Plant Stress Physiology

PLANT IRON DEFICIENCY METABOLOMICS

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BACKGROUND

Metabolites are the end products of cellular regulatory processes, and their levels can be regarded as the ultimate response of biological systems to genetic or environmental changes. In parallel to the terms 'transcriptome' and 'proteome', the set of metabolites synthesized by a biological system constitute its 'metabolome'. Yet, unlike other functional genomics approaches, the unbiased simultaneous identification and quantification of plant metabolites has been largely neglected. Fe deficiency lead to important changes in the plant metabolism, due to reduced photosynthetic rates that affects the C incorporation. The whole plant adapts to this situation by finding alternative ways to maintain the metabolic activity. The aim was to study the metabolic changes of sugar beet root tips and leaves, and tomato xylem under different Fe nutrition status.

EXPERIMENTAL

PLANT MATERIAL: Two week old sugar beet (cv. 'Orbis') and tomato (cv. 'Tres Cantos') plants were hydroponically grown for 10 days with 0 (-Fe) and 45 (+Fe; control) μM of Fe-EDTA in nutrient solution. Some Fe-deficient plants were Fe-resupplied with Fe-EDTA for 12, 24 and 72 h.

SAMPLING AND METABOLITE EXTRACTION: Sugar beet root tips and leaves, and tomato xylem were samples from at least six plants. Frozen samples were extracted with a 3:2:2 isopropanol:acetonitrile:water mixture in a vibration mill, centrifuged and the supernatant vacuum dried [1].

GC-MS ANALYSIS AND METABOLITE IDENTIFICATION: A mixture of internal retention index markers composed by different fatty acid markers were added to the dried extracts. Samples were derivatized in two steps with methoxiamine hydrochloride and MSTFA 1%, randomized and analyzed by GC-MS following the recommendations described by the Metabolomics Standards Initiative [1, 2]. Metabolites were identified using the SetupX/BinBase databases [3].

STATISTIC ANALYSIS: Metabolite data were normalized and analyzed using multivariate statistics techniques with the Statistica 8.0 software.

SUGAR BEET ROOT TIPS

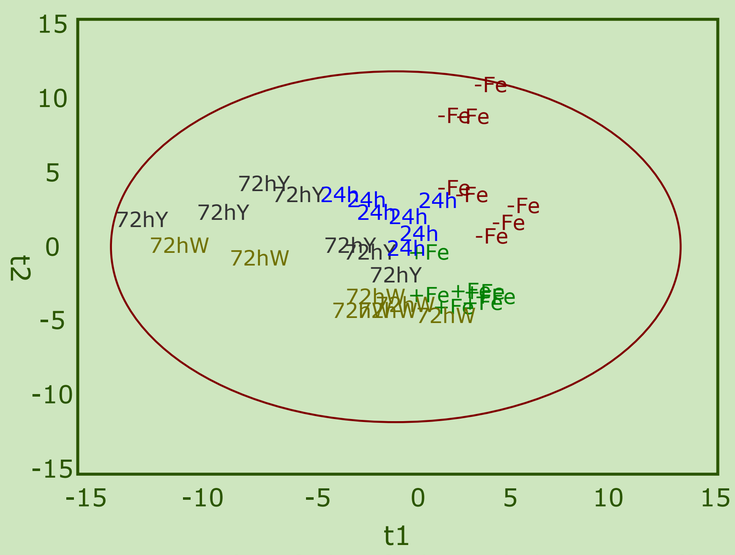

FIGURE 1. PLS score scatter plot of identified metabolites. Fe sufficient (+Fe), Fe-deficient (-Fe), 24 h Fe-resupplied (24h) and 72 h Fe-resupplied root tip yellow zone (72 h yellow) and new white zone (72 h white).

Seventy six metabolites were identified in root tips. A partial least square analysis (PLS) of the identified metabolites (Fig. 1) shows a good separation between +Fe and -Fe root tips. 24 h Fe-resupplied root tips fall between those of -Fe and +Fe root tips. 72 h Fe-resupplied root tips show a larger degree of variation due to sampling difficulties. An increase in organic acid metabolism (TCA cycle) with Fe-deficiency was observed. An activation of the raffinose series of oligosaccharides (RSOs), including raffinose, galactinol and myo-inositol was found in -Fe and 24-h Fe-resupplied plants. This activation has never been described before in plants under Fe deficiency, although is a common response in plants under other stresses.

REFERENCES: [1] Fiehn et al. (2008*) Plant J*. 53: 691. [2] Fiehn et al. (2007) Metabolomics. 3: 195. [3] Fiehn et al. (2005) *Proc. Lect. Notes Bioinformatics* 3615: 224. [4] López-Millán et al. (2000) Plant Physiol. 124: 873.

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SUGAR BEET LEAVES

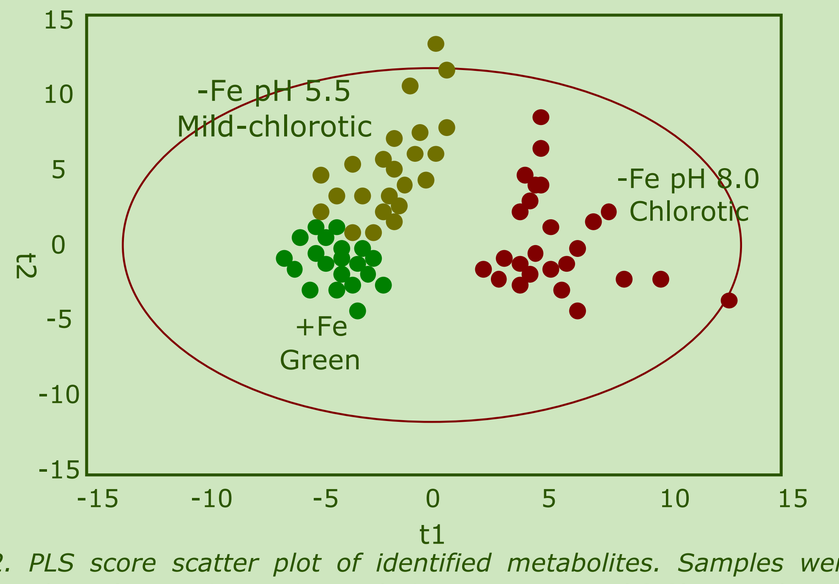

FIGURE 2. PLS score scatter plot of identified metabolites. Samples were taken from +Fe and -Fe (grown in nutrient solution at pH 5.5 and 8.0) sugar beet plants.

Plants growing under different treatments had different chlorophyll contents. The PLS analysis of the identified leaf metabolites (Fig. 2) shows a good separation of samples depending on Fe-chlorosis degree. A greater degree of variation among chlorotic samples is observed. The main changes were found for organic acids. Citric and oxalic acids and oxoproline were the most important variables in order to explain the separation between the different treatments. Both citric acid and oxoproline increased markedly in chlorotic plants. On the other hand, oxalic acid was decreased with Fe chlorosis.

TOMATO XYLEM SAP

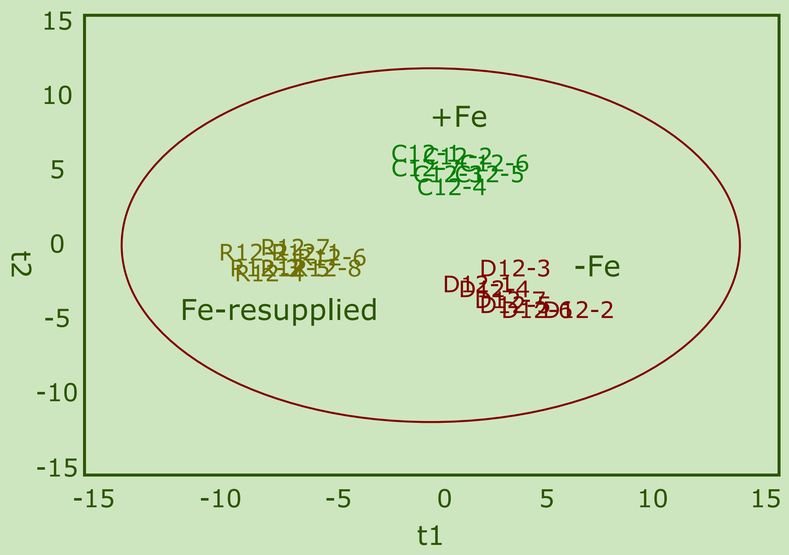

FIGURE 3. PLS score scatter plot of identified metabolites. Samples were taken from +Fe, -Fe and 12 h Fe-resupplied plants.

Samples from the three treatments separate very well indicating rapid and marked changes in the xylem sap of plants grown under different Fe-nutrition conditions. The greatest changes were found in TCA organic acids (citric, malic, succinic and fumaric), aminoacids (oxoproline), sugars (sucrose) as well as other carbohydrates (myoinositol and glyceric acid). These metabolites were among the most important to explain the separation found in the PLS scatter plot. For these metabolites, the highest signals were found as follows: oxoproline in green samples; myoinositol, sucrose, succinic, fumaric and glyceric acids in chlorotic samples; succinic, malic and citric acid in the resupplied samples.

CONCLUSIONS

Metabolomic analysis of tissues from plants grown under different Fe nutrition status showed metabolite alterations already known [4] like an activation of the TCA cycle and glycolysis. Some interesting results like an increase in the amount of the raffinose series of oligosaccharides, including raffinose, galactinol and myo-inositol, not previously described in plant Fe deficiency, were also observed. In all plant material studied, chlorotic and green plants always showed a good separation (Figs. 1-3). Metabolite changes were better observed in xylem sap than in root tips and leaves. After some time, Fe-resupplied plant metabolite levels come back to Fe-sufficient conditions.