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Combined label-free quantification techniques to detect water deficit induced changes in the *Medicago truncatula* proteome

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INTRODUCTION

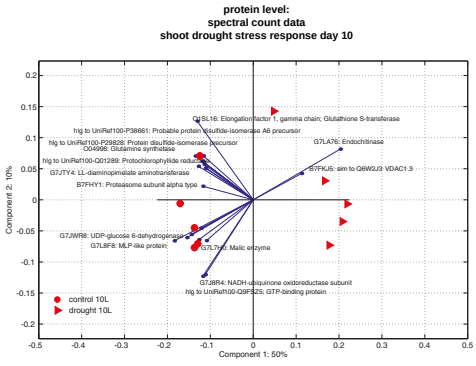
Shotgun proteomics is a powerful method for identification of vast amounts of proteins in complex sample mixtures. However, there exists a trade-off between the number of identifications and quantification robustness.
In an attempt to increase the depth of information obtained from mass spectrometry based proteomics we combined protein and peptide level quantification techniques: database-dependent spectral counting results are compared to results from database-independent peptide ion quantification. The peptide level analyses were performed using the recently pubished **ProtMAX** tool [1].
Legume species play an important role in global food security. Here we investigate the drought stress response of the shoot proteome of the model *Medicago truncatula* and the dynamic changes induced druing stress recovery.

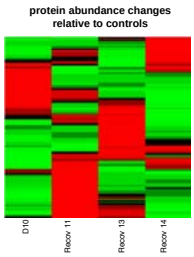
METHODS

Medicago truncatula cv. Jemalong was grown on vermiculite-perilte in a climatic chamber. 7 week old plants were subjected to slowly developing water defict by water-withholding. Protein extracts of shoot material were analysed using an LC-MS/MS Orbitrap based proteomics approach. MS/MS spectra were searched against the Medigaco spp. UniProt database using SEQUEST. Peptide-level quantifications were performed by mass accuracy precursor alignment using **ProtMAX** as recommended previously[1].

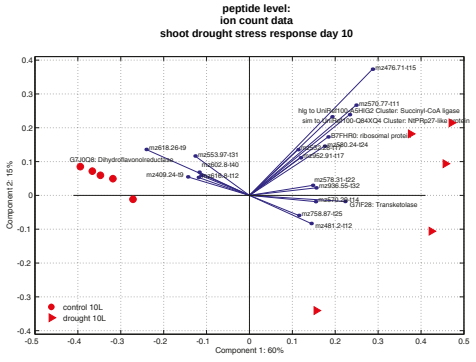
UNBIASED SEARCH

*** database-dependent:** counting fragment spectra yielding identifications.
Principal component anaysis of 435 protein groups identified via SEQUEST and infered using Proteome Discoverer. The biplot displays the 20 proteins with the highest loading on PC1 as vectors.



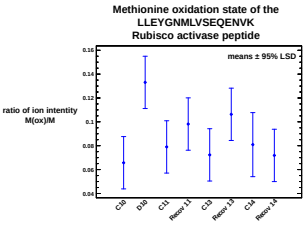


*** database-independent:** counting MS1 spectra of precursor ions within a predefined retention time window. **ProtMAX** aligned precursor ions (mz) within a predefined retention time window (t) along multiple MS-runs, resulting in 10643 bins. The features most relevant for the distinction of the drought responsive peptides from control levels are displayed as vectors. Most of the features are so far unknown. For 5 features we could infer a function based on previous identification via SEQEST.

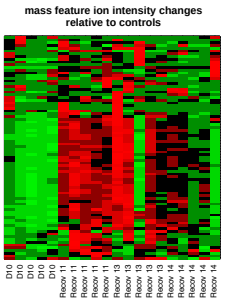


TARGET SEARCH

*** ion extraction of previously identified peptides:** adressing PTMs. As water deficit increases, CO2-fixation is impaired. Continued irradiation leads to an over-reduction of the photosynthetic electron transport chain and oxidative stres. Thus, oxidative protein modifications are of special interest in plant abiotic stress research. Methionine oxidation is a reversible PTM.



*** ion extraction of unidentified features:** putative peptide targets for de-novo sequencing strategies



CONCLUSION

Protein-level and peptide-level quantification techniques complement one another. On the peptide level we could obtain insights into the dynamics of oxidative peptide modifications during plant drought stress response. Protein level results provided information about the changes in relatively abundant proteins. Both techniques allow for the distinction of stressed and non-stressed plants. However, the identifiable relevant features detected by each strategy show no consensus. Most precursor ions exhibiting a possible regulatory role in plant responses to water deficit could not be identified via classical database-dependent approaches and will be subjected to further de-novo sequencing.

REFERENCES:
[1] Egelhofer V. et al., Using ProtMAX to create high-mass-accuracy precursor alignments from label-free quantitative mass spectromtry data generated in shotgun proteomics experiments. Nature Protocols, Vol 8 No3, 2013

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