**EFFECTS OF IRON DEFICIENCY AND IRON RESUPPLY ON METAL AND LIGAND CONCENTRATIONS IN XYLEM SAP, APOPLASTIC FLUID AND INTRACELLULAR EXTRACTS OF TOMATO PLANTS**

**Introduction**

The long-distance transport of metal micronutrients (e.g., Fe) is crucial for plant growth and reproduction. The path of metals to its final destination (e.g., the chloroplast) involves transport across multiple membranes mediated by different transporter proteins, as well as metal complexation by different ligands (e.g., nicotiamine and citrate; Fig. 1) in each compartment within the plant. A complete picture of this complex process is still lacking for any metal, in part because data on metal and ligand concentrations in plant fluids are still scarce [1].

The aim of this work was to investigate the effects of Fe deficiency and Fe resupply on the concentrations of metals and ligands in xylem sap and in apoplastic fluids and intracellular extracts of leaves (old and young) and roots in tomato.

**Materials and methods**

**Plant material**

Tomato (Solanum lycopersicum cv. FER) plants were grown in a growth chamber in half-strength Hoagland solution, including either 0 (Fe-deficient plants; -Fe) or 45 µM Fe(III)-EDTA (Fe-sufficient plants; +Fe) (Fig. 2). After 11 d, some Fe-deficient plants were treated with 45 µM Fe(II)-EDTA, and plants from the three treatments were sampled at different times during the following 48 h. Xylem sap was obtained using the plant de-topping technique as reported in López-Millán et al. [2]. Apoplastic fluid was sampled from leaves and roots by centrifugation as in López-Millán et al. [3], with some modifications. A symplastic soluble fraction (intracellular extracts; thereafter called SSF) was sampled from roots and leaves by centrifugation after freezing in liquid N. Contamination was assessed in xylem sap and apoplastic fluids by measuring cytosolic malate dehydrogenase activity [3].

**Iron, NA and organic acids determination**

For Fe determination, samples were digested with 65% HNO₃ at 60°C overnight and analyzed by ICP-MS. For NA determination, samples were filtered through 10 kDa filters and analyzed as in Xuan et al. [4] with modifications, using High Performance Liquid Chromatography (HPLC) coupled to a Time-Of-Flight (TOF) MS and Electrospray ionization (ESI) as ion source (Fig. 3). For organic acid determinations, samples were analyzed according to Relán-Alvarez et al. [5], using HPLC-ESI-MS/TOF/MS. Concentrations of NA and organic acids were quantified using external calibration with internal standardization.

**Results**

Iron deficiency led to the following major changes when compared with the controls: in roots, the apoplastic fluid and SSF were depleted in Fe and the SSF was enriched in citrate; in the xylem, an enrichment in citrate was found at some sampling times; in leaves, the SSF of old ones was enriched in NA and the apoplastic fluid of young ones was enriched in citrate.

Upon Fe resupply, changes in Fe concentration were very marked: at 12 h, an Fe wave was observed in all tissues except in the apoplastic fluid of the young leaves; at 24 h, Fe increased further in the root apoplastic fluid and in the apoplastic fluid and SSF from young leaves, and decreased in the xylem and apoplastic fluid of old leaves; at 48 h, there was a further decrease in xylem Fe and the apoplastic fluid of roots and young leaves.

Iron resupply also affected NA concentrations: at 12 h, a NA wave was observed in roots, with concentrations increasing in the root apoplastic fluid and SSF; at 24 h, NA decreased in the root SSF; at 48 h, NA decreased in root apoplastic fluid and increased in the SSF of old and young leaves.

Finally, Fe resupply also affected citrate concentrations: at 12 h, citrate decreased somewhat in the xylem; at 24 h, a citrate wave occurs, with concentrations increases in the root and leaf apoplastic fluids; at 48 h, citrate decreased in root and young leaf apoplastic fluid.

**Conclusion**

Results show that Fe movement to the upper parts of Fe-deficient plants resupplied with Fe is associated to an early NA flush in the root, followed by a citrate flush in the apoplast of both roots and leaves.

**References**


**Acknowledgements**

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**Table 1.** Fe, NA and citrate concentrations in the fluids and treatments studied. Asterisks denote a statistically significant difference using the -Fe plants as controls (Student’s t-test; p<0.05; n=4-20). N.D. indicates data could not be obtained due to matrix interferences.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Plant fluids</th>
<th>Fe (µM)</th>
<th>Resupply 12 h</th>
<th>Resupply 24 h</th>
<th>Resupply 48 h</th>
<th>NA (µM)</th>
<th>Resupply 12 h</th>
<th>Resupply 24 h</th>
<th>Resupply 48 h</th>
<th>Citrate (µM)</th>
<th>Resupply 12 h</th>
<th>Resupply 24 h</th>
<th>Resupply 48 h</th>
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<tbody>
<tr>
<td>+Fe</td>
<td>-Fe</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Young leaves apoplastic fluid</td>
<td>12 - 18</td>
<td>5 - 11</td>
<td>11.2</td>
<td>18.0*</td>
<td>10.8</td>
<td>10 - 20</td>
<td>7 - 13</td>
<td>7.1</td>
<td>9.7</td>
<td>8.1</td>
<td>180 - 210</td>
<td>370 - 450</td>
<td>241</td>
</tr>
<tr>
<td>Old leaves SSF</td>
<td>20 - 10</td>
<td>20 - 25</td>
<td>25.1</td>
<td>13.3</td>
<td>8.5</td>
<td>9 - 15</td>
<td>9 - 12</td>
<td>7.1</td>
<td>15.9</td>
<td>8.1</td>
<td>180 - 210</td>
<td>370 - 450</td>
<td>241</td>
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<tr>
<td>Xylem Sap</td>
<td>17 - 31</td>
<td>9 - 15</td>
<td>128.3*</td>
<td>78.3*</td>
<td>32.2</td>
<td>5 - 15</td>
<td>9 - 12</td>
<td>13.4</td>
<td>12.3</td>
<td>9.5</td>
<td>180 - 210</td>
<td>370 - 450</td>
<td>241</td>
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<tr>
<td>Root SSF</td>
<td>37 - 138</td>
<td>5 - 23</td>
<td>149.0*</td>
<td>129.3*</td>
<td>74.1</td>
<td>20 - 19</td>
<td>20 - 10</td>
<td>182.1*</td>
<td>47.0</td>
<td>42.5</td>
<td>180 - 170</td>
<td>270 - 340</td>
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<tr>
<td>Root apoplastic fluid</td>
<td>88 - 281</td>
<td>15 - 25</td>
<td>168.0*</td>
<td>402.2*</td>
<td>111.2</td>
<td>10 - 35</td>
<td>25 - 60</td>
<td>100.9*</td>
<td>57.2</td>
<td>45.3</td>
<td>1000 - 1400</td>
<td>1500 - 2500</td>
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</table>

**Figure 3.** Schematic representation of the effect of Fe deficiency and Fe resupply on Fe, NA and citrate concentrations in xylem sap, apoplastic fluid and intracellular extracts of tomato plants. *Citrato concentrations in leaf symplastic soluble fraction are not depicted, because data could not be obtained due to matrix interferences. The number of symbols in a given compartment and per a given analyte is proportional to their concentration in the corresponding plant fluid, with the following consideration: each citrate symbol corresponds to 200 µM and each NA or Fe symbol corresponds to 10 µM.**