S4-PO-04 PROTEOME ANALYSIS OF CHLOROPLAST INNER ENVELOPE: A COMBINED *IN VITRO* AND *IN SILICO* ASSAY IN *BRASSICA NAPUS*

Brigitta Müller[1]*, Barbara Kotán[1], Kehui Liu[2], Huilan Wu[2], Ferenc Fodor[1], Hong-Qing Ling[2], Ádám Solti[1]

[1]Department of Plant Physiology and Molecular Plant Biology, Institute of Biology, Faculty of Sciences, Eötvös Loránd University, Pázmány Péter sétány 1/C, Budapest, 1117, Hungary
[2]State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, No.1 West Beichen Road, Chaoyang District, Beijing, 100101 China.
* brigitta.lantos@ttk.elte.hu

Several experimental evidences are available on the composition and function of the chloroplast envelope membranes. Nevertheless, native protein complexes have not been documented well, yet. Proposed protein-protein interactions and complex formations of chloroplast envelope proteins involved in Fe uptake are hardly known, either. To provide an overall view on the protein repertoire of chloroplast envelope membranes and find the major proteins involved in chloroplast iron uptake, a combined bioinformatic and proteomic analysis were performed. Oilseed rape (*Brassica napus)* was used as model plant which is an optimal research object for both isolation technics and physiological measurement. Moreover it is closely related to *Arabidopsis thaliana* as well. Therefore, a comparative study of protein sequences can provide relevant and new information on *Brassica napus* envelope proteom. Chloroplast envelope proteome of the two parent species of *Brassica napus – Brassica rapa* (A genome) and *Brassica oleracea* (C genome) – were compared to *Arabidopsis thaliana* using previous experimental data of Gutierrez-Carbonell *et al*. (2014) and membrane protein data bases (http://aramemnon.uni-koeln.de; http://brassicadb.org) in order to predict orthologs in the *Brassica napus* chloroplast envelope proteome. 75.6% of the identified proteins in pea by Gutierez-Carbonell *et al*. (2014) was found in *Brassica napus* as predicted ortholog and 24.4% of the proteins may be excluded as predicted ortholog. The average rate of protein sequence similarities were 89.9% and 88.4% in case of *Brassica rapa* and *Brassica oleracea*, respectively. The comparison of protein sequences revealed that 8 out of 9 proteins related to chloroplast iron metabolism (FRO7, PIC1, MAR1, NAP14, YSL4, YSL6, CLPC1, CLPC1) also show significant homology (≥80%) between *Arabidopsis thaliana* and *Brassica* species, while any homologue protein to AtNiCo has less than 80% sequence similarity. The *in silico* analysis was confirmed with *in vitro* investigation as well. Proteins of *Brassica napus* purified chloroplast inner envelope vesicles were separated by two-dimensional polyacrylamide gel electrophoresis and significant spots of the inner envelope membrane proteins (9) were analysed by MALDI-TOF MS. All protein spots were compared to the results of Gutierez-Carbonell *et al*. (2014). Our data contributes to the identification of chloroplast iron metabolism involved proteins in *Brassica napus* genome.

Keywords: bioinformatics, *Brassica napu,* chloroplast, envelope proteome, iron

REFERENCES

Gutierrez-Carbonell E. et al. 2014 *J Prot Res 13:2941*

ACKNOWLEDGEMENTS. This work has been supported by the National Office for Research, Development and Innovation, Hungary (NKFIH, PD-112047).