The role of iron deficiency-induced release of flavins into the rizosphere – The case of *Beta vulgaris*

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Iron (Fe) deficiency is one of the major crop yield-limiting factors, especially in plants grown on calcareous soils. Some plant species tolerant to Fe deficiency, accumulate in roots and export to the rhizosphere riboflavin (Rbfl) and other highly soluble Rbfl derivatives (Rbfl sulfates in *Beta vulgaris*). The functional relevance of flavins in this context is still unknown. Flavins have redox properties and are capable to complex Fe, opening the possibility that they may solubilize plant-unavailable Fe forms in the root apoplast and the rhizosphere. The aim of this study was to investigate whether flavin root export is related to the high Fe efficiency of *Beta vulgaris.* Plants were grown hydroponically in a growth chamber under Fe deficiency, flavins were continuously removed from the solution using a recirculating pump system and a C_{18} cartridge and the effects of flavin removal on leaf chlorophyll and Fe concentrations were studied. Plants were grown with half-strength Hoagland nutrient solution (pH 5.5) with 45 μM Fe (III)–EDTA for 21 days. Then, Fe-sufficient (+Fe) plants were grown with 45 μM Fe(III)–EDTA (pH 5.5), Fe-deficient plants were grown with no added Fe (0 μM Fe; pH 5.5 adjusted daily) with (-Fe*) or without (-Fe) flavin removal for at least 11 days. During the treatment period, the chlorophyll level of fully developed young leaves was recorded with a chlorophyll meter (SPAD). At the end of the experiment young leaves were sampled and the Fe concentration determined by AAS. At different sampling times, Rbfl and Rbfl sulfates in the nutrient solutions were analyzed by HPLC-UV/Vis-MS. The C_{18} cartridge successfully retained all flavin compounds. By the 7th day of treatment, the leaves of –Fe* plants had SPAD readings lower than those of +Fe plants. By this time, the SPAD readings in –Fe plants (where flavins were not removed) were still similar to those of +Fe plants. However, by the 11th day of treatment, differences in SPAD readings were found among all treatments, with the highest and lowest SPAD values corresponding to +Fe and –Fe* plants, respectively. Therefore, the removal of flavins caused the early appearance of leaf chlorosis. The removal of flavins also caused lower leaf Fe concentrations in -Fe* plants when compared to those of both +Fe and -Fe plants, which were similar. Consequently, Fe deficiency-induced export of Rbfl sulfates by roots of *Beta vulgaris* plants improved Fe status, probably by enhancing reutilization of apoplastic Fe pools.

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