40 years of research on molecular biology
on plant genome
3-6th April Saint Petersburg

INRA-VIR Collaboration and modern state wheat and barley grain proteins investigation at INRA

Gérard BRANLARD

branlard@clermont.inra.fr

INRA

Clermont Ferrand

FRANCE

Genetic determination of protein quality in wheat grain

Genetic aspects of wheat storage proteins

Wheat storage proteins and quality

Quantitative variations of wheat storage proteins

Proteomics of wheat kernel

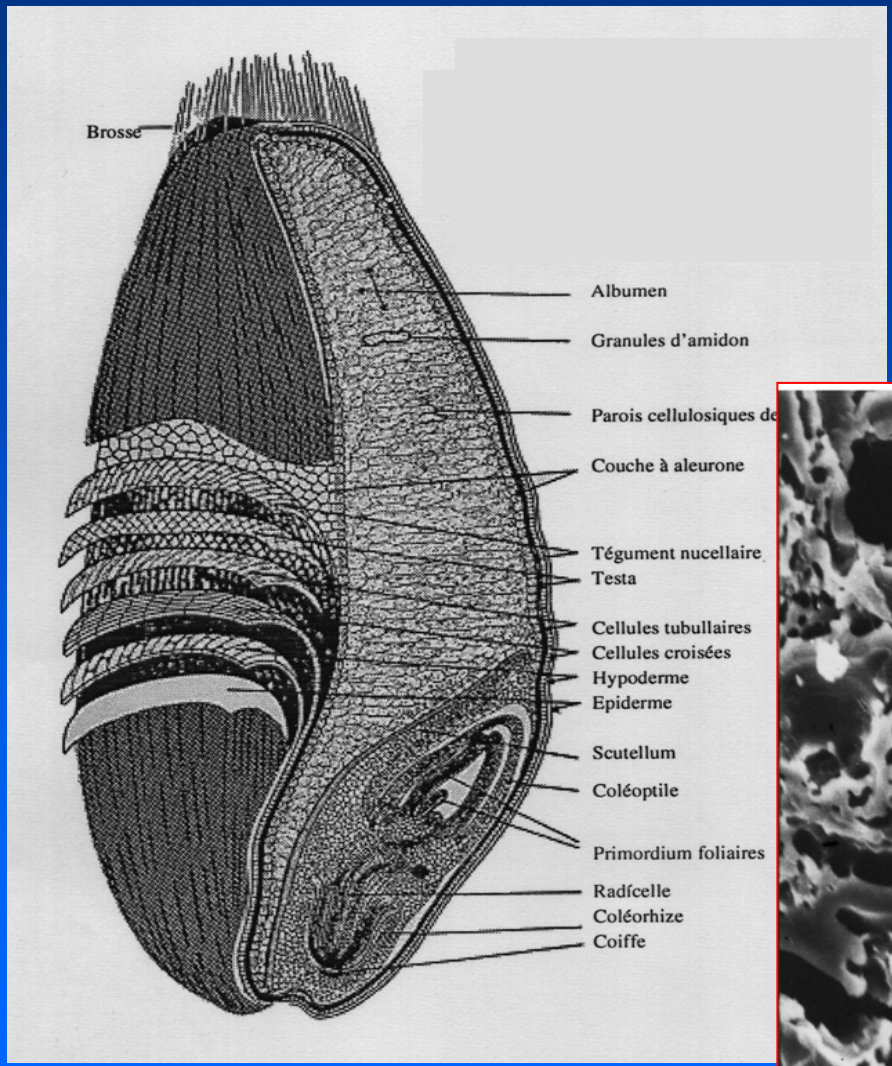
Proteomics on developmental kernel

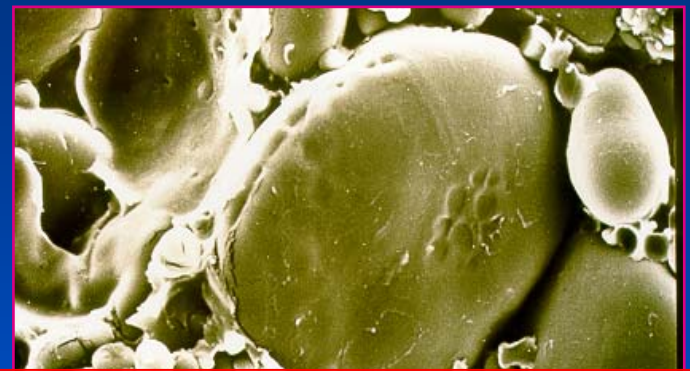
Endosperm responses to Heat stress

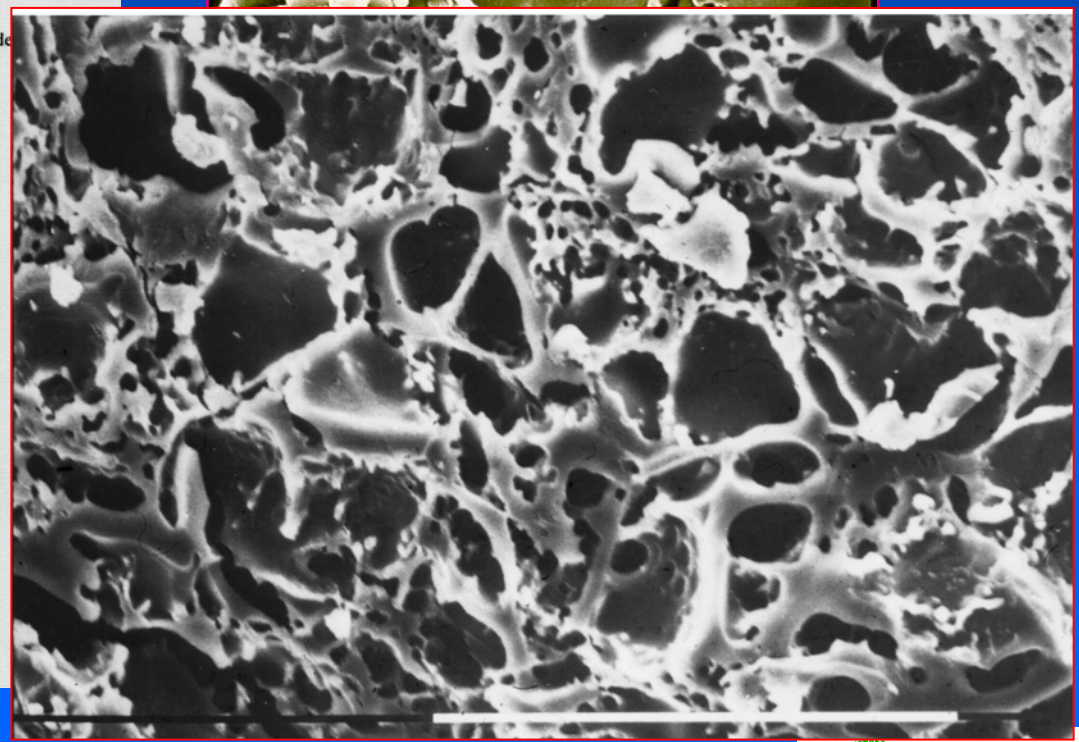
Some other proteins involved in quality

The Plate-forme for genotyping at INRA Clermont Ferrand

Wheat endosperm and Protein matrix







Wheat Endosperm Proteins

Protein type	Solubility	% flour protein	Characteristics
Albumins	Water	10	Enzymes and cell structure
Globulins	NaCl 0.5M	10	MWs 5 – 100 kDa
Gliadins	Alcohol	35-45	Monomeric MWs 30 – 80 kDa
Glutenins	SDS		Polymeric
HMW-GS	+ R. A.	10-15	MWs 75 -120 kDa
LMW-GS		20-30	MWs 25 - 45 kDa

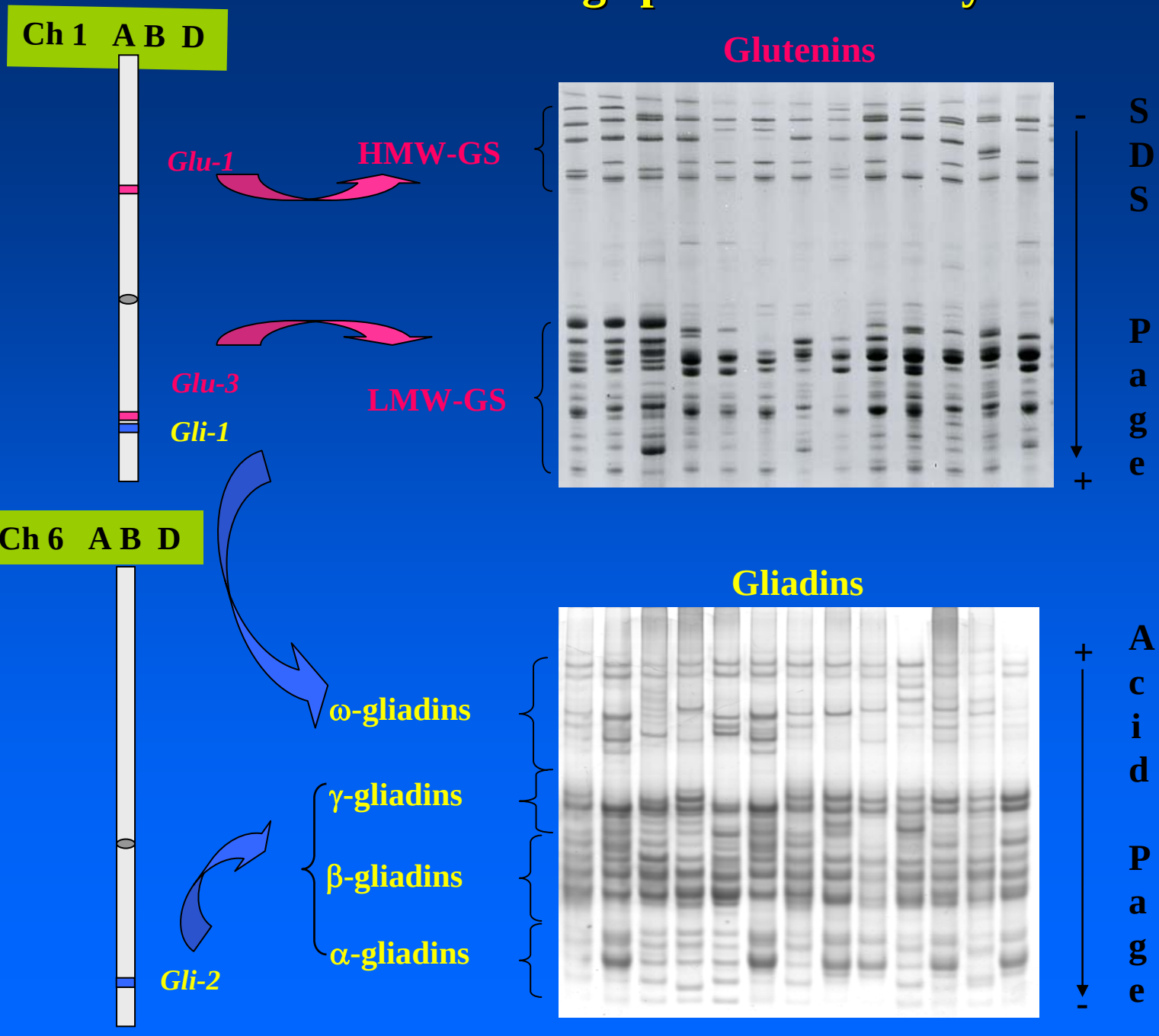
Soluble proteins

Storage proteins

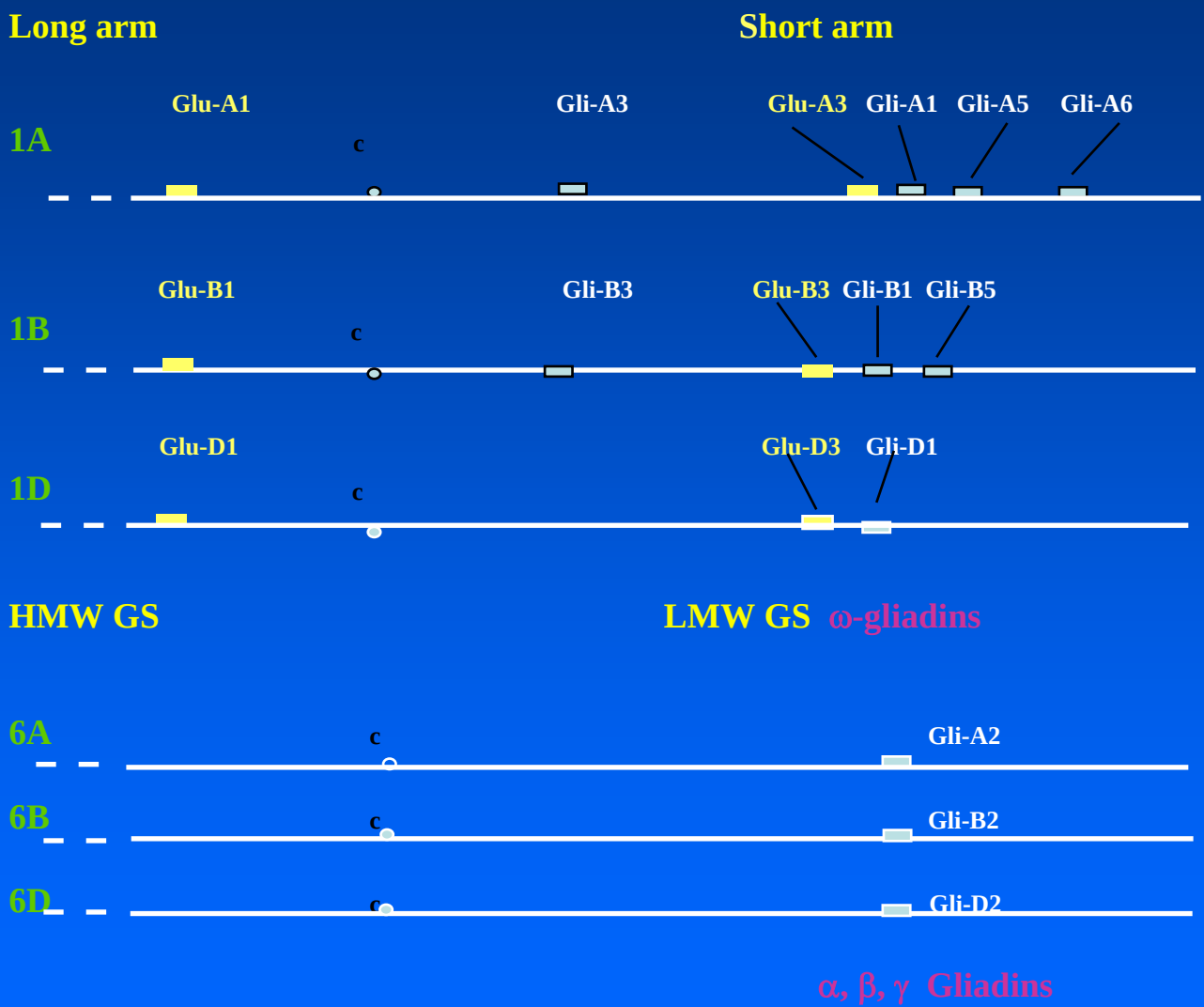
Genetic determination of protein quality in wheat grain

Genetic aspects of wheat storage proteins

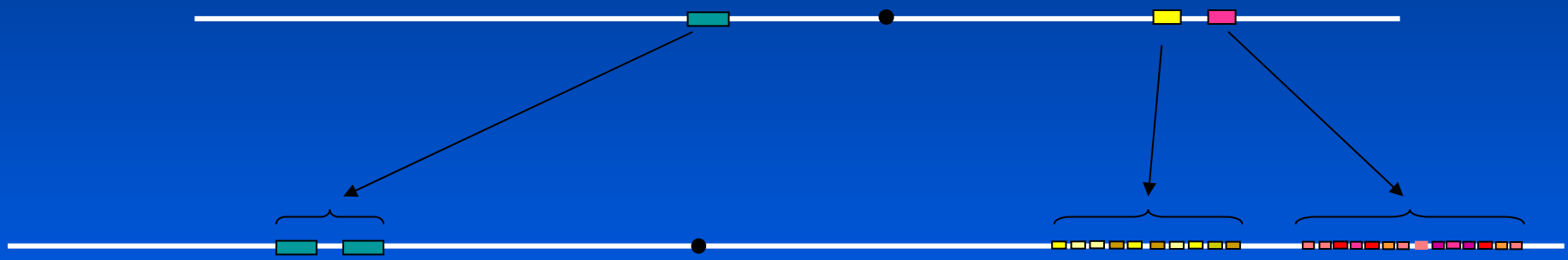
Wheat storage protein diversity



Main locus involved in synthesis of wheat endosperm storage proteins



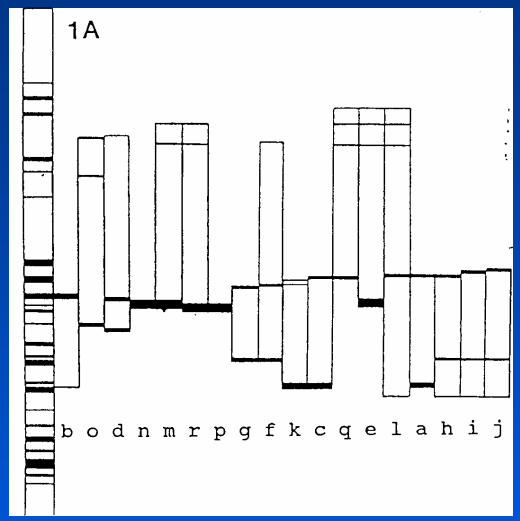
WSPs are encoded by clusters of genes

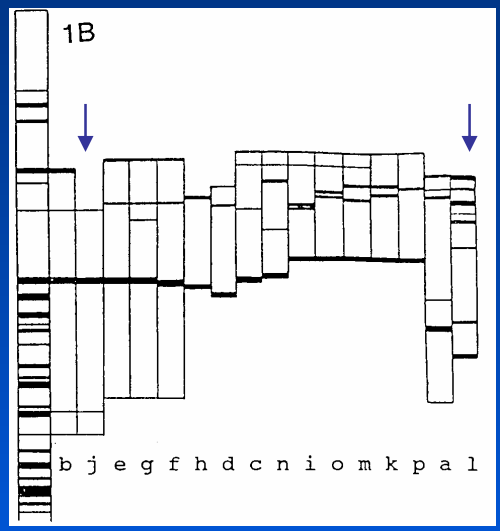


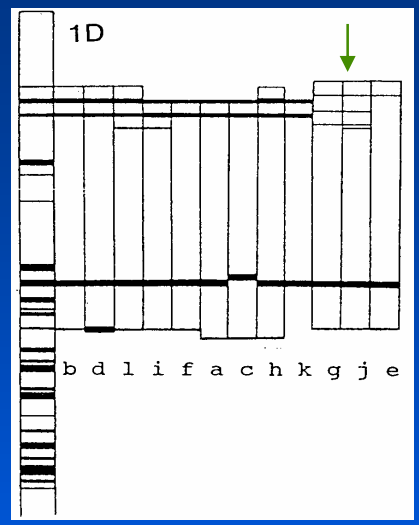
Glu 1x Glu 1y Glu 3 Gli 1

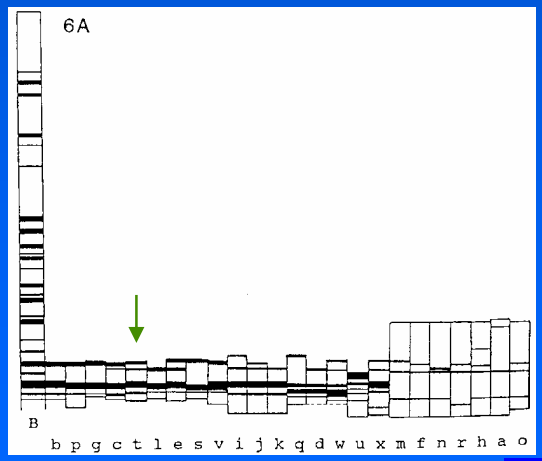
HMW-GS LMW-GS Gliadins

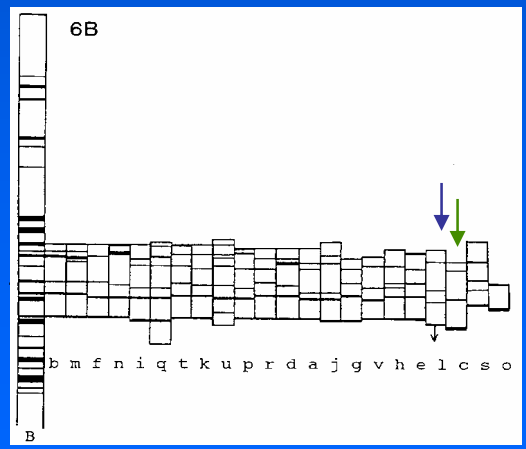
Allelic Diversity of Gliadins

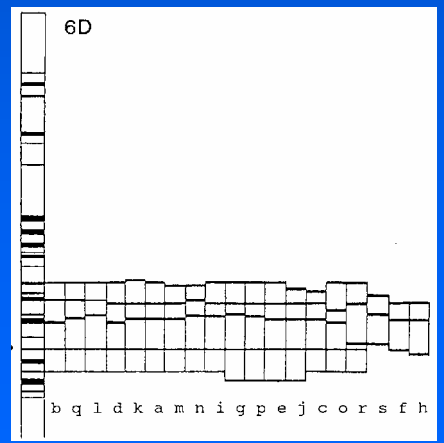






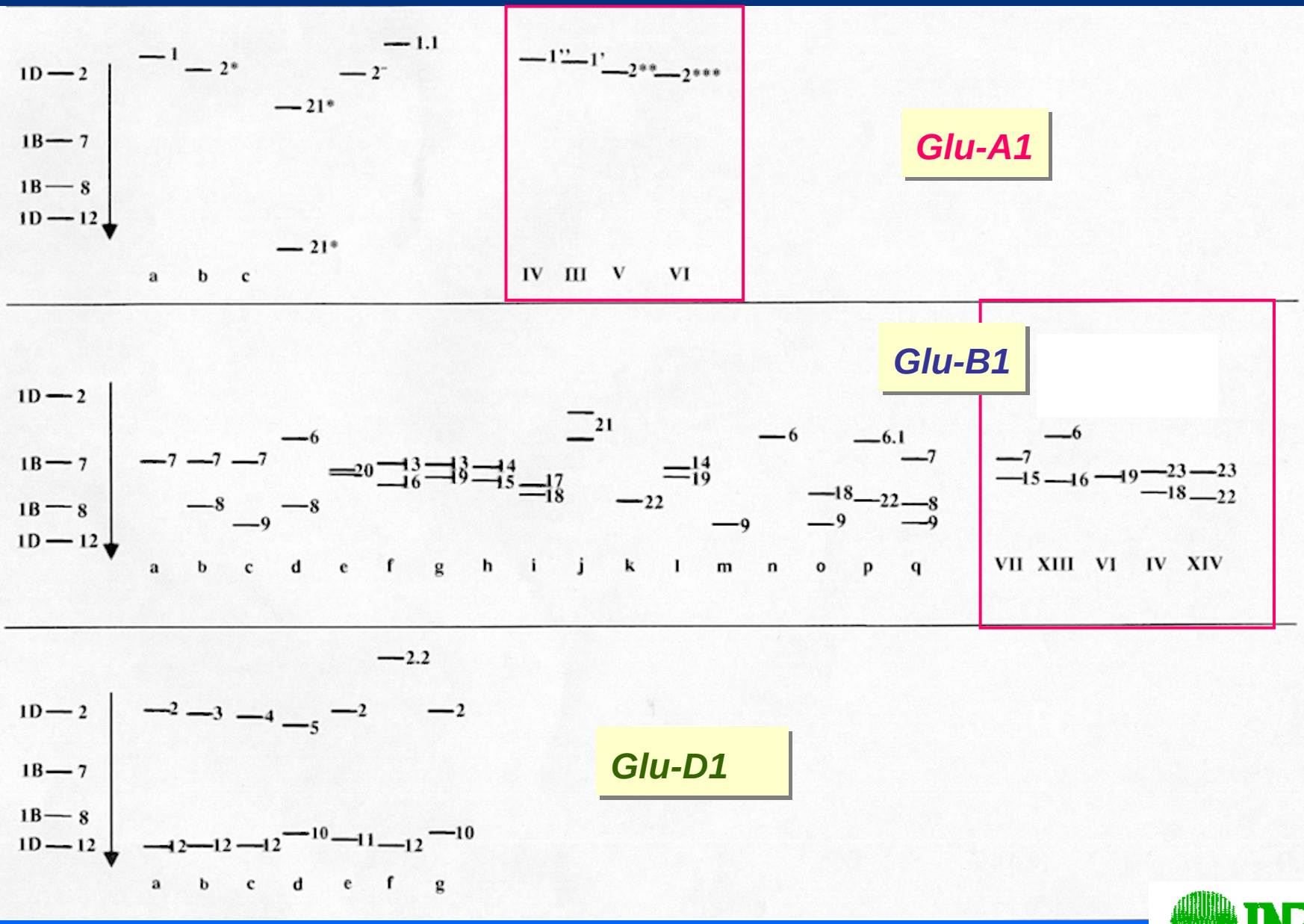




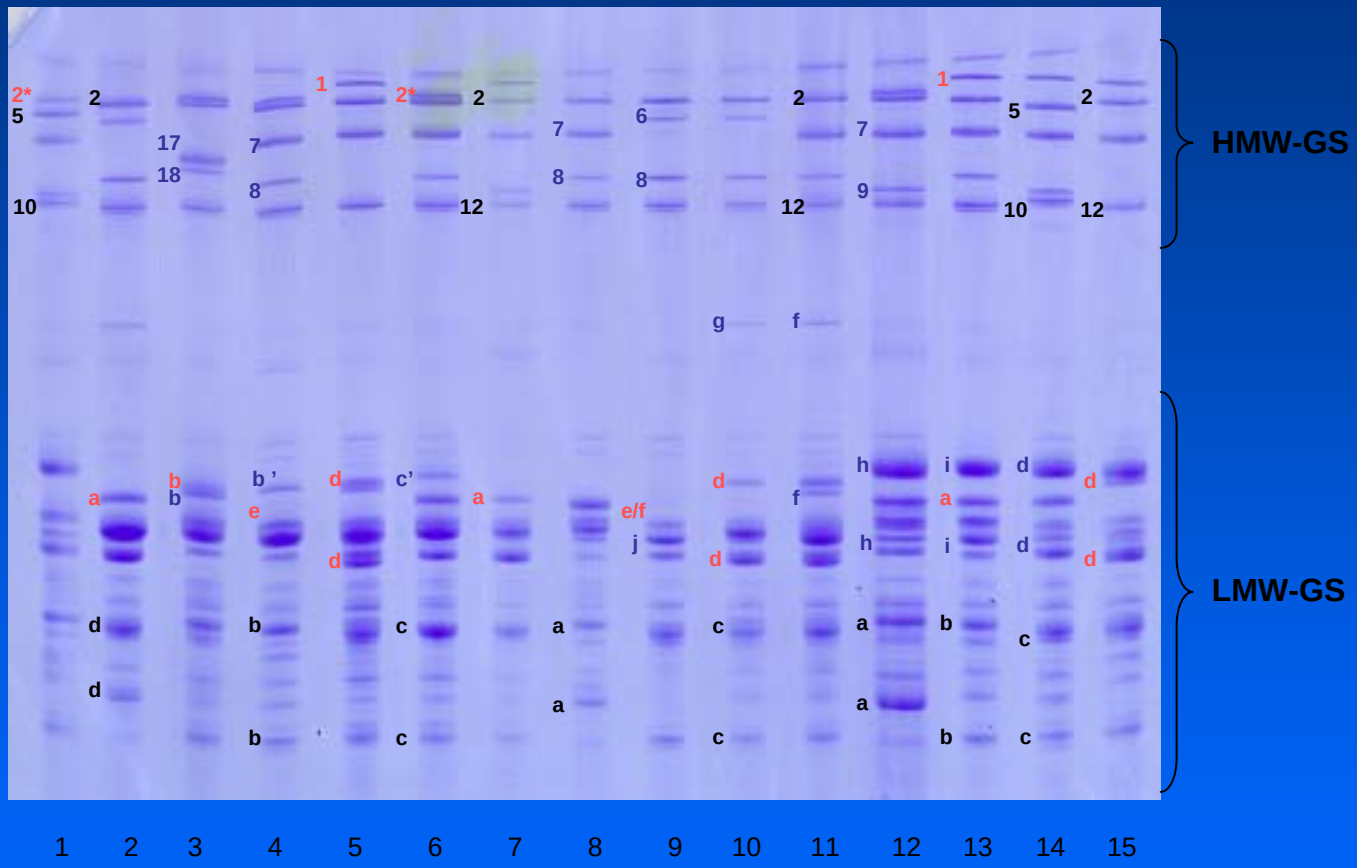


From Metakovsky 1994, J Genet.& Breeding

Main alleles encoding HMW-GS

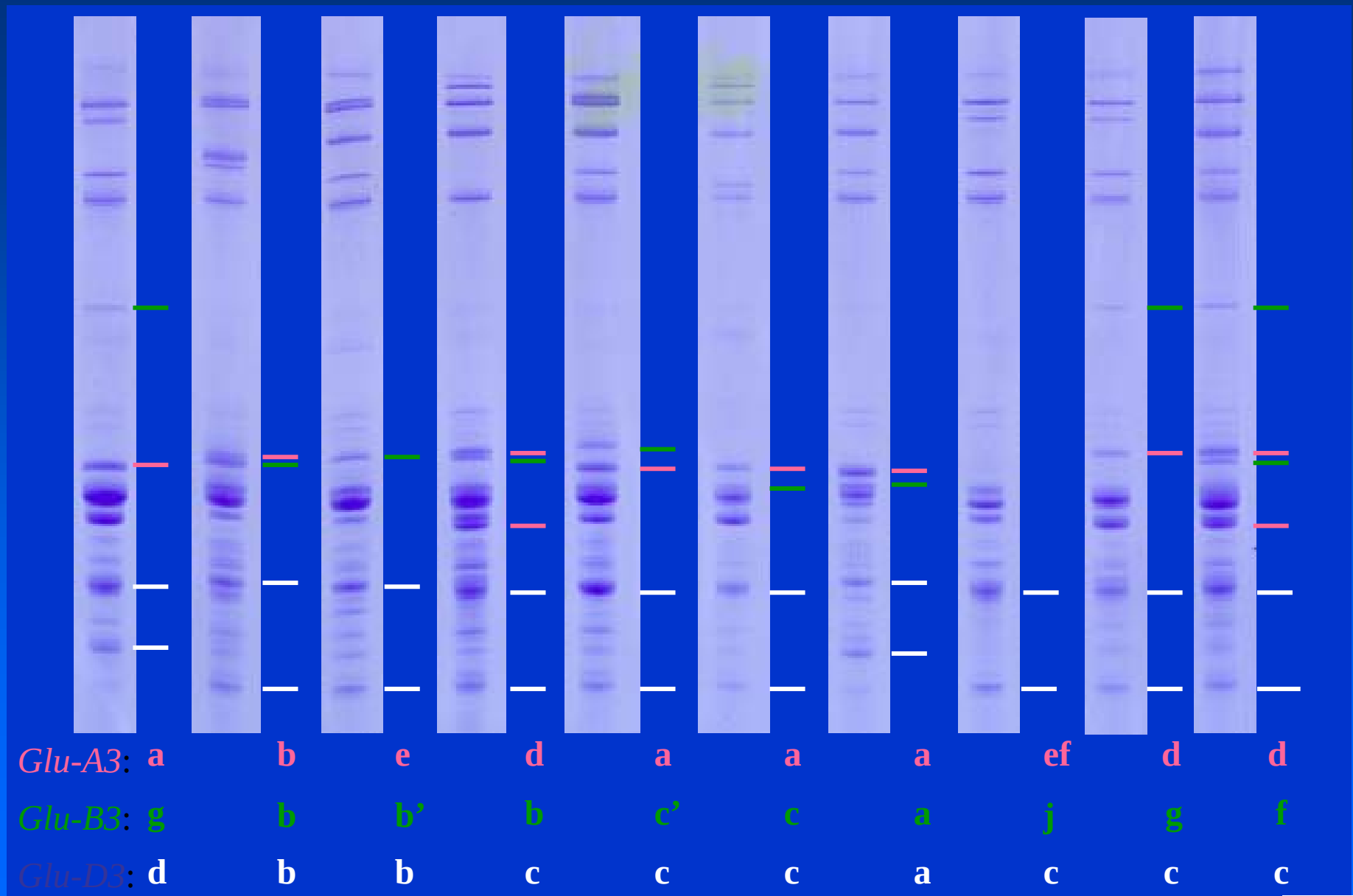


Some alleles of HMW-GS and LMW-GS



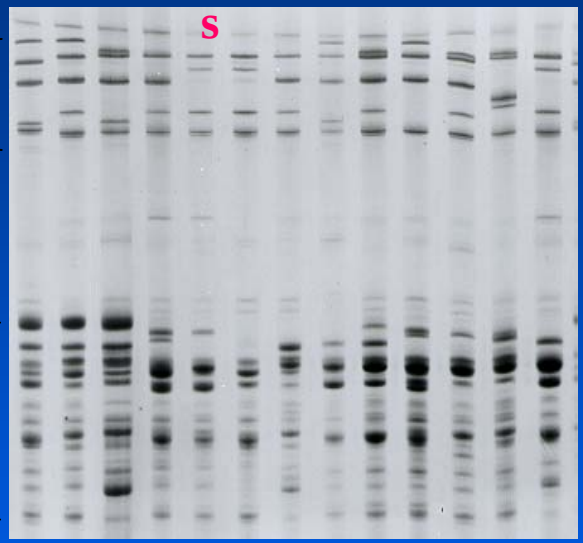
From: Branlard et al. *Genetic Resources and Crop Evolution* 2003, 50, 669-679

Identification of *Glu-A3*, *Glu-B3* and *Glu-D3* alleles



How these Wheat Storage Proteins are assembled ?

Glutenins



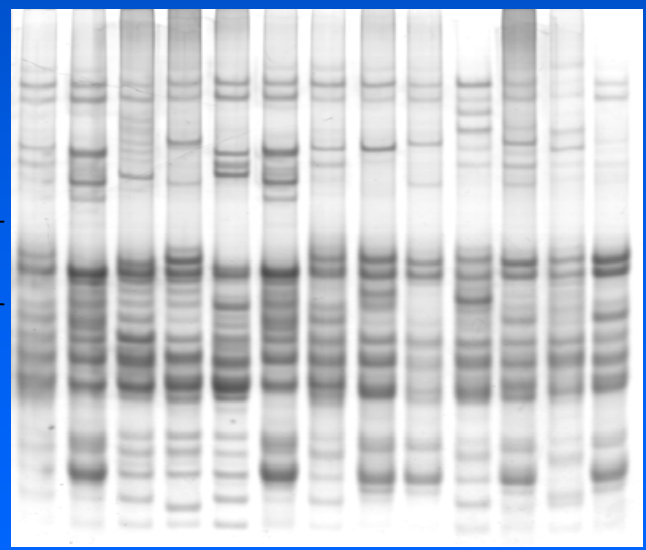
HMW-GS

LMW-GS

SDS Page

\- +

Gliadins



ω-gliadins

γ-gliadins

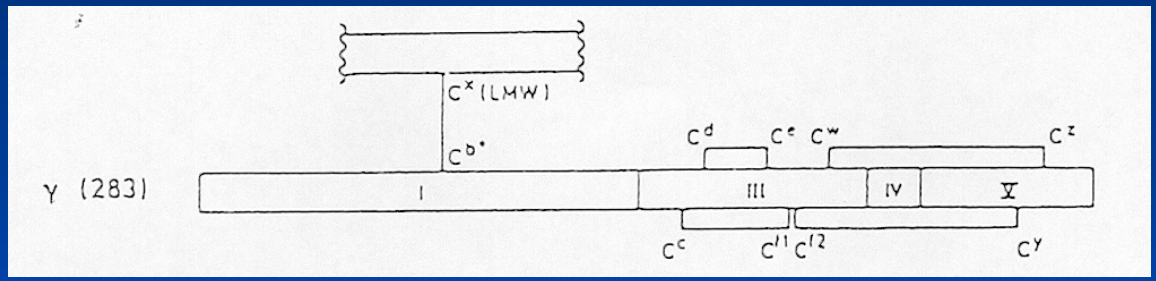
β-gliadins

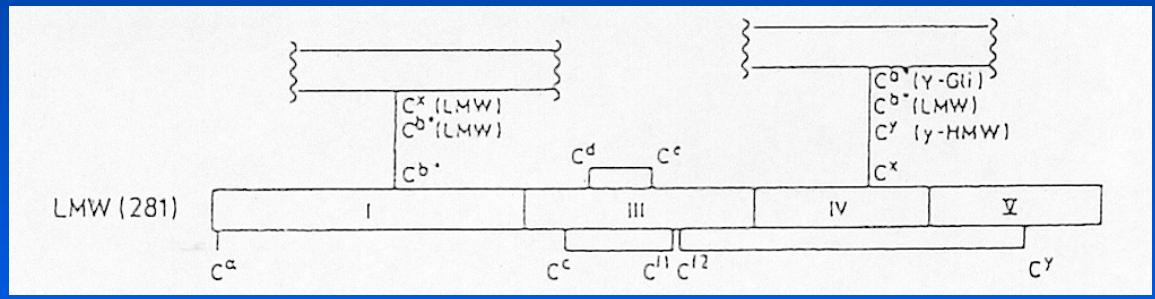
α-gliadins

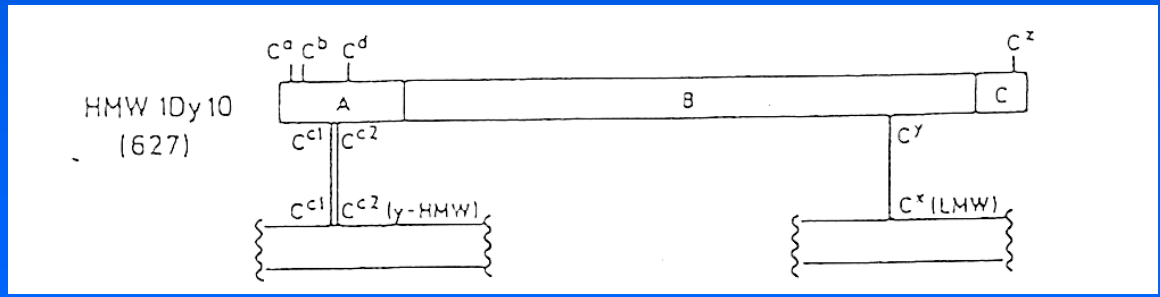
\+ -

Acid Page

Covalent SS links between wheat storage proteins



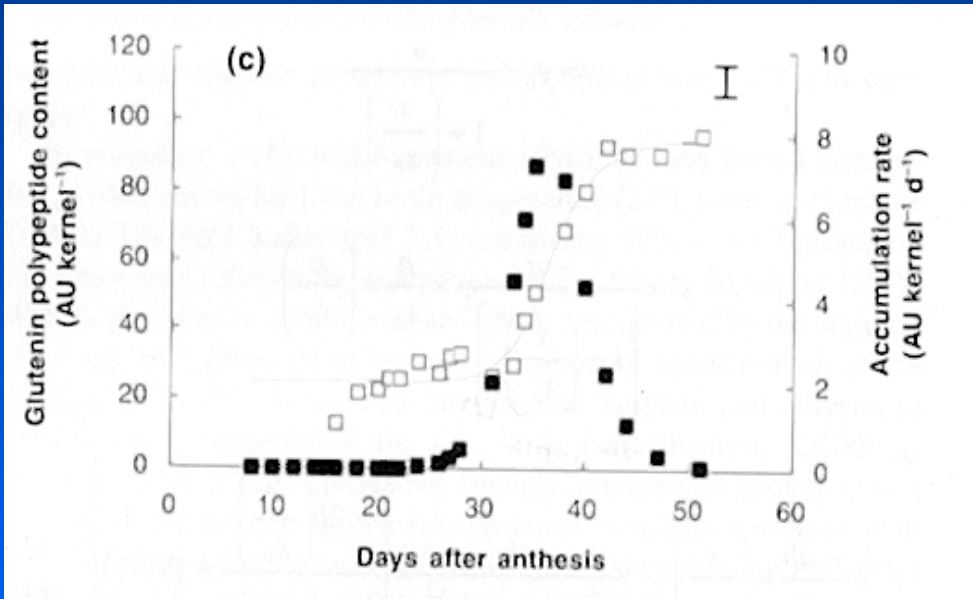




From Köhler P. Belitz H. Wieser H 1993 Z. Lebensm. Unters. Forsch 196,239-247

INRA

SDS insoluble glutenin polymer formation in developing grains of wheat, (cv: Soissons)



From Carceller JL, Aussenac T., Aust. J. Plant Physiol 2001, 28; 193-201

reproduced with permission

Genetic determination of protein quality in wheat grain

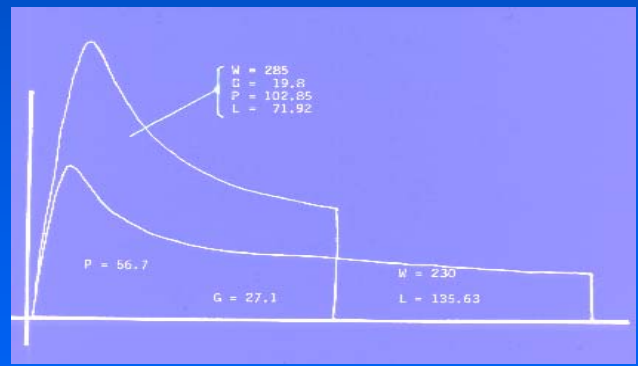
Genetic aspects of wheat storage proteins

Wheat storage proteins and quality

Wheat gluten proteins as part of the bread making quality

Comparison of alleles effects for phenotypic values of dough strength

Locus	Strength
GluA1	2* = 1 > null
GluB1	17-18 ≥ 13-16 ≥ 7-9 = 7-8 ≥ 7 = 6-8
GluD1	5-10 ≥ 3-12 = 2-12 ≥ 4-12
GluA3	a = d = f ≥ e
GluB3	b' ≥ d = c = c' = b = g > i > f ≥ j
GluD3	a ≥ b = d = c
GliA2	t ≥ k = r = f = g = j ≥ l = b = p
GliB2	m >b ≥ r ≥ h = o = g ≥ ae = l = ac
GliD2	m = e ≥ a = h = v = g = n



From: Branlard G.,Dardevet M., Saccomano R., Lagoutte F., Gourdon J. Euphytica, 2001, 119, 59-67

Comparison of alleles effects for phenotypic values of dough extensibility

Locus	Extensibility
GluA1	nsd
GluB1	13-16 ≥ 7-8 = 7-9 = 17-18 ≥ 7 ≥ 6-8
GluD1	nsd
GluA3	d = a = f ≥ e
GluB3	i ≥ b' ≥ c = c' = g > b = f = d > j
GluD3	nsd
GliA2	b = t ≥ k = g = l ≥ p = r = f = j
GliB2	ae ≥ m ≥ g = o = h = ac ≥ b = r = l
GliD2	nsd

(nsd: not significantly different)

From: Branlard G.,Dardevet M., Saccomano R., Lagoutte F., Gourdon J. Euphytica, 2001, 119, 59-67

Comparison of loci effects on quality parameters

HMW Glutenins

GluB1 ≥ GluD1 > GluA1

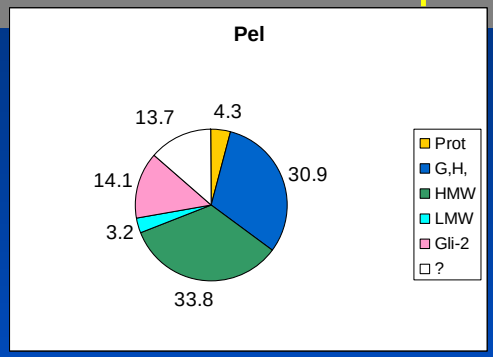
LMW Glutenins

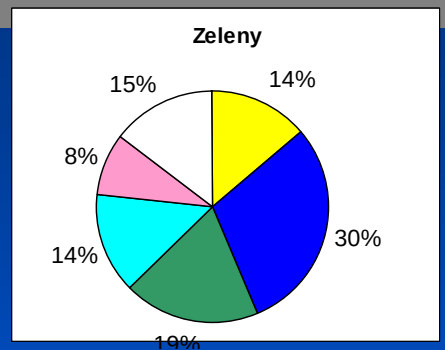
GluB3 > GluA3 > GluD3

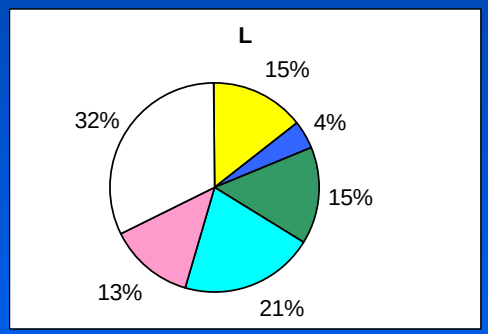
α, β, γ-gliadins

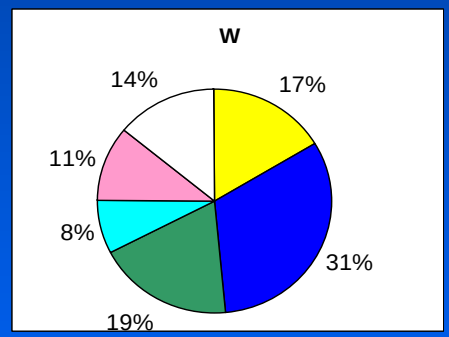
GliA2 > GliB2 > GliD2

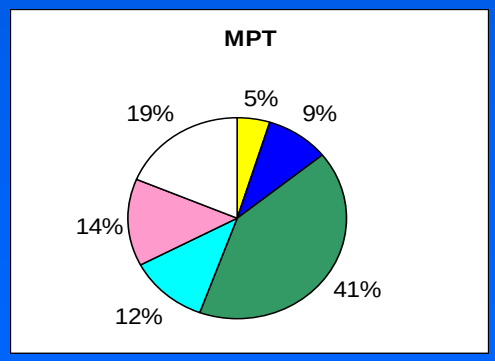
Part (R^2) of Protein content, G. Hardness, HMW, LMW GS and α, β, γ-gliadins in the genotypic variations of six bread wheat quality parameters

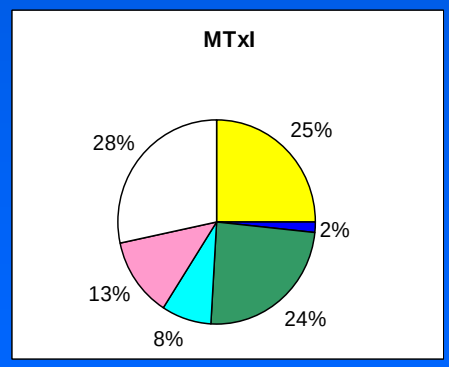












From: Branlard G.,Dardevet M., Saccomano R., Lagoutte F., Gourdon J.
Euphytica, 2001, 119, 59-67

Genetic determination of protein quality in wheat grain

Genetic aspects of wheat storage proteins

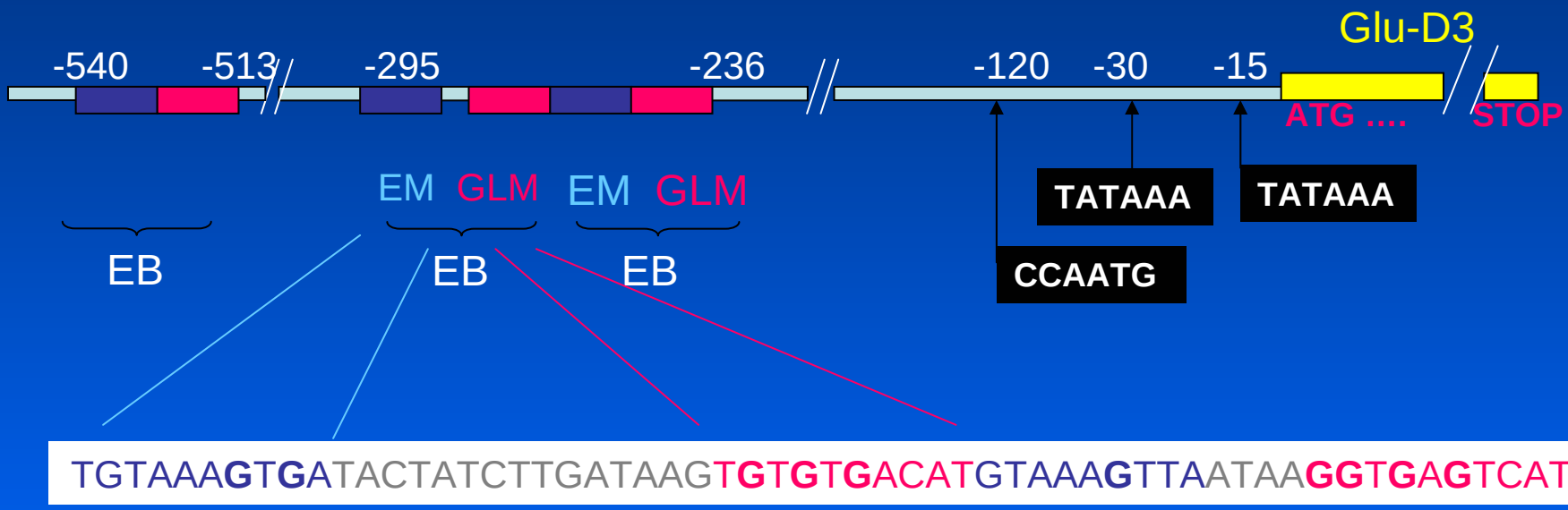
Wheat storage proteins and quality

Quantitative variations of wheat storage proteins

INRA

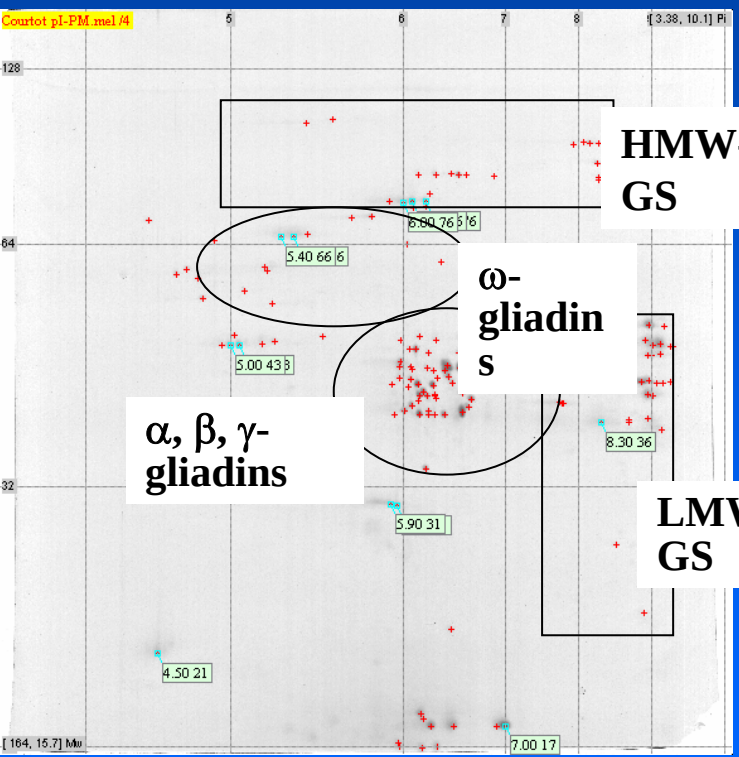
Regulation of the expression

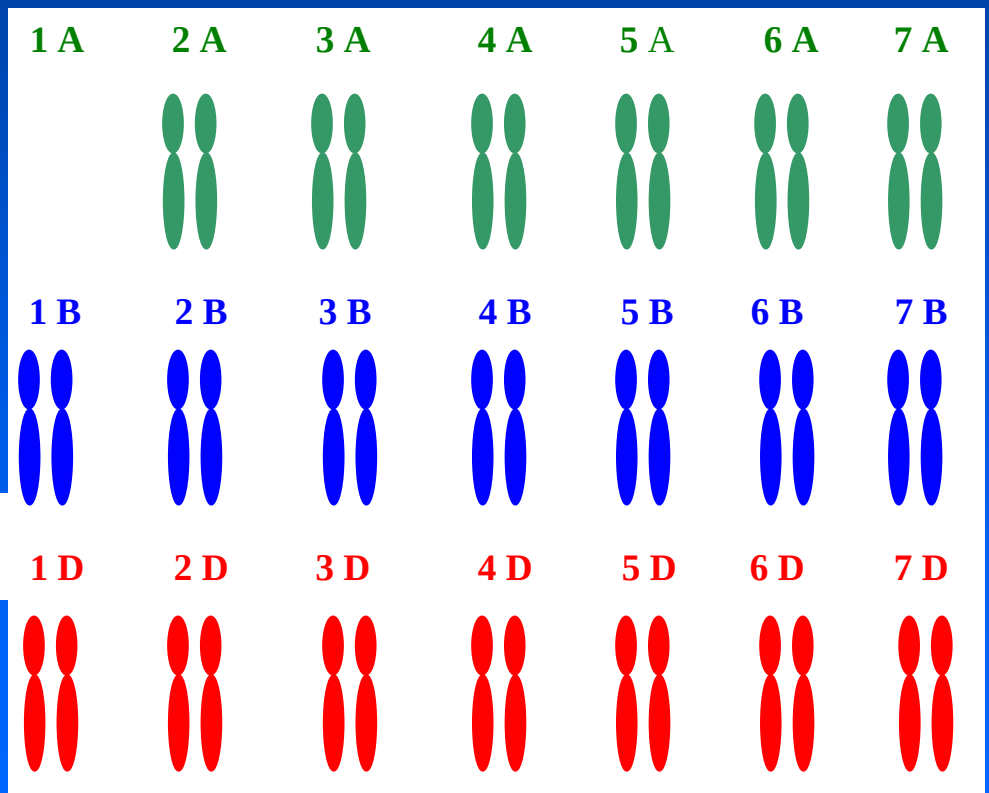
Promoter region of the LMW-GS Glu-3D



Regulation of the quantitative expression of the different loci

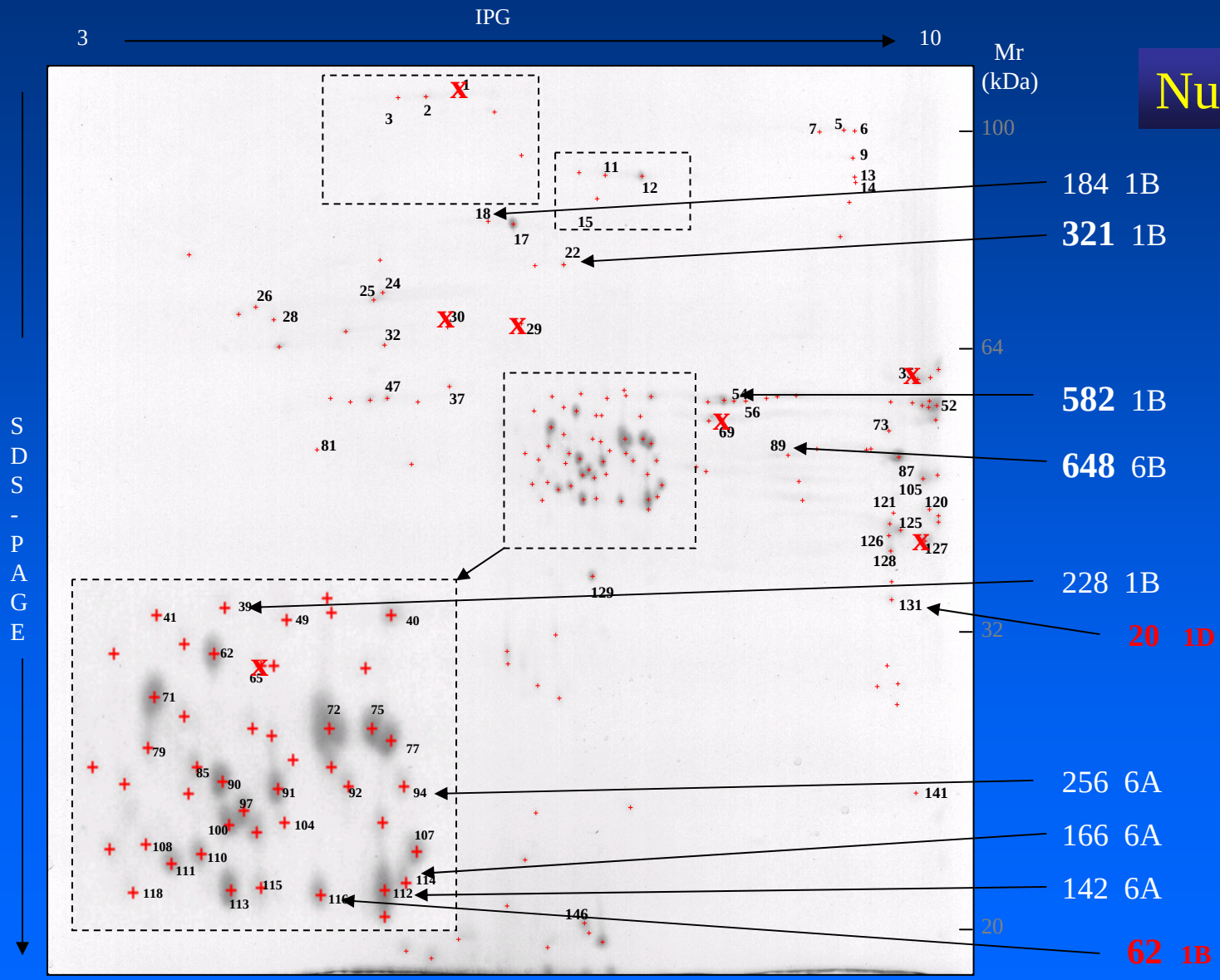
interactions between chromosomes (homologous and homeologous)





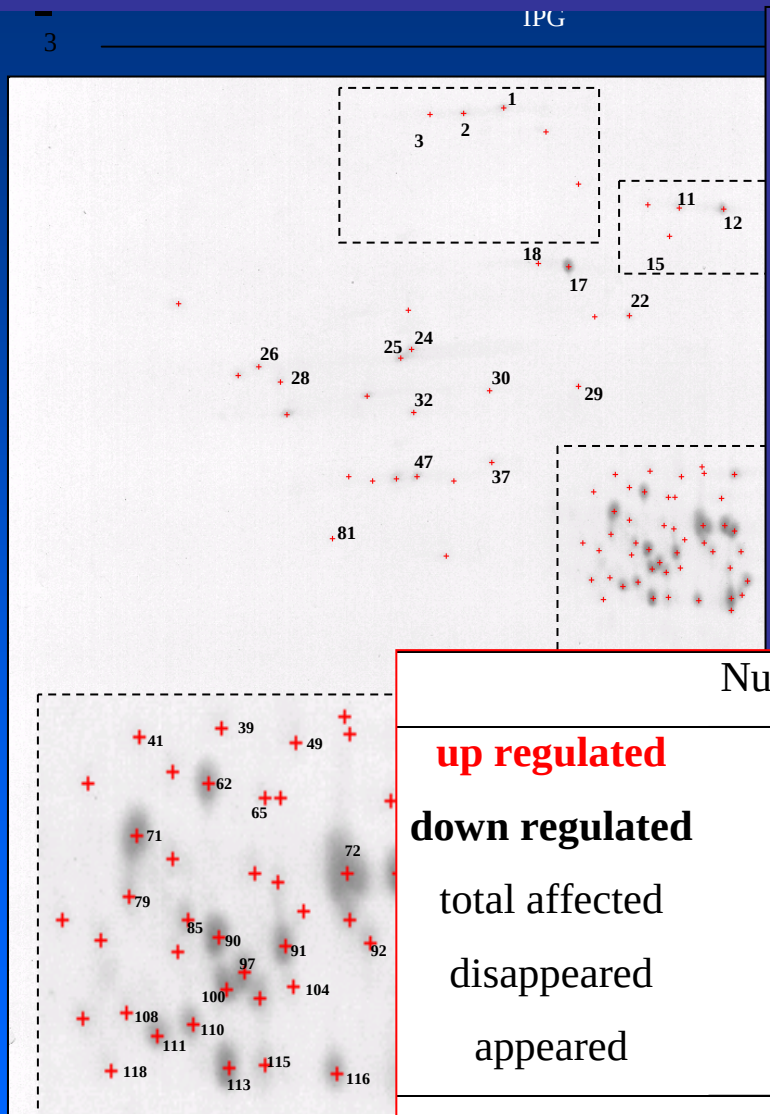
Regulation of the quantitative expression of the different loci

interactions between homeologous chromosomes. cv Courtot

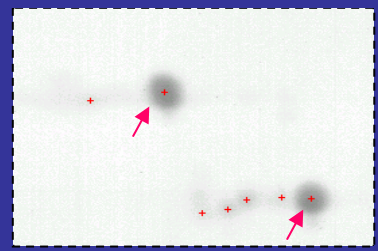


Regulation of the quantitative expression of the different loci

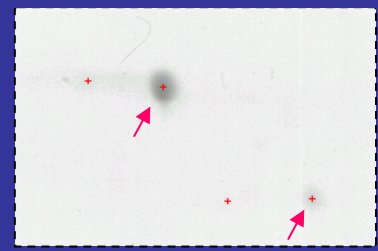
interactions between homolgous chr and homeologous chr.



Quantitiative differences

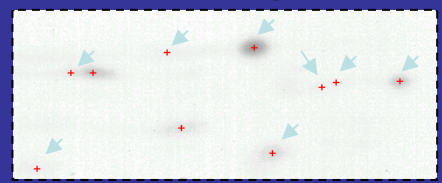


Courtot Nulli1D



Courtot Normal

Qualitative differences



	Null-1A	Null-1B	Null-1D	M-1A	M-1B	M-1D
up regulated	**8**	**15**	**14**	**17**	**16**	**26**
down regulated	**2**	**10**	**5**	**17**	**12**	**19**
total affected	10	25	19	**34**	**28**	**45**
disappeared	13	32	24	0	0	0
appeared	0	0	13	0	0	0

Dumur J, Jahier J, Bancel E, Laurière M, Bernard M, Branlard G 2004, Proteomics, 4, 2685-2695

INRA

Genetic determination of protein quality in wheat grain

Genetic aspects of wheat storage proteins

Wheat storage proteins and quality

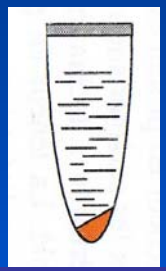
Quantitative variations of wheat storage proteins

Proteomics of wheat kernel

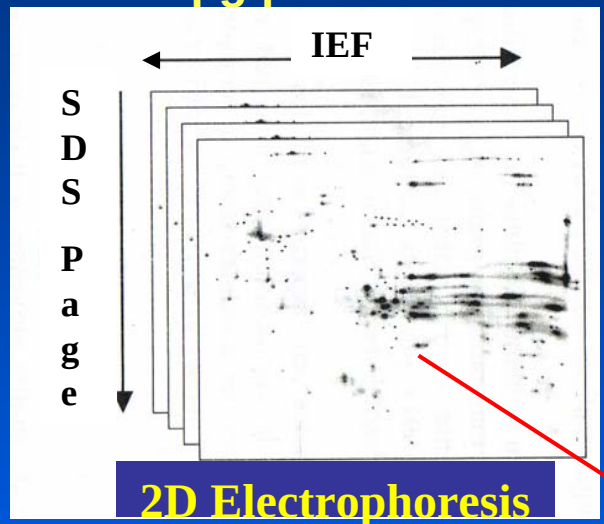
INRA

Methods: The main steps of proteomic

100-300 µg proteins loaded



Protein extraction



2D Electrophoresis

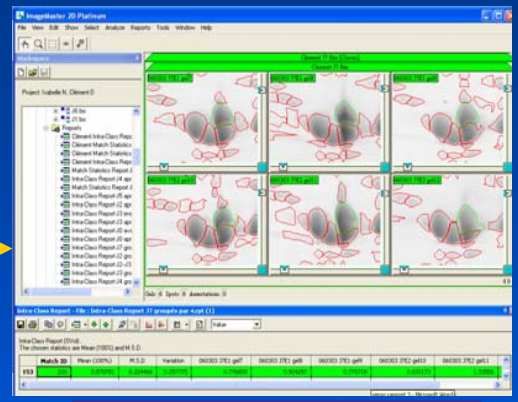
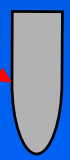
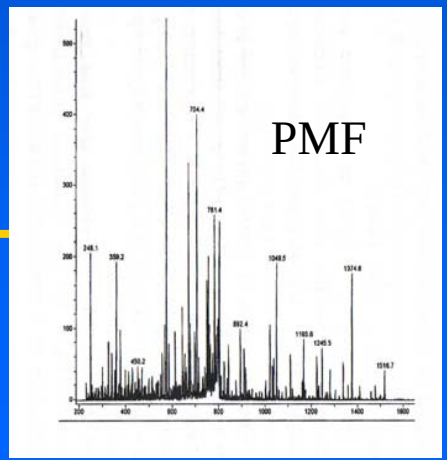


Image Analysis
Melanie 3/4
2D Platinum v 6.0



Trypsic Dig.

MALDI-TOF
ESI MS/MS



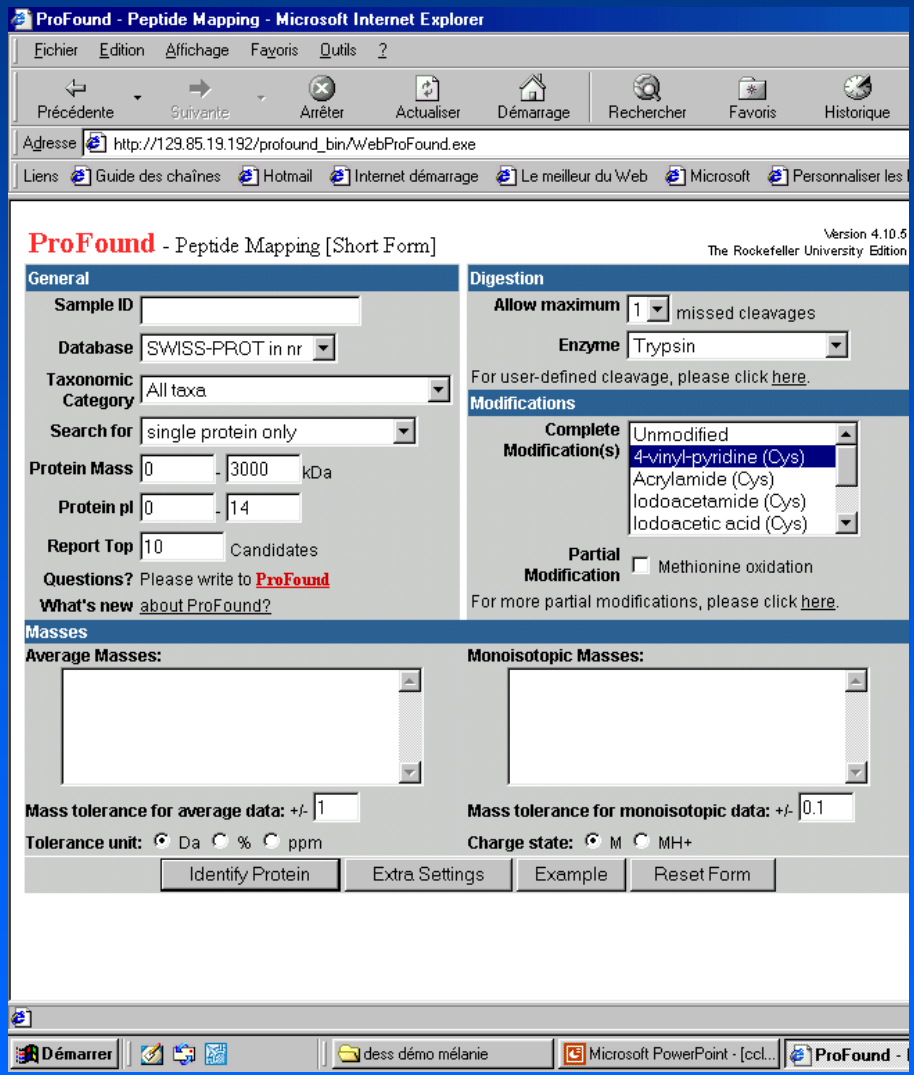
Swiss Prot
NCBI

Data Base
Interrogation

Mass Spectrometry

Methods (end)

Data Base interrogations



Main softwares used

Profound

Mascot

Msfit

Main Data bases used

SWISSPROT

NCBI

TrEMBL

Genetic determination of protein quality in wheat grain

Genetic aspects of wheat storage proteins

Wheat storage proteins and quality

Quantitative variations of wheat storage proteins

Proteomics of wheat kernel

Proteomics on developmental kernel

Wheat kernel sampling and 2DE

Bread wheat cultivar : Récital

Day / night temperature 18°C

Accurate notation of anthesis for each ears

0 1 3 6 9 12 15 18 ← Day after Anthesis

J0 J1 J2 J3 J4 J5 J6 J7 ← named stage

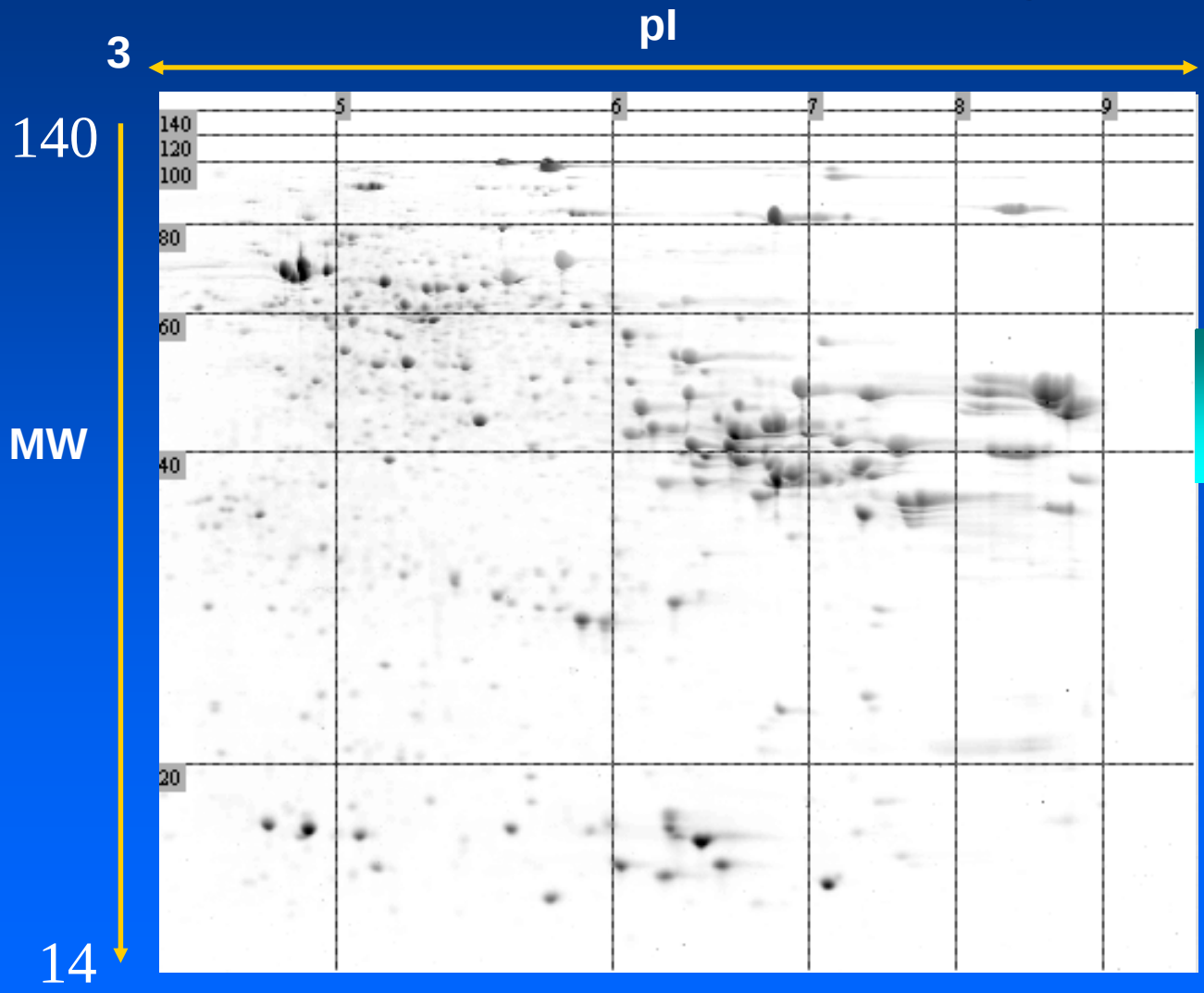
0 20 50 100 150 200 250 300 °CDay (Cumulative Day temperature.)

Total protein extraction from whole developing seed

6 2DE (IPG pH 3-10 x SDS PAGE) for each stage

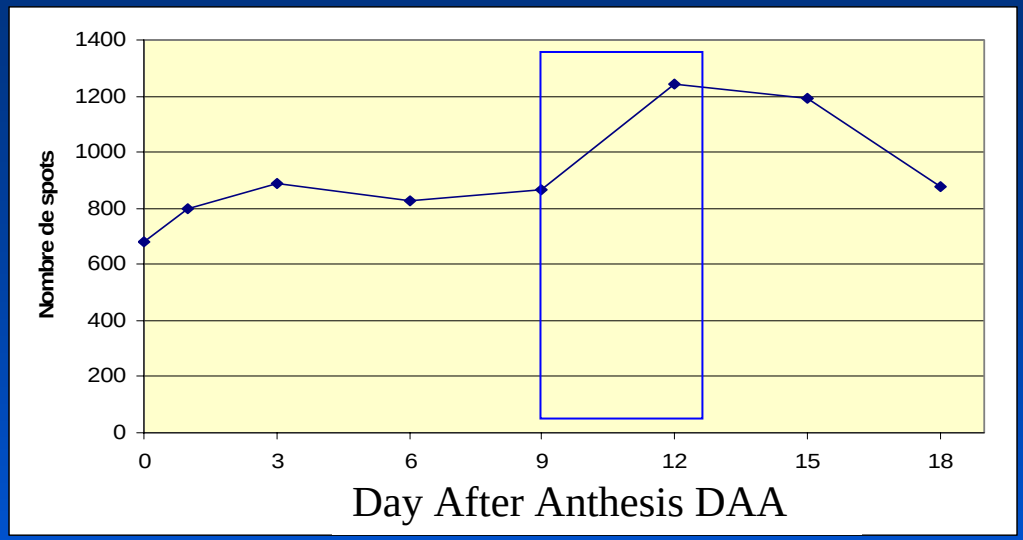
wheat grain development

Typical 2DE IPG x SDS PAGE of the Albumins-Globulins of developing wheat kernel (CBB staining)

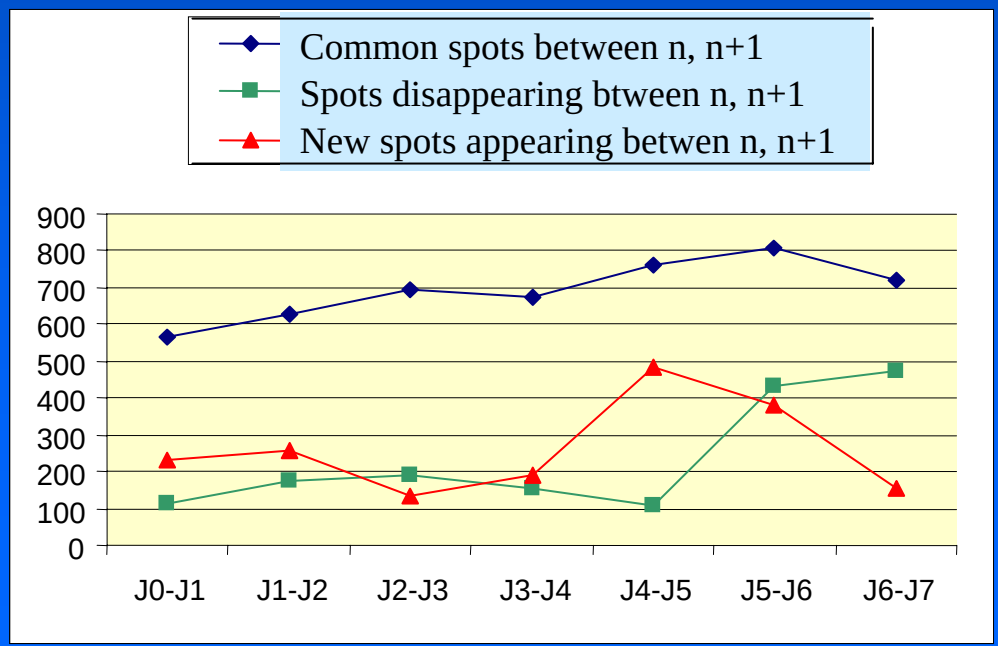


875 spots
DAA 18

Evolution of spots number in cours of time



The number of spots increased of 43% between 9 and 12 DAA

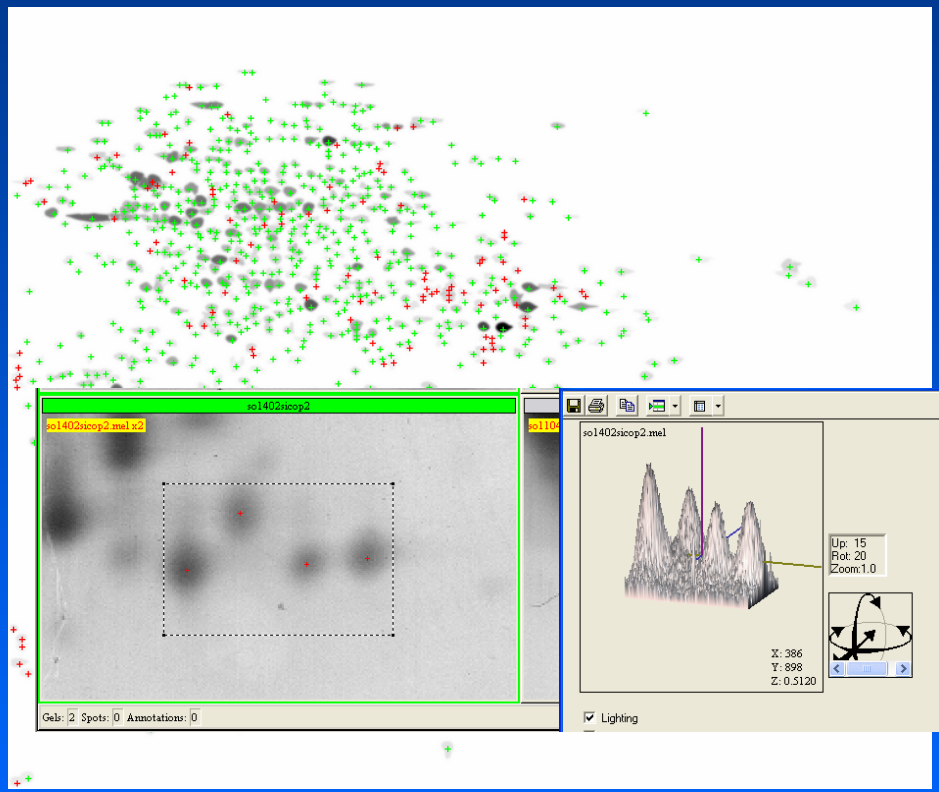


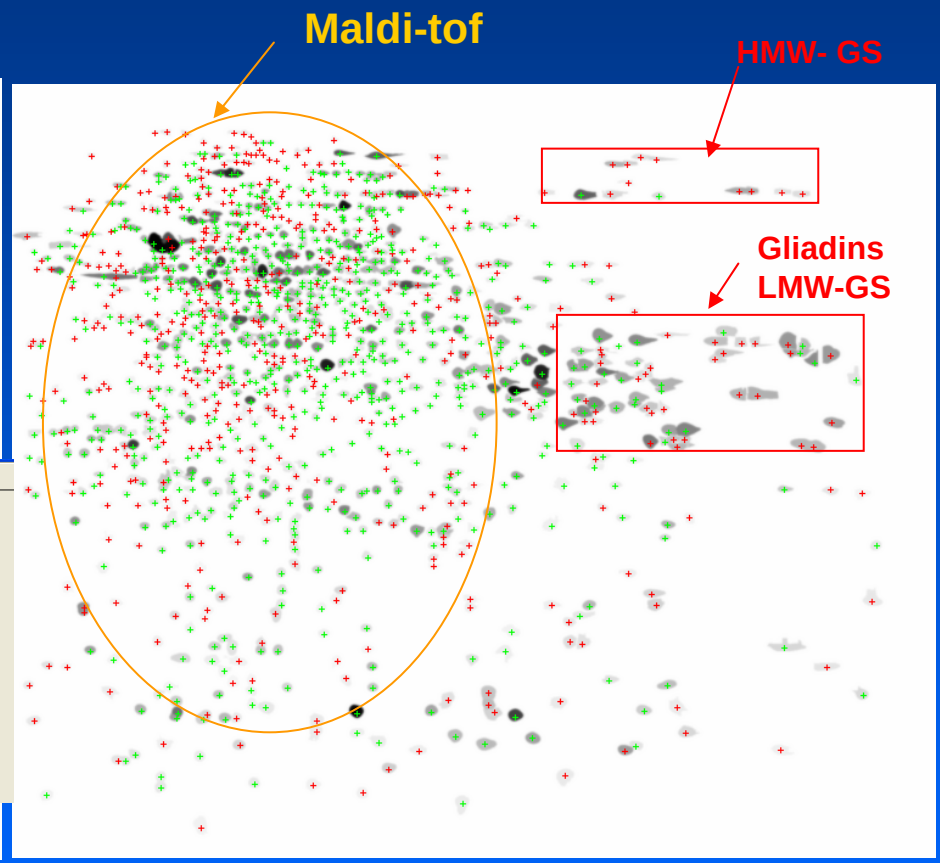
Parallele evolution of the number of new spots and spots desappearing

wheat grain development

Protemic differences between two consecutive stages

For example DAA 9 - DAA 12





DAA 9 : 865 Proteins

106 Desappearing proteins

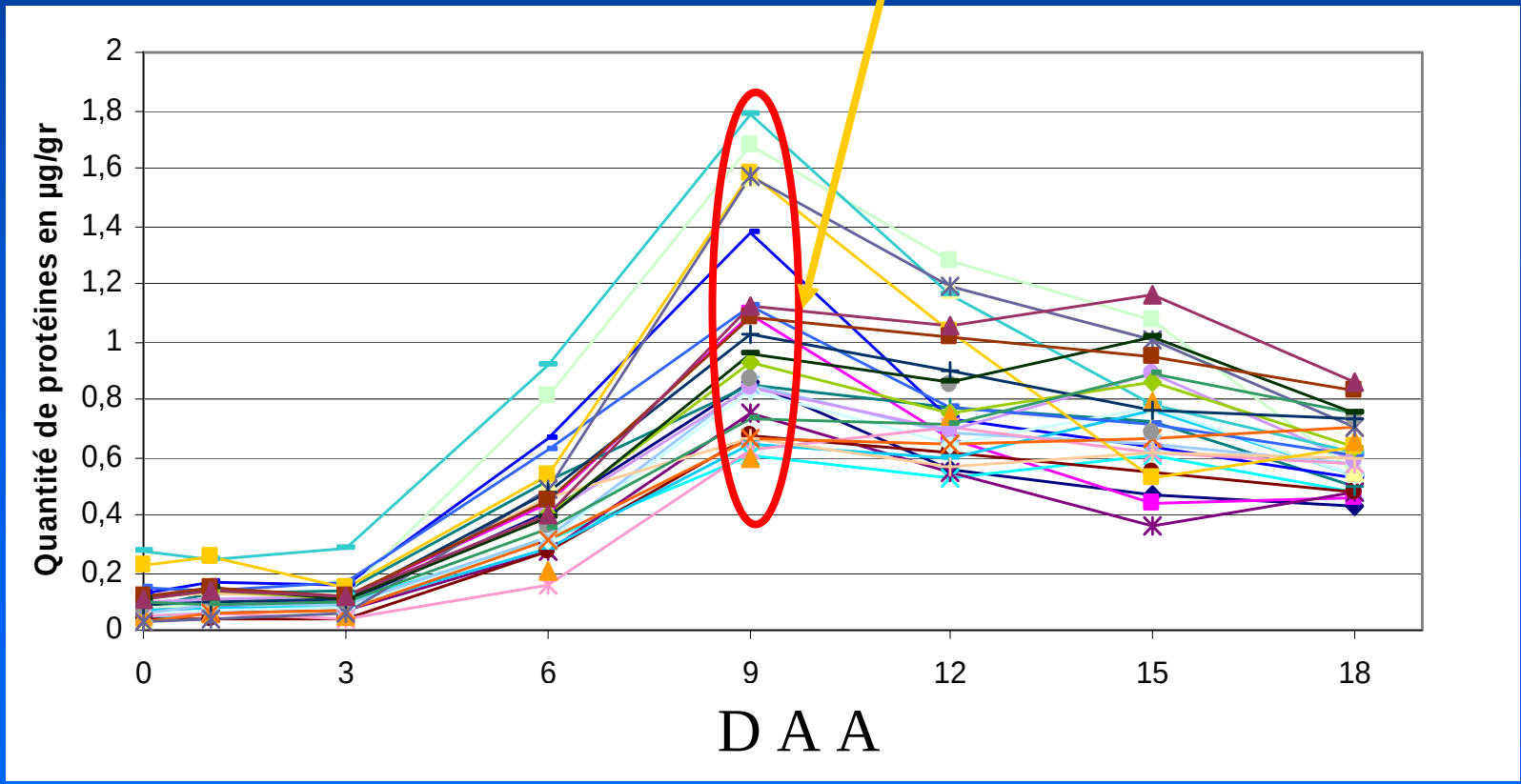
DAA 12 : 1242 proteins

483 New proteins

759 commun proteins

Evolution of the amount of protein per kernel (µg/kernel) for 285 spots commun to all stages (0 to 18 DAA)

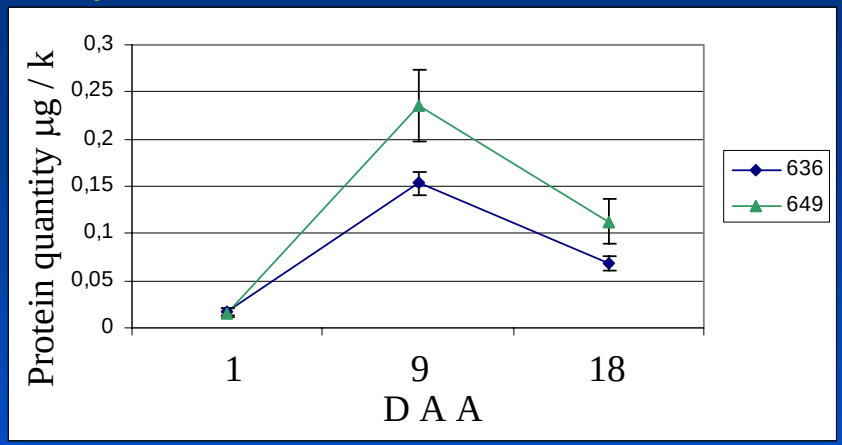
Peak at 9 DAA 9 : ~150°Cd)



wheat grain development

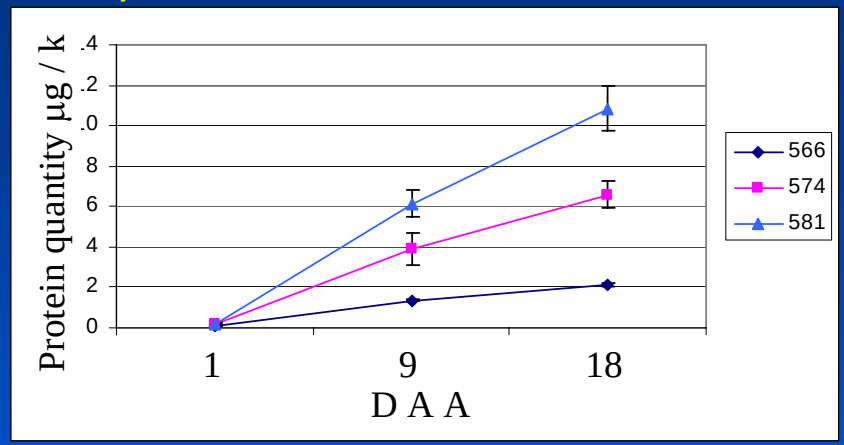
Main profiles found for the amount of protein (µg/kernel) accumulated, for the commun spots

78,2 %



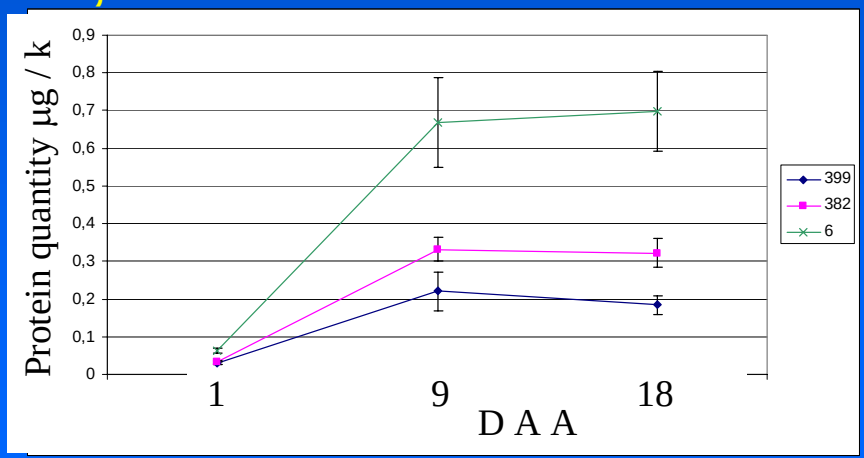
Cell division and cell structure

11,2 %



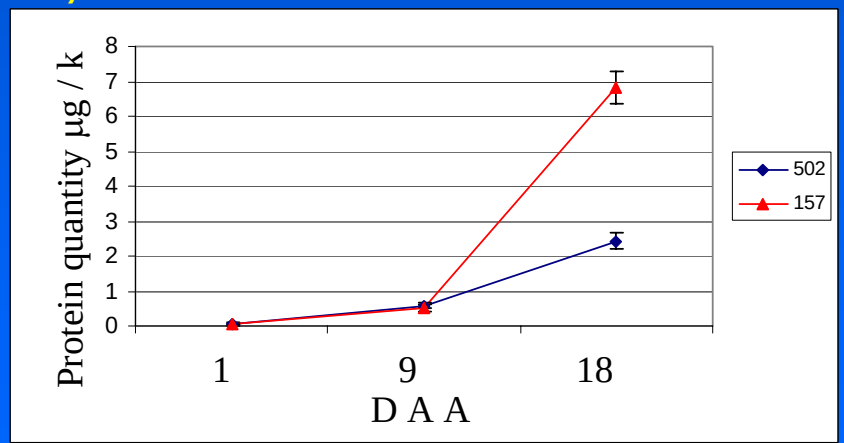
Enzymes for storage proteins and starch accumulation

8,8 %



Keeping house proteins

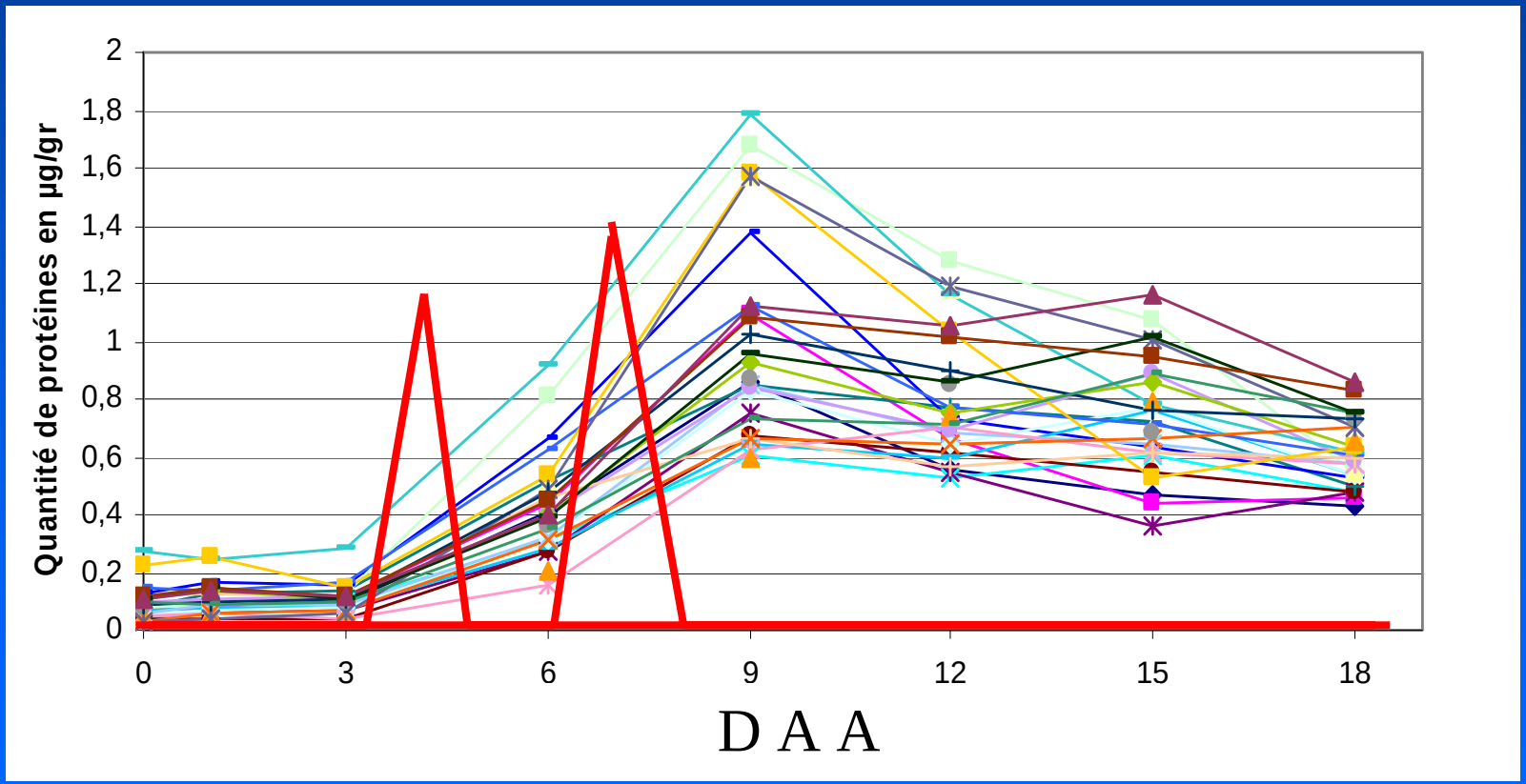
1,8 %



Enzymes for starch and storage protein polymerisation

INRA

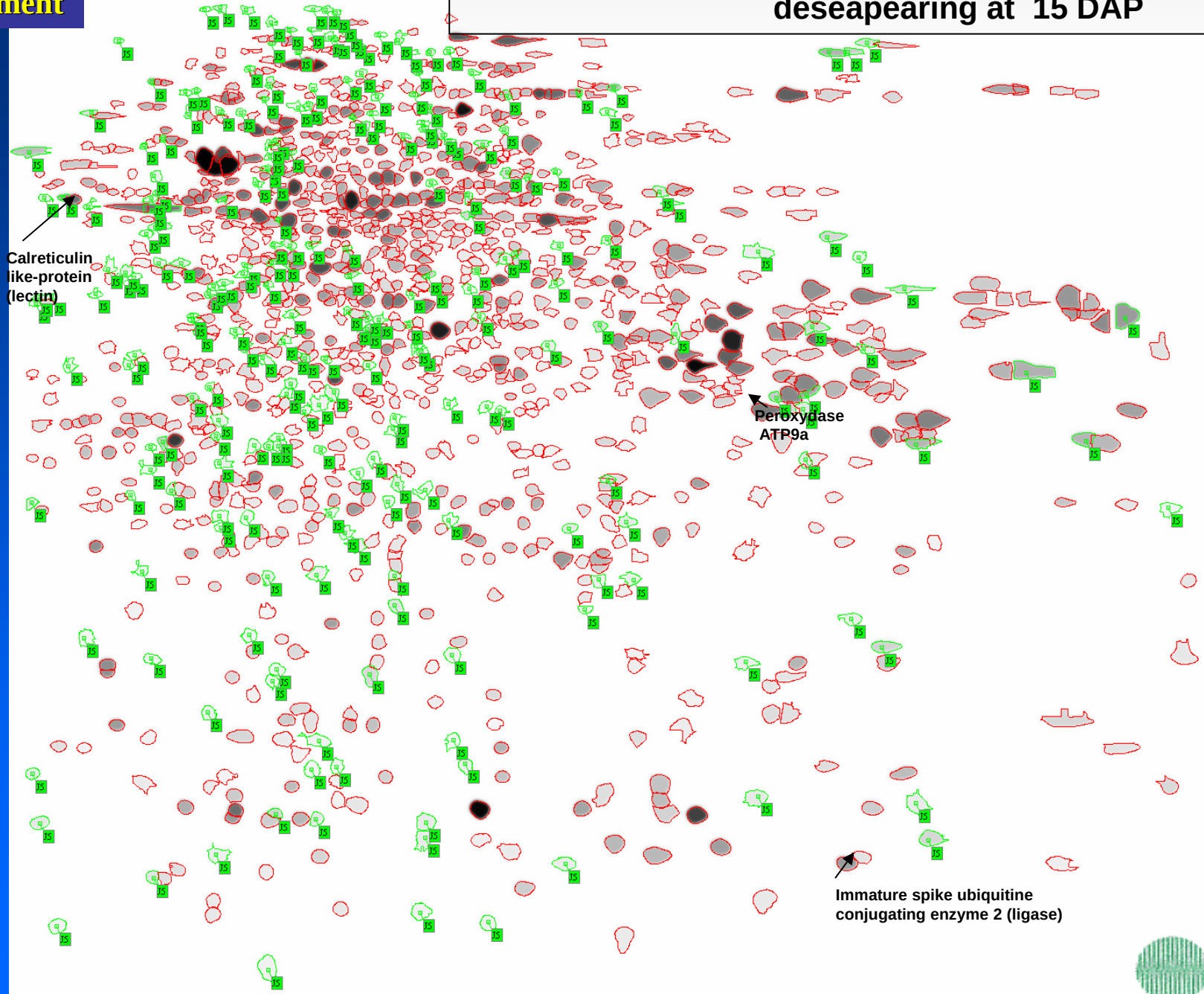
Another group of proteins



wheat grain development

Synthetic gel

245 Proteins spots appearing at 12 DAP and deseapearing at 15 DAP



INRA

Example of data collected from data bases and bio analysis

Pathway	Putative fonction	Biological process	Family protein	Developmental stages	Protein name	Organism	cell localization	Tissue	N° accession Swiss Prot	N° spot
Environmental Information Processing	Signal transduction	Coleoptile phototropism	NPH3 family	0 to 18 DAP	Root phototropism protein 2 (RPT2)	Arabidopsis thaliana	hypocotyl, guard cells, mesophyll cells		Q682S0	2626
Environmental Information Processing	Signal transduction	Protein synthesis	ribosomal protein	0 to 18 DAP	40S ribosomal protein SA, putative,	Oryza sativa	reticulum endoplasmic		ABF94326	2253
Environmental Information Processing	Signal transduction	Protein synthesis	ribosomal protein	0 to 18 DAP	putative 40S ribosomal protein	Oryza sativa			XP_479167	2275
Genetic Information Processing	Folding, Sorting and Degradation	Catalysis of the rearrangement of both intrachain and	isomerase	0 to 18 DAP	protein disulfide isomerase precursor	Triticum aestivum	reticulum endoplasmic	endosperm	P52589	1901
Metabolism	Carbohydrate metabolism	Calvin cycle	kinase	0 to 18 DAP	phosphoglycerate kinase	Triticum aestivum	Plastid; chloroplast	leaf	CAA51931	2267
Metabolism	Carbohydrate metabolism	Galactose metabolism	hydrolase	0 to 18 DAP	Alpha-Galactosidase	Oryza sativa	Endoplasmic reticulum		1UAS_A	2344
Metabolism	Carbohydrate metabolism	Glucose catabolism	phosphoglycerate mutase	0 to 18 DAP	phosphoglycerate mutase	Triticum aestivum	cytoplasm		AAP80633	1868
Metabolism	Carbohydrate metabolism	Starch synthesis	synthase	0 to 18 DAP	starch synthase I	Hordeum vulgare			AAF37876	1767
Metabolism	Carbohydrate metabolism	Xylose metabolism	xylose isomerase family	0 to 18 DAP	Xylose isomerase	Hordeum vulgare			Q40082	1970
Metabolism	Carbon utilization	alcohol metabolism	oxidoreductase	0 to 18 DAP	alcohol dehydrogenas	Hordeum vulgare	cytoplasm		AAC19351	2276
Metabolism	Carbon utilization	Regulation of photosystem	decarboxylase	0 to 18 DAP	isocitrate dehydrogenas	Medicago sativa	chloroplast		Q40345	2284

Genetic determination of protein quality in wheat grain

Genetic aspects of wheat storage proteins

Wheat storage proteins and quality

Quantitative variations of wheat storage proteins

Proteomics of wheat kernel

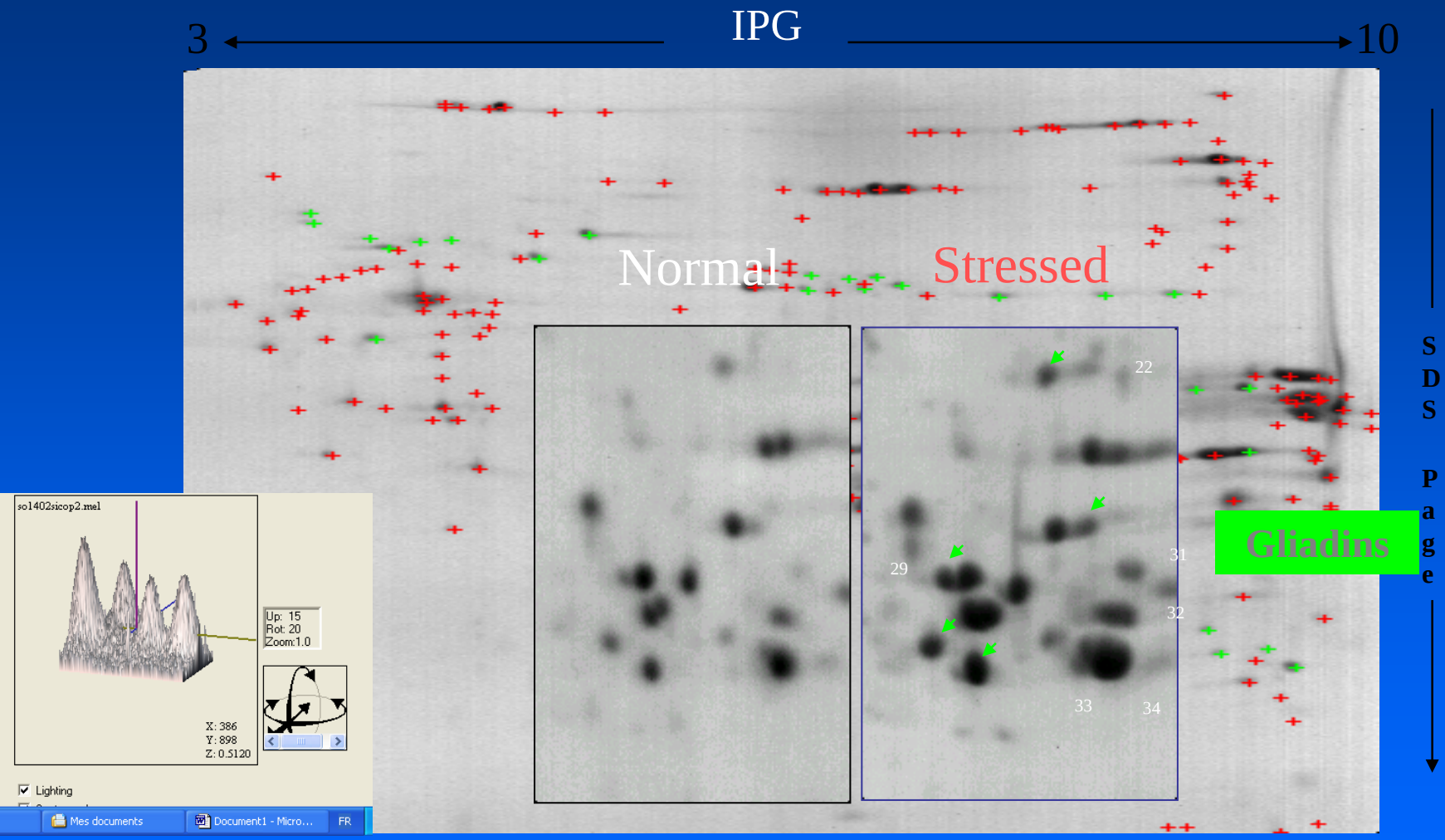
Proteomics on developmental kernel

Endosperm responses to Heat stress

INRA

Differential proteomics : quantitative analysis between two types of samples

Effect of warm temperature on wheat proteome at kernel maturity on cv Thésée

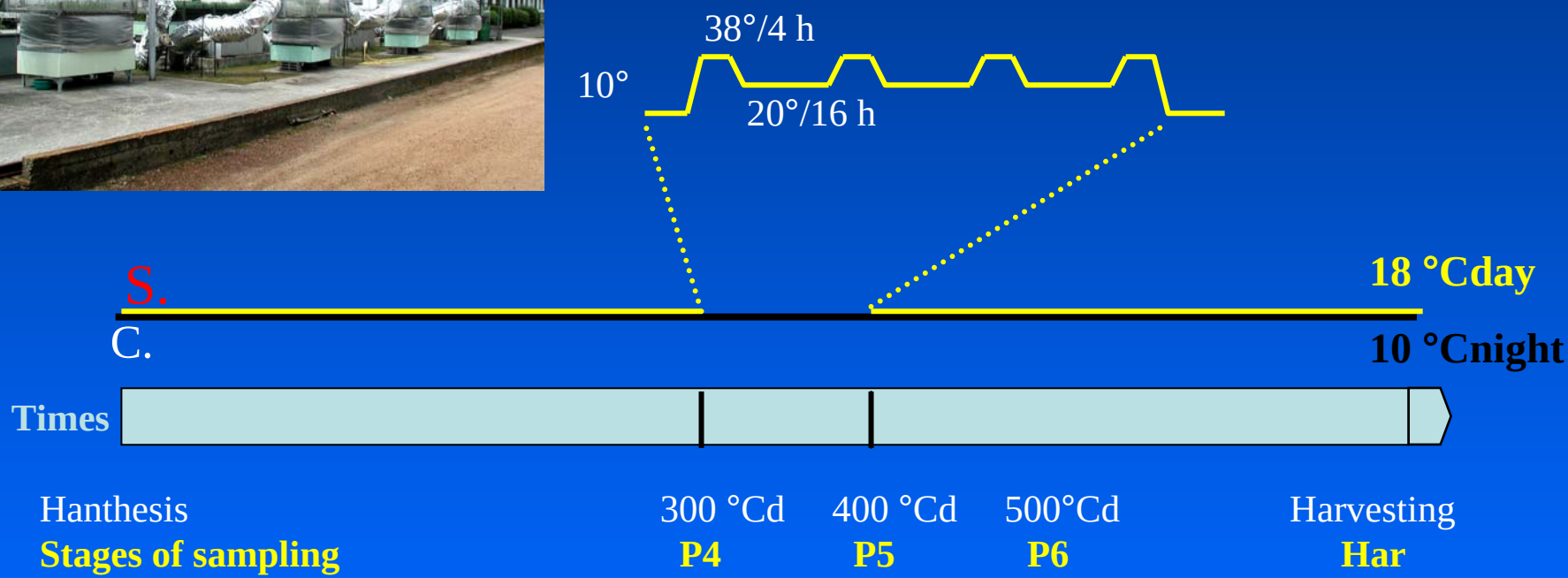


Common Spots of Higher amount at 34/10 ($p<0.005$)

Heat Stress

Experiment 2

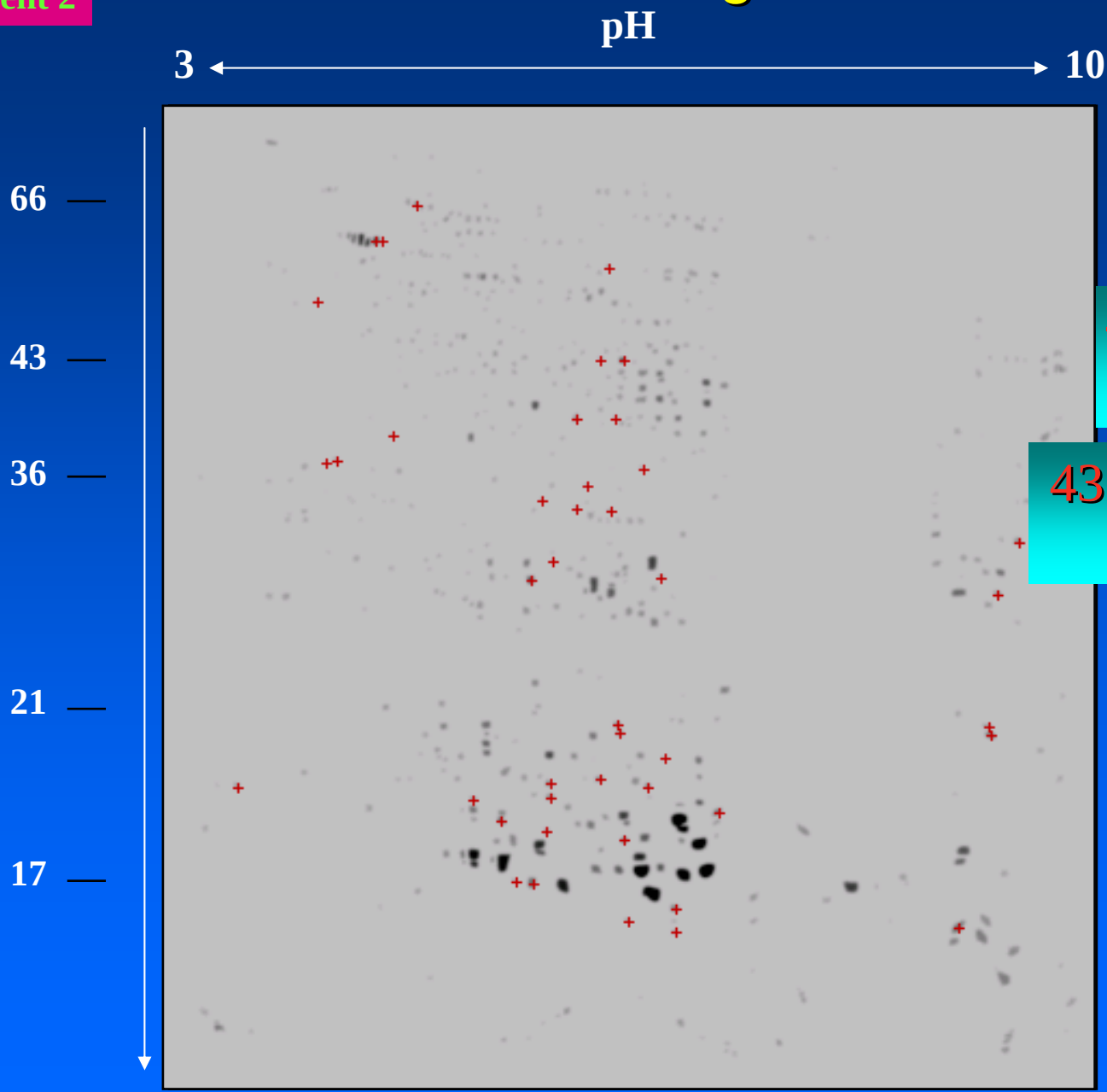
Thermal conditions for control and stressed wheat : cv Récital



Heat Stress

Experiment 2

2DE of the Albumins-globulins Récital (P5)



42 induced spots

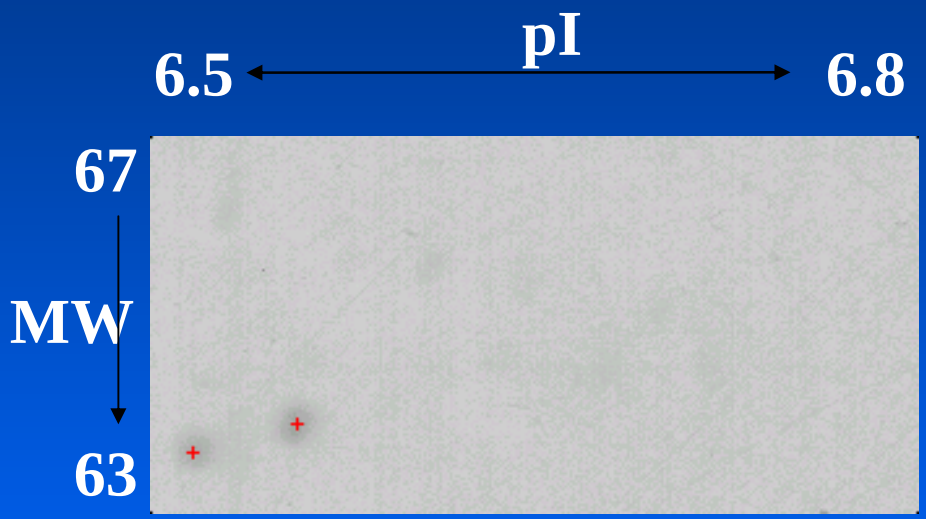
24 up-regulated spots

43 down-regulated spots

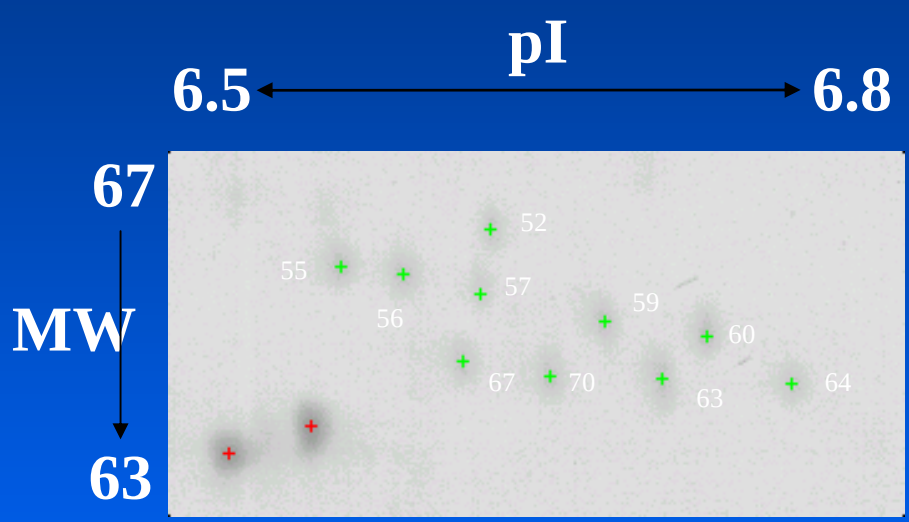
INRA

Qualitative variations (presence/absence) of endosperm proteins at stage P5 (after 4 days of heat stress)

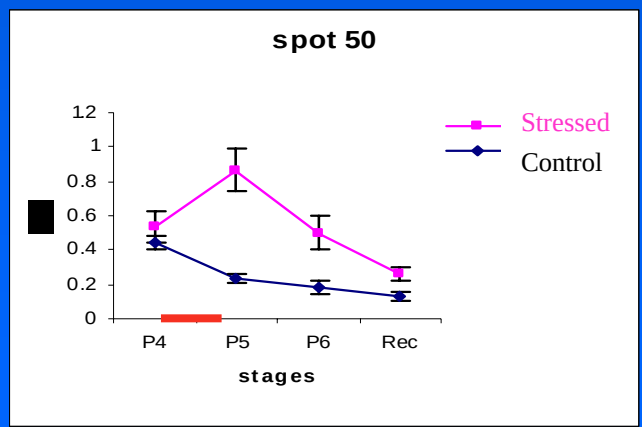
Control



Stressed



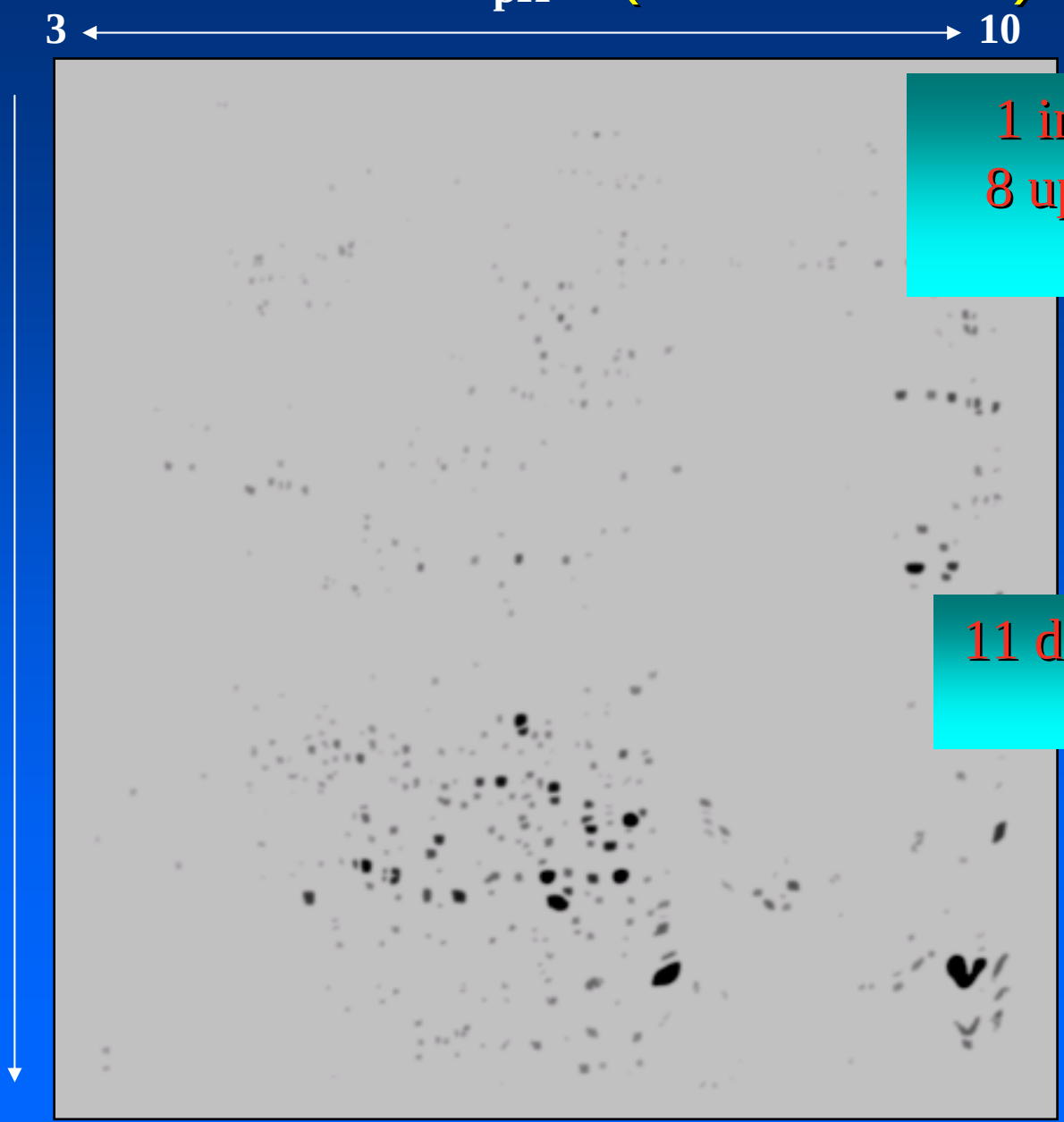
HSP70 : Bind ATP. Also involved in the folding and oligomerization of newly synthesized polypeptides, involved in the assembly disassembly of multimeric structures.



Heat Stress

Experiment 2

2DE of the Albumins-globulins Récital (Harvest time)



1 induced +
8 up-regulated
spots

11 down-regulated
spots

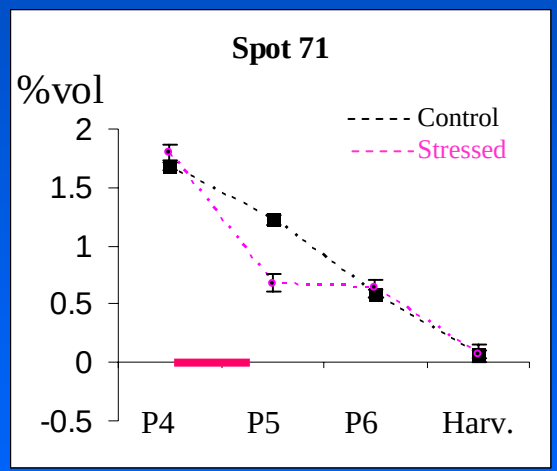
INRA

Identification of some proteins and variation during grain formation

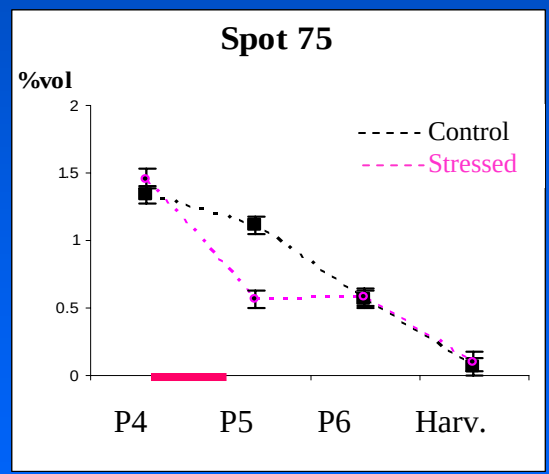
(Continuation)

Glutathion synthase } oxydo-reduction, polymerisation
Glutathion transferase } of proteins, detoxication etc…

Protein disulfite isomerase: Binding proteins, protein folding, chaperone



PDI

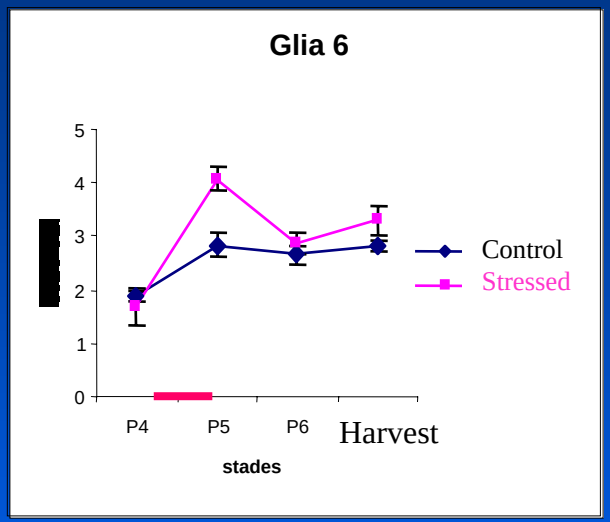


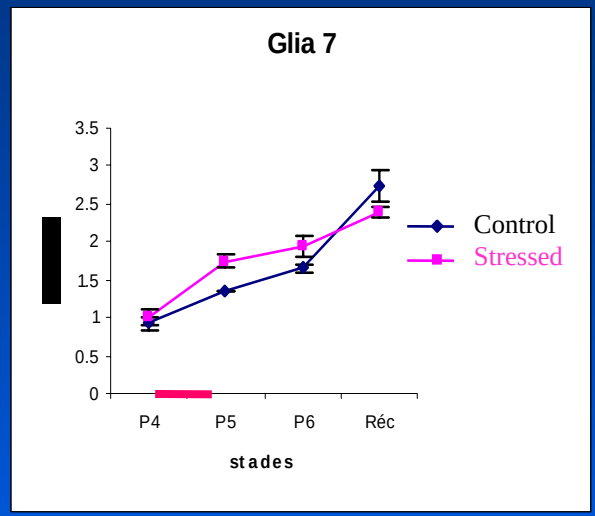
Heat Stress

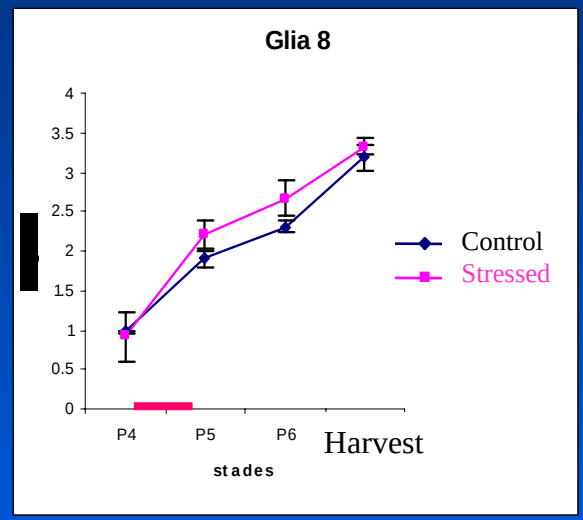
Experiment 2

Identification of some proteins and variation during grain formation

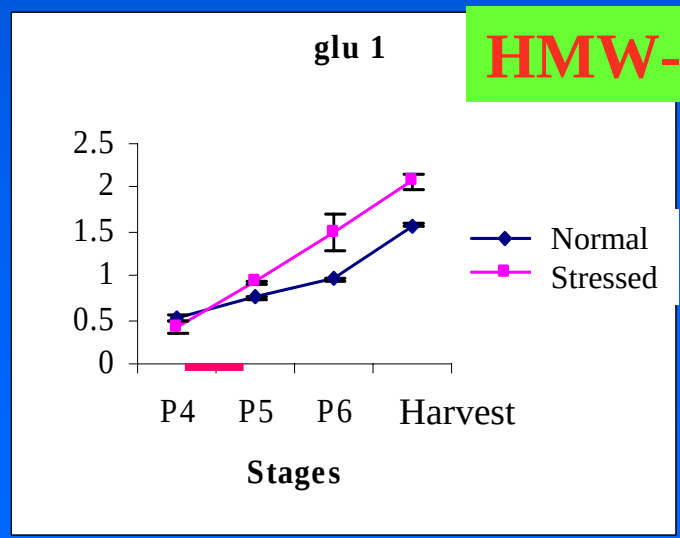
α-Gliadins







HMW-GS



Genetic determination of protein quality in wheat grain

Genetic aspects of wheat storage proteins

Wheat storage proteins and quality

Quantitative variations of wheat storage proteins

Proteomics of wheat kernel

Proteomics on developmental kernel

Endosperm responses to Heat stress

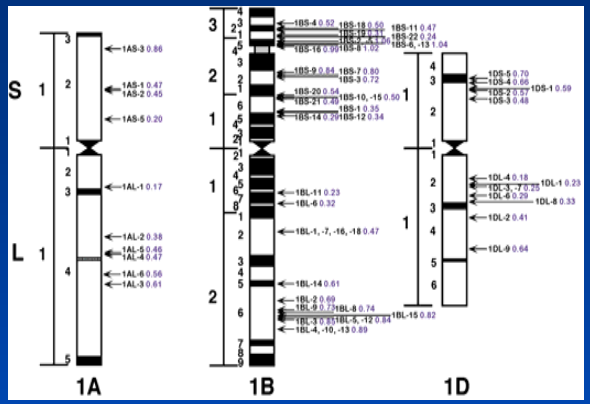
Some other proteins involved in quality

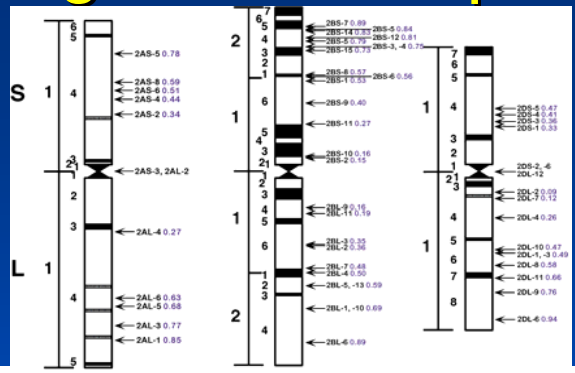
INRA

Mapping Albumins -Globulins

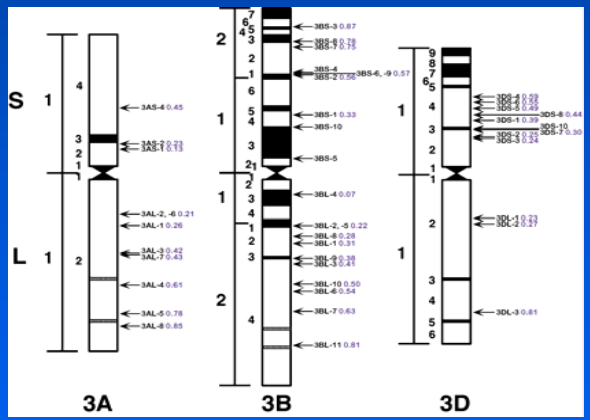
Current mapping of tissue specific proteins using Chinese Spring deletion lines

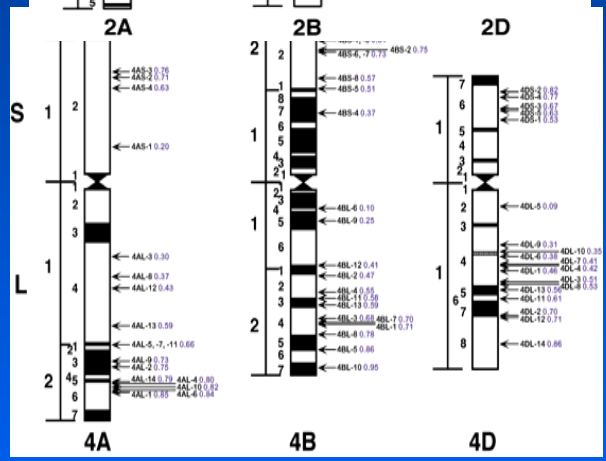
From B. Gill KSU, USA



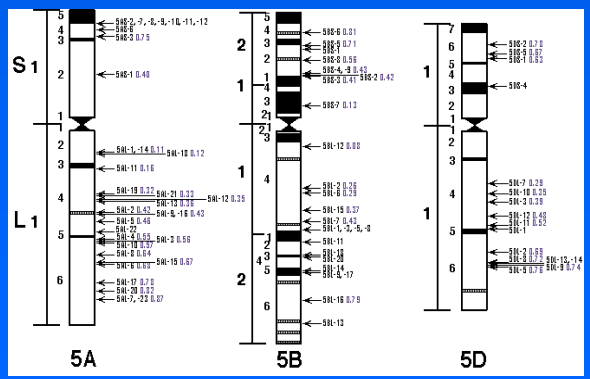


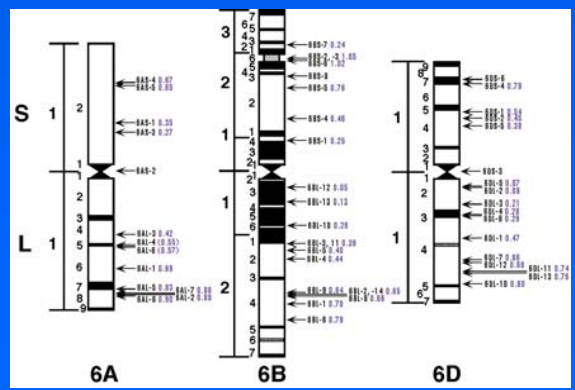
Proteins of the aleurone layer

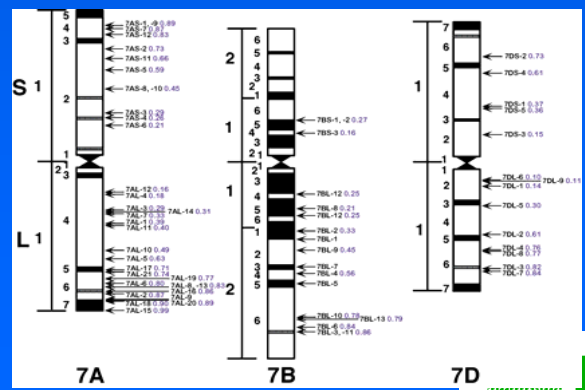




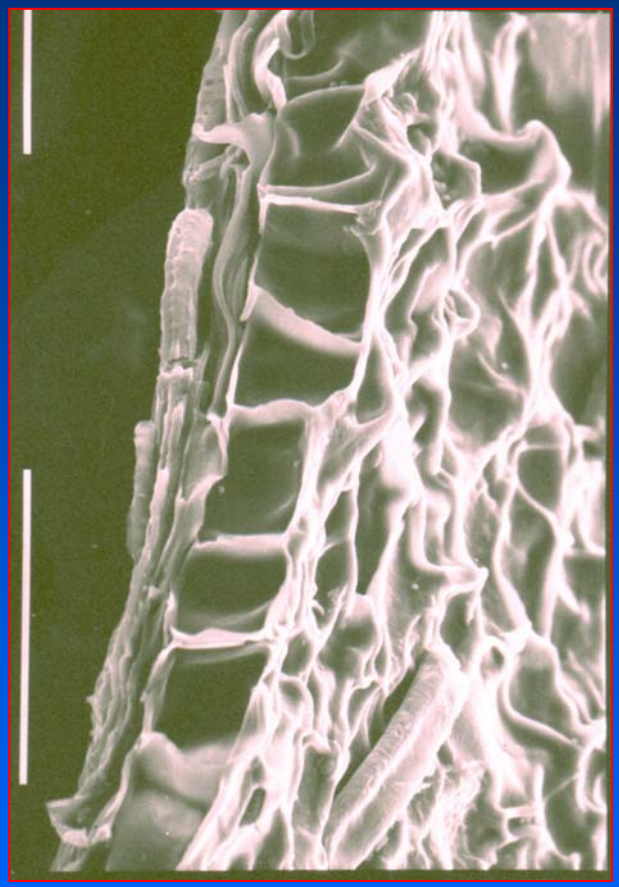
Albumins-Globulins of the endosperm



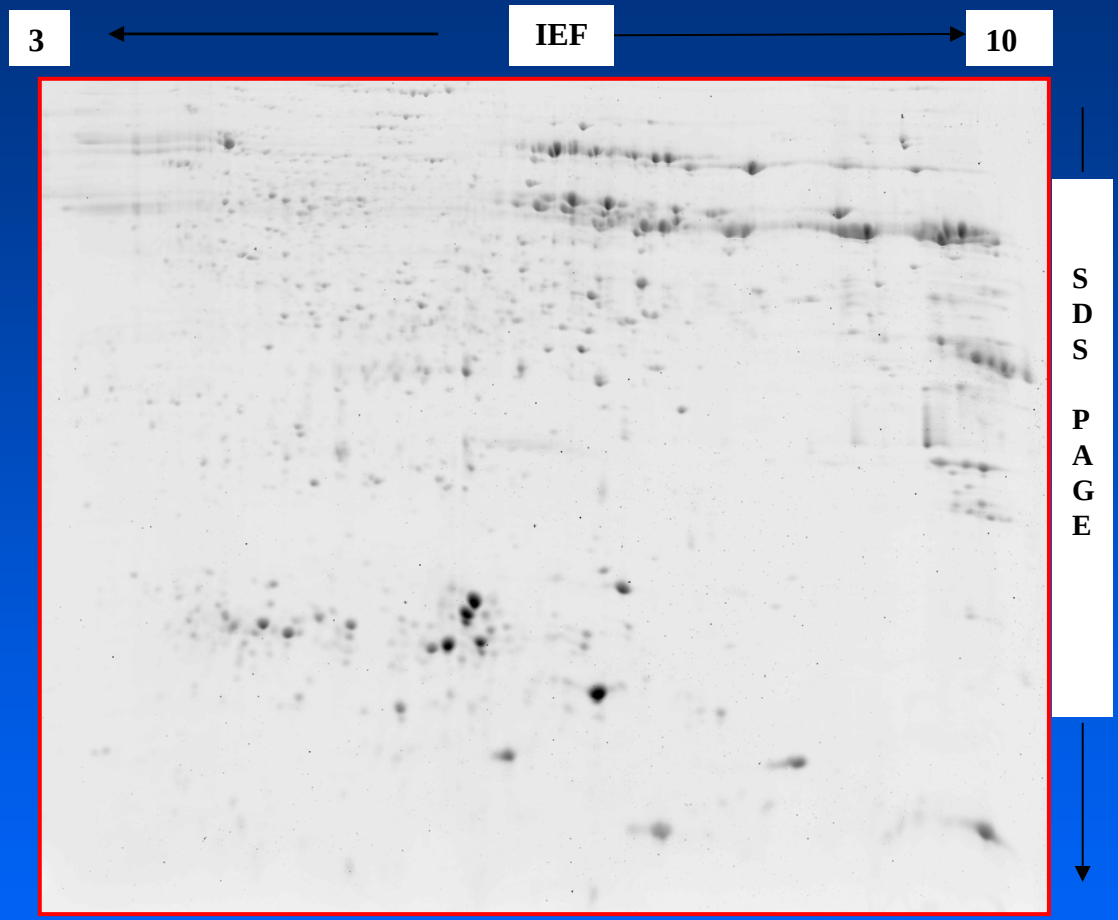




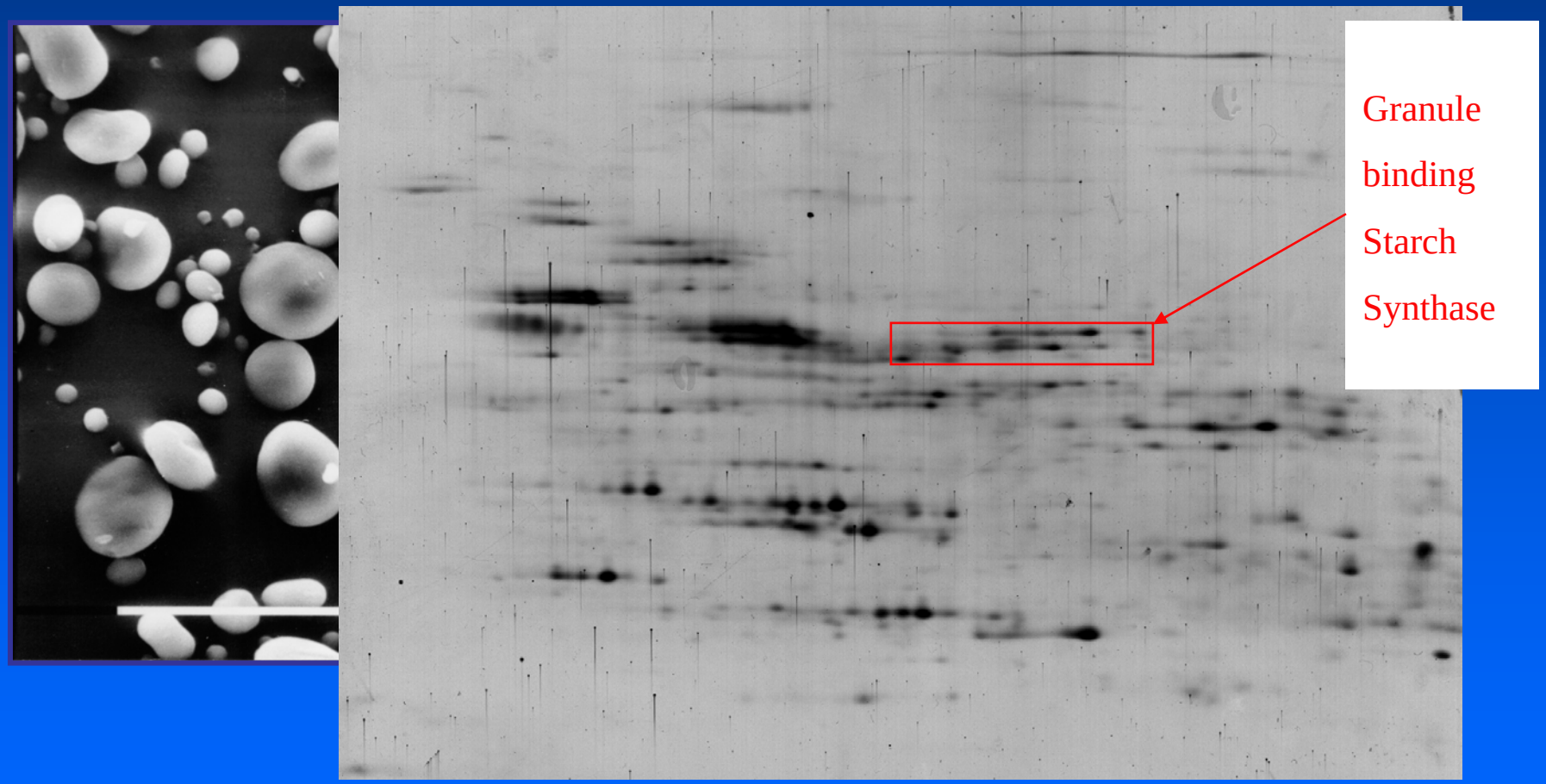
Proteomics of aleurone layer



(+ nucelus): e = 100 microns



Proteomics of wheat starch granules: Diversity of the inner or strongly attached proteins to starch granules



From: Branlard G. 2000, IAA, 5, 91-97

Conclusion

- Proteomic analysis of soluble proteins allowed to identify in total approx 400 soluble proteins and genes coding 260 spots have already been located on 21 Chr.

- Proteomic approach will be an unavoidable tool for studies on:
  - gene expression
  - functional genomic (assoc. with transcriptome)
  - gene regulations
  - genome interactions
  - relations between genotype and phenotype
  - plant physiology and environmental influence
  - etc...

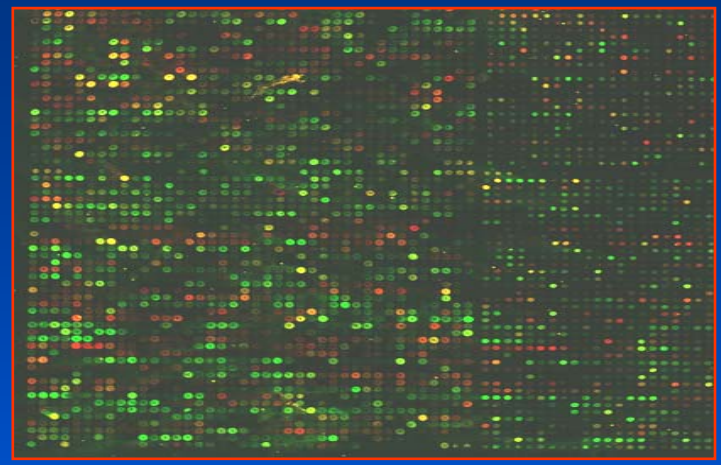
- Proteomic can be carried out on different cell components or specific tissues. A proteomic DB is currently in preparation

INRA

Question: How to find-out the key genes that govern the kernel protein content for future quality wheat ?

Micro-array provides a complex picture of the numerous DNA sequences which could be directly or not associated to the character

Micro-array is a bottom-up approach for studying gene expression



Proteomic approach is an unavoidable tool for studies on:

- gene expression, functional genomic, gene regulations
- relation between genotype and phenotype
- etc...

Proteomic is the top-down approach particularly useful for studies on plant physiology and environmental influence

INRA

Genetic determination of protein quality in wheat grain

Genetic aspects of wheat storage proteins

Wheat storage proteins and quality

Quantitative variations of wheat storage proteins

Proteomics of wheat kernel

Proteomics on developmental kernel

Endosperm responses to Heat stress

Some other proteins involved in quality

The Plate-forme for genotyping at INRA Clermont Ferrand

INRA

Specific rooms were setting – up

210 m^2 of laboratories, Inaugurated in june 2006

- Cold room
- room DNA-Extractions
- room pre-PCR 1 (ADN)
- room pre-PCR 2 (Mix)
- room post-PCR
- room RT-PCR
- room Developement Technology
- Room Informatique – Data analysis
- Office

Tools on the plate-forme

- **1 LIMS (Laboratory Information Management System)**
  (SQL-LIMS APPLIED BIOSYSTEMS - installation in progress)
- **3 Computers:** 2 Linux (HP Bi processeurs 2.4 GHz);
  1 Windows (Dell Bi processeurs 3 GHz)
- **3 capillary sequencers** ABI 3100
  (APPLIED BIOSYSTEMS)
- **1 plateforme RT-PCR** ABI PRISM 7900HT-384 wells
  (APPLIED BIOSYSTEMS)
- **1 plate-forme for automatic pipeting** Genesis 150 (TECAN)
- **3 plate-forme for automatic pipeting heads 96** BiomekFX / BiomekNX / Multimek (BECKMAN)
- **1 robot for pipeting** Biomek2000 (BECKMAN)
- **3 thermocyclers** PTC-225 : **12 blocs 384** (MJ RESEARCH)

Other researches carried out at Inra Clermont-Ferrand

Wheat Genetic resources : characterization, evolution : F Balfourier

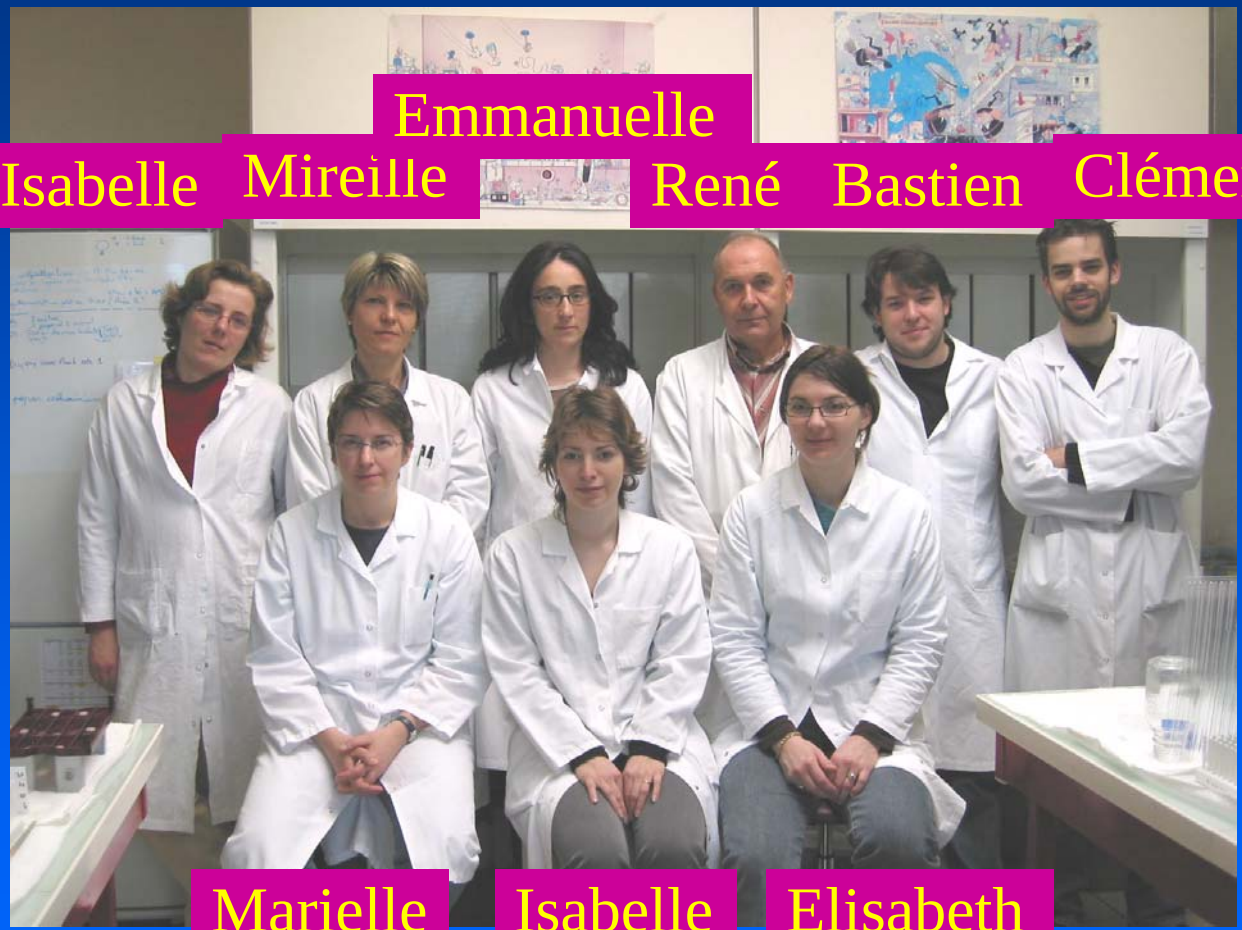
Stability of wheat quality : Nitrogen use efficiency : J Legouis

Identification of genes involved in wheat quality

- **SNP and Transcriptional Factors : C Ravel**
- **Synteny, transcriptionnal genetics : J Salse**

Genomics and physical mapping of Chr 3B : C. Feuillet

Aknowlegements



Thank you