KEY FACTS: What, why, when, where, how, who…?

- Wheat has been one of the most important food grain sources for humans throughout history.
- This cereal is grown on more land area than any other crop (>218 million Ha) and over 700 million tonnes are harvested annually.
- Glutenins and gliadins are the main protein components of technological characteristics of wheat flour.
- *Glo-2* genes are closely linked to the HMW glutenin genes at the *Glu-1* locus.

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Expression and variation of *Glo-2* locus in bread wheat (*Triticum aestivum* L.)

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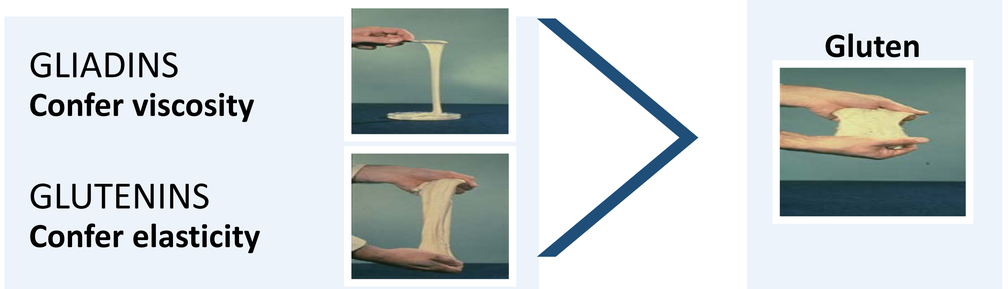
Wheat is the most widely grown cereal crop in the world and the wheat seed is the single greatest source of protein in the human diet. Wheat prolamins are subdivided into gliadins and glutenins according to their polymerisation properties and represent about 80 % of the total protein in the wheat grain. *Glo-2* genes are closely linked to the HMW glutenin genes at the *Glu-1* locus. Analysis of their sequence raises the possibility that the product of these genes are incorporated into the gluten matrix.

Wheat endosperm proteins

The glutenins and gliadins are the two main protein components that determine in a complementary way the technological characteristics of wheat flour. They are crucial to the quality of the gluten network formed during the baking process and seen as a major end-use determinant [1].



Material & Methods

Eight bread wheat cultivars were used, respectively: 'Chara', 'Chinese Spring', 'Egret', 'Gabo', 'Glenlea', 'Halberd', 'Janz' and 'Suneca'. The bread wheat samples were selected on the basis of different bread making quality and differences in the *Glu-D1* locus. The lines represented respectively 2+12 subunits (Cultivars 'Chara', 'Chinese Spring', 'Gabo' and 'Janz') and 5+10 (Cultivars 'Egret', 'Glenlea', 'Halberd' and 'Suneca'). The 'Chinese Spring' nullitetrasomic lines N1AT1B, N1AT1D, N1BT1A, N1BT1D, N1DT1A and N1DT1B were used as control for chromosome assignment of the PCR products.

Genomic DNA from kernels was prepared using NucleoSpin® Kit (Macherey-Nagel) following the procedure of application manual. PCR was performed in a Hybaid thermocycler and reactions were performed in a final volume containing 150-200 ng genomic DNA using HotStar Taq polymerase (1 U, Qiagen), 10x PCR buffer, 1.25 mM of dNTP's, and 5 pm of each PCR primer Glo2F (5'-ATATGGG(C/T)A(A/G)GTTCGTCTTCT-3') and Glo2R1D (5'-CTAGTAGTACTGGTCGGCGGT-3').

Individual amplification products were treated according to QIAquick PCR Purification Kit Protocol of the QIAquick Spin Handbook (Qiagen). Individual amplification products were then sequenced using the appropriate PCR primers using BigDye terminator mix (Applied Biosystems Foster City, Calif.) as per the manufacturer's instructions.

The synthetic peptides based on the glo-2 sequences (in red, see below) were used to raise antisera after coupling to ovalbumin. Only the peptide RHGSSGERQQEQGC produced suitable antisera that was used for Western blotting.

Analysis of PCR and sequencing analyses

Comparison of globulin gene sequences obtained from the different wheat cultivars showed one single nucleotide polymorphism (SNP). HMWG 2+12 subunits carry an adenine at Glo2 position 20 whereas varieties with 5+10 subunits have a guanine residue. The D genome is an important determinant of flour functional quality, and wheats possessing Dx5 + Dy10 genes are associated with high quality, while the composition Dx2 + Dy12 is associated with poor dough quality. The position and the similarity in sequences between the globulin and HMW glutenin genes indicates the presence of these two genes as part of the original duplication creating the paralogous x- and y-type HMW glutenin genes, and a paralogous *Glo-2* gene and a fragmented globulin adjacent to the Dx gene.

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CS          ATGGGTAGGTTCGTCTTCTTCGCGGTGTTCCTGGCGGCCCTGGTAGCCGTCTCCGCCGCC
Glenlea     ATGGGTAGGTTCGTCTTCTTCGCGGTGTTCCTGGCGGCCCTGGTGGCCGTCTCCGCCGCC
Halberd     ATGGGTAGGTTCGTCTTCTTCGCGGTGTTCCTGGCGGCCCTGGTGGCCGTCTCCGCCGCC
Janz        ATGGGTAGTTTCGTCTTCTTCGCGGTGTTCCTGGCGGCCCTGGTAGCCGTCTCCGCCGCC
Gabo        ATGGGTAGGTTCGTCTTCTTCGCGGTGTTCCTGGCGGCCCTGGTAGCCGTCTCCGCCGCC
Chara       ATGGGTAAGGTCGTCTTCTTCGCGGTGTTCCTGGCGGCCCTGGTAGCCGTCTCCGCCGCC
Egret       ATGGGGAGGGTCGTCTTCTTCGCGGTGTTCCTGGCGGCCCTGGTGGCCGTCTCCGCCGCC
Suneca      ATGGGTAAGGTCGTCTTCTTCGCGGTGTTCCTGGCGGCCCTGGTGGCCGTCTCCGCCGCC
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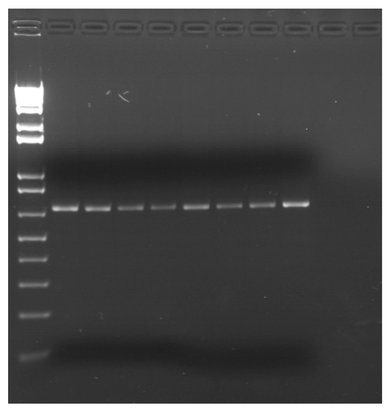
Comparative alignment of the Glo-2 PCR fragment nucleotide sequences for the eight varieties studied from the start of translation.

Comparison of deduced protein sequences

To verify the applicability of PCR-based methods in identification of Glo-2 genes, samples of eight bread wheat cultivars were analyzed using primers Glo-2F and Glo-2RD, as described above. In all the cases the 650-700 bp amplification product was present.

Cultivar	1Ax	1BX	1By	1Dx	1Dy
Chara	2*	7	8	2	12
Chinese Spring	N	7	8	2	12
Egret	N	7	8	5	10
Gabo	2*	17	18	2	12
Glenlea	2*	7	8	5	10
Halberd	1	7	9	5	10
Janz	1	7	8	2	12
Suneca	1	17	18	5	10

Nomenclature of Payne and Lawrence (1983) [2].



PCR amplification of primers Glo-2F and Glo-2RD specific for globulin gene encoded by *Glo-2* locus. Lanes M, SPP1 DNA ladder, 1 'Chara', 2 'Chinese Spring' 3 'Egret', 4 'Gabo', 5 'Glenlea', 6 'Halberd', 7 'Janz', 8 'Suneca', 9 'Chinese Spring' N1DT1A and 10 Water control.

Conclusions

These results indicated that SNP markers could be produced from Glo-2 sequences which could be used for tracing HMW glutenin alleles or to follow attributes determined by the Glo-2 proteins themselves. These results may allow subsequent development of PCR-based markers to facilitate marker-assisted selection (MAS) of specific *Glo-2* alleles and HMW glutenins. This knowledge contributes to the strategic conservation of wheat genetic resources and improvement of wheat breeding to meet the challenges of the 21st century.

Production of monospecific antisera and accumulation of globin during development

Accumulation of the putative product of the Glo-2 genes was investigated using purified antisera raised against the encoded polypeptide. The aim of this work was to investigate the accumulation in the endosperm of protein corresponding to the EST-deduced sequence. The polypeptide could not be detected at 10 DAF but could be detected from 25 DAF in the soluble phase and was present in the mature grain. Overloading of protein samples enabled the polypeptide to be detected in the insoluble phase as well.

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MGRFVFFAVFLAALVAGSAAQGVLEQSLTDAQCRGEVQEKPLLACRQILEQQLTGRAGEGAVGVPLFHAQ
WGARERCCRQLESVSRECRCAALRGMVRDYEQSMPPLGEGRHGSSGERQQEQGCSGESTEPEQRQEVQGG
QYGSETGGGQQQGGGYHGVTVGRGGQRQGQVLCHKRPQRQQGEGFSGEGAQQKPQAGRVRLTKVRLPTAC
RIEPQECSVFTADQYY.
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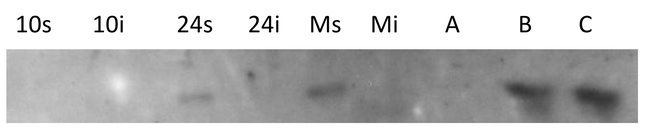
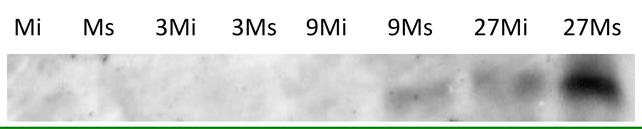
Deduced sequence of globin-2 *Aegilops tauschii* showing the regions used to raise antisera in red.



Western blot of soluble(s) and insoluble (i) fractions. The numbers refer to days after anthesis. A, B, C are 3 times 10s, 24s and Ms respectively.



Western blot of 3 fold increases of insoluble and soluble fractions at maturity in order to determine relative amounts of globin-2 .The amounts loaded in (a) and (b) are not identical.

References:

1. Wieser H (2007) Chemistry of gluten proteins. Food Microbiol 24 (2):115-119.

3. Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex loci, Glu-A1, Glu-B1 and Glu-D1 which coded for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Research Communications 11:29-35.