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

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Abstract

When grown in hydroponics under Fe deficiency, some Strategy I plant species develop yellow roots and cause a yellowing of the solution [1-2]. 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 [3]. The function these compounds play in plant Fe efficiency is still not known, although roles in facilitating electron flow to the root Fe reductase and as antimicrobial agents in the rhizosphere have been hypothesized [4]. Any of these mechanisms may contribute to increase plant Fe efficiency. The aim of this work was to study flavin compounds present in roots of Fe-deficient *Medicago truncatula*. Plants were grown in Fe-sufficient nutrient solution (45 µM Fe) and in two Fe-deficient (0 µM Fe) nutrient solutions, either with $CaCO_3$ (pH 8.0) or without $CaCO_3$ (pH 5.5). 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 $CaCO_3$. Flavin compounds in the nutrient solution were concentrated in C18 Sep-Pack cartridges and eluted in methanol, and those in roots were extracted by grinding them with 100 mM ammonium acetate, pH 6.1. Flavin derivatives in root extracts and nutrient solution concentrates were separated by high performance liquid chromatography, and identification was carried out by ultraviolet-visible photodiode array spectrophotometry and electrospray ionization mass spectrometry, using time of flight (TOF) and quadrupole time of flight (QTOF) instruments. Root flavin accumulation and excretion depended on the plant Fe status and the presence of $CaCO_3$ in the nutrient solution. In root extracts from Fe-sufficient plants only riboflavin was detected, whereas in roots of plants grown in both Fe deficiency treatments riboflavin and three different riboflavin derivatives were detected. Two of these derivatives were identified as 7α-hydroxyriboflavin and (E)-5-(4,5-dimethyl-2-((3R,4S)-2,3,4,5-tetrahydroxypentylamino)phenylimino)pyrimidine-2,4(3H,5H)-dione, the latter compound originated from the partial rupture of the riboflavin's isoalloxazine ring. In nutrient solutions, riboflavin and derivatives were detected only in Fe deficiency treatments, and the concentrations were much higher in nutrient solutions without $CaCO_3$ than in those with $CaCO_3$. As a conclusion, Fe-deficient *M. truncatula* roots accumulated and excreted riboflavin and three riboflavin derivatives different from those previously reported in plants. Further investigation is under way to identify the third flavin compound found.

[1] Welkie and Miller (1960) *Plant Physiol* **35:** 516
[2] Susín *et al.* (1994) *Planta* **193:** 514
[3] Susín *et al.* (1993) *J Biol Chem* **268:** 20958
[4] López-Millán *et al.* (2000) *Plant Physiol* **124**: 885

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