



Agricultural Institute of Slovenia

Use of proteomics in breeding for drought tolerance in common bean

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Outline

- Introduction:
 - drought and common bean
- Proteomic analysis of drought stress response:
 - Leaf proteome
 - Stem proteome
 - Glycoproteins
- Conclusions and future perspectives

Drought stress in plants

- Drought influences **growth, development** and **productivity** of plants
- Plants experience drought when the **water supply is limited** or when the **transpiration rate becomes high**

Breeding tolerant cultivars:

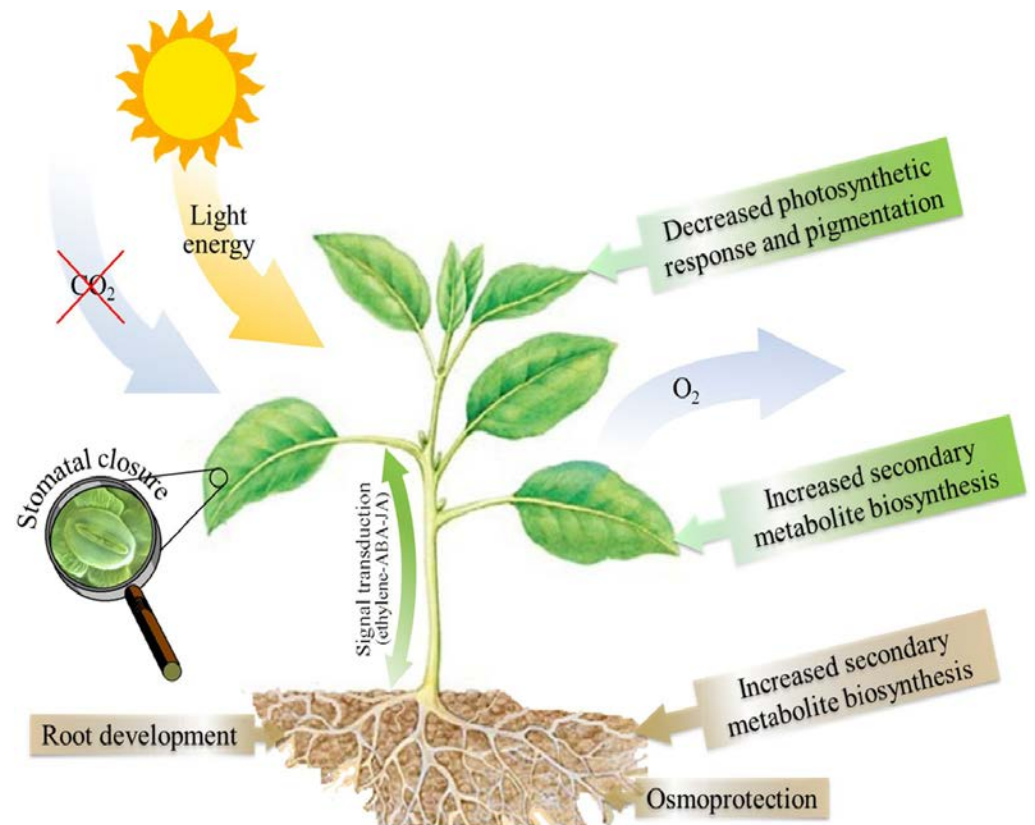
- Tolerance to drought – a complex trait controlled by several small effect genes or QTLs



Drought stress in plants

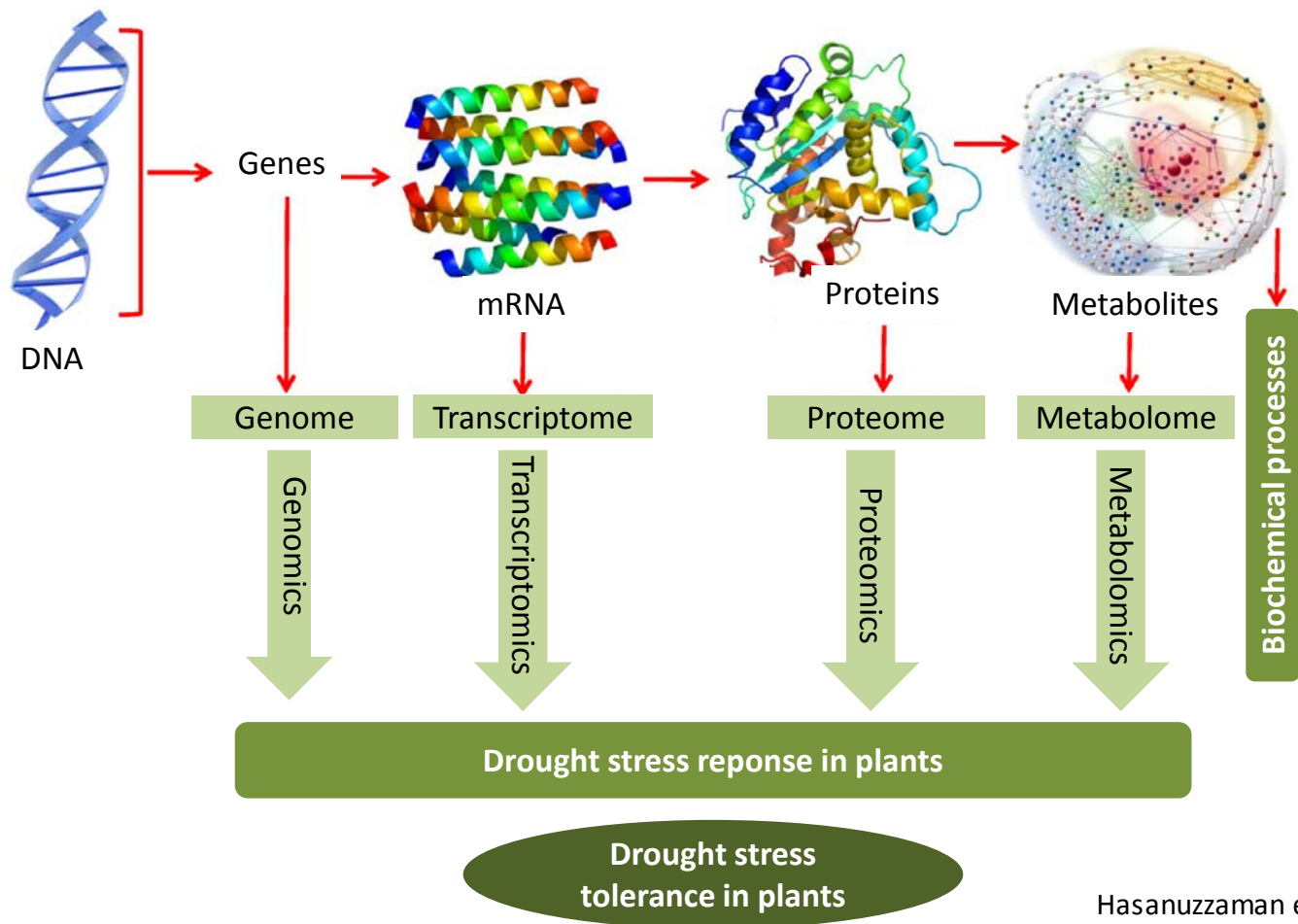
Plant response to stress as individual cells and synergistically as a whole organism.

- Physiological level:
 - Photosynthesis
 - Stomatal closure
 - Osmotic adjustment
 - Root development...
- Cellular level:
 - Hormones, ROS, sugars, starch
- Molecular/biochemical level:
 - Genes, Gene expression
 - Functional, Regulatory proteins



Zingaretti et al., 2013

Drought stress in plants



Hasanuzzaman et al., 2013

Common bean (*Phaseolus vulgaris* L.)

- The most important grain legume for direct human consumption:
 - high nutritional value (high content of proteins, dietary fibres, minerals,...)
 - antioxidant activity,...
- Sensitive to drought - 60 % of the world's common bean is cultivated under rainfed conditions.
- Mechanisms of tolerance is not well characterized yet due to genetic complexity and diverse, often ambiguous phenotypic effects.



Understanding the response of common bean to drought is the first step in breeding of tolerant cultivars



Beans in Slovenia

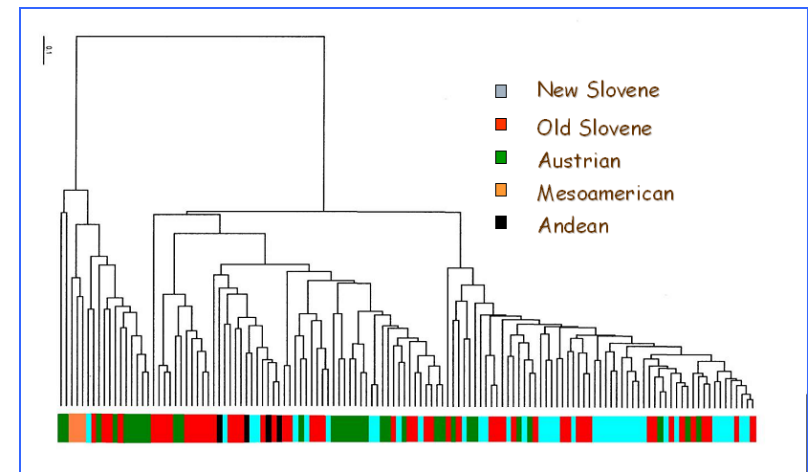
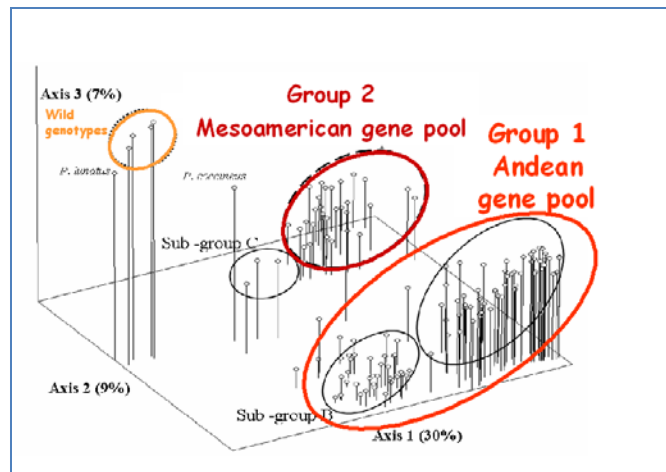
Collection of *Phaseolus* accessions in the Slovene Plant Gene Bank:

- 1035 accessions collected in Slovenia:
 - >800 *Phaseolus vulgaris* L. ssp. *vulgaris* var. *vulgaris*,
 - >180 *Phaseolus vulgaris* L. ssp. *vulgaris* var. *nanus*,
 - 53 *Phaseolus coccineus* L.
- 61 accessions from international expeditions
- 39 accessions from other gene banks worldwide



Evaluation of common bean

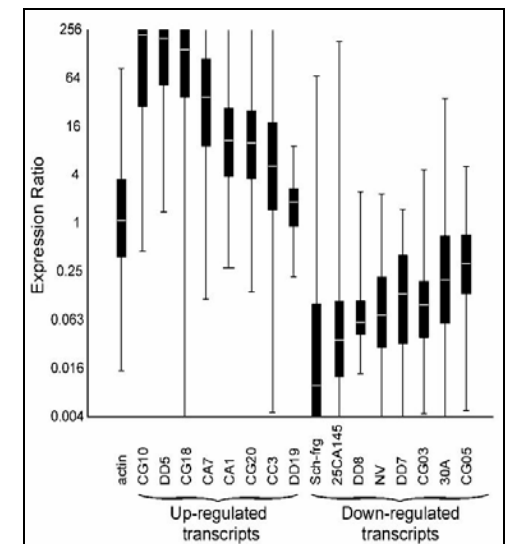
- **Diversity studies** (morphological, biochemical, molecular markers)
- **Temporal changes in genetic diversity** and studies of **dissemination pathways** in Central Europe and Balkans
- **Disease resistance** (*Colletotrichum lindemuthianum*)



Evaluation of common bean

Drought tolerance:

- Physiological response :
 - RWC, water potential, antioxidant activities, photosynthetic parameters, ..
- Identification of genes whose expression is altered under conditions of drought
- Proteases under drought:
 - Identification of novel subtilases from leaves



Proteomics



- Application in many biological processes with:
 - **identification of proteins**
 - determination of the **protein expression profile** during various conditions
 - **analysis of post-translational modifications**,
 - studies of **protein–protein interactions**
 - **subcellular proteomics**
- Useful in revealing plant mechanisms of drought stress response, tolerance and for identification of candidate biomarkers for application in molecular breeding
- Its integration into the field of crop science is helping to enrich genome annotation efforts and accelerate the improvement of crop productivity

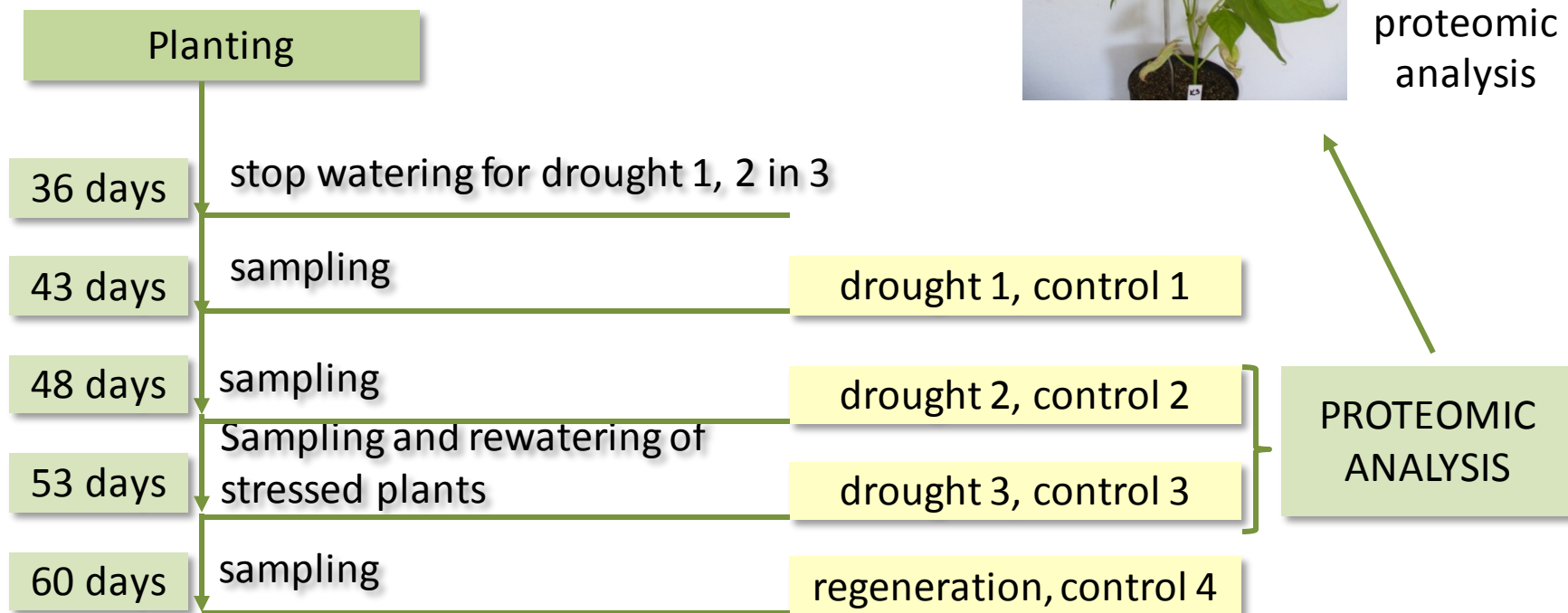
Experiment

The aim:

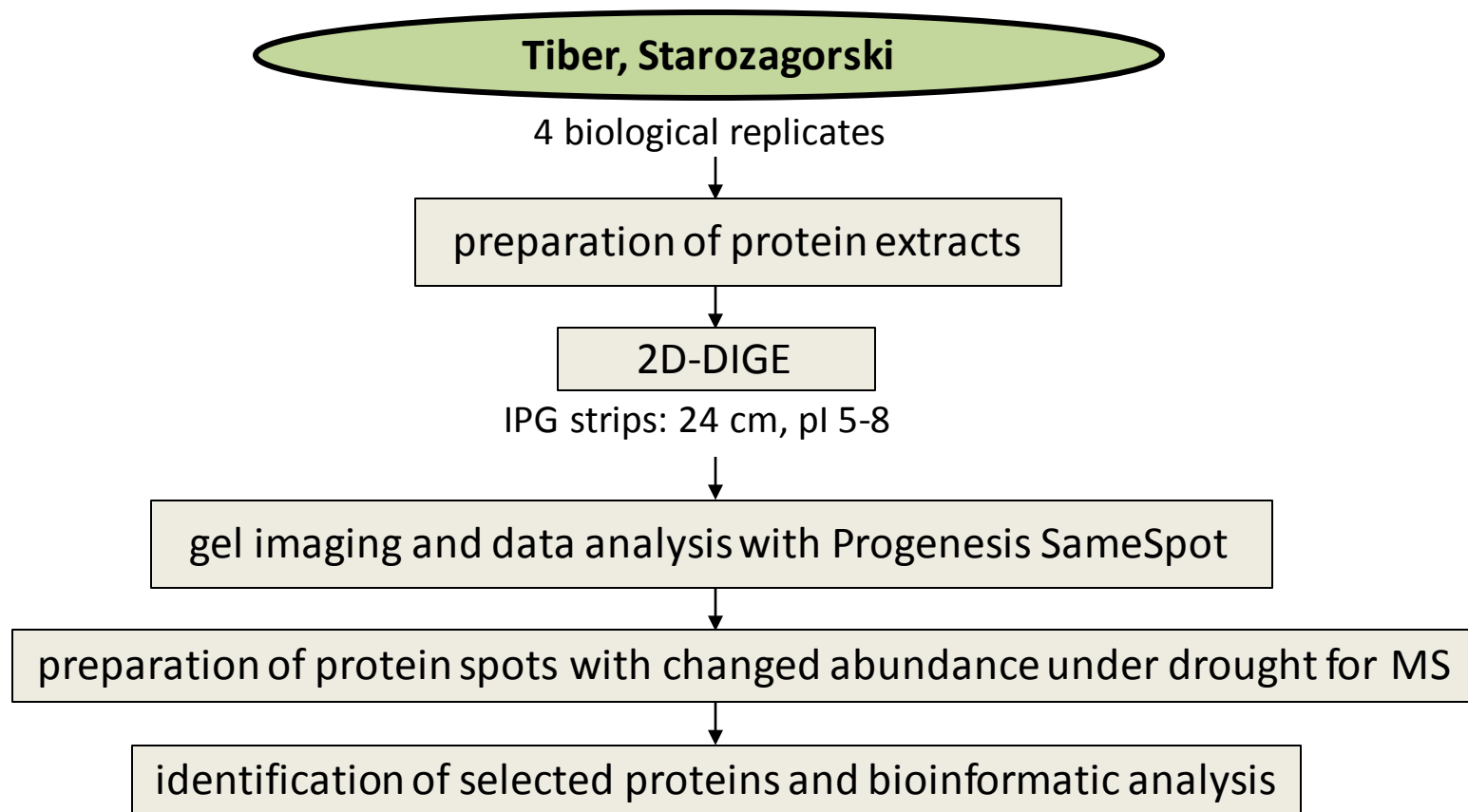
To identify drought-responsive proteins in leaves of two cultivars differing in their response to drought – Tiber and Starozagorski čern



Sampling of third trifoliate leaves for proteomic analysis

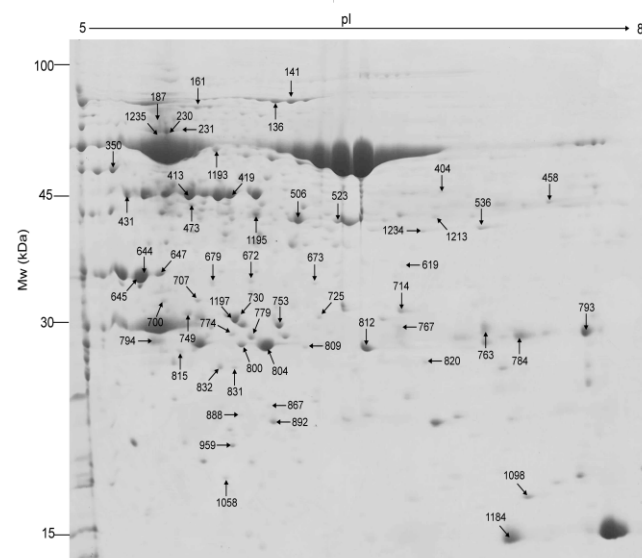


Experiment



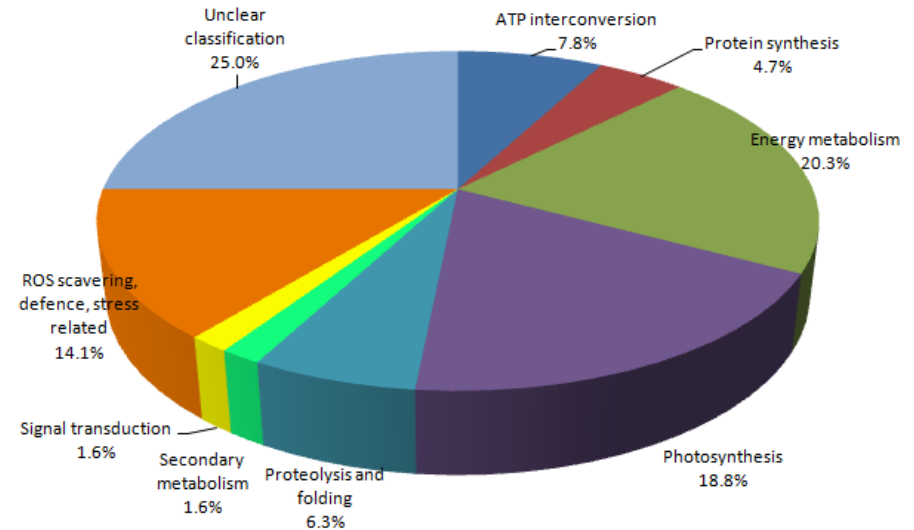
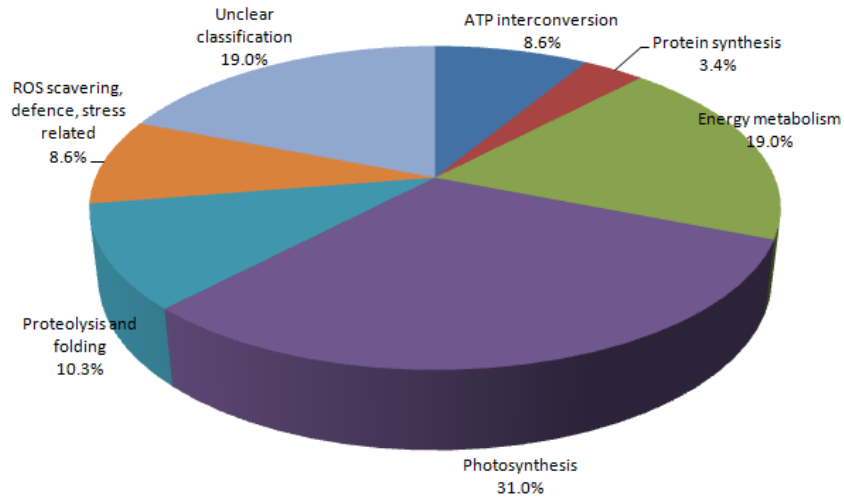
Results

	Tiber	Starozagorski
total number of protein spots	400	543
number of protein spots with changed abundance under drought	62 (42 ↑, 20 ↓)	68 (36 ↑, 32 ↓)
number of identified proteins	58 (39 ↑, 19 ↓)	64 (32 ↑, 32 ↓)



2D electrophoresis gel of protein extracts for cultivar Tiber

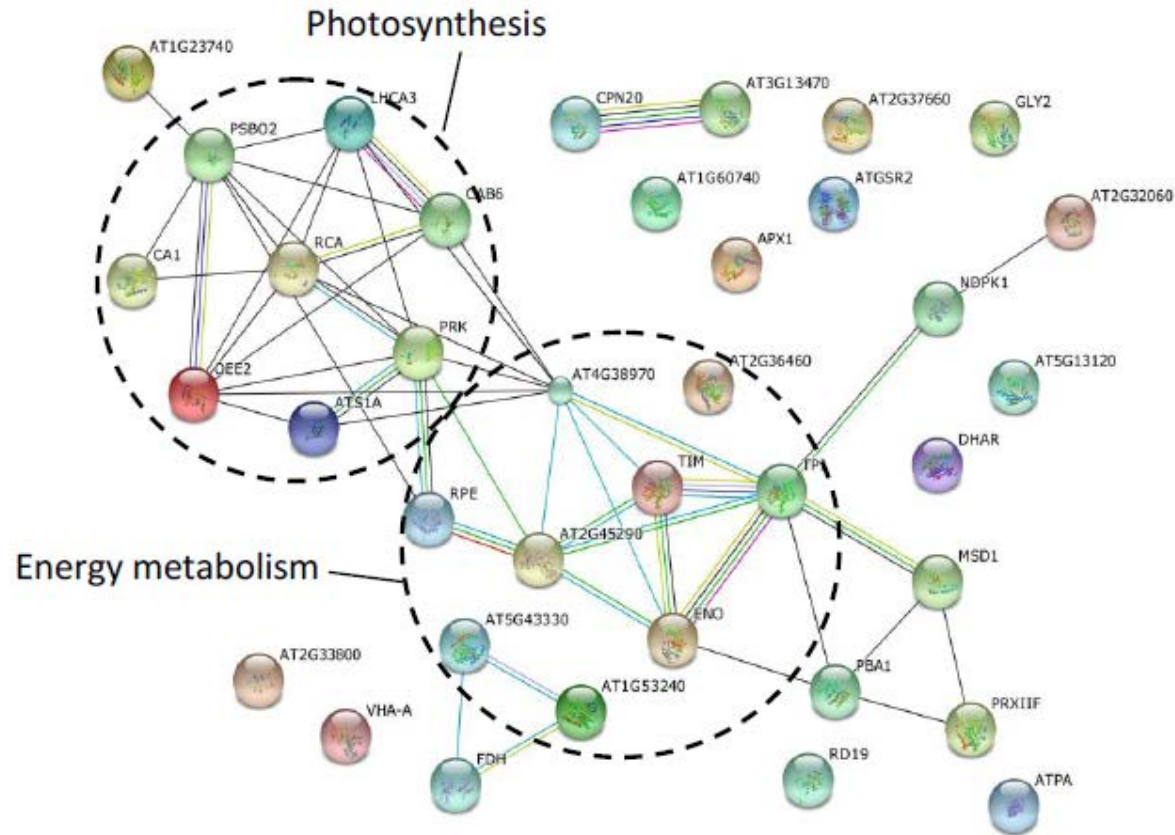
Functional classification of identified proteins



Tiber (left) and Starozagorski (right)

Zdražnik *et al.* 2013. *J Proteomics* 78: 254-272

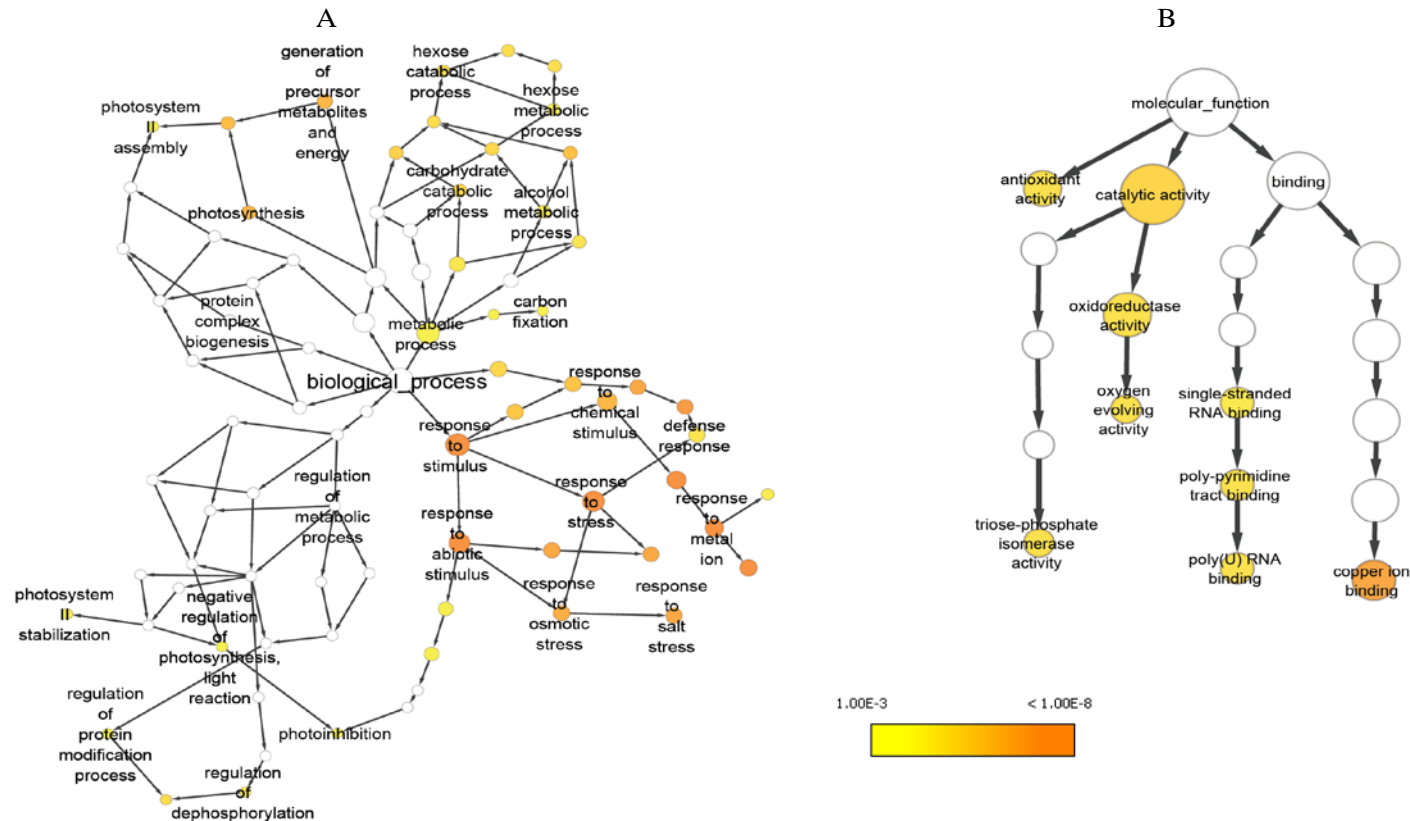
Analysis of protein-protein interaction of identified proteins



Cultivar Tiber

Zdražnik *et al.* 2013. *J Proteomics* 78: 254-272

Biological pathways and molecular function network



Biological pathway (A) and molecular function (B) networks generated by BiNGO. GO categories of TAIR homologous proteins for cultivar Tiber.

Leaf proteome: results and conclusions

- One third of identified proteins were detected in multiple spots → posttranslational modifications, isoforms, degradation

- **Proteins involved in photosynthesis:**

↓ abundance in Starozagorski

↑ in ↓ abundance of proteins in Tiber

oxygen evolving enhancer proteins!

} negative effect on proteins of photosynthetic apparatus; greater extent in Starozagorski

- **Stress response related proteins:**

↑ abundance in Tiber

↑ abundance in Starozagorski

(exception quinone oxidoreductase-like protein and thioredoxin)

Leaf proteome: results and conclusions

- **Proteins related to synthesis, folding and proteolysis:**

↑ abundance of proteins in both cultivars

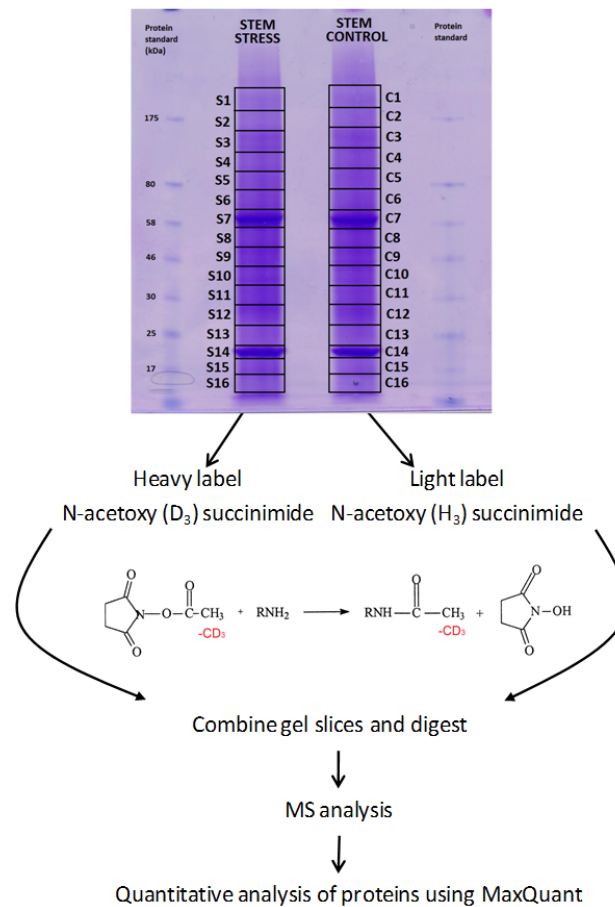
- ↑ abundance of proteins related to **secondary metabolism** and **signal transduction pathways** only in **Starozagorski**



- Certain identified proteins could be used as **markers in the selection process for drought tolerance in common bean** (proteins with contrasting abundance patterns between cultivars) → **needs to be confirmed by a proteomic comparison of the two cultivars**

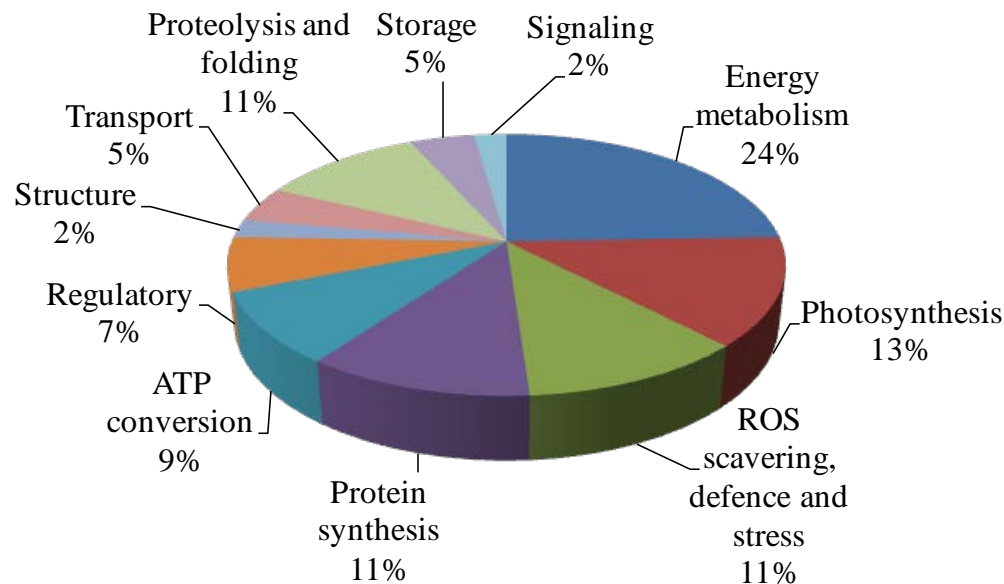
Drought stress: stem proteome

- Cultivar Tiber
- SDS-PAGE separation of isotope labeled protein extracts from stems.
- 16 protein slices of drought stress samples and 16 slices of control samples excised from the gel.
- Proteins were in-gel labeled, digested with trypsin and analyzed by LC-MS/MS.



Stem proteome: results

A total of 560 proteins identified, after filtering narrowed down to 222.
45 proteins changed in abundance between control and stressed samples.



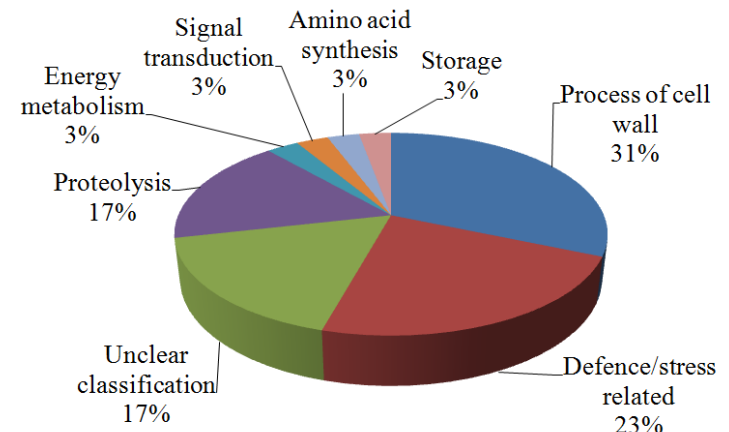
Functional classification of quantified proteins

Glycoproteins

Posttranslational modifications play an important role in tolerance and response to abiotic stress. Glycosylation - a widespread protein posttranslational modification that effect protein structure and its function.

Aim: analysis of the N-glycosylation during drought stress

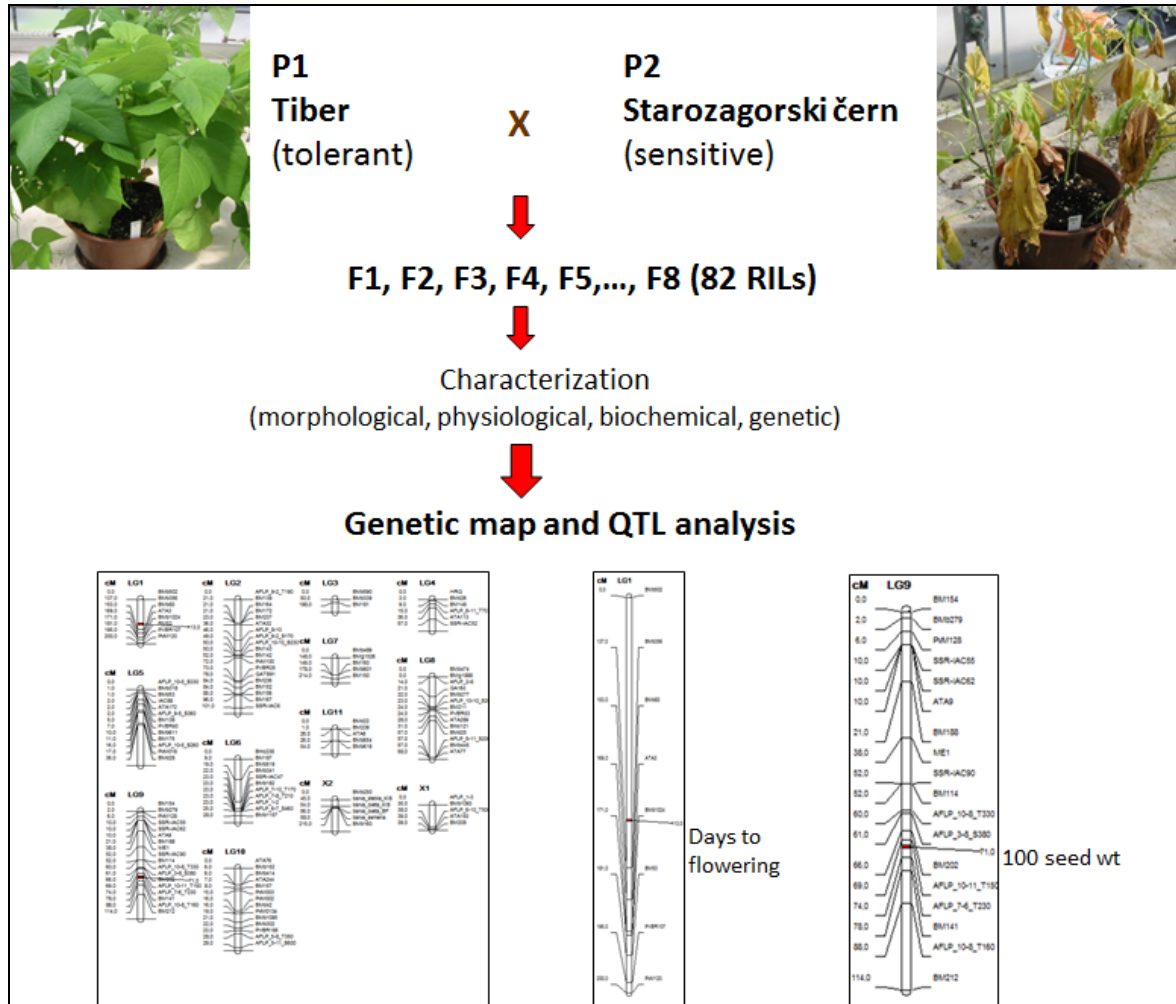
- Cultivar Tiber, Lectin affinity chromatography, SDS separation, LC-MS/MS analysis
- Drought stress has the most negative effect on proteins involved in the biochemical metabolism of the cell wall.
- The abundance of proteins involved in defence against stress, proteolysis, energy metabolism were also influenced by drought. These protein groups fit into the general concept of plant response to drought stress.
- Structures of high mannose, complex and hybrid types of N-glycans were found by manual inspection from spectra.



Classification of proteins into functional groups.

Genetic map and QTL analysis

Poster 51



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Thank you for your attention!

Foto: J. Verbič