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 at 24 h 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 3:2:1 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 methoxamine 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/Brinbase databases [3].

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

CONCLUSIONS
Metabolomic analysis of tissues from plants grown under different Fe-nutrition conditions. The greatest changes were found in TCA organic acids (citric, malic, succinic and fumaric), aminoacids (oxoproline), 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 glycric 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: oxoprolnine in green samples; myoinositol, sucrose, succinic, fumaric and glycric acids in chlorotic samples; succinic, malic and citric acid in the resupplied samples.


ACKNOWLEDGMENTS: This work was supported by the Spanish Ministry of Science and Innovation (projects AGL2006-1416 and AGL2007-61948, co-financed with FEDER), the European Commission (Contract no. P6-FOOD-CT2006-016297), and the Aragón Government (group 403).

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).

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 oxoprolnine were the most important variables in order to explain the separation between the different treatments. Both citric acid and oxoprolnine increased markedly in chlorotic plants. On the other hand, oxalic acid was decreased with Fe chlorosis.

SUGAR BEET ROOT TIPS

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 (oxoprolnine), sugars (sucrose) as well as other carbohydrates (myoinositol and glycric 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: oxoprolnine in green samples; myoinositol, sucrose, succinic, fumaric and glycric acids in chlorotic samples; succinic, malic and citric acid in the resupplied samples.