Changes in the xylem sap metabolome of tomato and lupin with Fe deficiency

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Background

Plant xylem sap contains mainly water and minerals, but also contains low concentrations of a wide array of organic compounds, including amino acids, organic acids, hormones, polyamines and proteins. Changes in xylem sap composition caused by abiotic stresses such as Fe deficiency are important for plant growth and development, and for instance it is well known that signals traveling via xylem from roots to shoots modulate plant growth and transpiration [1]. In previous studies dealing with changes in xylem sap composition in response to Fe deficiency, only a small number of compounds have been determined simultaneously [2, 3]. In this study, an untargeted metabolomic analysis was carried out to quantify the changes in xylem sap constituents under Fe-deficiency and Fe-resupply.

Experimental

❑ PLANT MATERIAL: Xylem sap samples were obtained by detopping Fe-sufficient (grown with 45 μM Fe(III)-EDTA), Fe-deficient (grown with 0 μM Fe) tomato (*Lycopersicum esculentum* Mill.) and lupin (*Lupinus albus* L.) plants. Also, xylem was obtained from Fe-sufficient and Fe-deficient tomato plants, 6, 12, 18 and 24 hours after Fe-resupply with 45 μM Fe(III)-EDTA.

❑ METABOLOMIC ANALYSIS: metabolites were extracted from xylem samples as described elsewhere [4]. After extraction, samples dried with a Speedvac. A mixture of internal retention index markers composed by different fatty acids markers were added to the dried extracts and then a derivatization process in two steps was carried out. Firstly, 20 μL of methoxiamine hydrocloride dissolved in pyridine was added and extracts were shaken for 1.5 h at 30 ºC. Secondly, 90 μL of MSTFA 1% TMCS was added and shaken for another 30 min at 37 ºC. Then, the derivatized xylem sap metabolites were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) (Agilent GC coupled to a LecoTOF mass spectrometer) following the recommendations described by the Metabolomics Standards Initiative [5]. Mass chromatograms were deconvoluted using the Leco ChromaTOF software and peaks were exported to the BinBase database [6] and identified using the Fiehn Library (http://fiehnlab.ucdavis.edu/Metabolite-Library-2007). Results were further analyzed using different statistical techniques.

Changes in the xylem sap metabolome of lupin and tomato under Fe deficiency

TABLE 1. Effect of Fe-deficiency on the lupin and tomato xylem metabolome. Green (lupine) and red (tomato) numbers in brackets indicate the concentration fold-change regarding to the Fe-sufficient control plants.

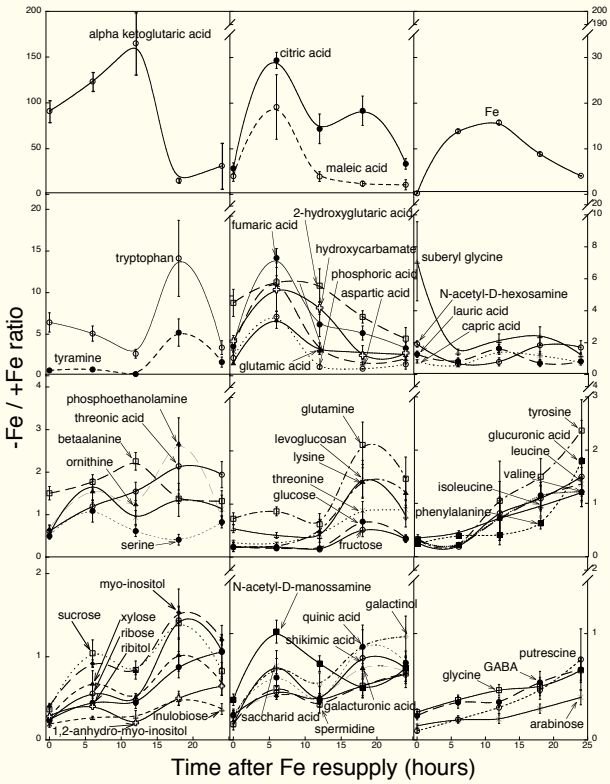
Metabolites affected by Fe-deficiency and fold-change (-Fe/+Fe) over the control values		
In lupin & tomato	Only in lupin	Only in tomato
2-Hydroxyglutaric acid (**1.6**; **4.6**)	5'-Deoxy-5'-methylthioadenosine (0.4)	Myo-inositol (0.4)
Aconitic acid (**2.6**; **2.8**)	Asparagine (0.7)	Citric acid (**5.7**)
Alanine (0.4; 0.4)	Galactose-6-phosphate (**0.2**)	Fructosa (0.2)
Alpha ketoglutaric acid (**1.7**; **91.0**)	Glucose-6-phosphate (**0.2**)	Rhamnose (0.3)
Arabinose (1.4; 0.1)	Glutamic acid (0.6)	Galactinol (**0.2**)
Beta alanine (0.4; 1.5)	Glutamina (0.7)	Glucose (**0.2**)
Fumaric acid (1.9; **1.8**)	Glyceric acid (0.6)	Glucuronic acid (**0.2**)
GABA (0.4; **0.2**)	Glycerol-alpha-phosphate (0.4)	Glycerol (0.5)
Galacturonic acid (1.8; **0.2**)	Guanosine (0.4)	Hydroxycarbamate (**2.2**)
Glycine (0.5; 0.3)	Inositol-4-monophosphate (0.3)	Inulobiose (**0.2**)
Isoleucine (0.5; 0.3)	Methionine sulfoxide (0.4)	Leucine (0.3)
Lysine (0.6; **0.2**)	Oxoproline (0.4)	Lyxitol (0.4)
Maleic acid (2.5; 4.0)	Phenylalanine (0.7)	Malic acid (**6.2**)
Methionine (0.7; 0.3)	Proline (0.5)	N-Acetyl-D-hexosamine (**1.9**)
N-Acetyl-D-mannosamine (0.5; 0.4)	Trehalose (0.4)	Ribitol (**0.2**)
Putrescine (**0.1**; **0.1**)		Saccharic acid (**0.1**)
Quinic acid (0.6; **0.2**)		Serine (0.5)
Ribose (**1.7**; **0.2**)		Suberyl glycine (**7.1**)
Shikimic acid (0.5; **0.2**)		Urea (0.4)
Spermidine (0.5; **0.1**)		
Threonine (0.6; 0.3)		
Tryptophan (0.5; **6.4**)		
Tyrosine (0.5; 0.3)		
Sucrose (0.5; 0.4)		
Succinic acid (**2.4**; **4.7**)		
Valine (0.7; 0.3)		

❑ 83 and 80 metabolites were identified in the xylem sap of lupin and tomato plants, respectively. In both species Fe-deficiency affected the relative concentration of more than 40 metabolites, with 26 being common in the two species (including several carboxylates, amino acids, and ribose).

❑ Major changes were found in tomato, with the highest –Fe/Fe ratio found for alpha-ketoglutaric acid (91.1), suberyl glycine (7.1), triptophan (6.4), malic acid (6.2), citric acid (5.7) and 2-hydroxyglutaric acid (4.6). In lupin changes were less pronounced, with the highest –Fe/Fe ratios being for aconitic and maleic acids (2.6 and 2.5, respectively).

Changes in the xylem sap metabolome of Fe-deficient tomato plants after Fe-resupply

FIGURE 2. Time course of the fold-change (-Fe/+Fe) for xylem metabolites and Fe concentrations in Fe-deficient tomato plants after Fe-resupply.



❑ Fe-resupply to Fe-deficient tomato plants led to changes in the xylem metabolome that showed complex trends over the Fe-resupply period depending on the specific metabolite. Metabolite concentration trends differed from that of xylem Fe concentrations. The highest increases in the –Fe/+Fe ratios were generally observed at 6 and/or 18 h after Fe-resupply, and corresponded to those metabolites that had increased more by Fe-deficiency (see Table 1). After 1 d of Fe-resupply, the relative xylem metabolite concentrations approached those of Fe-sufficient plants (i.e., -Fe/+Fe ratios values close to 1).

Conclusions

❑ Fe-deficiency brought about some expected (i.e. increases of the concentrations of many tricarboxylic acid cycle intermediates) as well as previously unreported (i.e. decreases of GABA, putrescine and spermidine) changes in the metabolite concentrations in the xylem sap of lupin and tomato plants.

❑ Fe-resupply to Fe-deficient tomato plants led to further changes, including major increases in organic acids in the first h after Fe resupply. Twenty-four h after Fe-resupply, many of the compounds which showed changes in the –Fe/+Fe ratios approached values found in the Fe-sufficient controls.

References

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