Quantitative gel-free phosphoproteomic approach in elicited *Arabidopsis thaliana* cells

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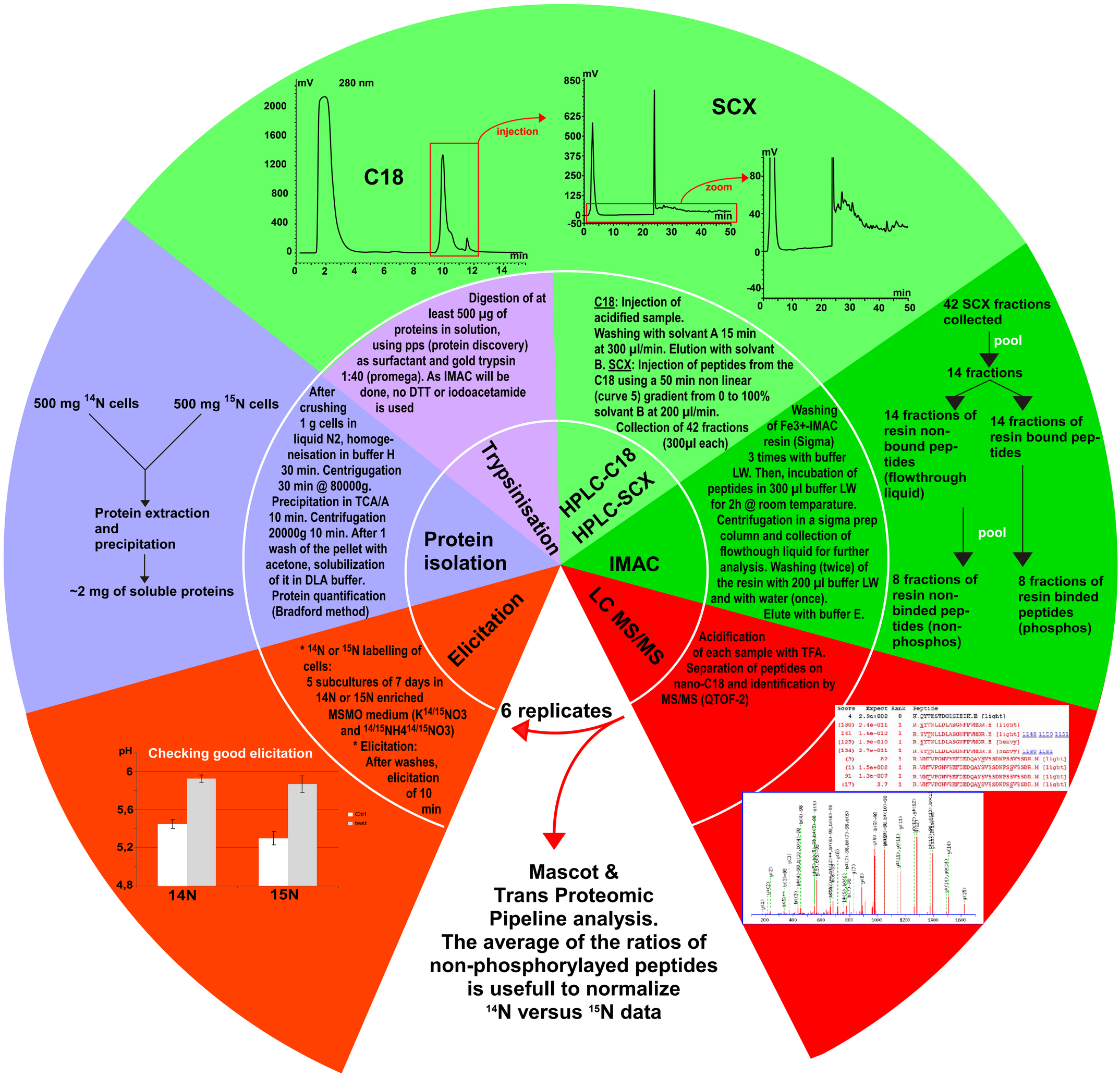
Characterization of signal transduction pathways involved in plant-pathogen interactions and plant cell defence responses is challenging. For such a goal, full 15N metabolic labelling gel-free quantitative phosphoproteomic approach is promising. The main objective is here to set up a reproducible method for studying effects of any cell treatment at the phosphoproteome scale.

Arabidopsis thaliana cells cultures were grown for at least 5 subcultures periods of 7 days on either ^{15}N or ^{14}N Murashige and Skoog medium prior to elicitation. Soluble proteins were extracted from plant cells and precipitated. Overnight trypsinisation of denatured proteins was then made in solution. After that, a HPLC-C18 step was chosen to clean the sample. To make an effective enrichment procedure, a reduction of the complexity of the peptides mixture is necessary. It was done by running HPLC-SCX wherein 42 fractions were collected per run. These fractions were enriched in phosphopeptides via a commercial Fe^{3+}-IMAC resin. Peptides were then sequenced by LC-MS/MS. As finding a real "housekeeping protein" is problematic, flowthrough fractions of the IMAC were kept in order to normalize 14N-labelled versus 15N-labelled peptides. Analysis of QTOF output data was possible thanks to the freely available Trans Proteomic Pipeline and/or the Mascot software.



HPLC and IMAC steps were first validated with a mix of caseins as protein mixture (phosphos and dephosphos).

We are now able to identify proteins that are (de)phosphorylated due to cell treatment. In general, 40 proteins are identified as phosphorylated per run/ replicate

Buffers:
*H: Tris-HCl 50mM; EDTA 2mM; DTT 5mM, PVPP 0.6%; PMSF 1mM; protease inhibitor cocktail (ROCHE #04693159001); phosphatase inhibitor cocktail (CALBIOCHEM #524627); pH 5.7
*DLA: thiourea 2M; Urea 7M; CHAPS 4%; Tris 30mM; pH 8.5
*LW: 250mM HAc; 30% ACN
*E: 0.4M NH_4OH; 30% ACN

TCA/A: TCA in acetone

Solvants
*C18: *A*: 0.1% HAc / *B*: 0.1% HAc; 70% ACN
*SCX: *A*: 30% ACN; 0.5% Formic acid; pH 2.6 / *B*: 30% ACN; 0.5% Formic Acid; 400mM Ammonium formate; pH 4.7

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