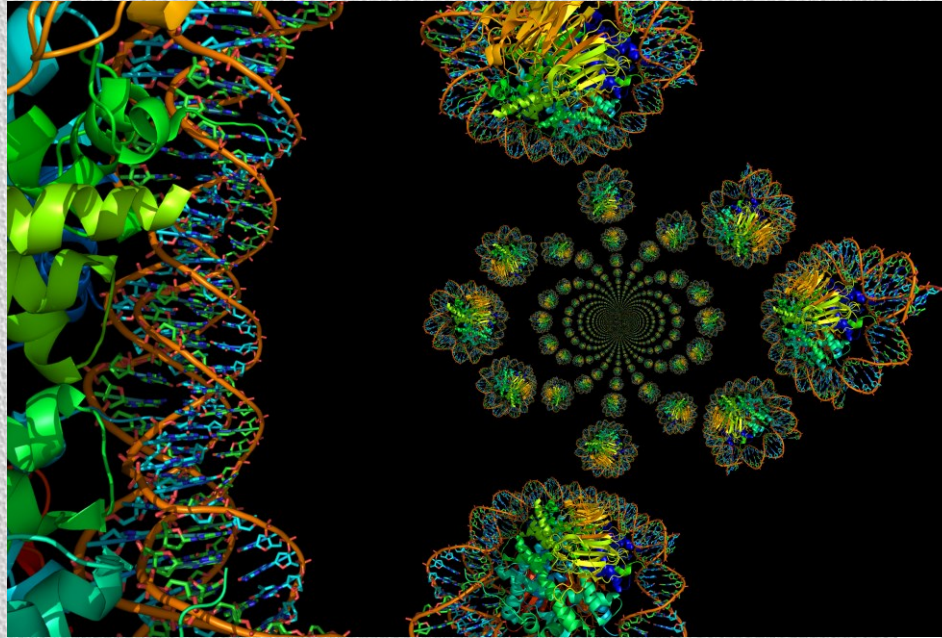


Proteomics in wheat gluten research: where are we standing and where are we going?



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Proteomics

1994 is considered the birth year of proteomics

Is the efficient and high-throughput identification of proteins in tissues and cells

First papers on the proteomics of plants only appeared in 2001



Proteomics

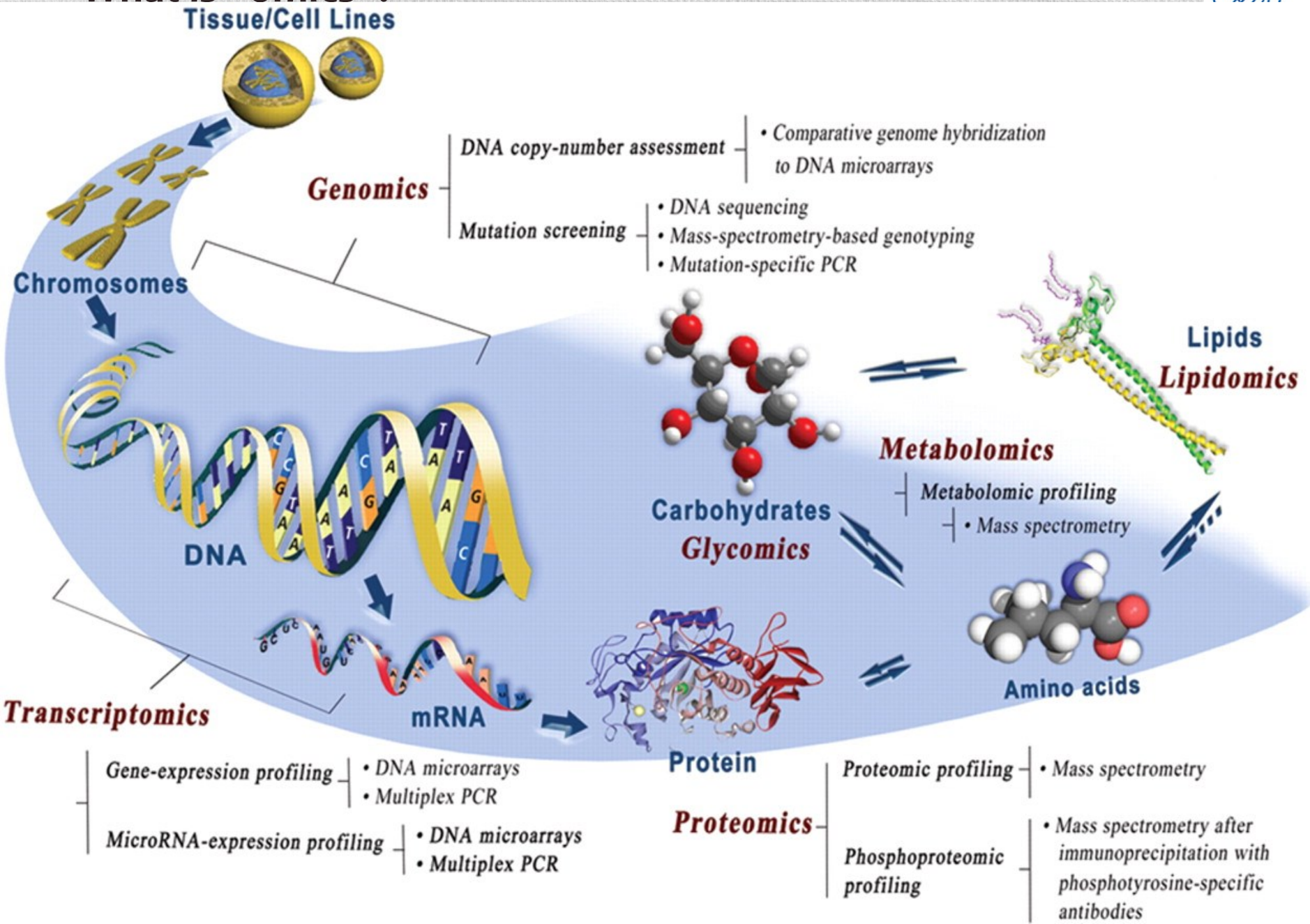
Comes from PROTEins
expressed by a genOME

Bioinformatics and
computational science are
connecting proteomics to
other “omics”

Genomics and proteomics
are leading in discovery of
novel genes



What is “omics”?



Some other research in the wheat proteome: current status





Work in early 2000 reported on use of proteomics for cultivar identification, using particularly some heat shock proteins (Skylas et al., 2001)



Proteomic analysis reported for diseases such as Fusarium head blight, Septoria and leaf, stem and yellow rust



Defense proteins in response to diseases



Proteomic analysis looking at carbohydrate metabolism



Proteomic analysis for abiotic stress in leaf and root material



Abiotic stresses include heat, drought, light, salt, heavy metals and minerals

“OMICS” IN WHEAT GRAIN

GENOME (genes for grain quality)

ENVIRONMENT (climate, nutrients, soil composition)



TRANSCRIPTOME (mRNA of expressed genes)



PROTEOME (polypeptides)



FUNCTIONAL PROTEINS (storage and metabolic proteins)



GRAIN COMPOSITION



PROCESSING (functional properties)



PRODUCTS (consumer acceptance)



Chromatographic methods largely been used for analysis of gluten proteins until recently

Difficulties to separate gluten proteins with these methods, so 2D electrophoresis has become method of choice for gluten protein identification

2DE is followed by mass spectrometry for specific protein identification



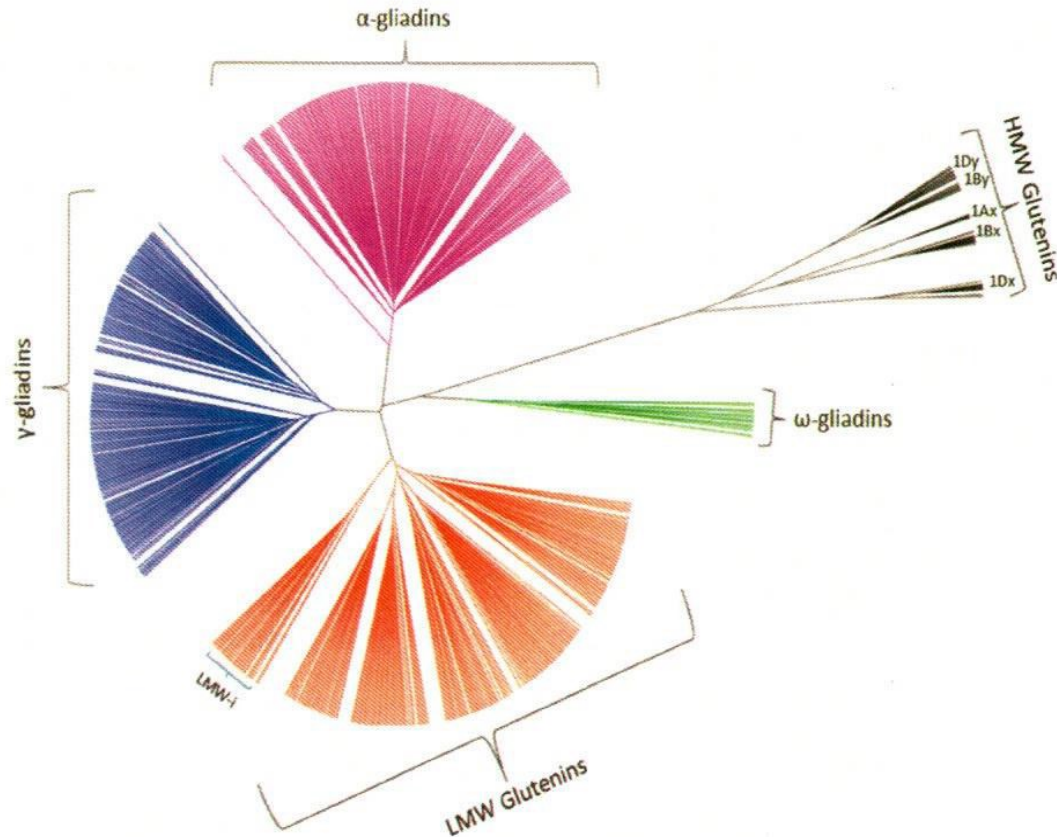
Spots are excised, digested with trypsin or other proteases

Analysis with mass spectrometry

Spectra are then matched to theoretic data generated from a database of protein sequences

- Vensel et al. (2011) optimized MS/MS methods for identification of gluten proteins
- Databases such as Uniprot focusses on curation of plant derived protein sequences from *Arabidopsis thaliana* and *Oryza sativa* and contains 37 000 sequences
- Mascot and Swissprot databases are also used
- Using these databases for wheat can sometimes be challenging, as wheat is a polyploid crop

- Curated custom wheat gluten protein database developed and released in 2017 (Bromilow, 2017. J. Proteomics)
- Called GluPro V 1. 0 (manual database)
- Is based on full length cDNA sequences exclusively from hexaploid wheat, which can also be used for durum wheat
- They have combined all known gluten protein sequences
- 55 HMW-GS, 224 LMW-GS, 185 α gliadins, 154 γ gliadin, 12 ω gliadin
- Consistent with 2DE PAGE profiling



Phylogenetic tree for the complete curated wheat gluten database GluPro. The tree was created using the BLOSUM 62 algorithm in Jalview and then manipulated in FigTree. The three prolamin groups α -, γ - and ω -gliadins and the HMW and LMW glutenins are coloured pink, blue, green, grey and orange respectively. Where further sub-classifications cluster distinctly on the tree these are labelled, this is observed for the LMW-i and LMW-s subgroups of the LMW glutenins and the Ax, Bx, By, Dx and Dy subgroups of the HMW glutenins.



kDa

200.0

116.3

97.4

66.3

55.4

36.5

31.0

21.5

14.4

6.0

4.5

5.0

5.5

5.9

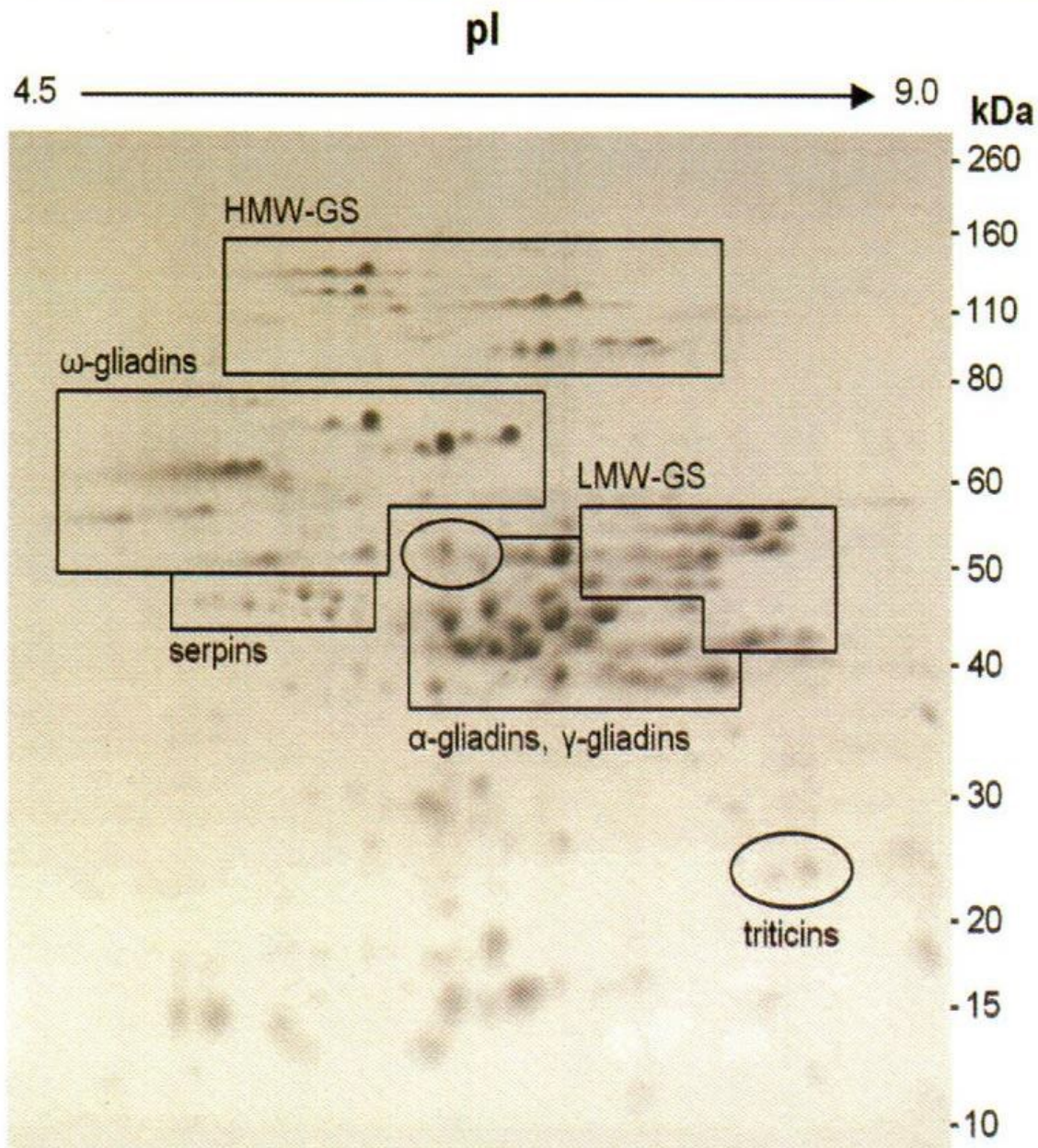
6.6

7.0

8.5

pI

Key to protein spot numbers in 2DE of a protein extract from white flour. LMW-GS are labelled in blue, alpha-gliadins in red and gamma-gliadins in green. All other proteins are labelled in black. The spots are identified in Tables 3, 4, 5, 6, 7, 8, 9, 10.



2-DE pattern of total protein extract obtained from sonication in 0.5% SDS. The major groups of gluten proteins are indicated. Molecular weight is shown on the vertical axis and isoelectric point is indicated by the horizontal axis.

- Although gel systems are still most frequently used, newer non-gel systems are also now used
- QTOF-LTQ reported by Bromilow et al. (2017)
- Can sequence longer peptide sequences
- Able to identify more gluten specific peptides than reported previously using only MS methods

Proteomics has in the last years been successfully used to:

- **Catalogue major proteins in wheat grains**
- **Determine changes in the grain proteome during germination and grain development**
- **Determine responses of the proteome to biotic and abiotic stresses**



Protein
cataloguing

Many
papers
reported on
presence
and types of
proteins

HMW-GS,
LMW-GS, α ,
 ω and γ
gliadins

Various
methods
used for
extraction
and analysis

Developmental
studies

Accumulation
profiles of
proteins
throughout
development in
endosperm

Provides
insights into
molecular
processes
taking place
during grain
development

Establishes
detailed picture
of flour protein
composition
critical for
understanding
flour quality

Major abiotic stresses

Drought

Heat

Combined
heat and
drought

Fertilizer



These studies have varied using whole grain, endosperm and white flour

Different protein extraction methods and different methods of protein identification were reported

Studies reported changes in α , β , ω and γ gliadins and LMW and HMW-GS



Changes in relative amounts of protein spots

Up and down regulation of proteins due to stress conditions

Some authors extracted only a specific fraction for analysis, such as gliadins, albumins/globulins, HMW or LMW-GS

Case studies:

- Hurkman et al. (2013) compared the effects of high temperature and fertilizer singly and in combination on the entire complement of gliadins and glutenins in flour from mature grain
- 122 gluten protein spots representing 19 gluten protein gene sequences responded significantly to fertilizer: five HMW-GS, six α -gliadins, and four ω -gliadins increased and three LMW-GS and one γ -gliadin decreased
- Zörb et al. (2010) reported that high N supply with low or high sulphur supply changed 41 and 66 of gliadin and glutenin proteins

Case study:

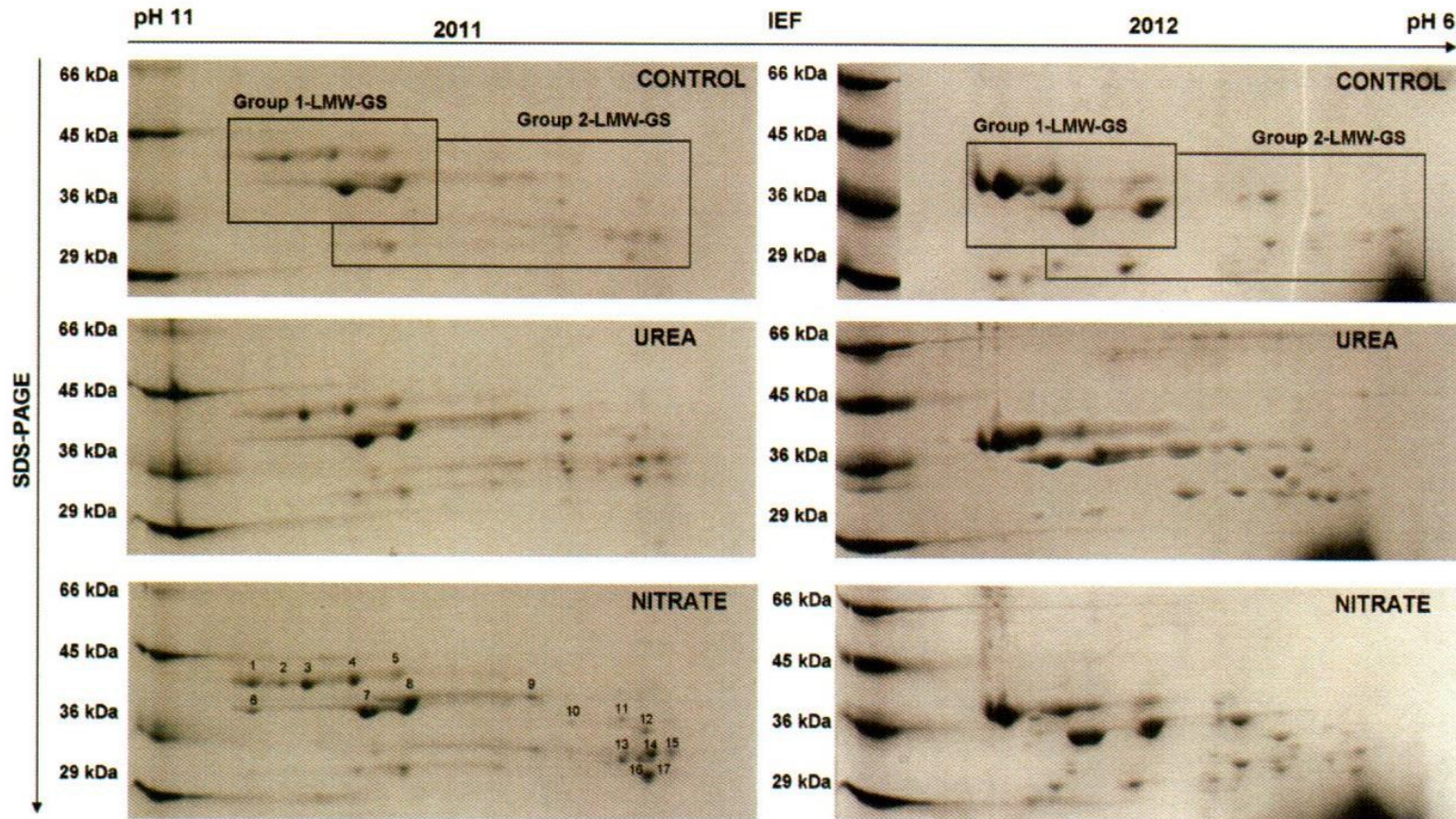


Fig 8. 2D-GE pattern of the LMW-GS from durum wheat (cv Achille) in 2011 and 2012. Comparison of 2D-GE maps of the LMW-GS fractions extracted from mature grains of durum wheat fertilized with urea (UREA) and calcium nitrate (NITRATE) at the rate of 150 kg N ha⁻¹ plus unfertilized CONTROL in 2011 and 2012. The marked spots were analysed by LC/MS for protein identification.

doi:10.1371/journal.pone.0156007.g008

Visioli et al., 2016 (PLOS One)

Wheat allergies

- Coeliac disease is the most common inflammatory condition in developed countries and affects about 1% of the population
- Is an immune-mediated enteropathy due to abnormal immune response to gluten in genetically susceptible people carrying HLA-DQ2 or DQ8 haplotype (Martinez-Estosa et al., 2016)
- 98.6% of coeliac toxic proteins have a minimum of 9 amino acids, which are likely to elicit toxic response

- α gliadins have consistently high content of coeliac toxic motifs
- γ and ω gliadins also have toxic motifs
- Proteomics can be used to target breeding for presence of low numbers of toxic motifs
- Proteomics to be used to test gluten free products for absence of toxic motifs (Bromilow et al., 2017)

Other

- Gobaa et al. (2008) showed 1BL.1RS translocation to cause large changes in endosperm proteome
- Fernando et al. (2015) reported decreases in HMW-GS spots with subsequent reduction in loaf volume under elevated CO₂ conditions
- Studies on pre-harvest sprouting in wheat (Kamal et al., 2009) showed an effect on some glutenin and gliadin spots, among other proteins

The future

- Proteomics is still a relatively new science, which is developing rapidly
- It has the potential to contribute significantly to marker-assisted selection in breeding
- In terms of gluten proteins it can be used to select for good flour quality and against allergen sequences
- Technology is improving consistently
- Databases are developing and are being expanded
- It will further contribute to studies on effects of biotic and abiotic stresses in the wheat proteome

THANKS FOR YOUR ATTENTION!

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