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Nuclear Magnetic Resonance Study of the Metabolomic Changes Induced by Iron Deficiency in Soybean Leaves

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INTRODUCTION

Iron (Fe) is an essential nutrient for plants. However, Fe deficiency chlorosis (IDC) problems are a major concern in agriculture: low concentration or low availability of Fe in soils leads to reduced plant growth, and to reduced yields and crop quality. Although several strategies have been used to mitigate IDC problems, no successful solutions, either economically inexpensive or environmentally safe, have been found (Abadía *et al*., 2011). A better understanding of the plants' response to Fe deficiency, including at the metabolic level, can contribute to the design of strategies to ameliorate IDC problems. Here, NMR spectroscopy was used to study the metabolic response of soybean leaves to Fe deficiency. We used ^{1}H NMR to analyze extracts of leaf tissue, as well as ^{1}H high resolution magic angle spinning (HRMAS) to directly analyze leaf samples in the solid state.

METHODS

Plant growth and collection

Glycine max cv. Williams 82 seeds were germinated for 6 days, and then transferred to hydroponics in a controlled environment chamber (16 h day 25 ºC, 8 h night 18 ºC, 70% relative humidity, 350 mmol/m^{2}s photon flux). Plants were grown for 2 weeks in a permanently aerated nutrient solution (Vasconcelos *et al*., 2006) changed weekly. Fe-sufficient conditions were provided by adding 20 mM Fe(III)-EDDHA, while no Fe was added to Fe-deficient conditions. After 2 weeks plants were scored for IDC using a scale of 1-6 (Vasconcelos *et al*., 2006). Trifoliate leaves of each plant were pooled and immediately frozen in liquid N_2, and stored at -80 ºC until further processing.

Preparation of leaf methanolic extracts for ^{1}H-NMR analysis

Powdered biomass was extracted in 80% methanol-d4 in 90 mM KH_2PO_4 buffer (D_2O, pH 6.0) containing 0.1% TSP. Samples were extracted for 1 h at room temperature and sonicated for 30 min. Supernatant was recovered after centrifugation and stored at 4 ^{o}C until NMR analysis.

NMR analysis

Immediately before HRMAS analysis, 20-40 mg of frozen powdered biomass were placed into a rotor for analysis as described previously (Duarte *et al*., 2010). For ^{1}H NMR, conditions of analysis are described in Lima *et al*. (2010).

Statistical analysis

Data matrices of integrated regions of 0.005 ppm width (excluding water region), were analyzed by Principal Component Analysis (PCA) and PLS-DA using SIMCA-P version 11.5 software.

RESULTS AND DISCUSSION

^{1}H NMR spectroscopy has the ability to detect a broad range of compounds in a single experiment, in a rapid non-destructive way, therefore giving a representative view of the metabolome describing complex biological

systems (Lima *et al*., 2010). The characterization of the metabolic profile of soybean leaves by both ^{1}H NMR and HRMAS revealed the occurrence of Fe deficiency-induced metabolic changes. In particular, the PCA of the ^{1}H NMR spectra of leaf extracts (Fig. 1) revealed specific metabolite changes, related to Fe deficiency (in PC1, 81.5%) and to different degrees of IDC symptoms (in PC2, 6.9%). A similar analysis based on whole leaf metabolic profiles (not shown) provided information on changes affecting non-extracted components.

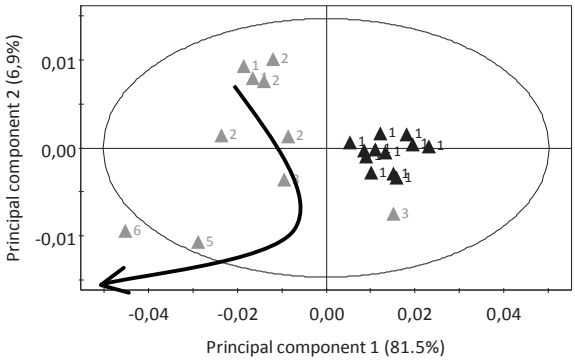

Fig. 1. PCA scores scatter plot showing separation of Fe-sufficient (black) and Fe-deficient (grey) leaves by PC1 (81.5% of total variance). Numbers represent degrees of IDC symptoms: 1-full green, 2-mild chlorosis, 3-full yellow/veins still green, 5-full chlorosis with necrosis, 6-tip necrosis/chlorotic axillary shoots developing. Arrow indicates progression of IDC symptoms along PC2.

CONCLUSIONS

NMR metabolomics techniques were able to unveil Fe deficiency-induced metabolic changes in soybean leaves. Analysis of soybean leaf extracts by ^{1}H NMR revealed metabolic profile differences among Fe deficient leaves showing diverse degrees of IDC symptoms. NMR of whole leaves provided complementary information on non-extracted leaf components.

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