



Root excretion and accumulation of riboflavin derivatives in iron-deficient *Medicago truncatula*

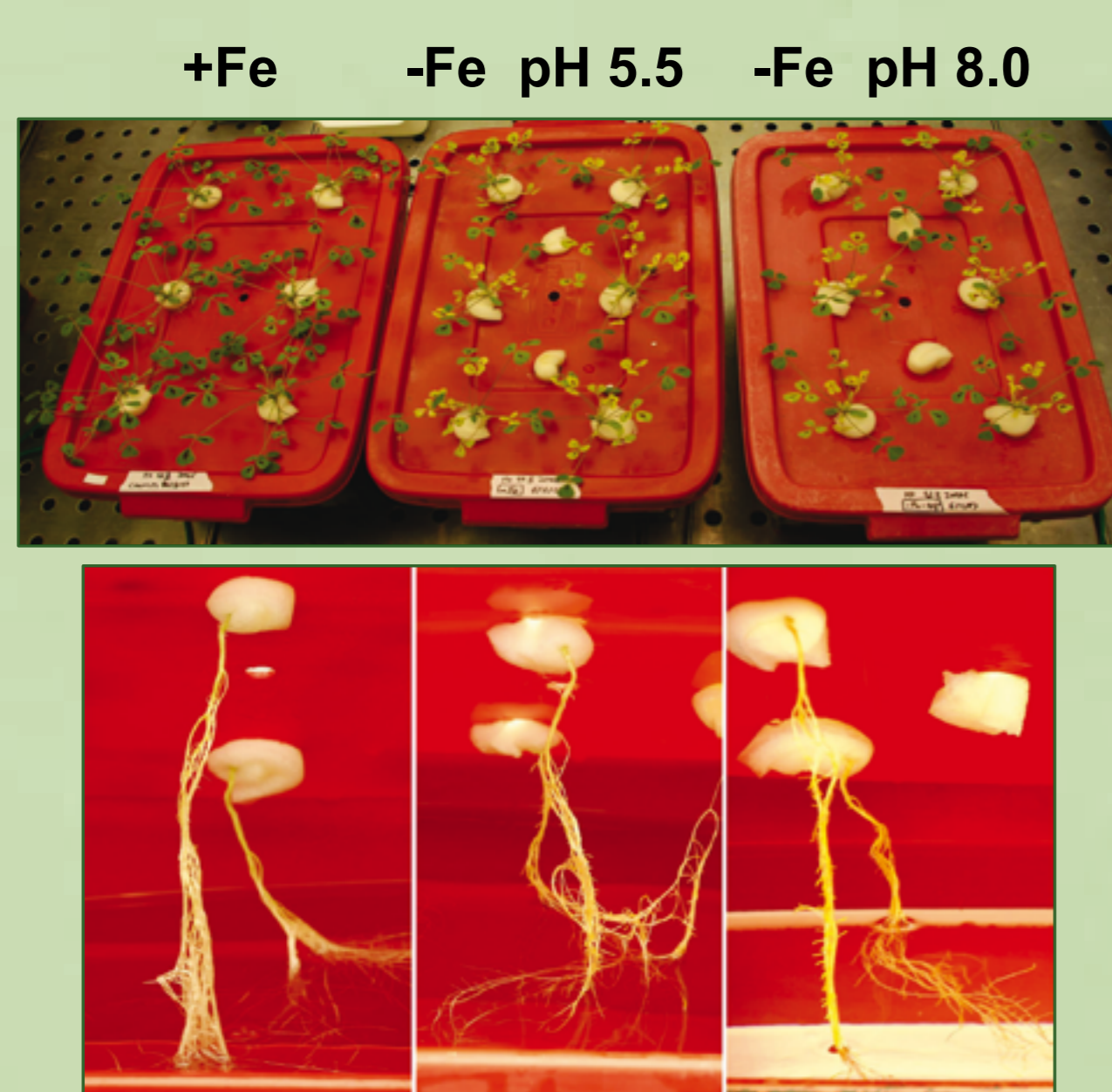
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Introduction

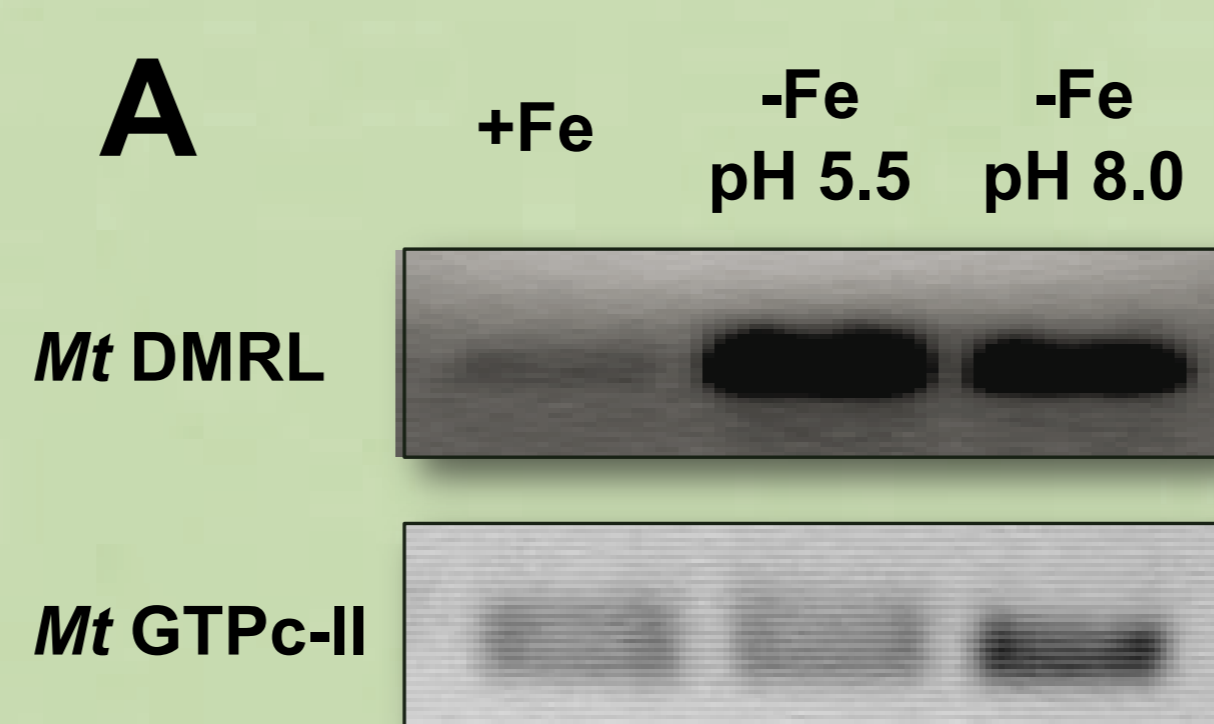
When grown in hydroponics under Fe deficiency, some Strategy I plant species develop yellow roots and cause a yellowing of the solution. This phenomenon, first reported in the 60's, is due to root accumulation and excretion of riboflavin and/or riboflavin derivatives such as riboflavin sulphates. The function these compounds play in plant Fe efficiency is still not known, although roles in facilitating electron flow to the root Fe reductase, facilitating symbiosis nodule formation, and as antimicrobial agents in the rhizosphere have been hypothesized. Any of these functions may contribute to increase plant Fe efficiency. The aim of this work was to study the influence of Fe deficiency in riboflavin biosynthetic pathway at genomic, proteomic and metabolite level in *Medicago truncatula* roots, including flavin compounds production and excretion.

Plant Material

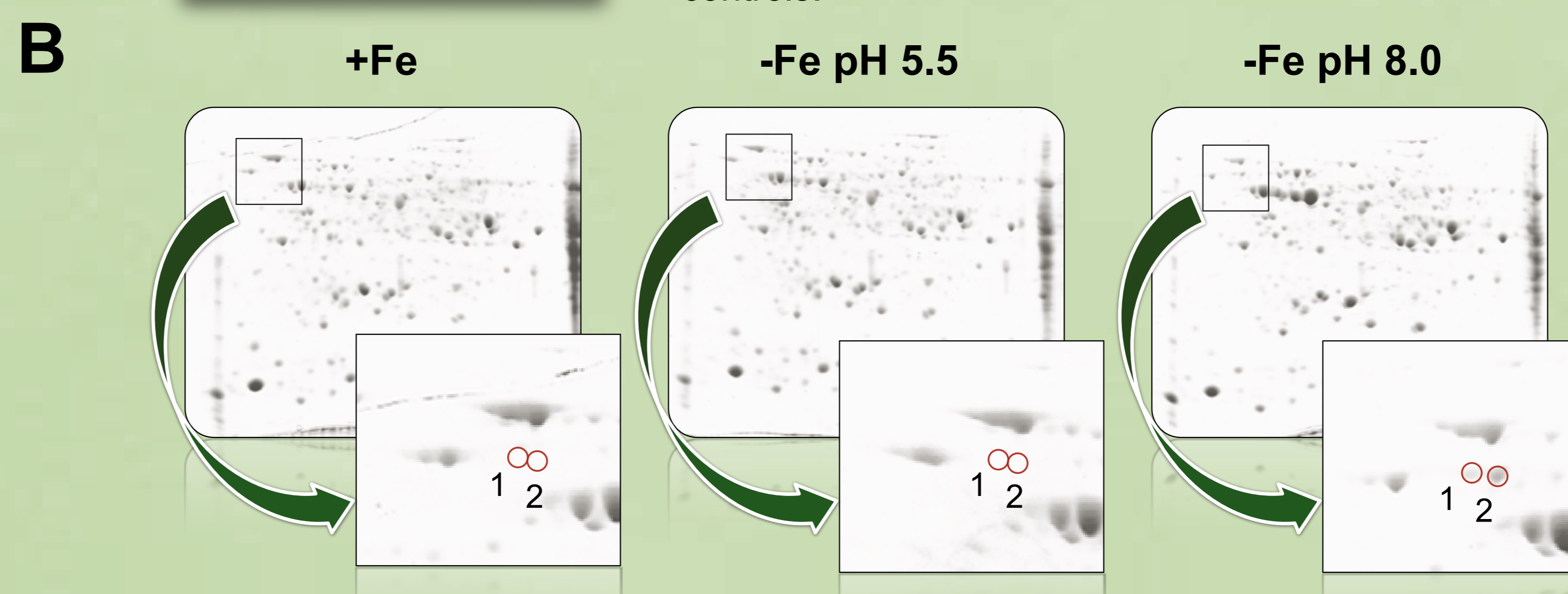


Plants were grown in Fe-sufficient nutrient solution (+Fe) and in two Fe-deficient (-Fe) nutrient solutions, either without CaCO₃ (pH 5.5) or with CaCO₃ (pH 8.0). Roots from Fe-sufficient plants were white and roots from Fe deficient plants were yellow. Root morphology in the two Fe-deficient treatments was different, with swollen yellow tips at pH 8.0, and swollen tips (only some of them yellow) and yellow patches along their length at pH 5.5. A yellow colour was observed only in the Fe-deficient nutrient solution without CaCO₃.

Gene expression and proteomics data



Our gene expression data (A) indicate an up-regulation in both Fe-deficiency treatments of GTP cyclohydrolase II (*MtribA-TC100898*) and DMRL synthase (*MtGL-TC107317*), two key enzymes of riboflavin biosynthesis pathway. This information was supported by our 2D-IEF-SDS-PAGE proteomic data (B), where we identified a large increase in GTP cyclohydrolase II production in Fe-deficient root protein extracts when compared to controls.



Spot N°	Protein Identification	ID	Score (MASCOT)	Peptide Count	Ions Sequenced	MW / pI
1	GTP cyclohydrolase II	gi 33308405	303	8	4	58 / 5.71
2	GTP cyclohydrolase II	gi 33308405	366	10	6	58 / 5.71

Flavin compounds production and excretion patterns

Root flavin accumulation and excretion depended on both plant Fe status and the pH of the nutrient solution. In root extracts from Fe-sufficient plants, HPLC-UV/VIS data showed only riboflavin (Rb), whereas riboflavin and three different flavin compounds (FC1, FC2, FC3) were detected in roots of plants grown in both Fe deficiency treatments (A). Our data indicate an extreme influence of pH of nutrient solution in the production/excretion pattern of this compounds under Fe-deficiency. Fe-deficient plants grown at pH 5.5 secreted 41% of the flavin compounds produced, whereas those grown at pH 8.0 only excreted 6% (B). These flavin compounds were putatively identified by HPLC-MS and HPLC-MS/MS spectrometry as those represented in C.

