

**TITLE: Carboxylate metabolism changes induced by Fe deficiency in barley, a Strategy II plant species.**

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**ABSTRACT:** The effects of iron (Fe) deficiency on carboxylate metabolism have been investigated in barley (*Hordeum vulgare* L.) using two cultivars, Steptoe and Morex, which differ in their Fe-efficiency response. In both cultivars, root extracts of plants grown in Fe-deficient conditions showed higher activities of enzymes related to organic acid metabolism, including citrate synthase, malate dehydrogenase and phosphoenolpyruvate carboxylase, compared to activities measured in root extracts of Fe-sufficient plants. Accordingly, the concentration of total carboxylates was higher in Fe-deficient roots of both cultivars, with citrate concentration showing the largest increase. In xylem sap, the concentration of total carboxylates was also higher with Fe deficiency in both cultivars, with citrate and malate being the major organic acids. Leaf extracts of Fe-deficient plants also showed increases in citric acid concentration and in the activities of glucose-6-phosphate dehydrogenase and fumarase activities and decreases in aconitase activity. Results indicate that changes in root carboxylate metabolism previously reported in Strategy I species also occur in barley, a Strategy II plant species, supporting the existence of an anaplerotic carbon fixation *via* increases in the root activities of these enzymes, with citrate playing a major role. However, these changes occur less intensively than in Strategy I plants. Activities of the anaerobic metabolism enzymes pyruvate decarboxylase and lactate dehydrogenase did not change in barley roots with Fe deficiency, conversely to what occurs in Strategy I plants, suggesting that these changes may be Strategy I-specific. No significant differences were observed in overall carboxylate metabolism between cultivars, for plants challenged with high or low Fe treatments, suggesting that carboxylate metabolism changes are not behind the Fe-efficiency differences between these cultivars. Citrate synthase was the only measured enzyme with constitutively higher activity in Steptoe, relative to Morex leaf extracts.

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