

## The chemical forms of Fe used as *in vivo* substrate in uptake process of chloroplast and enzymatic characteristics of cFRO

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Iron is required for several essential plant cell functions and metabolic processes especially photosynthesis. Consequently chloroplasts are the major iron sinks contain 80-90% of iron taken up by plant cells. The limited availability of Fe for chloroplasts causes chlorosis on the leaves and significant decrease in biomass production. Obviously, chloroplast iron uptake is a relevant question from scientific and agricultural viewpoint as well.

We tested the iron uptake capacity of chloroplasts with several unstudied natural and synthetic iron complexes and chelates purposes to elucidate the substrate preference of the Fe uptake machinery of chloroplast. Iron uptake was followed by a bathophenanthroline- (BPDS-) based method. Iron uptake machinery of intact chloroplasts seems to prefer Fe(III)-citrate complexes to Fe(II)-citrate and Fe(III)-malate as iron source, furthermore chloroplast iron uptake from Fe(III)-NA was negligible compared to Fe(III)-citrate. We also compared chloroplast iron uptake rate using different Fe(III):citrate ratio. Impact of iron deficiency on chloroplasts iron uptake rate was tested and significant decrease was observed in the presence of Fe(III)-citrate however the iron uptake rate was not affected in the case of iron surplus.

Chloroplast iron uptake process requires a ferric chelate oxidoreductase enzyme (cFRO) to reduce ferric-chelates to soluble ferrous form (FCR reaction). In its absence chloroplasts are not able to gather iron from the cytoplasm effectively. However, the detailed mechanism of action of this protein has not been characterized so far. We studied the exact localisation and enzymatic characteristics of cFRO, and the effect of iron deficiency on its activity. Using purified chloroplast envelope fractions, FCR reaction was clearly associated to chloroplast inner envelope membranes. We measured a significantly higher cFRO activity in the presence of NADPH compared to NADH. Iron deficiency results in a more efficient iron uptake from the cytoplasm into the chloroplasts by enhancing the affinity of cFRO to ferric-chelates.

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