Preferred session: ORAL
Selected topics: Dynamics of iron pools between organelles

Iron uptake machinery of chloroplasts tends to utilise stoichiometric ferric-citrate complexes in *Brassica napus*

Brigitta Müller[1], Hong Diep Pham[1], Krisztina Kovács[2], Yusuf Kavak[1], Balázs Gyuris[1], Máté Sági-Kazár[1], Viktória Soós[1], Waqas Ahmad[1], Helga Zelenyánszki[1], Kálmán Szenthe[3], Ferenc Fodor[1], Ádám Solti[1*]

[1]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Budapest, Hungary
[2]Laboratory of Nuclear Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary
[3]RT-Europe Nonprofit Research Ltd., Mosonmagyaróvár, Hungary
**adam.solti@ttk.elte.hu*

In plant shoots, the majority of iron is found in the chloroplasts, incorporated into the photosynthetic, sulphur assimilatory and Fe-S cluster biogenesis apparatuses. Although some members of their Fe transport machinery related to both reduction-based and nicotianamine-complex uptake systems have already been identified, the *in vivo* substrate preference of the system remained unknown.

To clarify the mechanism of action and the substrate preference of the uptake system, intact chloroplasts of oilseed rape (*Brassica napus*) were subjected to Fe uptake assays using natural and artificial Fe complexes and chelates: Fe(III)-citrate 1:1.1 and 1:10, Fe(III)-malate 1:1.1, Fe(II)- and Fe(III)-nicotianamine 1:1.2, Fe(III)-EDTA 1:1 and Fe(III)-*o,o*'EDDHA 1:1. Iron complexes were typified by the chemical microenvironment of Fe in the compounds by Mössbauer spectroscopy. Iron uptake by chloroplasts was assessed by determining chloroplast iron content spectrophotometrically. Putative homologue genes of major, Fe uptake related, chloroplast envelope membrane proteins were identified in *Brassica napus* using the Brassica Database and NCBI. The expression of *BnFro7* (*Bra037953*), *BnMar1* (*Bra020559*), *BnNico* (*Bra037287*), *BnPic1* (*Bra036409*), and *BnYsl4* (*XM_009141995.2*) was studied using *ß-tubulin* (*XM_009125342.1*) and *18S rRNA* (*KT225373*) as for reference genes in leaves subjected to chloroplast Fe uptake assays. Chloroplast inner envelope ferric chelate reductase activity of the isolated chloroplasts were also monitored using Fe(III)-EDTA.

Chloroplasts preferred stoichiometric Fe(III)-citrate compared to Fe(III)-citrate 1:10 and Fe(III)-malate complexes. Moreover, uptake from Fe(III)-NA and Fe(II)-NA but also from Fe(III)-EDTA and Fe(III)-*o,o*'EDDHA sources were negligible (with significantly higher K_M) compared to Fe(III)-citrate complexes. For these latter complexes, the light-inducible component was also missing. Regarding the components of the chloroplast Fe uptake system, genes of the reduction-based Fe uptake system showed high expression only. Nevertheless, the Fe-nicotianamine transport related chloroplast transporter *BnYsl4* was mainly expressed in generative tissues. In conclusion, chloroplasts in leaves can only effectively utilize stoichiometric Fe(III)-citrate complexes in their Fe uptake.

This work was supported by the grant financed by the National Research, Development and Innovation Office, Hungary (NKFIH K-124159). Á.Solti was also supported by the Bolyai János Research Scholarship of the Hungarian Academy of Sciences (BO/00207/15/4).