4th ICC Latin American Cereals Conference

13th International Gluten Workshop

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Mexico City, Mexico

LACC IGW

PROTEOMICS TO ASSESS THE QUALITY OF PORTUGUESE BREAD WHEAT

Gilberto Igrejas

UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

40 ANOS utad

utad DEPARTAMENTO Genética e Biotecnologia

FUNCTIONAL GENOMICS and PROTEOMICS UNIT

LAQV @ REQUIMTE

SPECIAL THANKS

- INRA-UMR GDEC/UBP (FRANCE)
  - **Dr. Gérard BRANLARD**
  - **Dr. Emmanuelle BANCEL**
  - **Dr. Mireille DARDEVET**
  - **Dr. Catherine RAVEL**

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Júlio NUNES-MIRANDA

- UPM-ETSIA (SPAIN)
  - **Dr. José Maria CARRILLO and**
  - **Dr. Marta RODRIGUEZ-QUIJANO.**

- BIOSCOPE | LAQV-REQUIMTE
  - **Dr. José Luís CAPELO and group (PORTUGAL).**

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- CSIRO-Plant Industry (AUSTRALIA)
  - **Dr. Evans LAGUDAH**

- **Florimond Desprez Seed Company(FRANCE).**
- **Ministery of Fisheries and Agriculture (PORTUGAL).**

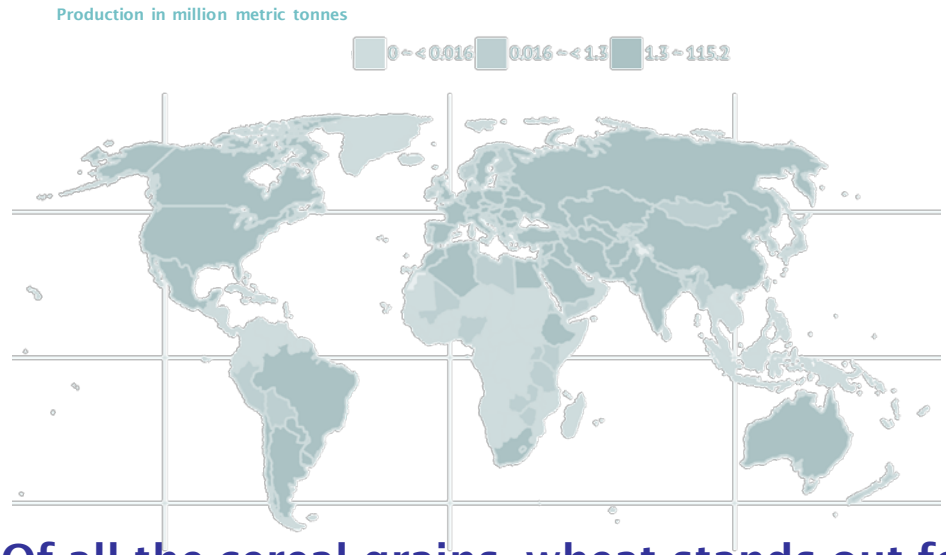
Take home messages

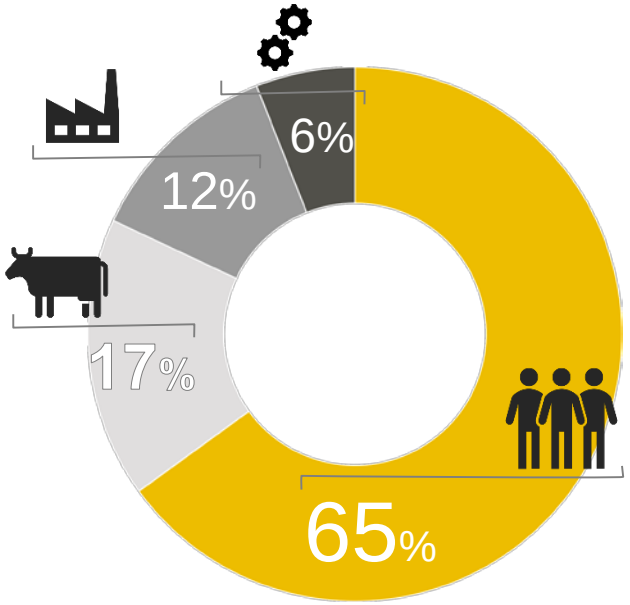
«*Nothing is more prolific than wheat*» Pliny the elder (23-79 AD), *Naturalis Historia*

Wheat price is not an issue of the past

- **Combine the rich molecular biology with traditions to tell stories of wheat to teach real human history**

Wheat in the world

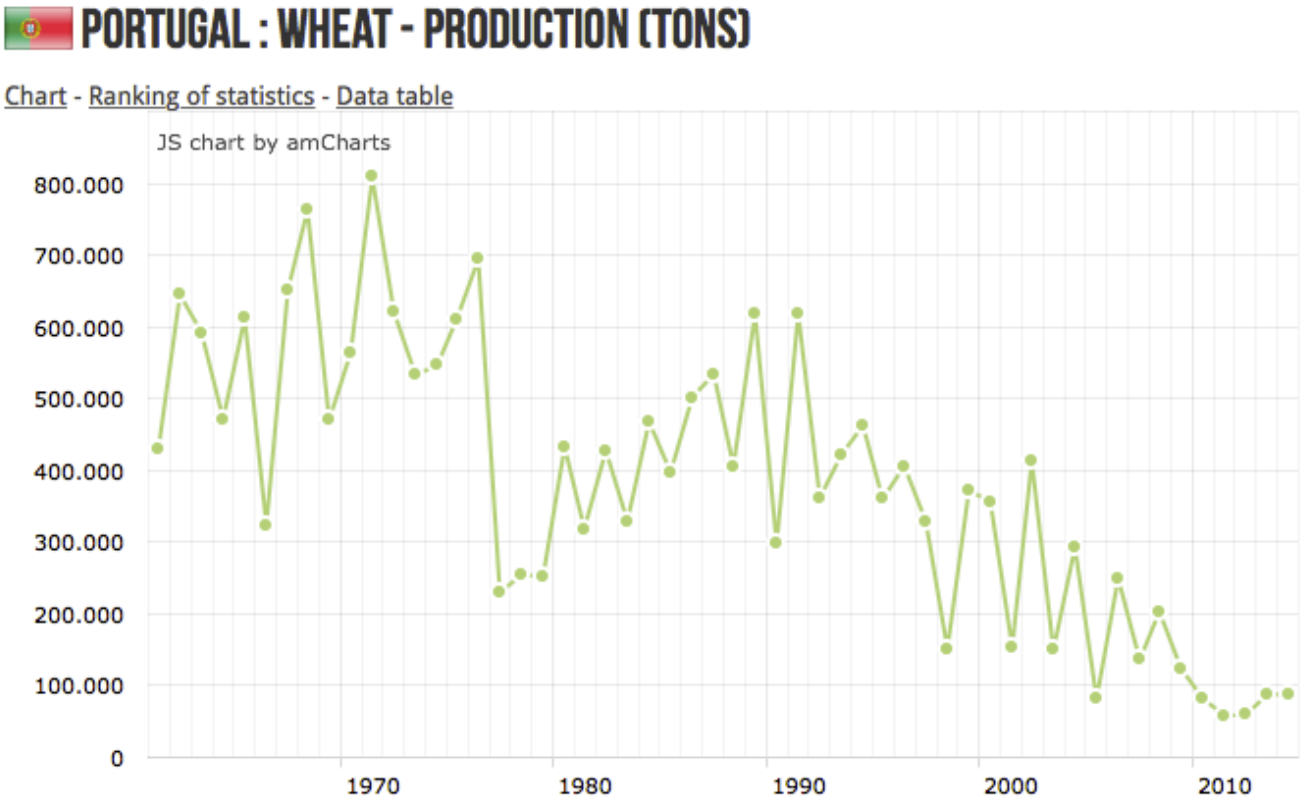




Of all the cereal grains, wheat stands out for being a major source of protein and energy for the human diet:

- More grown (730 million tons)
- High consumption worldwide
- The most transacted

Portugal wheat production

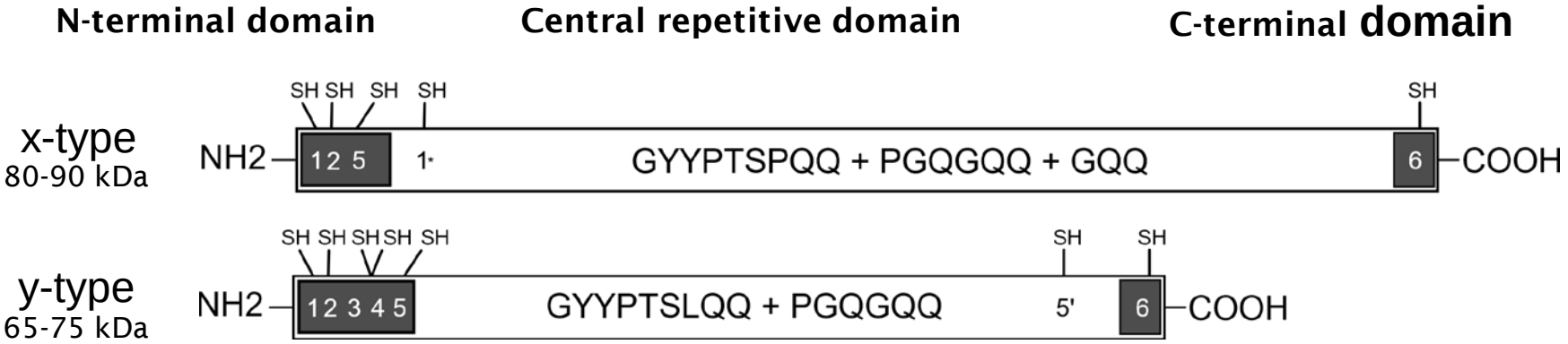


Wheat grain storage proteins (gluten-forming proteins)

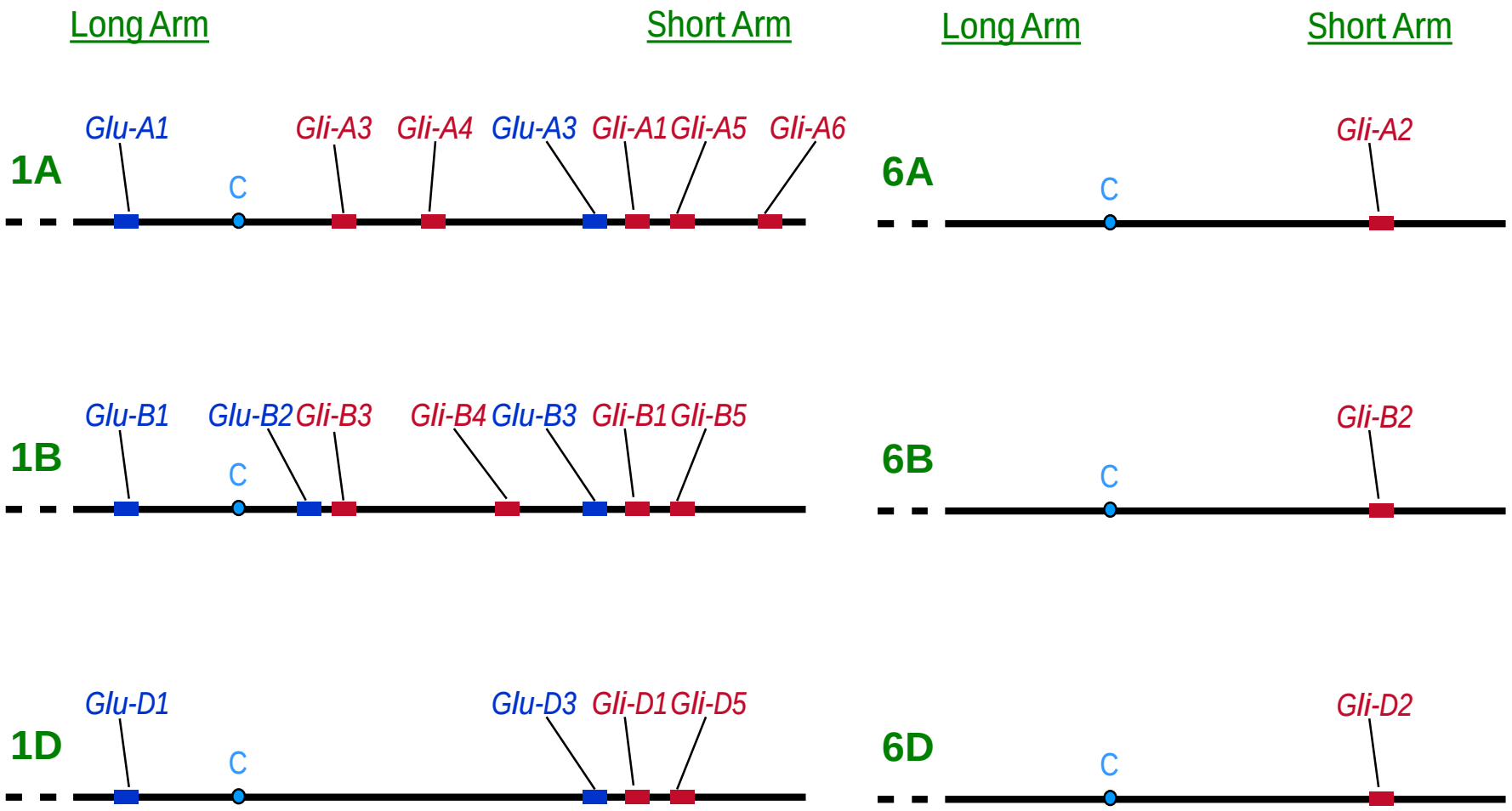
- Monomeric Gliadins
  - ω-gliadins
  - γ-gliadins
  - α/β-gliadins
- **Polymeric Glutenins**
  - LMW glutenin subunits
    Low Molecular Weight
  - **HMW glutenin subunits**
    High Molecular Weight

≈ **80% total grain proteins**

❖ **major determinants of gluten elasticity** by promoting the formation of larger glutenin polymers



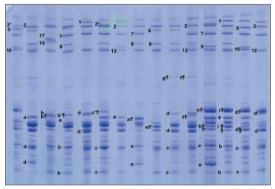
Genetic control of storage proteins

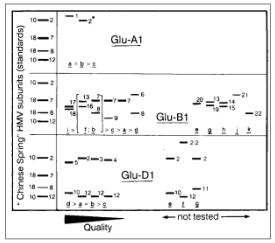


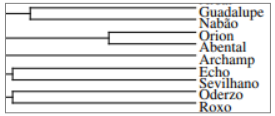
HMW-GS

LMW-GS and Gliadins ω

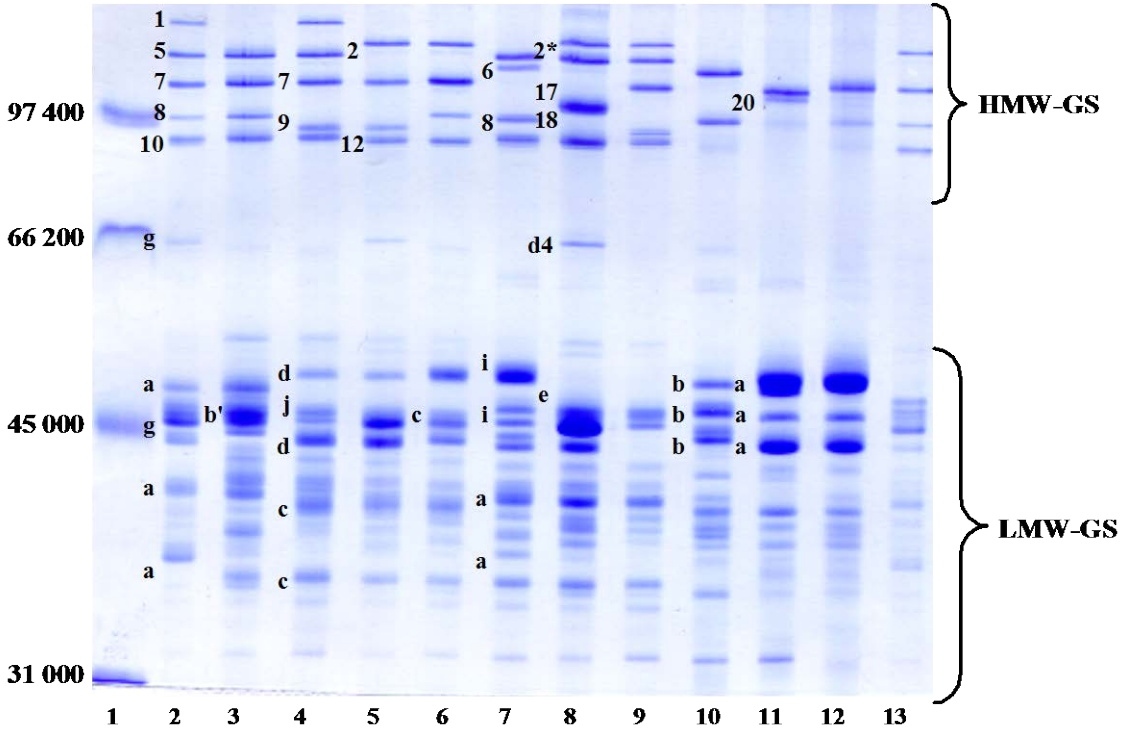
Gliadins α, β and γ







HMW-GS, LMW-GS and gliadins of seeds belonging to the National Catalogue of Varieties



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The high and low molecular weight glutenin subunits and ω-gliadin composition of bread and durum wheats commonly grown in Portugal

G. Igrejas[1, 2], H. Guedes-Pinto[1], V. Carnide[1] and G. Branlard[2, 3]

[1] Departamento de Genetica e Biotecnologia, Universidade de Tras-os-Montes e Alto Douro, P, 5001 Vila Real Codex, Portugal;
[2] INRA, Station d'Amelioration des Plantes, 234, Avenue du Brezet, F, 63039 Clermont-Ferrand Cedex 02, France;
[3] Corresponding author

With 2 figures and 4 tables

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RESEARCH ARTICLE

Towards allelic diversity in the storage proteins of old and currently growing tetraploid and hexaploid wheats in Portugal

Miguel Ribeiro · Carlos Carvalho · Valdemar Carnide · H. Guedes-Pinto · Gilberto Igrejas

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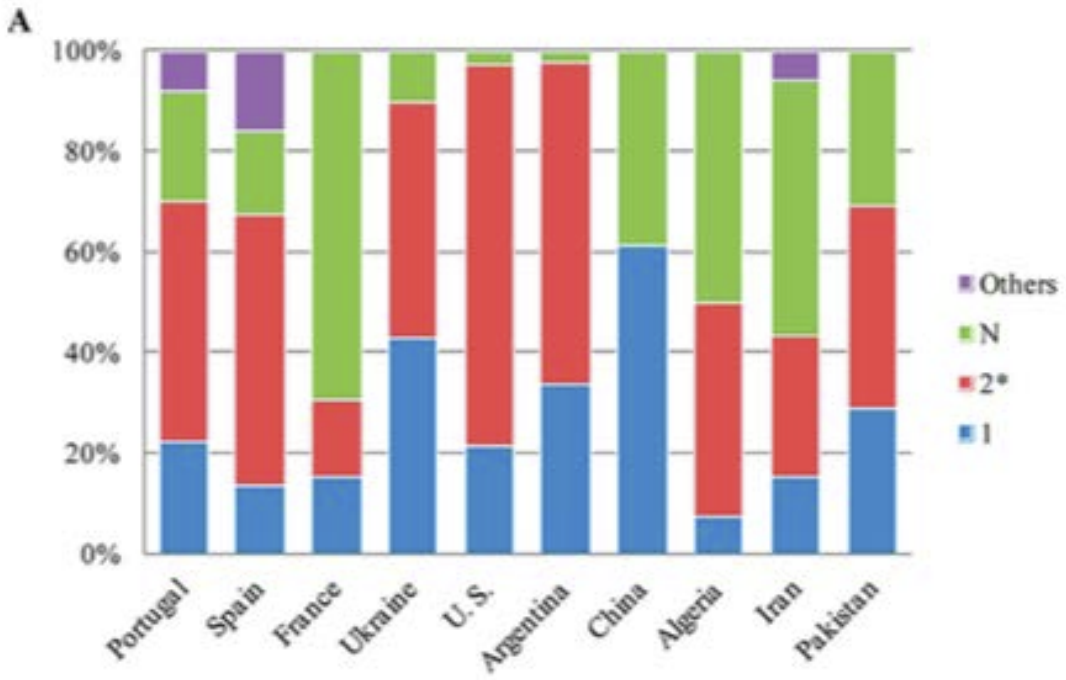
Glu-A1



Journal of proteome research

Reviews

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One Hundred Years of Grain Omics: Identifying the Glutens That Feed the World

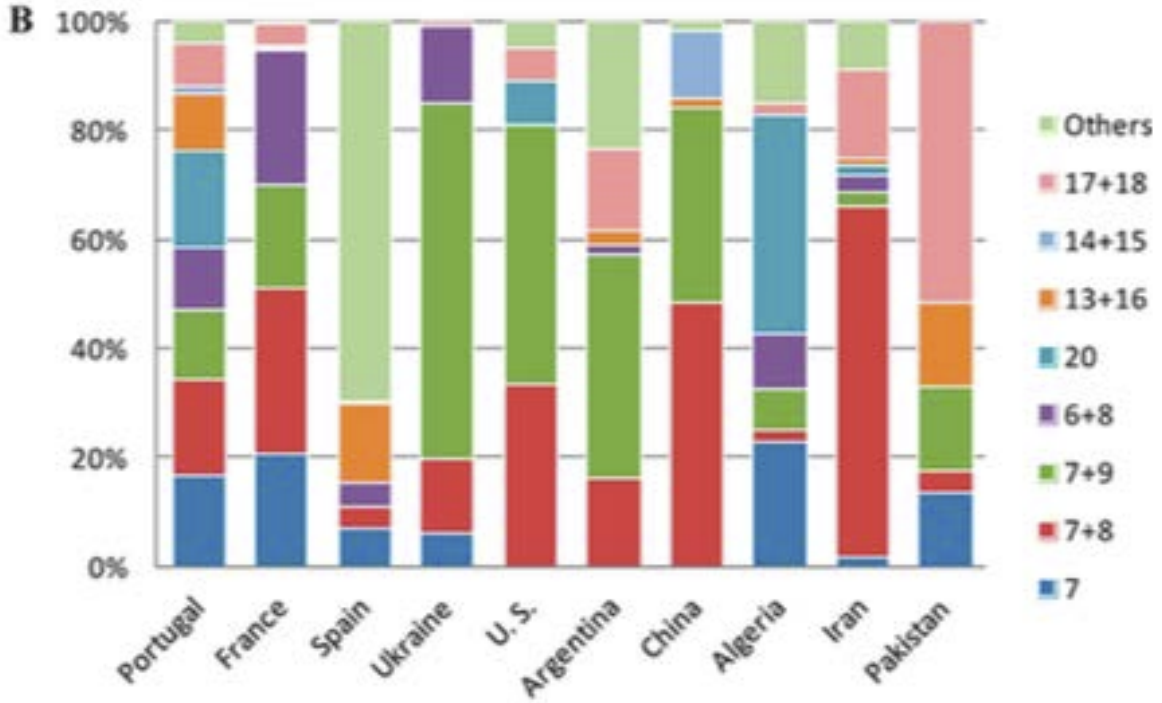
Miguel Ribeiro,[†,‡,⊥] Júlio D. Nunes-Miranda,[†,‡,⊥] Gérard Branlard,[§] Jose Maria Carrillo,[∥] Marta Rodriguez-Quijano,[∥] and Gilberto Igrejas*[,†,‡]

[†]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

[‡]Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

[§]Institut National de la Recherche Agronomique GDEC/UBP, UMR 1095, 234 av du Brezet, 63100 Clermont-Ferrand, France

[∥]Unidad de Genética y Mejora de plantas Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos Universidad Politécnica de Madrid, Madrid, España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France
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Argentina
China
Algeria
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Pakistan
Others
17+18
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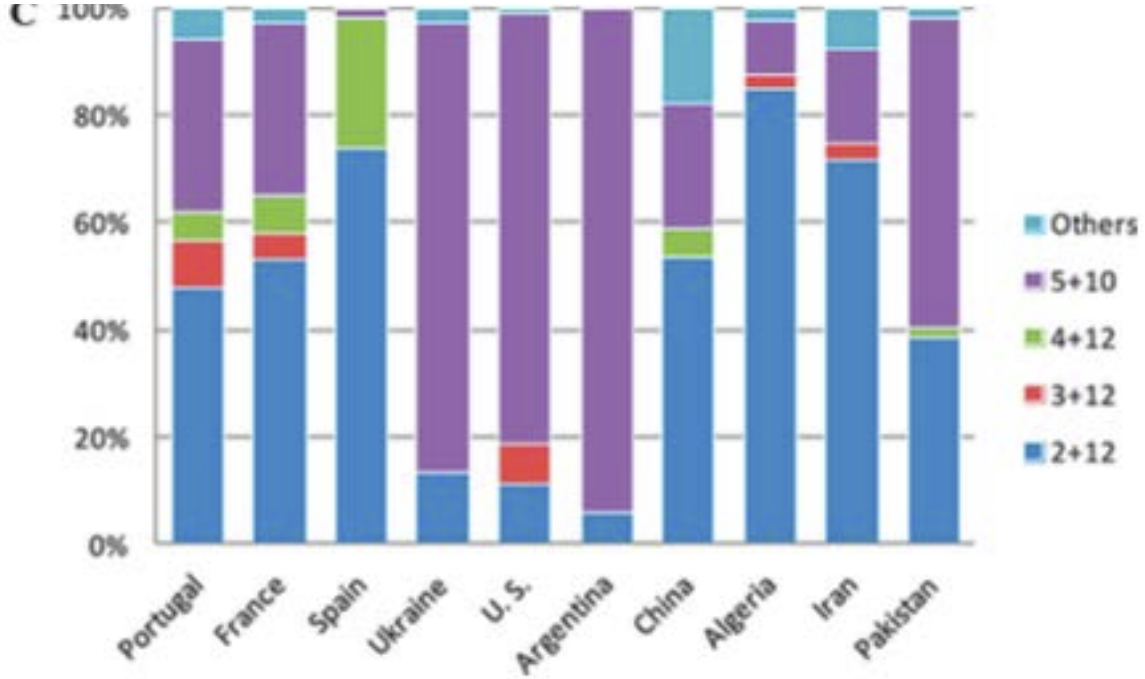
20
6+8
7+9
7+8
7

Glu-B1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Spain
Ukraine
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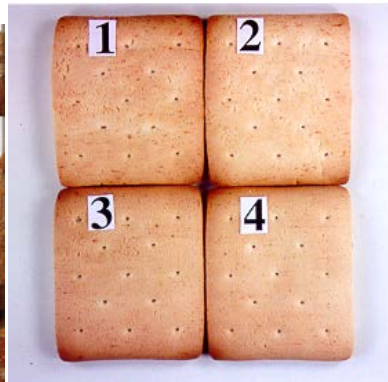
Argentina
China
Algeria
Iran
Pakistan
Others
5+10
4+12
3+12
2+12

Glu-D1

Storage proteins and score of quality

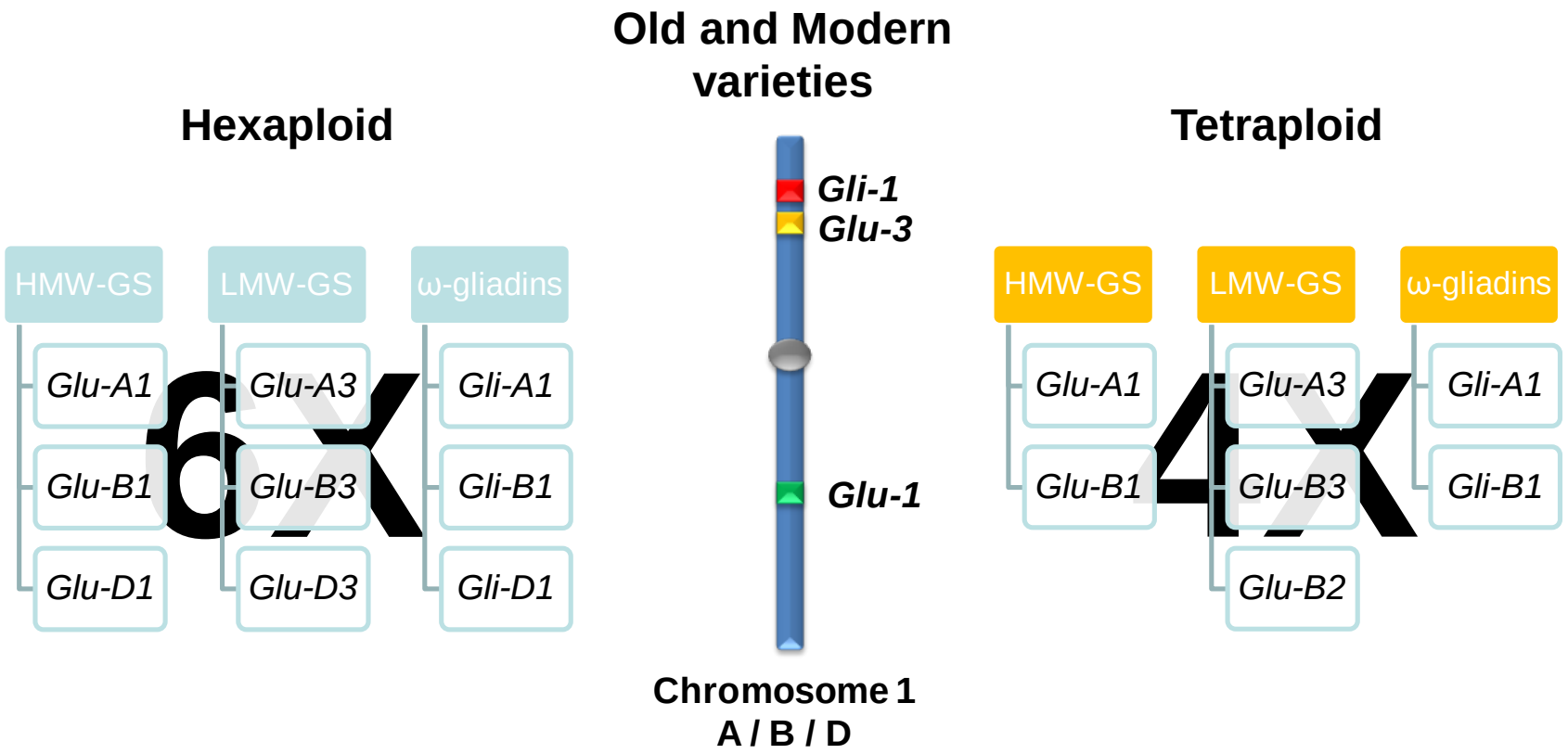
Locus	**Strenght**
• *GluA1*	2* = 1 > nul
• *GluB1*	17-18 ≥ 13-16 ≥ 7-9 = 7-8 ≥ 7 = 6-8
• *GluD1*	5-10 ≥ 3-12 = 2-12 ≥ 4-12
• *GluA3*	a = d = f ≥ e
• *GluB3*	b' ≥ d = c = c' = b = g > i > f ≥ j
• *GluD3*	a ≥ b = d = c
• *GliA2*	t ≥ k = r = f = g = j ≥ l = b = p
• *GliB2*	m >b ≥ r ≥ h = o = g ≥ ae = l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

They are the major genotypic determinants of the strength of wheat dough.
Possessing a key role in the rheological behavior of the flour during the baking process.



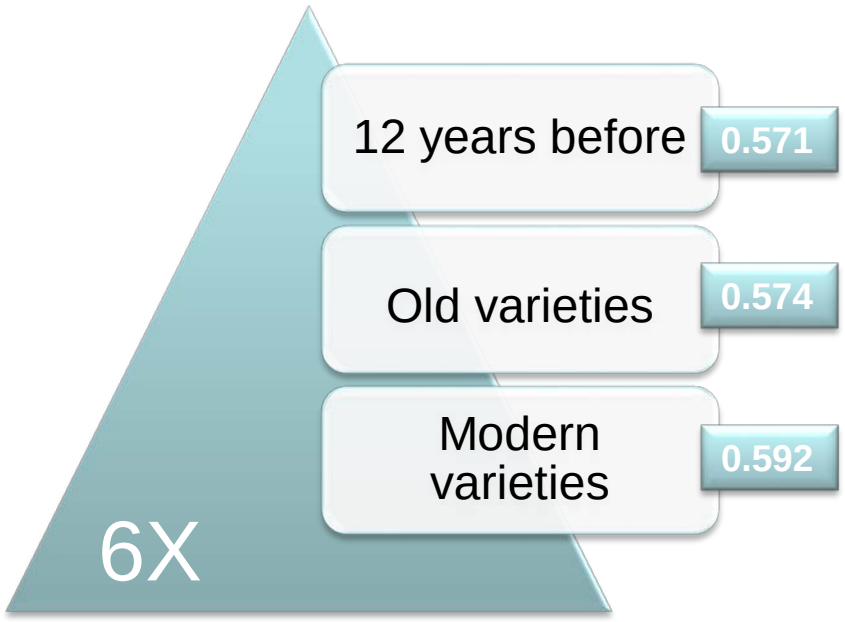
Genetic Resources

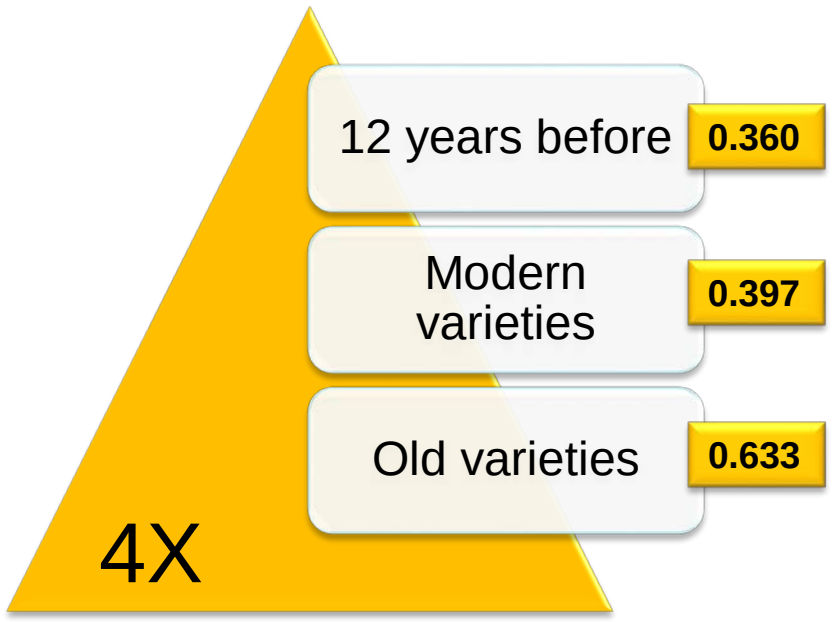
Monitoring genetic resources through analysis of storage protein alleles – Glutenins and Gliadins



Ribeiro et al. (2011). Towards allelic diversity in the storage proteins of old and currently growing tetraploid and hexaploid wheats in Portugal. *Genetic Resources and Crop Evolution* 58(7): 1051-1073.

Genetic Resources



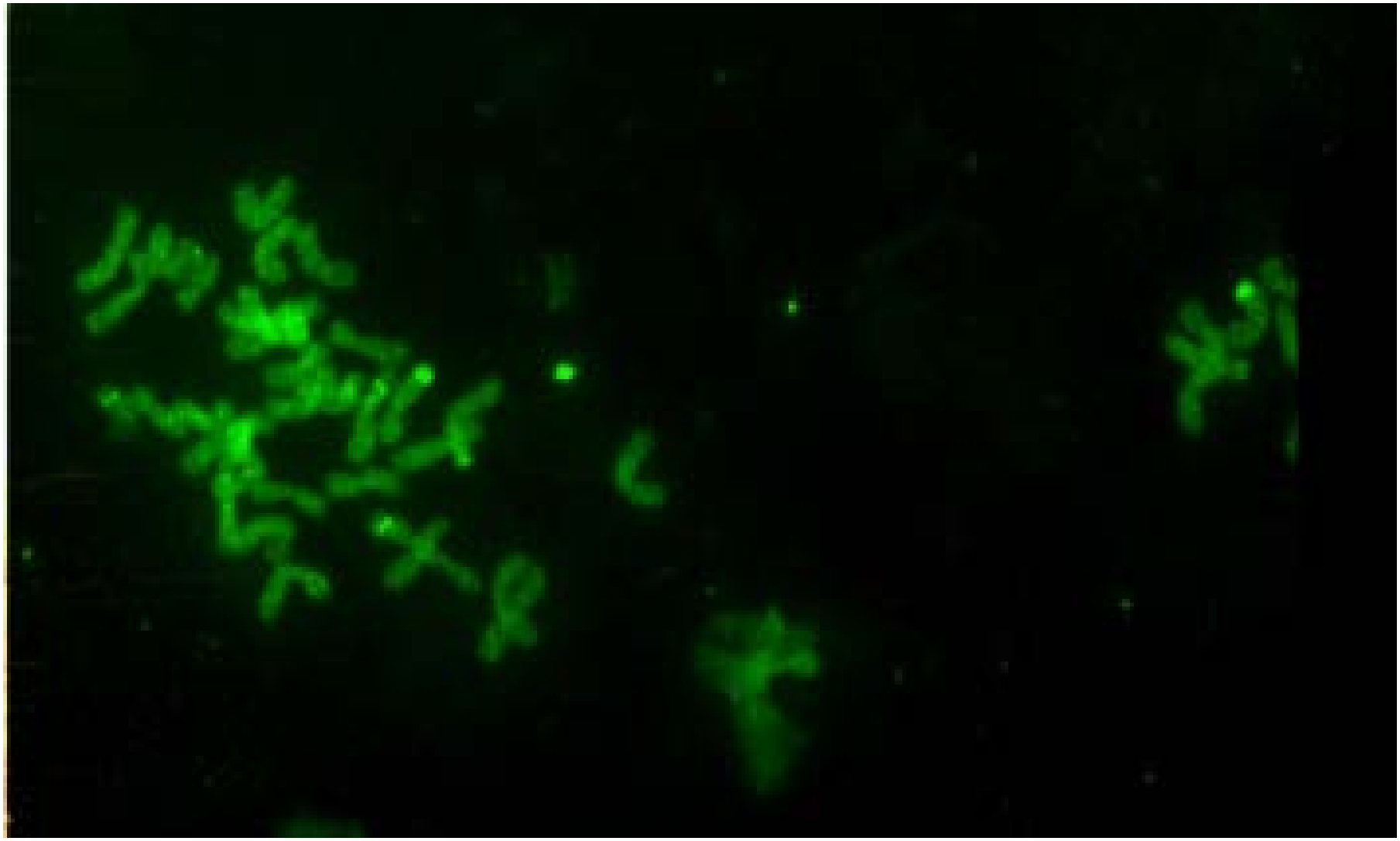


Genetic Resources

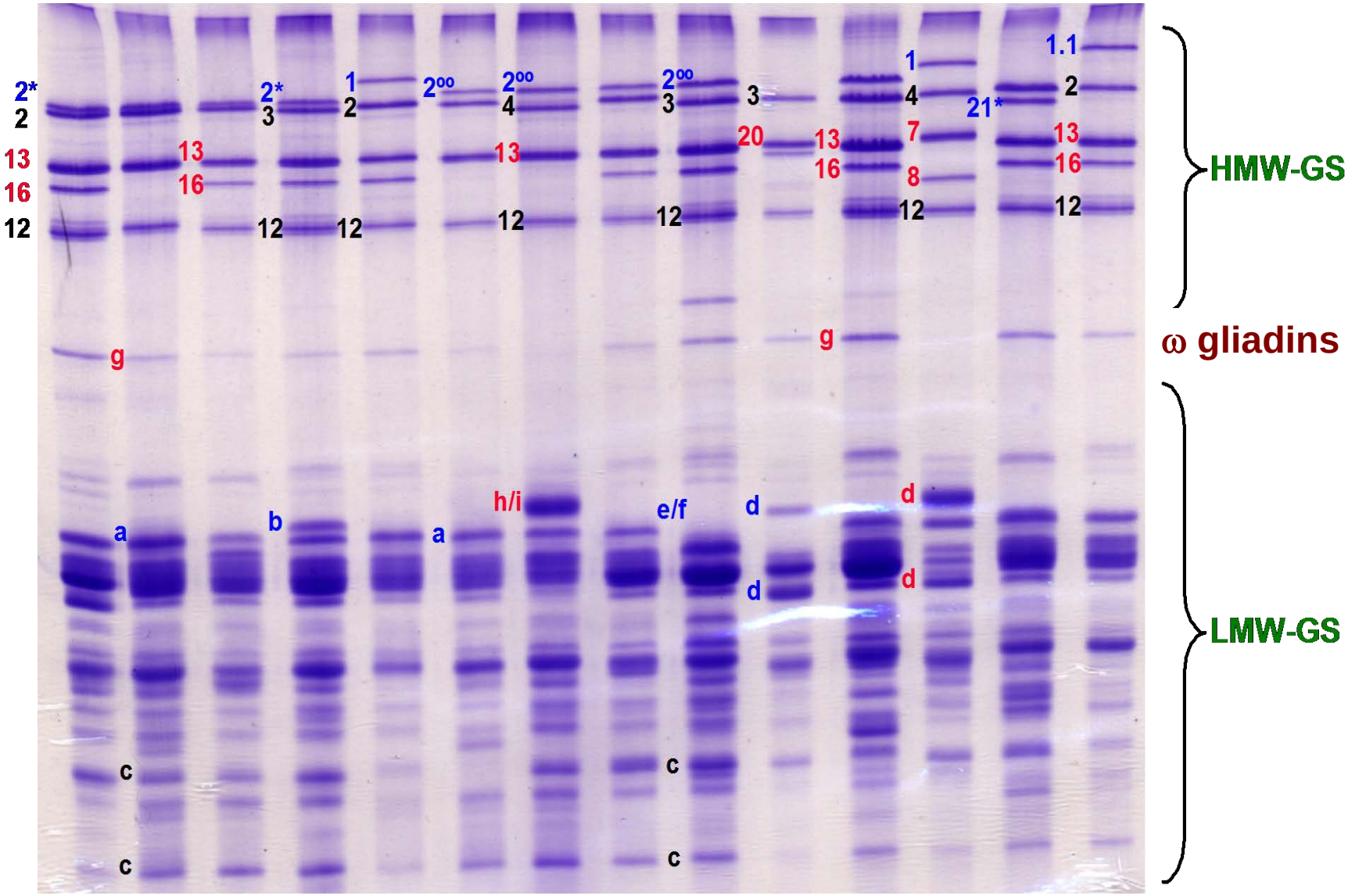
- **Modern varieties showed that the prevalence of certain alleles is due to its association with technological quality as a consequence of selection in the breeding programs**

- **Regarding the HMW-GS, approximately 90% of the combinations observed corresponded to good bread-making quality**

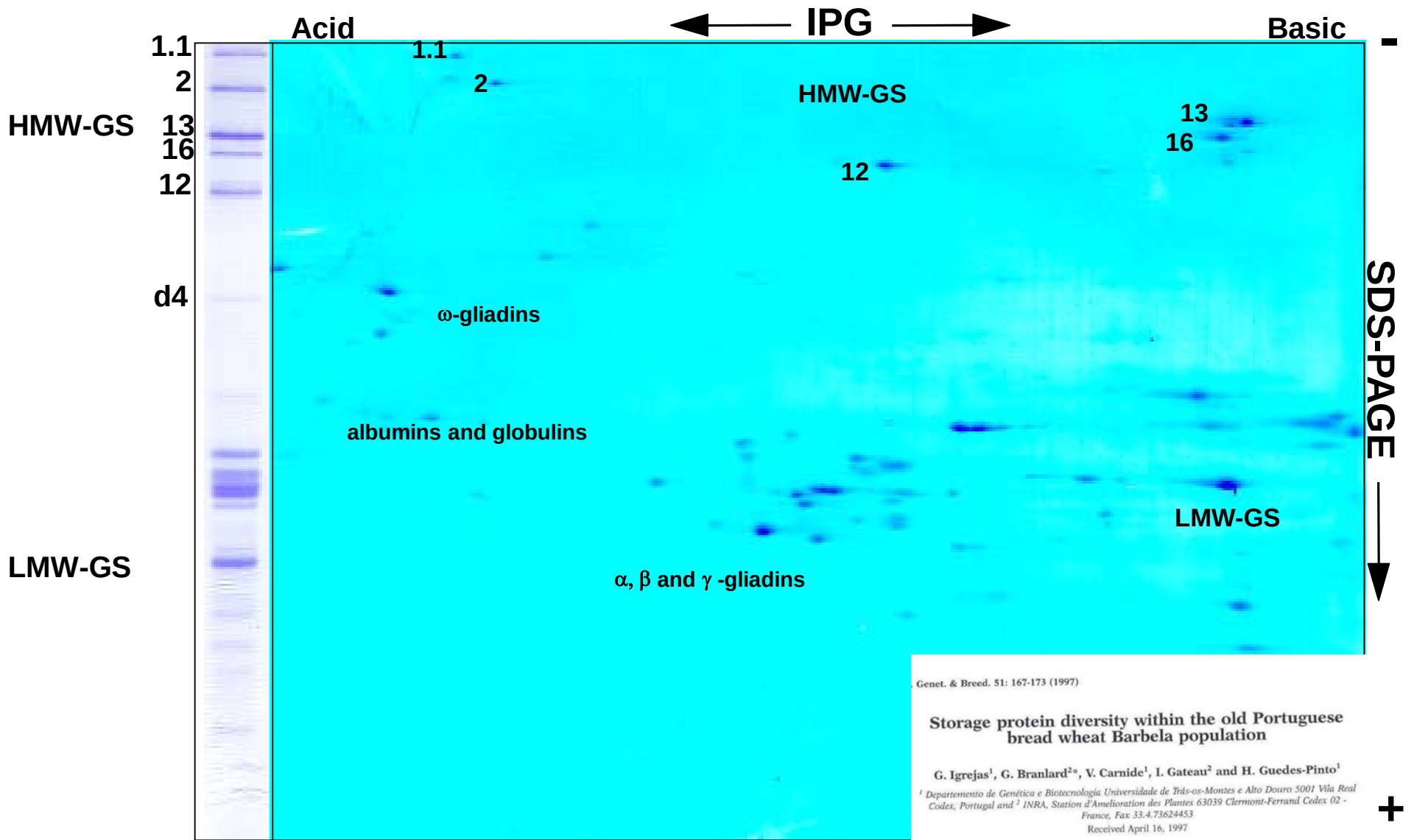
Barbela Bread Wheat



Different HMW-GS and LMW-GS of wheat lines



New HMW-GS in Portuguese wheat landrace 'Barbela‘



. Genet. & Breed. 51: 167-173 (1997)

Storage protein diversity within the old Portuguese bread wheat Barbela population

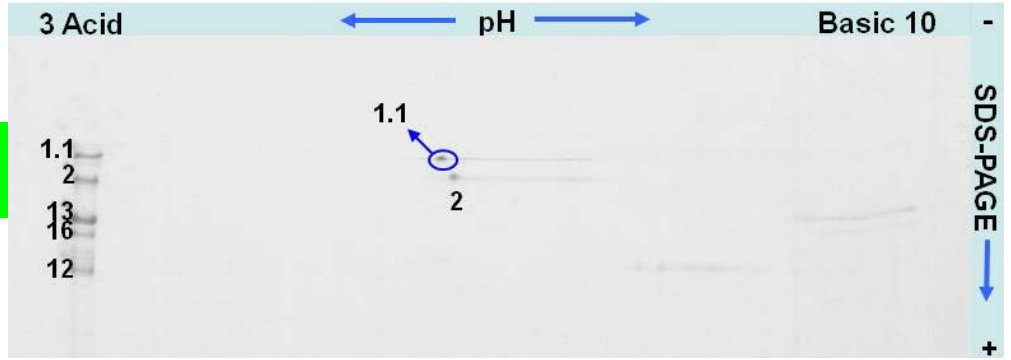
G. Igrejas[1], G. Branlard[2]*, V. Carnide[1], I. Gateau[2] and H. Guedes-Pinto[1]

[1] Departemento de Genética e Biotecnologia Universidade de Trás-os-Montes e Alto Douro 5001 Vila Real Codex, Portugal and [2] INRA, Station d'Amelioration des Plantes 63039 Clermont-Ferrand Cedex 02 - France, Fax 33.4.73624453

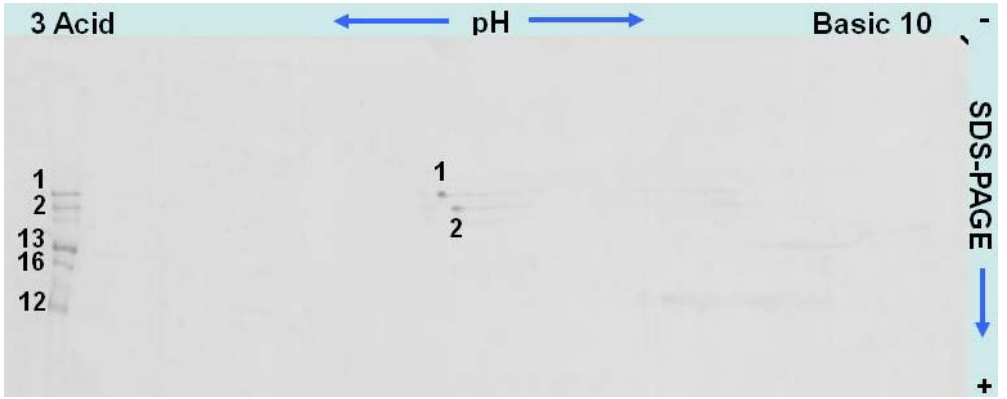
Received April 16, 1997

IEF x SDS-PAGE

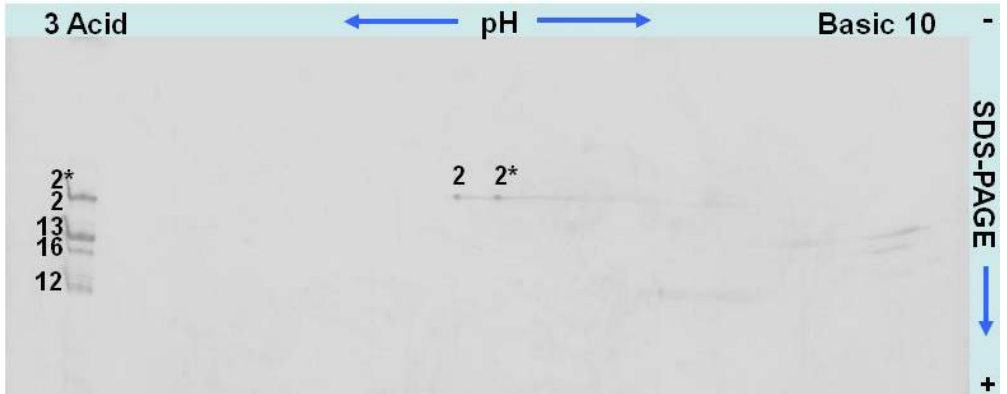
‘Barbela 28’



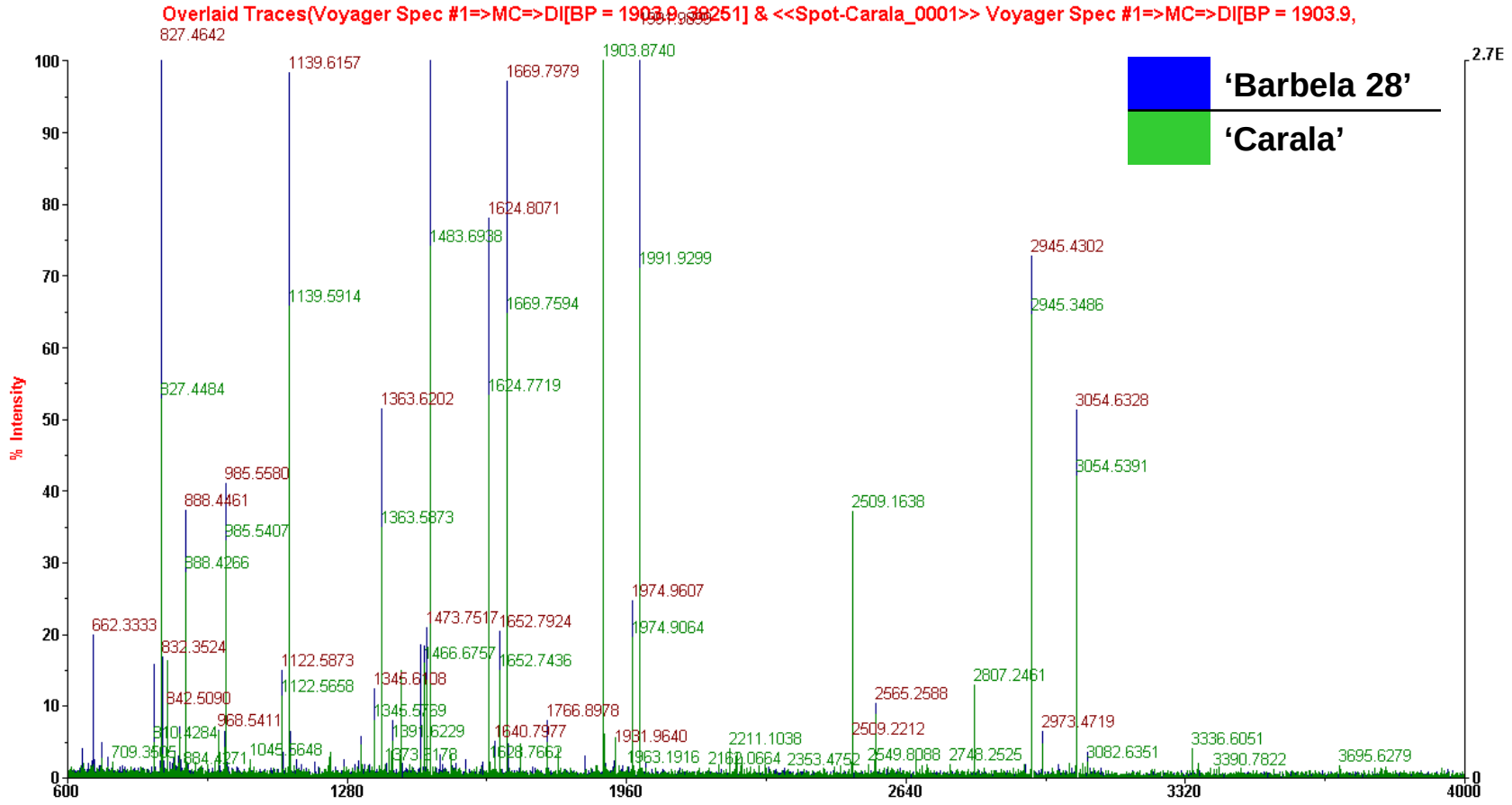
‘Carala’



‘Atlas 66’



Mass Spectrometry



1Ax1.1 Mw and pI

M*r* Subunit 1.1 = 93.648 Da

Higher molecular weight reported for HMW-GS encoded by *Glu-A1* locus

Impact on the amount and size of GMP formed; important in determining the quality

pI Subunit 1.1 = 5.7

More acidic pI reported for HMW-GS encoded by *Glu-A1* locus

BankIt1453293 1Ax1.1 JN172932

Table 1. Molecular Weights of HMW-GS from Common Wheat (*Triticum aestivum* L.) Identified and Measured by MALDI-TOF Mass Spectrometry

locus	gene	subunit	molecular mass (Da) deduced from coding gene[a] (% RSD[b])	molecular mass (Da) measured by MALDI-TOF-MS[a] (% RSD[b])	error %	ref
		1.1	91508 (0.00)	91858 (0)	0.38	41
Glu-A1	1Ax	1	87678 (0.00)	87882 (0.16)	0.23	45, 62−65
		2*	86320 (0.01)	86715 (0.52)	0.46	45, 47, 63−67
		6	86414 (0.00)	86520 (0.02)	0.12	45, 65, 67
		7	82609 (0.15)	82743 (0.37)	0.16	62−64, 66−68
		7*	unknown (−)	82279 (0.00)		47, 65
		7b*	unknown (−)	82600 (0.00)		64, 67
	1Bx	7[OE]	83134 (0.00)	82900 (0.00)	−0.28	64, 65, 67
		13	83249 (0.00)	83132 (0.16)	−0.14	48, 64, 65, 67
		14	84012 (0.00)	83139 (0.81)	−1.05	45, 48, 64, 65, 67
		17	78607 (0.00)	78422 (0.28)	−0.24	45, 48, 64, 67
		20	83913 (0.00)	83435 (0.53)	−0.57	45, 64, 67, 68
Glu-B1						
		8	75156 (0.00)	75312 (0.27)	0.21	45, 64, 65, 67
		8a*	unknown (−)	74800 (0.00)		67
		8b*	unknown (−)	75000 (0.00)		67
		9	73516 (0.00)	73426 (0.29)	−0.12	45, 47, 62, 64, 65, 67
	1By	15	75733 (0.00)	75175 (0.18)	−0.74	45, 48, 64, 65
		16	77282 (0.00)	77050 (0.19)	−0.30	48, 65, 67
		18	unknown (−)	75229 (0.15)		45, 48, 64, 67
		19	unknown (−)	75565 (0.00)		45
		20	75733 (0.00)	75448 (0.12)	−0.38	45, 64, 67
		1.5	86807 (0.00)	86649 (0.07)	−0.18	45
		2	87022 (0.00)	87218 (0.27)	0.22	45, 48, 62, 64, 65, 67
		2.2	107000 (0.00)	86620 (0.00)	−23.53	45
	1Dx	2.2*	101000 (0.00)	86340 (0.00)	−16.98	45
		3	86664 (0.00)	86400 (0.00)	−0.31	67
Glu-D1		4	87655 (0.00)	86460 (1.23)	−1.38	45, 67
		5	87940 (0.34)	88148 (0.18)	0.24	45, 48, 63−65
	1Dy	10	67478 (0.01)	67750 (0.28)	0.40	45, 47, 48, 62−65, 67, 69
		12	68652 (0.00)	68795 (0.35)	0.21	45, 48, 62, 64, 65, 67, 69

[a]Average HMW-GS molecular mass calculated from the different values cited in the references. [b]%RSD, relative standard deviation.

Mass Spectrometry

1.1 VS 1

```
  1 MTKRLVLFAA VVVALVALTA AEGEASGQLQ CERELQEHSL KACRQVVDQQ
 51 LRDVSPECQP VGGGPVARQY EQQVVVPPKG GSFYPGETTP PQQLQQSILW
101 GIPALLRRYY LSVTSPQQVS YYPGQASSQR PGQGQQPGQG QQEYYLTSPQ
151 QSGQWQQPGQ GQAGYYPTSP QQSGQEQPGY YPTSPWQPEQ LQQPTQGQQR
201 QQPGQGQQLR QGQQGQQSGQ GQPRYYPTSS QQPGQLQQLA QGQQGQQPER
251 GQQGQQSGQG QQLGQGQQGQ QPGQKQQSGQ GQQGYYPISP QQLGQGQQSG
301 QGQLGYYPTS PQQSGQGQSG YYPTSAQQPG QLQQSTQEQQ LGQEQQDQQS
351 GQGRQGQQSG QRQQDQQSGQ GQQPGQRQPG YYSTSPQQLG QGQPRYYPTS
401 PQQPGQEQQP RQLQQPEQGQ QGQQPEQGQQ GQQPGQGEQG QQPGQGQQGQ
451 QPGQGQPGYY PTSPQQSGQG QPGYYPTSPQ QSGQLQQPAQ GQQPGQEQQG
501 QQPGQGQQGQ QPGQGQQPGQ GQPGYYPTSP QQSGQEQQLE QWQQSGQGQP
551 GHYPTSPLQP GQGQPGYYPT SPQQIGQGQQ PGQLQQPTQG QQGQQPGQGQ
601 QGQQPGQGQQ GQQPGQGQQP GQGQPGYYPT SLQQSGQGQQ PGQWQQPGQG
651 LPGYYPTSSL QPEQGQQGYY PTSQQQPGQG PQPGQWQQSG QGQQGYYPTS
701 PQQSGQGQQP GQWLQPGQWL QSGYYLTSPQ QLGQGQQPRQ WLQPRQGQQG
751 YYPTSPQQSG QGQQLGQGQQ GYYPTSPQQS GQGQQGYDSP YHVSAEHQAA
801 SLKVAKAQQL AAQLPAMCRL EGGDALLASQ
```

- **Score:** 184
- **Similarity:** 27%

- **Also found a great similarity with the subunit 2***

Mass Spectrometry

Repetitive Motifs:

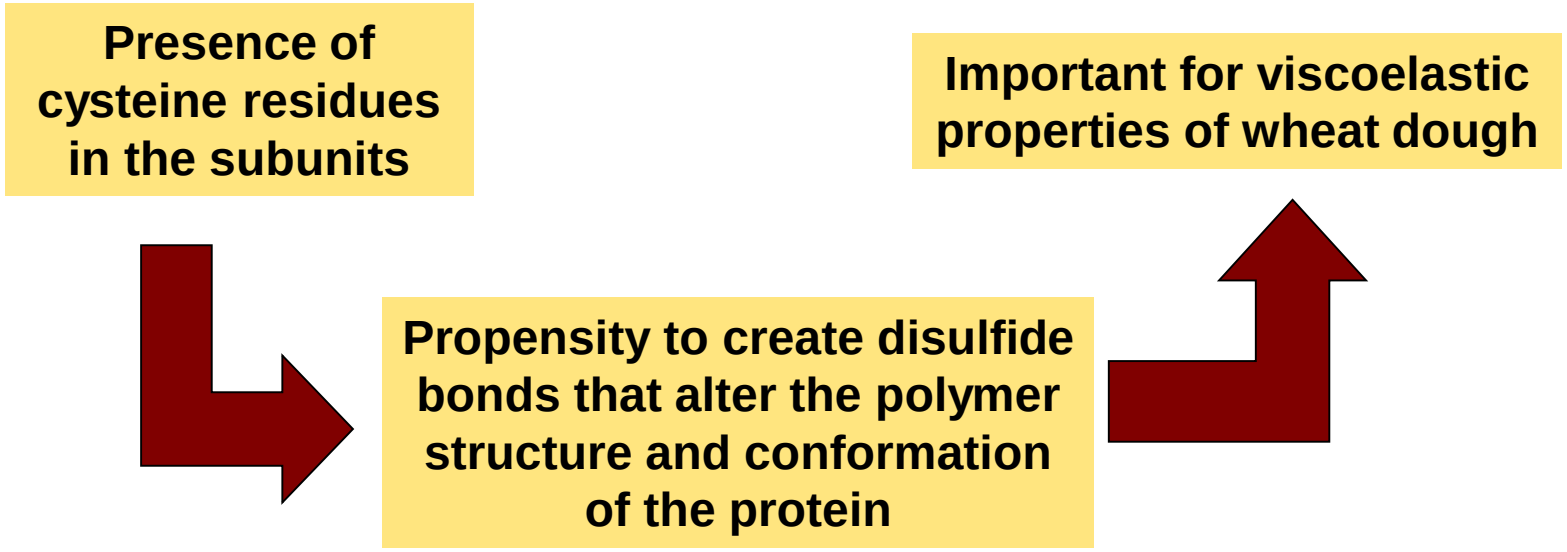
- Tripeptides: GQQ
- Hexapeptides: PGQGQQ e PGQLQQ
- Nonapeptides: LRQGQQGQQ
- Tripeptides + hexapeptides: PGQGQQGQQ
- Nonapeptides + hexapeptides: PGQGQQLRQGQQGQQ

Appearance of several glutamine residues

Amino acid related to the elastic properties of the dough due to the extensive array of interchain hydrogen bonds that form

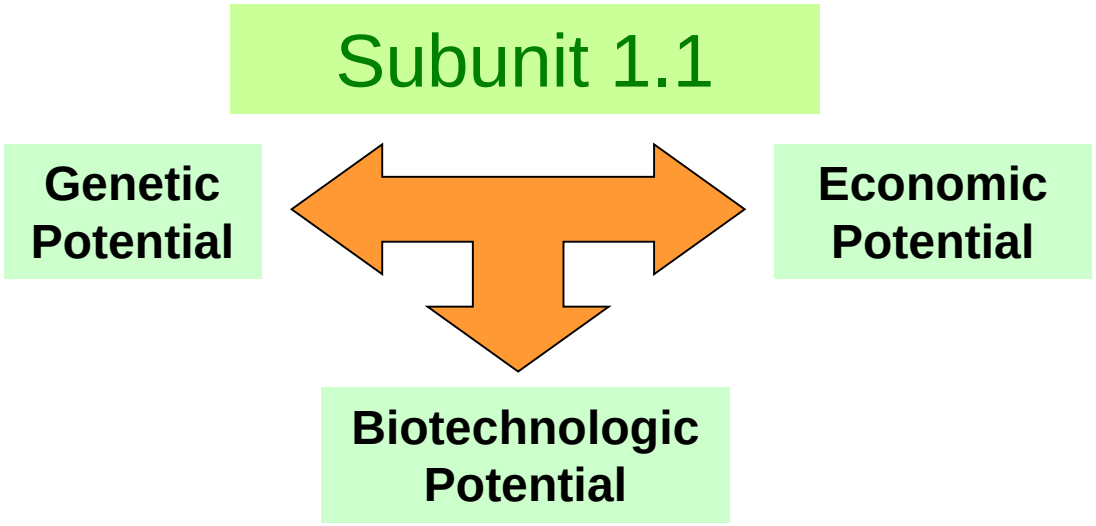
Mass Spectrometry

- The subunit 1.1 present the same number of cysteines in the N- (3) and C-terminal (1) domains as the other subunits encoded by the same locus



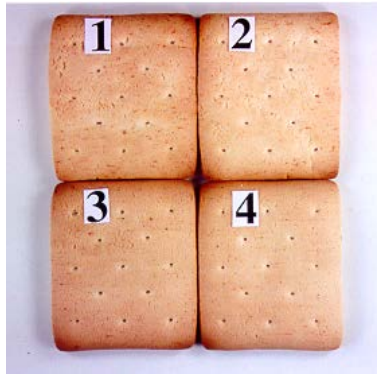
HMW-GS 1Ax1.1

- The extensibility of the dough is a crucial parameter for the production of biscuits and is known to be a character of low heritability.



Technological tests

- In recent years, the quality differences among cultivars have gained increasing importance in wheat world trade due to important economic and social trends globally.



Quality Tests

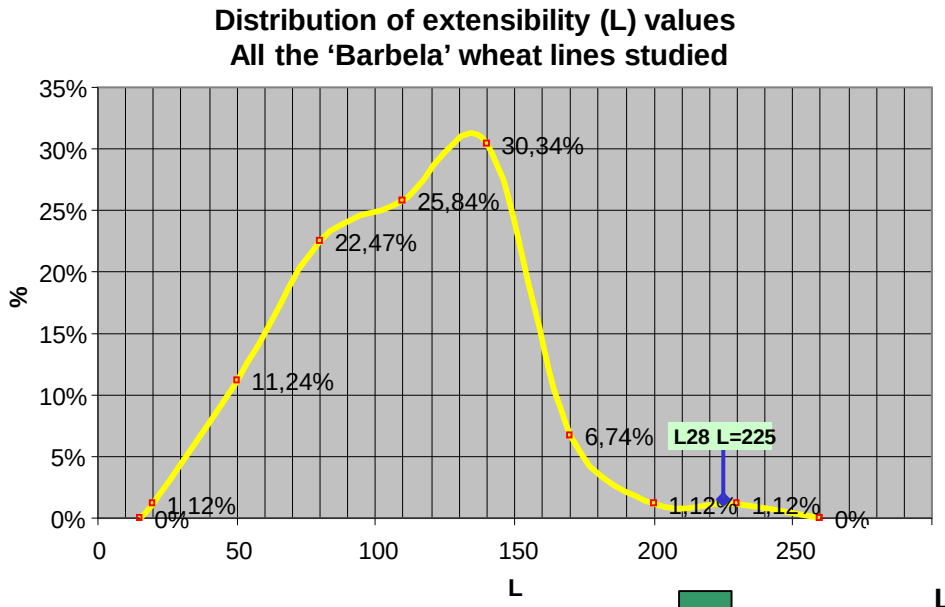
- **Storage proteins**
  - ***Glu-1***
  - ***Gli-1***
  - ***Glu-3***

- **Sweet-biscuit**
  - Speed and energy of dough extrusion,
  - Cooking time
  - Dough temperature
  - Biscuit mass before and after cooking,
  - Length, thickness, width, density and surface appearance of the biscuit.

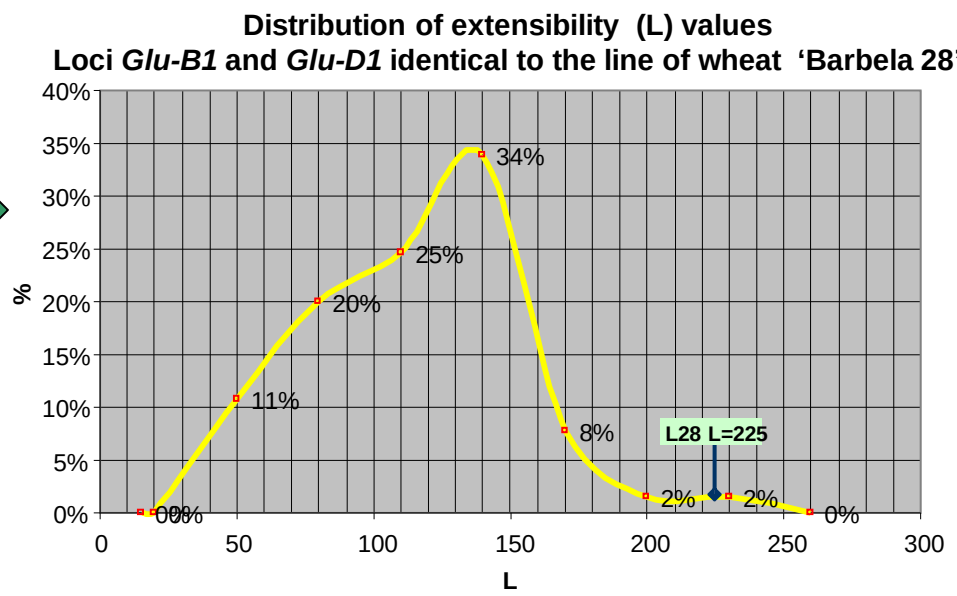
- **Chopin alveograph**
- **Mixograph**
- **Viscosity of the pentosans**
- **Zeleny test**
- **Laser granulometry**
- **Starch damage**

- **Flour yield**
- **Protein content**
- **Grain hardness**
- **Thousand kernel weight of grains**

Results of Chopin Alveograh

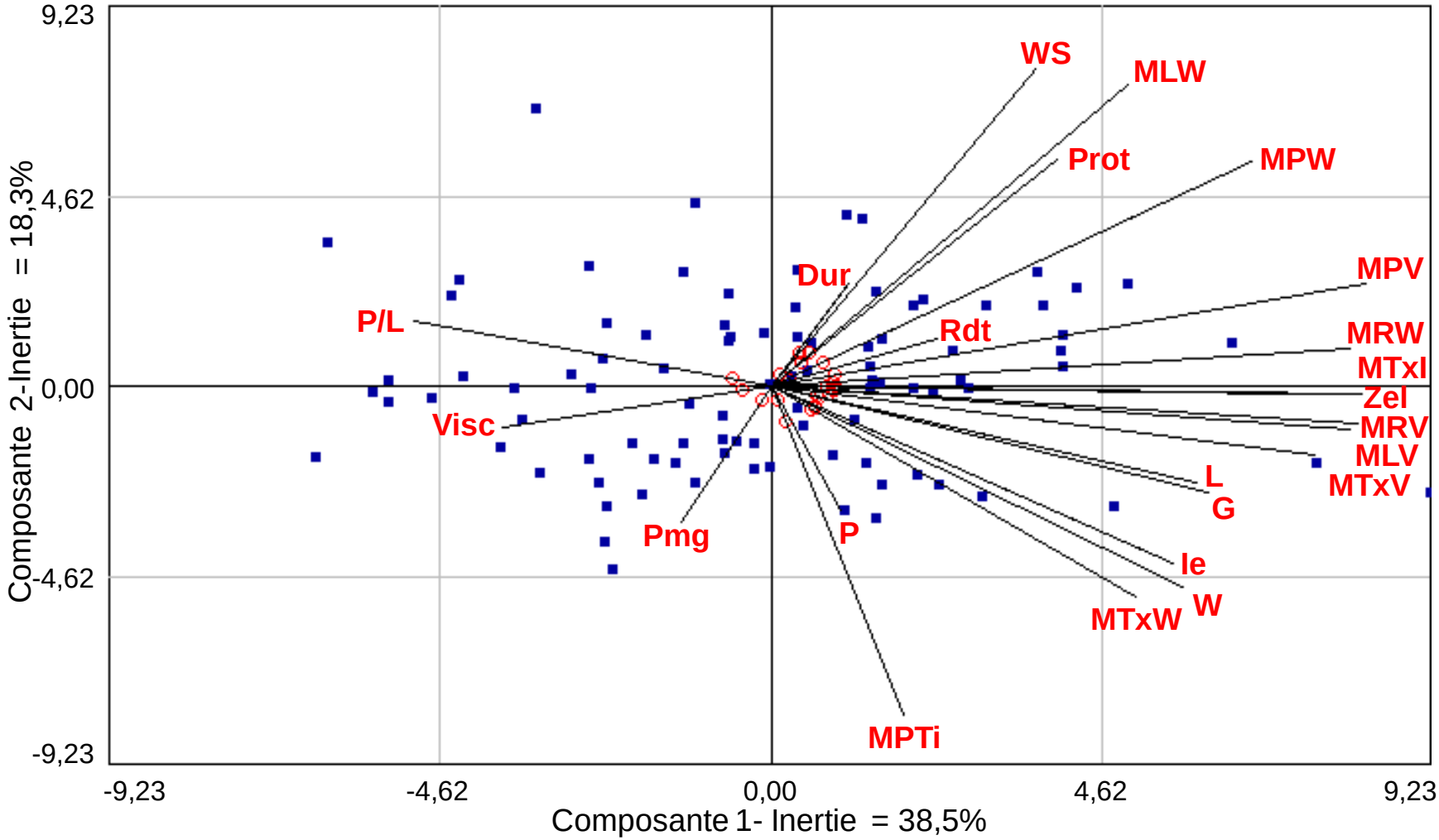


- This line represents 1.12% of the population for extensibility values greater than **225 mm**



- Selection of wheat lines with identical composition to the line evaluated to the loci *Glu-B1* and *Glu-D1.*


1
2
3
4


9,23
4,62
0,00
-4,62
-9,23
Composante 2-Inertie = 18,3%
-9,23
-4,62
0,00
4,62
9,23
Composante 1- Inertie = 38,5%
WS
MLW
Prot
MPW
MPV
MRW
MTxI
Zel
MRV
MLV
MTxV
L
G
Ie
W
MTxW
Dur
Rdt
P/L
Visc
Pmg
P
MPTi

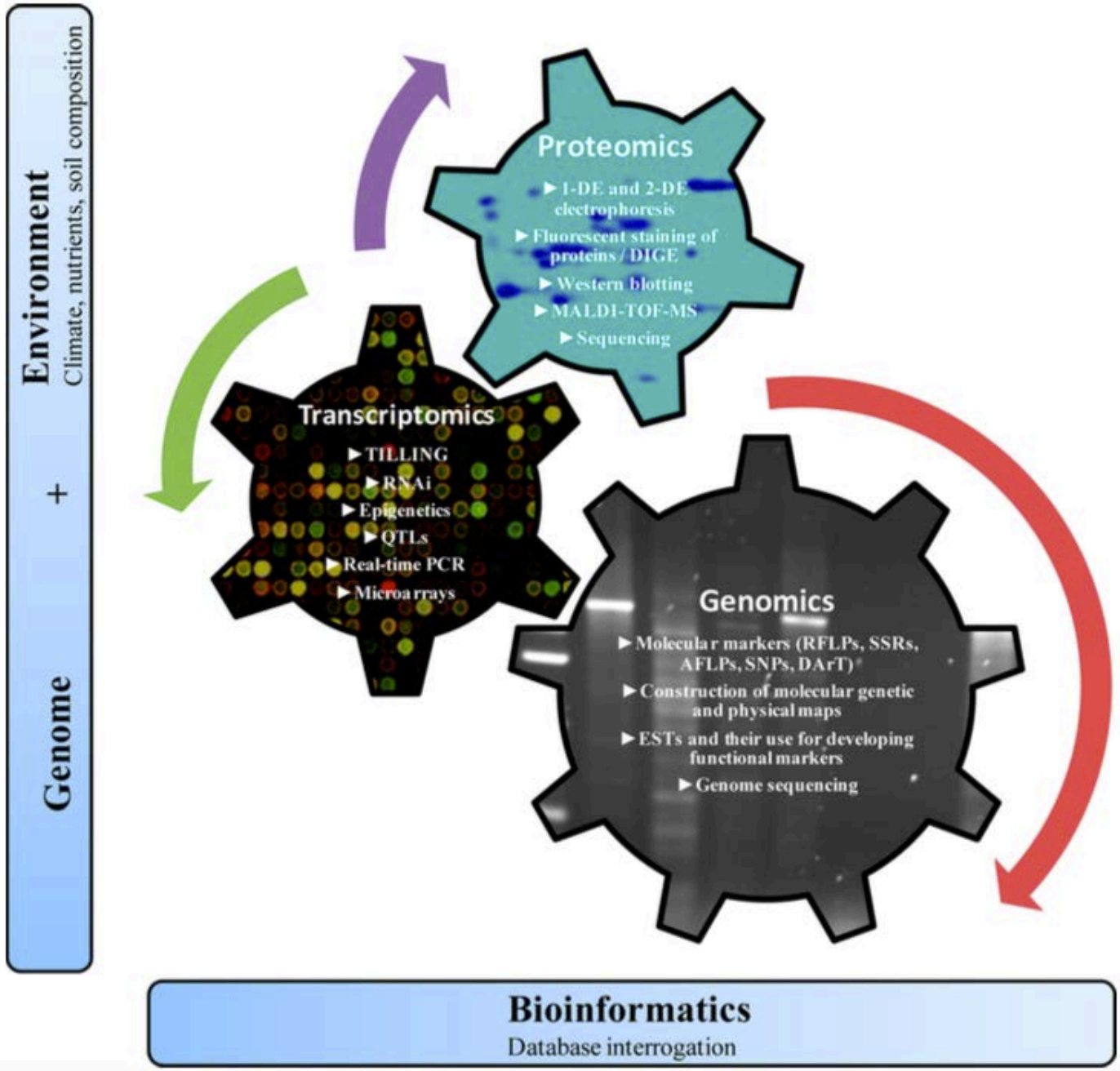


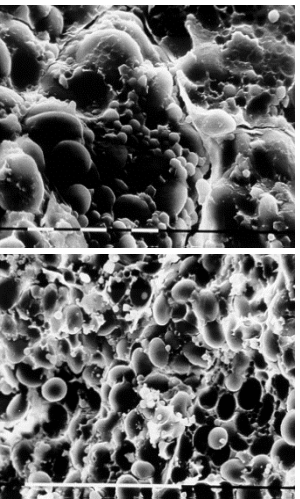
Figure 1. Integration of omics technologies to elucidate the interactions of the wheat genome and proteome with the environment.

From Proteomics to Genomics

Analysis of 12 sequences for the design of primers:

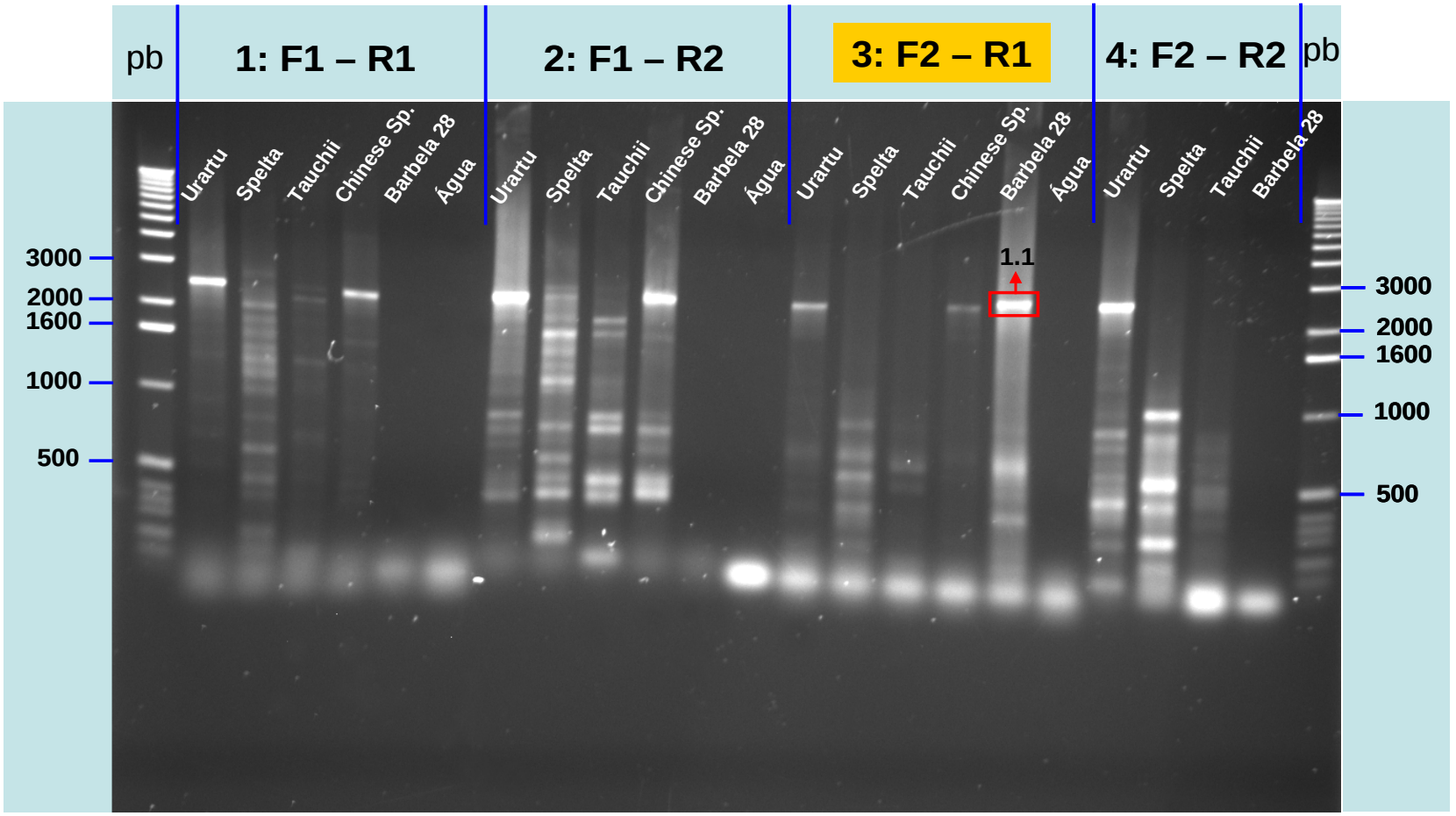
- *Glu-Ax*: 1; 2*; 2^{oo} and *Glu-Ay*
- *Glu-Bx*: 13; 20; 7 and *Glu-By*: 16; 8; 15; 9
- *Glu-Dx* and *Glu-Dy*: 12*; 10

Together with data from mass spectrometry



Primers Forward	gluA**F1**: 5'-CCGACAGTCCACCGAGATGA-3'
	gluA**F2**: 5'-CGAGATGACTAAGCGGTTGGTT-3'
Primers Reverse	gluA**R1**: 5'-GAGTTCTATCACTGGCTGGCCA-3'
	gluA**R2**: 5'-ATAGCTAAGGTGCATGCATGCC-3'

PCR



Amplification of a sequence of about 2,600 bp in ‘Barbela 28’ wheat line

Coding sequence of HMW-GS 1.1

The sequence obtained (GenBank accession JN172932) showed a high identity with those present in public databases for subunits encoded by the same locus, such as the subunit 1.

1.1 VS 1

95 % of Identity

1.1 VS 1

- The 1.1 ORF showed about 108 more base pairs than the ORF of subunit 1

- Several differences were found between the two sequences:

- 46 single-base substitutions
- 12 two-base substitutions
- 9 three-base substitutions
- 1 four-base substitution
- 1 six-base substitution
- 1 single-base insertion

- 4 two-base insertions
- 1 four-base insertion
- 6 single-base deletions
- 4 two-base deletions
- 2 four-base deletions
- 1 five-base deletion

1.1 HMW-GS Aminoacidic sequence

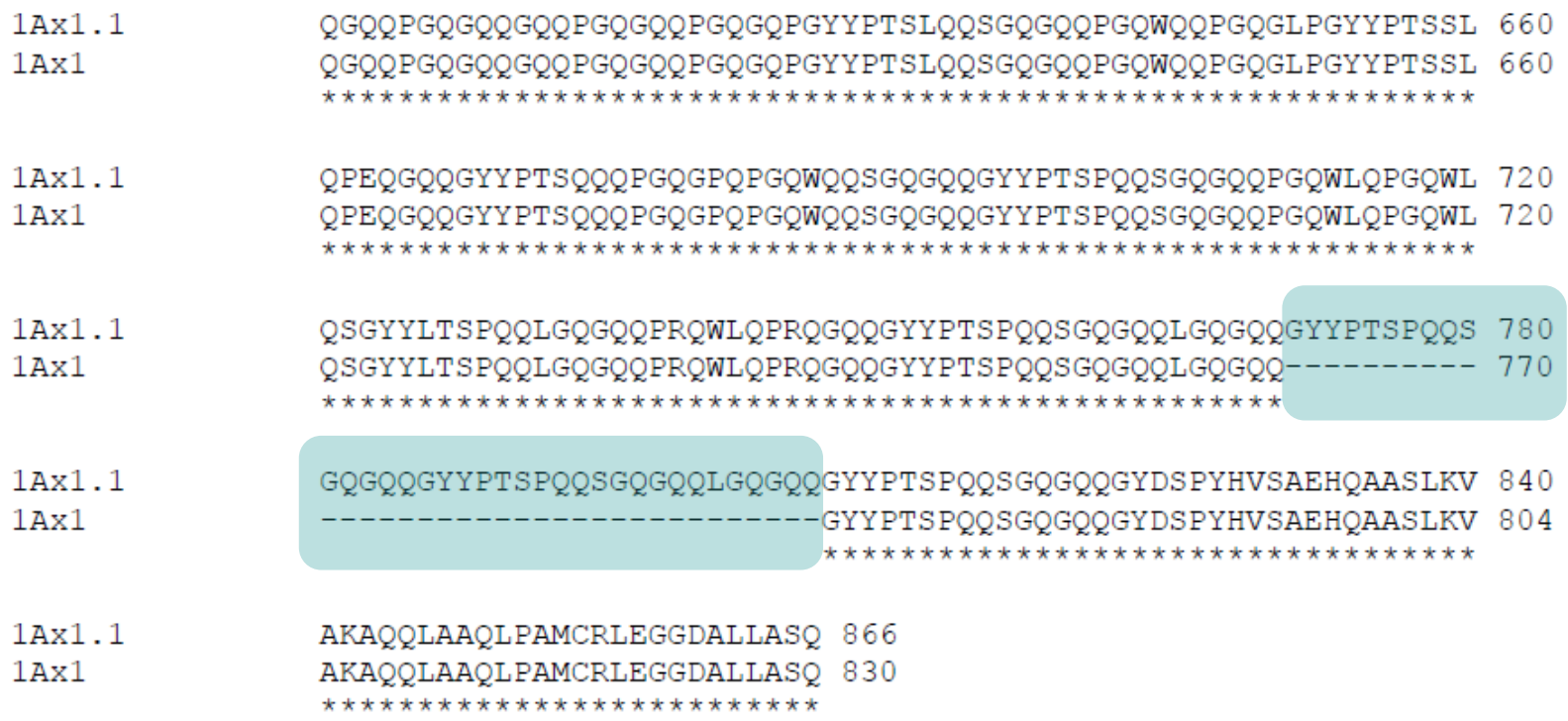
- HMW-GS 1.1 shows a length of 866 amino acids, being HMW-GS 1 thirty-six amino acids shorter.
- The predicted amino acid composition of N- and C-terminal domains of subunit 1.1 is identical to the subunit 1, as expected due to the results previously obtained by mass spectrometry.
- All the subunit 1 cysteines were conserved in subunit 1.1 and no extra cysteine residue was observed in the central repetitive domain of 1.1.
- In addition, 2 highly conserved substitutions were observed at positions +29 and +162 in the N-terminal and C-terminal domains respectively.

1.1 HMW-GS Aminoacidic sequence

- An insertion of 36 amino acids was observed in the end of the central repetitive domain at position +771.

- These mutations led to some changes in the HMW-GS 1.1 repetitive motifs, namely tripeptide (GQQ), hexapeptide (LGQGQQ) and nonapeptide (GYYPTSPQQ) motifs in the 36-aa insertion, compared with HMW-GS 1.

1.1 VS 1



36-aa insertion

‘Barbela’ landrace used in a breeding programme

Year	crosses
2013	Barbela x French elite wheat A (med-hard, strong dough)
	Barbela x French elite wheat B (med-hard, extensible dough)
2014	(Barbela x Elite A) x Elite A, BC1
	(Barbela x Elite B) x Elite B, BC1
2015	BC1S1
2016	BC1S2
2017	BC1S3, HMW glutenin selection
2018	BC1S4, HMW glutenins and hardness selection, indirect quality tests
2019	BC1S5, yield trials and advanced quality tests (W Chopin, gluten,…)

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Final Considerations

- The very high molecular weight fraction of glutenins is considered important in determining wheat flour quality. Thus, the very high molecular weight of this subunit can obviously have positive effects on the formation and size of the glutenin macropolymer (GMP).

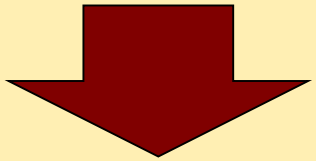
- The GMP and its rheological properties are extensively reported to be good quality predictors.

- GMP content is strongly related with the maximum resistance (Rmax) and extensibility of dough.

This relationship appears to be intrinsically linked to the high extensibility of the dough provided by HMW-GS 1.1

Final Considerations

- In summary, this project provided a better understanding of the molecular and genetic basis of this unique HMW-GS.



- The knowledge gained can now be used in breeding programs, particularly in the ongoing breeding cultivars with high quality, to develop the extensibility of wheat in terms of food biotechnology.

- **Omics of wheat endosperm: a tool to find genes involved in kernel composition and quality.**

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