agropolis fondation

IProPhen

IProPhen - Integration of proteomic resources for molecular phenotyping of plant growth in response to environmental and climate change

ABSTRACT

Keywords : Plant, Ecophysio/architecture/phenotyping, Operation, Climate change, Phenotoping, Protein/proteomic

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Project leader : Michel Rossignol
Project leader's institution : INRA-INRAE
Project leader's RU : BPMP

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GOAL

Our goal was to build proteomics resources suitable to characterize simultaneously the various transporters present in the Arabidopsis plasma membrane in order to study their responses to environmental stresses and correlate them to macroscopic phenotypic features of aerial parts of plants. A targeted proteomics strategy relying on mass spectrometry in the Multiple Reaction Monitoring (MRM) mode was selected for its high specificity and sensitivity. This method requires three consecutive steps: the bioinformatics identification of peptides of interest together with the prediction of their behavior in LC-MS/MS, their synthesis in light or heavy form (by incorporation of heavy isotopes) and tuning of the mass spectrometer for every peptide.
► Identification of peptides of interest.
Three main types of transporters were selected for this proof of concept study: the H+-ATPases proton pumps as primary transporters (AHA family), the influx or efflux nitrogen (as well nitrate as ammonium) transporters (AMT, NRT and POT families) as example of secondary transporters, and aquaporins as channels (PIP family). For these different families, bioinformatics analysis first allowed to identify ca 800 tryptic peptides as unique in the Arabidopsis genome, and being thus usable to specifically identify isoforms in a given family. Their propensity to be analyzable by LC-MS/MS was then calculated using the various predictors. The resulting data were then inspected by partner teams expert for each transporter family in order to select the most pertinent protein and peptides on a knowledge basis (available expression data, known post-translational modifications, availability of mutants). Finally, a total of 30 transporters was selected for MRM analysis.
► Peptide synthesis.
Thirty-two peptides covering the 30 accessions (5 AHAs, 13 PIPs, 5 AMTs, 5 NRTs and 2 POTs) were synthesized in light and heavy forms.
► MS optimization.

For every peptide, optimal sensitivity requires tuning both ionization and fragmentation instrumental parameters. Under optimized conditions, response curves of peptides must then be checked for robust statistical significance of detection and quantification thresholds (LOD, LOQ). In addition, different quantification methods (using either light or heavy peptides) were compared. The method finally selected as the more robust relies on (i) spiking samples with known amount of the mixture of heavy peptides and (ii) using a digest of biological samples as a matrix for establishing dilution curves of heavy peptides.

RESULTS

In terms of resource (identification of usable sequences, availability of heavy peptides, peptide-dependent optimized conditions), this work resulted in a tool allowing the simultaneous characterization of the 28 accessions, excepted for the ammonium transporter AMT1.1. For this latter, most of the 7 peptides unique in the Arabidopsis genome display unfavorable predicted features, whereas others may co-exist under various states due to post-translational or chemical modifications. The less unfavorable peptide was initially selected but turned out to be convenient only in quite simple mixtures (such as that of the synthetic peptides used here) and no longer robustly detectable in a complex matrix (like a digest of membrane proteins). This peptide was discarded. Such situation is illustrative of a potential limitation of the strategy for highly homologous multigene families where the identification of favorable sequences may be problematic.
In terms of molecular phenotyping, due to the late availability of some synthetic peptides, the resource was little used with plants submitted to simultaneous macroscopic phenotyping under salt stress. Molecular phenotyping was performed, at the root level, on plants grown in hydroponics and exposed to a short salt stress (150 mM NaCl, 4 hours). Three AHAs, all the 13 PIPs, 2 AMTs and 2 NRTs were simultaneously identified and quantified in roots, with accumulation differences covering 3 orders of magnitude. The abundance of 11 among them appeared to be significantly lowered under salt stress. These results are novel in several concerns:
- for a number of transporters, they correspond to the first experimental observation as proteins (available antibodies not allowing until now to resolve isoforms);
- they constitute the fist simultaneous analysis of several transporter families covering the main types (primary transporters, secondary active transporters, channels);
- they enable functional analysis (expression regulation) accessible to date only at the transcript level. In this view, excepted for a limited number of accessions, results (i) show a weak disconnection with available data for the accumulation of PIPs transcripts in roots, and (ii) demonstrate for all families a fair overall correlation between transcriptome and proteome responses to salt stress.

PERSPECTIVES

This project allowed developing a unique resource for the simultaneous molecular phenotyping of various transporter families. This resource is innovative, including outside the plant area, and reusable. It has been already taken to profit for investigating the effect of nycthemeral status on the expression of PIPs in the leaf. In addition, the approach is generic and, at the possible reserve of specific cases within some multigene families, is portable at larger scale as well to address other transporter families as more generally for targeted proteomics strategies.