Value-added products from biomass (BIO-VALUE)

BIO-VALUE

A Strategic Platform for Research and Innovation on biorefining

UNIVERSITY OF COPENHAGEN

Sejet planteforædling

Project 1, Jan Van Hecke

Interactions between Nitrogen metabolism and cell wall properties in winter wheat straw

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Goals

Part I:

- Deeper understanding of the role of nitrogen for straw productivity and structure
  - ➢ Nitrogen pools at different growth stages (N-Audit)
  - ➢ Cell wall structure as affected by straw N status

Part II:

- Identify and modify important cell wall proteins (CWPs) for tailored biomass
  - ➢ Proteomics, genetics

Field trial setup

- 1 Genotype (high straw yielding)

- 5 amounts of N fertilization (60, 100, 160, 220, 280 kg/ha)

- Harvest at different growth stages (GS, Zadoks *et al.*, 1974)
  - end of anthesis (GS 68)
  - soft dough stage (GS 85)
  - harvest time (GS 92)

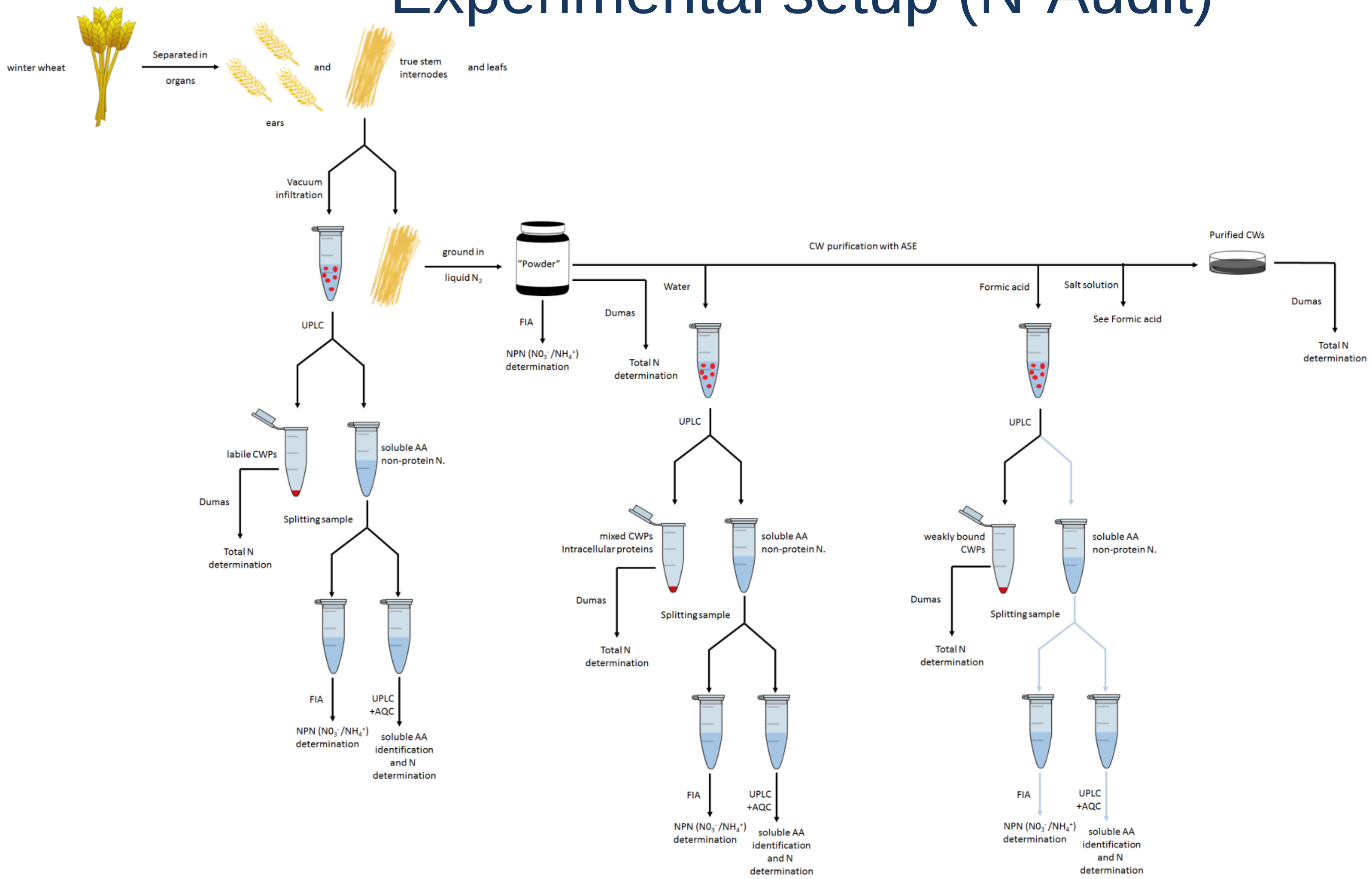
- Stripped into organs (true stem, ear, leafs)

Part I: Nitrogen pool identification (N-Audit)

Extraction and measurement of N containing substances:

- Non-protein N containing substances (NPN)
- Intracellular proteins
- Free Amino Acids
- CWPs

Experimental setup (N-Audit)



Results

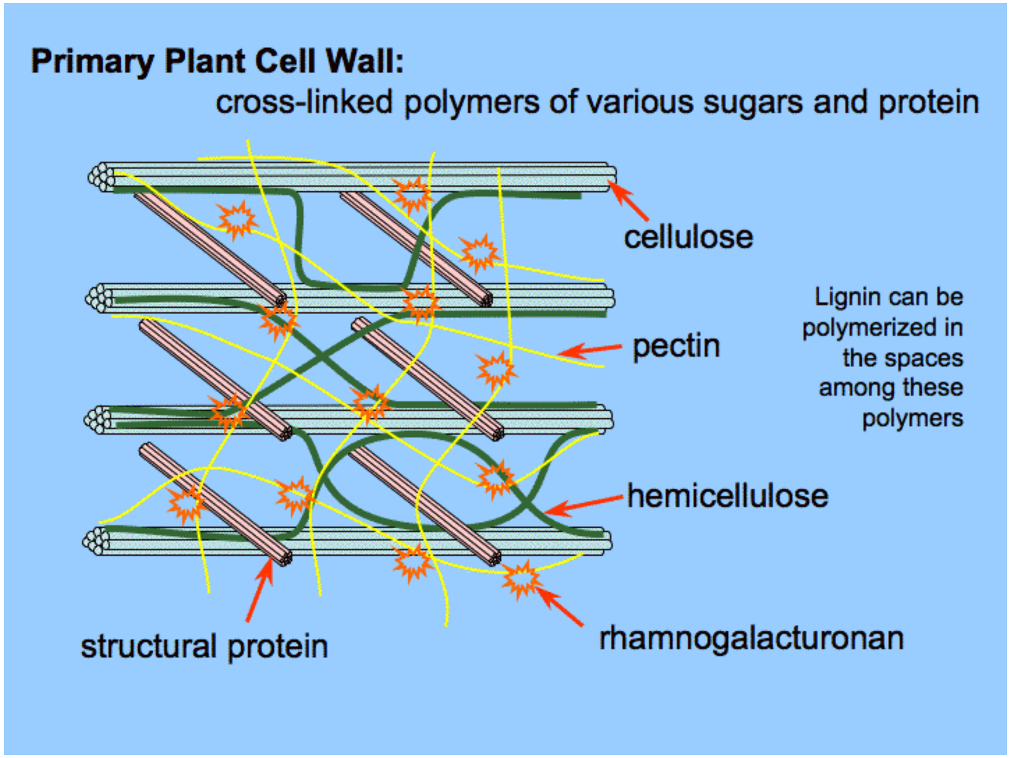
- Identification of the different N pools
  - most abundant N containing substance
  - most remobilized N containing substance
- Identification of straw physical characteristics (microscopy)

Prospects

- Finding genotypes with desired straw properties (Sejet)
  (e.g. more lodging resistant straw; better N remobilization from straw)
- Possibility for genetic modifications of pathways
  (knock down/out; overexpression)

Part II

A close look at the cell wall



Grass cell wall composition

Table 1

Approximate composition[a] (% dry weight) of typical dicot and grass primary and secondary cell walls

	Primary wall		Secondary wall	
	Grass	Dicot	Grass	Dicot
Cellulose	20–30[b,c]	15–30[c,d,e]	35–45[c,f]	45–50[c]
Hemicelluloses				
Xylans	20–40[d]	5[c]	40–50[c,g]	20–30[c,g]
MLG	10–30[d]	Absent	Minor	Absent
XyG	1–5[c,d,g]	20–25[g]	Minor	Minor
Mannans and glucomannans	Minor	5–10[d]	Minor	3–5[g]
Pectins	5[c]	20–35[d]	0.1[c]	0.1[c]
Structural proteins	1[d]	10[d,e]	Minor	Minor
Phenolics				
Ferulic acid and ρ-coumaric acid	1–5[c,d]	Minor (except order Caryophyllales)	0.5–1.5[c]	Minor (except order Caryophyllales)
Lignin	Minor	Minor	20[c]	7–10[c]
Silica			5–15[c]	Variable

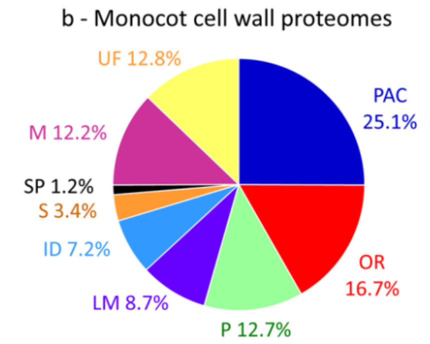
[a] Numbers in this table were taken from several sources to provide rough approximations of generalized cell wall composition from typical dicots and grasses. Some of the numbers are averages or ranges based on multiple sources.

- Cell wall proteins (CWPs)
  - Only Nitrogen containing substance in CW
  - Important for straw strength

Vogel, J. : Unique aspects of the grass cell wall. Current opinion in plant biology 11, 301–7 (2008).

CWPs

- Different classification of CWPs have been proposed
  - by functional classes (Albenne *et al.*, 2013)
    - Enzymes
    - Structural proteins etc.



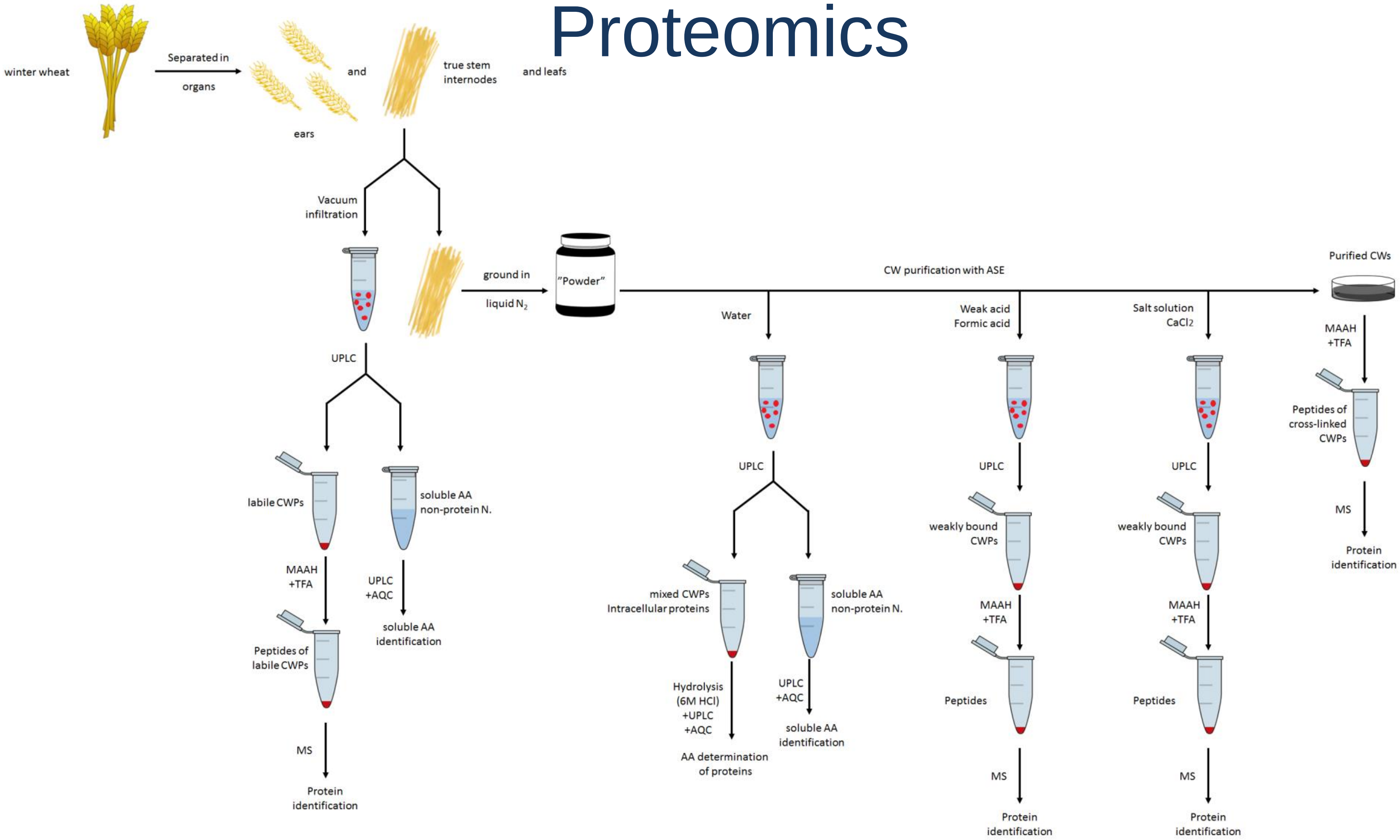
Pooled proteomics data from different papers
Albenne *et al.*, 2014

  - by interaction with CW (Jamet *et al.*, 2008)
    - labile, weakly and covalent bound CWPs

CWPs
the forefront of plant research

- Diverse functions / roles (Somerville *et al.*, 2004)
  - responses to abiotic and biotic stresses (e.g. flooding)
  - **cell structure** maintenance
    - ➢ Target for tailored biomass in bio-refinery

Experimental setup
Proteomics

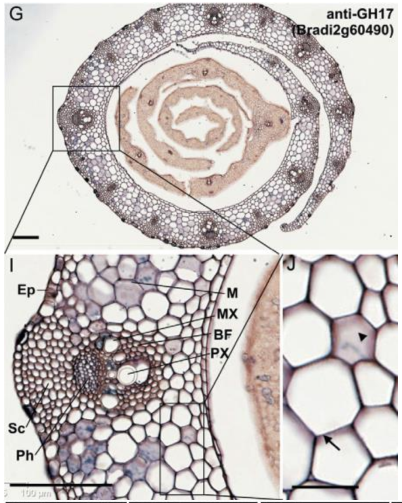


Results (short term)

- Identification of the
  - most abundant CWPs
  - most recalcitrant CWPs

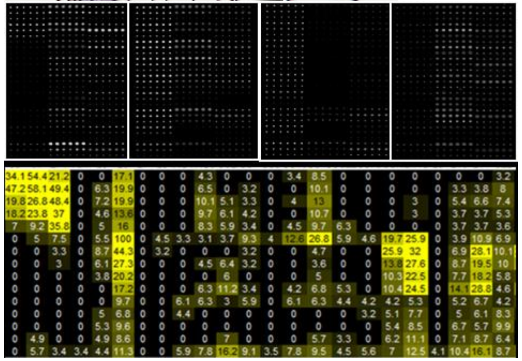
Results (long term)

- Localization of CWPs of interest
  - ➢ Antibody staining



Anti-GH17 screen, *B. distachyon* Douché T. *et al.*, 2013

- High throughput screening of plants
  - ➢ CoMPP



Comprehensive **M**icroarray **P**olymer **P**rofiling
Taken from the Plant Glycobiology Homepage KU

- Functional characterization
  - ➢ genetic modifications of CWPs in model plant (knock down/out; overexpression; *B. distachyon*)

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Thank you for listening!

References

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Final Goal (Brachypodium) Genetic modifications

- Knock down/out of poorly remobilized CWPs
  - Increased NUE (Reduced not remobilized N, N fertilization)
  - Changed CW-properties (increased digestibility)
- Overexpression of highly remobilized CWPs
  - Creation of sink (reduced N leaching, increased nutritional value)