

WORKSHOP SCIENTIFIC REPORT

Action number: **CA16212; INDEPTH**

Workshop title: **Training School on Plant Nuclear Proteomics (TS4)**

Workshop start and end date: **July 8-12, 2019**

Organizers: **Marek ŠEBELA and Aleš PEČINKA**

Location: **Faculty of Science, Palacký University in Olomouc and Institute of Experimental Botany, Czech Academy of Sciences, Olomouc, Czech Republic**

PURPOSE OF THE WORKSHOP:

The training school TS4 was organized as a part of the planned activities of the Working Group 2 ("Transcriptional regulation through association of chromatin domains with nuclear Compartments").

The aim was to show participants the whole procedure of plant nuclear proteome analysis starting with a flow cytometric sorting of barley nuclei followed by their DNase treatment (to remove DNA), protein extraction, gel electrophoresis, in-gel digestion by trypsin, peptide purification and liquid chromatography coupled with tandem mass spectrometry plus data processing.

The duration of TS4 was 5 days. The participants (17 people in total) were divided into two groups; the number of instructors was 7. Each group performed laboratory experiments and attended related lectures with a mutual exchange of place. This arrangement reflected the existence of a limited space for experiments.

All participants were selected based on their motivation letters and CVs. Only one candidate was refused as Palestine is not a member state of the COST actions. The others were accepted, their personal data and provided information were found relevant and the total number of registered was lower than the capacity of TS4.

DESCRIPTION OF WORK CARRIED OUT DURING THE WORKSHOP

The training school included the following lectures and laboratory experiments:

- Lectures:
 - 1) Mass spectrometry of proteins I and II
 - 2) Flow cytometry
 - 3) Sample processing in proteomics
 - 4) Electrospray mass spectrometry
 - 5) Data processing from mass spectrometry

- Experiments:
 - 1) Sample preparation and flow sorting
 - 2) Nuclei treatment by DNase
 - 3) Protein electrophoresis
 - 4) In-gel digestion
 - 5) Peptide desalting and microgradient separation

- 6) Peptide mass fingerprinting, MALDI MS/MS
- 7) nLC-MALDI
- 8) nLC-ESI-MS/MS

All participants were received printed and electronic information materials including lectures, full texts of relevant scientific articles and protocols/manuals for laboratory experiments.

DESCRIPTION OF THE MAIN OUTPUTS OF THE WORKSHOP

TS4 was organized in a scheme, which allowed all participants to get a deep view inside the strategy of plant nuclear proteomics. They could listen lectures providing a theoretical background to all experiments conducted in a hands-on arrangement. The feedback was continuous as results of all experiments were evaluated immediately. TS4 was considered very useful by all participants, the organizer did not receive any complains or negative feedback.