric acid fruit content in chromosome 12, some segregating for frameshift Indels (Kevei et al. 2015) in parental genomes, E9 or L5, (Mut.). The Locus reference, its relative root expression (Exp.) in Heinz cultivar (Max: maximum, H: high, M: medium, VL: very low,

L: low and N: no data), and the number of genes counted from the QTL peak (Ord.) are also shown.

Supplementary Table 9- List of genes within QTLs detected for frequency of fruits with blossom end rot (pFNber), some segregating for frameshift Indels (Kevei et al. 2015) in parental genomes, E9 or L5, (Mut.). The Locus reference, its relative root expression (Exp.) in Heinz cultivar (Max: maximum, H: high, M: medium, VL: very low, L: low and N: no data), and the number of genes counted from the QTL peak (Ord.) are also shown.

Supplementary Fig. 10- Overrepresented molecular functions within blossom end rot QTLs by means of the PANTHER Classification System (<http://www.pantherdb.org/>) using the Fisher's Exact with FDR multiple test correction.

“QTL and candidate gene analyses of rootstock-mediated tomato fruit yield and quality traits under low iron stress” submitted by Asins et al. (IVIA, Apartado Oficial, 46113 Moncada, Valencia, Spain; mjasins@ivia.es) to EUPHYTICA.

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