Effects of HMW-& LMW-glutenins and grain hardness on size of gluten polymers

Véronique S. Lesage, L. Rhazi, T. Aussenac, B. Meleard, Gerard G. Branlard

11th International Gluten Workshop

Program and Abstracts

Beijing, China
August 12-15, 2012

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Organizers

Chinese Academy of Agricultural Sciences/Institute of Crop Science/National Wheat Improvement Center
Chinese Academy of Sciences/Institute of Genetics and Developmental Biology/State Key Laboratory of Plant Cell and Chromosome Engineering
International Maize and Wheat Improvement Center (CIMMYT)

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Conference Exhibitors

Brabender
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11th International Gluten Workshop

Aug 12-15, 2012, Fragrant Hill Empark Hotel, Beijing, China

Sunday, Aug12, 2012

8:00-17:00 Registration at hotel lobby, participants collect conference bag with documents

Monday, Aug 13, 2012

8:30-9:00 Opening ceremony and group photo

Session 1 Capturing discoveries from genomics, proteomics, and transcriptomics

Chairs: Angela Juhasz and Daowen Wang

9:30-10:00 Keynote presentation, wheat A genome sequencing and its application for quality modification, Daowen Wang, Chinese Academy of Sciences, China

10:00-10:30 Keynote presentation, proteome bioinformatics and genetics for associating proteins with grain phenotype, Rudi Appels, Murdoch University, Australia

10:30-11:00 Keynote presentation, creating a detailed map of the wheat flour proteome: a critical step in understanding the effects of environment on flour quality and immunogenic potential, Susan Altenbach, USDA-ARS, USA

11:00-11:20 Monitoring of storage proteins: accumulation and impact of high temperature in wheat kernel using proteomics, Gerard Branlard, INRA, France

11:20-11:40 The effects of nitrogen application on transcriptome, protein composition, and bread making quality of UK wheat varieties, Yongfang Wan, Rothamsted Research, UK

11:40-12:00 Proteomic analysis of mature wheat kernels of transgenic bread wheat expressing PGIP infected *Fusarium graminearum*, Renato D'Ovidio, University of Tuscia, Italy

12:00-12:20 Daily changes in the phosphoproteome of the Dinoflagellate Lingulodinium, Bolin Liu, China National Seed Group, China

12:20-12:40 Towards understanding genetic basis of chapatti (Indian flat bread) making quality, Monika Garg, National Agri-Food Biotechnology Institute, India

12:40-13:40 Lunch

Session 2 Biosynthesis, structure, and functional analysis of storage protein

Chairs: Gerard Branlard and Guangyuan He

13:45-14:15 Keynote presentation, an asparagine residue affects LMW-GS maturation processing, Stefania Masci, Universita della Tuscia, Italy

14:15-14:45 Keynote presentation, studying the intracellular trafficking and tissue distribution of gluten proteins by epitope-tagging, Paola Tosi, Rothamsted Research, UK

14:45-15:05 Trafficking pathway of wheat storage proteins in transgenic rice endosperm, Maria Oszvald, Eotvos University, Hungary

15:05-15:25 Virus-induced gene-silencing in wheat spikes and grains and its application in functional analysis of HMW-GS-encoding genes, Huixian Zhao, Northwest A & F University, China

15:25-15:45 Gluten protein structures: variation in wheat grain and at various applications, Eva Johansson, Swedish University of Agricultural Sciences, Sweden

15:45-16:05 A model for the transcriptional regulation of low molecular weight glutenin genes in wheat, Angela Juhasz, Hungarian Academy of Sciences, Hungary

16:05-16:25 Molecular mechanisms of HMW glutenin subunits from $1S^{l}$ genome positively affecting wheat breadmaking quality, Shunli Wang, Capital Normal University, China

16:25-16:45 Isolation and characterization of key factors involved in glutenin trafficking in rice endosperm cells, Yihua Wang, Nanjing Agricultural University, China

16:50-22:30 Tea House Cultural Show including light dinner

Tuesday, Aug 14, 2012

Session 3 Improvement of end use qualities by genetic and alternative approaches

Chairs: Ferenc Bekes and Xianchun Xia

8:30-9:00 Keynote presentation, transferring research findings into industry application, Craig Morris, USDA-ARS, USA

9:00-9:30 Keynote presentation, wheat quality improvement in China, progress and prospects, Zhonghu He, Chinese Academy of Agricultural Sciences/CIMMYT, China

9:30-9:50 Transformation of common wheat (*Triticum aestivum* L.) with *avenin-like b* gene improves functional properties, Guangyuan He, Huazhong University of Science and Technology, China

9:50-10:10 Update on low-molecular-weight glutenin subunit identification, John Rogers, Universidad Nacional del Centro de la Provincia de Buenos Aires, Argentina

10:10-10:30 Low-molecular-weight glutenin subunit gene family in micro core collection of Chinese wheat germplasm, Dongcheng Liu, Chinese Academy of Sciences, China

10:30-10:50 Tea Break

Session 3 Continued

Chairs: Roberto J Peña and Aimin Zhang

10:50-11:10 Low molecular weight glutenin subunits and gliadins in 150 double haploid wheat lines derived from a cross between Kariega and Avocet, MarykeTine Labuschagne, University of the Free State, South Africa

11:10-11:30 Comparison of glutenin subunit composition among Australian and North American wheat classes, Tatsuya Ikeda, NARO/Western Region Agricultural Research Center, Japan

11:30-11:50 High frequency of abnormal high and low molecular weight glutenin alleles in Chinese wheat landraces of the Yangtze-River region, Dongfa Sun, Huazhong Agricultural University, China

11:50-12:10 Understanding the effects of HMW-GS and LMW-GS on processing quality using near-isogenic lines and development of functional markers for LMW-GS in wheat, Xianchun Xia, Chinese Academy of Agricultural Sciences, China

12:10-12:30 Estimating dough properties and end-product quality from flour composition, Ferenc Bekes, CSIRO, Australia

12:30-12:50 MALDI-TOF approach to measure cysteine number of wheat glutenin subunits, Wujun Ma, Murdoch University/Western Australian Department of Agriculture and Food, Australia

12:50-13:50 Lunch

Session 3 Continued

Chairs: Tatsuya Ikeda and Wujun Ma

13:50-14:10 Reliability of gluten-related small-scale - tests to estimate dough visco elasticity and bread loaf volume, Roberto J. Peña, CIMMYT, Mexico

14:10-14:30 New possibilities in micro-scale wheat quality characterisation: micro-gluten determination and starch isolation, Sándor Tömösközi, Budapest University of Technology and Economics, Hungary

14:30-14:50 Advances in quality property improvement and study of winter wheat in China, Yimin Wei, Chinese Academy of Agricultural Sciences, China

14:50-15:10 Oxidation of gluten peptide, Tuula Sontag-Strohm, University of Helsinki, Finland

15:10-15:30 Study on starch and noodle quality of wheat Wx near-isogenic lines in two different genetic backgrounds, Shunhe Cheng, Jiangsu Lixiahe Agricultural Research Institute, China

15:30-15:50 Characteristics and evaluation parameters associated with cooking quality of Chinese fresh noodle, Yan Zhang, Chinese Academy of Agricultural Sciences, China

15:50-18:30 Tea Break and Poster Session

18:00-19:00 Dinner

<u>Wednesday, Aug 15, 2012</u>

Session 4 Starch and health attributes of the wheat grain

Chairs: Craig Morris and Yimin Wei

8:10-8:40 Keynote presentation, starch biosynthesis in rice grains: natural variation and genetic improvement, Xiaoquan Liu, Yangzhou University, China

8:40-9:00 Bio-fortification for high micronutrient s in wheat breeding program in China, Yong Zhang, Chinese Academy of Agricultural Sciences, China

9:00-9:20 Allergen potential of non-prolamin seed proteins in Brachypodium distachyon, Angela Juhasz, Hungarian Academy of Sciences, Hungary

9:20-9:40 Blood pressure lowering peptides from wheat gluten, Xin Huang, University of Helsinki, Finland

9:40-10:00 How Reliable are the measurements of residual gluten in gluten-free foods? Päivi Maria Kanerva, University of Helsinki, Finland

10:00-10:20 Tea Break

Session 4 Continued

Chairs: Tuula Sontag-Strohm and Zhonghu He

10:20-10:40 Effects of HMW- & LMW-glutenins and grain hardness on size of gluten polymers, V.S. Lesage, INRA, France

10:40-11:00 Study on starch quality in the grain of wheat and naked barley, Zhifen Pan, Chinese Academy of Sciences, China

11:00-11:20 Structural and mechanical properties of compression-molded wheat gluten, gliadin and glutenin enriched films, Faiza Rasheed, The Swedish University of Agriculture Sciences, Sweden

11:20-11:40 Wheat and lupin protein interaction at baking: modifying extractability from lupin-wheat bread, IslamShahi, The University of Western Australia, Australia

11:40-12:00 Closing remarks

12:10-13:30 Lunch

13:30-17:00 Visit the Chinese Academy of Agricultural Sciences, and bus departs at 13:30 in front of hotel

Oral Presentation

Session 1. Capturing discoveries from genomics, proteomics, and transcriptomics

Wheat A genome sequencing and its application for quality modification

H.Q. Ling, A. Zhang, D. Wang, D. Liu, J.Y. Wang, H. Sun, H.J. Fan, Y.W. Li, Z.S. Li, Y. Zhao, D.W. Wang, K.P. Zhang, Y.S. Yang, J.J. Wang, and L. Dong

The State Key Lab of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.

Wheat is one of the most important food crops in the world, and is more widely cultivated and consumed than other major cereals. Because of its immense importance for human subsistence, major international efforts are being devoted to study and to improve the agronomic traits of wheat, such as yield, quality, nutrient use efficiency, and resistance to biotic and abiotic stresses. Wheat quality includes multiple aspects (i.e., processing, milling, flour color, starch, functional nutrient, etc), each involving the action of multiple genes and being influenced by environmental conditions. Although conventional genetic and breeding studies have contributed substantially to understand and improve wheat quality traits, they usually focus on small number of genes, and may not be highly efficient in revealing the functional complexities underlying the different types of quality traits. Functional genomics studies, conducted at genome wide level, offer high prospects for dissecting, understanding, and modifying complex agronomic traits. However, this kind of studies is currently not possible for wheat owing to the lack of complete genome sequence information. The huge and complex polyploid genomes in common and durum wheats have made complete genome sequencing difficult in these species. Thus, alternative and complementary strategies are needed for obtaining genome sequencing information for wheat. Our State Key Laboratory has chosen to sequence the A genome of *Triticum urartu* (AA, 2n = 2x = 14), which is the progenitor of the A genomes in many polyploid wheats (including common and durum wheats). Using next generation sequencing facility, coupled with transcriptome sequencing of multiple organs, a draft genome sequence of *Triticum urartu* has been generated. In this presentation, the salient features of *Triticum urartu* A genome will be briefly described. The major emphasis of the talk will be placed on several lines of ongoing research concerning the application of *Triticum urartu* genome sequencing information and resources in studying and improving wheat quality traits.

Proteome bioinformatics and genetics for associating proteins with grain phenotype

Rudi Appels[1], Paula Moolhuijzen[2], Brett Chapman[2], Wujun Ma[2], Matthew Bellgard[2], Yueming Yan[3], Shunli Wang[3], Angela Juhasz[4], and Frank Bekes[5]

[1]Centre for Comparative Genomics, Murdoch University, Perth WA 6150, Australia; [2]Centre for Comparative Genomics, Murdoch University, Perth WA 6150, Australia; [3]Capital Normal University, Beijing 100048, China; [4]Agricultural Institute, Centre for Agricultural Research of the Hungarian Academy of Sciences, Martonvásár 246, Hungary; [5]FBFD Pty Ltd, Beecroft, NSW 2119, Australia.

The unique transcripts of the wheat gene index (Dana Faba Cancer Institute, DFCI) and coding sequences predicted from the wheat genome survey sequences (International Wheat Genome Sequencing Consortium, IWGSC) were resources used to establish a dataset of predicted protein entities with their respective molecular weights estimated. This dataset was then cross-referenced to available data for the molecular weights of proteins reported in the literature as well as from our own analyses of grain protein in samples from structured mapping populations. The chromosomal locations from the mapping population of 225 doubled haploid lines using high throughput MALDI-TOF analyses could be compared to predicted locations of proteins determined from the chromosome arm-based analysis of the wheat genome survey sequences. The detailed study highlighted some technical challenges in resolving proteins close to each other in molecular weight which can now be addressed with newer mass spectroscopy technologies. The available dataset of predicted proteins was also analyzed for epitopes of potential interest for identifying grain proteins that may cause health issues in consumers of wheat-based products.

Creating a detailed map of the wheat flour proteome: a critical step in understanding the effects of environment on flour quality and immunogenic potential

S.B. Altenbach, F.M. Dupont, W.H. Vensel, C.K. Tanaka, and W.J. Hurkman

USDA-ARS Western Regional Research Center, Albany, CA, USA.

The wheat flour proteome is dominated by the gluten proteins, a complex group of closely related proteins that confer functional properties critical for the production of bread, noodles and various baked goods from wheat flour. The gluten proteins consist of the monomeric gliadins, separated into alpha/beta-, gamma- and omega-gliadins, and the polymeric glutenins, consisting of low molecular weight glutenin subunits (LMW-GS) linked by disulfide bonds to high molecular weight glutenin subunits (HMW-GS). There are many proteins with very similar sequences within each group and all have highly repetitive sequences with an abundance of glutamine and proline. It is important to be able to distinguish individual gluten proteins because small changes in their sequences can have consequences for both flour quality and immunogenic potential. However, it has been very difficult to identify gluten proteins by tandem mass spectrometry (MS/MS) and most studies report very low sequence coverage of these proteins. Using improved methods, a comprehensive proteomic map of wheat flour from the US bread wheat Butte 86 was developed in which 230 proteins were associated with specific gene sequences. Spectra were generated from proteins digested separately with trypsin, chymotrypsin, and thermolysin and the spectra were used to interrogate an enhanced wheat database that included cultivar-specific sequences. Five HMW-GS, 22 LMW-GS, 13 gamma gliadins, seven omega gliadins and 23 alpha gliadins were distinguished, many with sequence coverage greater than 50%. The increased sequence coverage made it possible to identify gliadins that contained extra cysteine residues and to distinguish alpha-gliadins containing sequences that trigger celiac disease from similar proteins that do not contain these sequences. In addition to the gluten proteins, three farinins, three purinins, three triticins, eight globulins, three grain softness-related proteins, 16 alpha amylase/protease inhibitors, nine serpins, three beta-amylases, three tritins, one xylanase inhibitor, and 33 distinct enzymes were identified in the flour. Proportions of the different protein types were determined. This detailed proteomic map made it possible to delineate the precise effects of fertilizer and temperature on the entire array of wheat flour proteins in Butte 86.

Monitoring of storage proteins: accumulation and impact of high temperature in wheat kernel using proteomics

A. Tasleem-Tahir, E. Bancel, P. Martre, and G. Branlard

INRA, UMR 1095 GDEC, 5 Chemin de Beaulieu, Clermont Ferrand 63039, France.

Several studies have shown that proteomics is the dedicated tool to analyse the accumulation of the numerous proteins in the starchy developing endosperm. First, a general survey of the major outcomes from studies on the proteomics of developing wheat endosperm will be presented.

Then proteomics studies carried out on manually extracted starchy endosperm of three cultivars Recital, Arche and Tamaro from 200 ℃ days to full maturity will be reported with a particular focus on gluten proteins. The albumin-globulin of the developing endosperm of Recital were first extracted and studied over 21 stages of development. The storage proteins were then extracted using CHAPS –Urea for 8 stages of development from 200 to 1000 cumulative ℃days. Proteins were separated on 2DE immobiline pH (3-11) 24 cm x SDS-PAGE and revealed using Coomassie G blue staining procedure. A total of 832 spots were revealed using image analysis. Among these spots 498 had significant quantitative or qualitative (presence/absence) variation during endosperm development and maturation. Among those 498 spots, nearly 200 were specifically storage proteins: gliadins and glutenins. Based on the quantitative variation of these storage proteins principal component analysis revealed four major phases during accumulation of storage proteins. Hierarchical clustering analysis revealed 6 major profiles for the storage proteins evidencing differences in kinetics of protein synthesis. Identification of individual spot of gliadins and glutenins shown that among these two families, proteins did not have similar accumulation profiles. Variation of the gliadins to glutenins ratio was also analysed during endosperm development and in relation to the amount of total grain protein. The storage protein profiles were compared to profiles revealed in the albumin-globulin proteins identified in the developing endosperm of cv Recital. This integrative proteomics approach allowed getting the major comprehensive features that take place over accumulation of gluten proteins till grain maturity.

Similar analysis was carried out for the two other bread wheat cultivars Arche and Tamaro (of medium and very high quality, respectively). Two temperature regimes were applied over grain formation and protein accumulation: heat treated 28/15 ℃ vs control 23/11 ℃ for day/night temperature. Endosperm storage proteins were extracted from kernels at similar thermal time after anthesis, for control and heat treated plants. Although significant differences could be observed between stressed and control over accumulation time for gliadins to glutenins ratio, for both cultivars this ratio was not different among the temperature treatment at full maturity. Interestingly, the very high quality cultivar Tamaro had far less gliadins to glutenins ratio fluctuations than Arche cultivar. This finding indicates that differences between cultivars exist for genetic regulation of the gluten proteins synthesis, which require further investigations.

The effects of nitrogen application on transcriptome, protein composition, and bread making quality of UK wheat varieties

Yongfang Wan[1], Malcolm J. Hawkesford[1], Peter R. Shewry[1], Rowan Mitchell[1], Gemma Chope[2], and Dhan Bhandari[3]

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High grain protein content required for good breadmaking is associated with lower grain yield. Increased yields require high inputs of nitrogen fertilizer which is not sustainable in terms of cost, energy requirement for fertilizer production and environmental footprint. This study examines the responsiveness of wheat cultivars to different nitrogen levels and also the effects of environments on gene expression, protein composition and breadmaking quality.

Six wheat varieties (Hereward, Cordiale, Marksman, Istabraq, Malacca, and Xi-19) were grown at five UK sites, at three nitrogen levels: 100 kg/ha, 200kg/ha, and 350kg/ha over three years (2009-2011). High nitrogen input generally caused an increase in the grain yields and protein contents of all six cultivars. However, there was also a large effect of site and year. Hereward consistently showed high protein content with lower grain yield, while Istabraq showed low protein content with high grain yield which is suitable for animal feed and biofuel. Marksman and Cordiale combined high protein content with high grain yield—an effect known as grain protein deviation. The molecular biological basis of the protein deviation was further investigated. Gene expression in caryopses at 21days post anthesis was profiled using Affymetrix chips. 105 transcripts that were significantly up-regulated by nitrogen were observed. These nitrogen responsive transcripts consisted of genes involved storage protein, defence, photosynthesis, as well as some of unknown function. The differences in expression profiles were validated by realtime RT- PCR.

Protein composition profiles were determined by SDS-PAGE and SE-HPLC from developing caryopses at 21, 28, 35, and 42 days post anthesis, and for mature grains. Nitrogen supply had strong influences on protein quantity and composition. The proportions of HMW glutenin subunits and ω-gliadins were increased at higher nitrogen levels, while those of LMW glutenin subunits and remaining gliadins were decreased. The effects of nitrogen fertilizer on individual subunit proportion within the HMW-glutenin subunit and ω- gliadin groups were independent of the variety, site, and year. A higher ratio of (F1+F2)/(F3+F4) corresponding to ratio of polymeric glutenins/monomeric gliadins was detected in developing and mature grains by SE-HPLC in Hereward, Malacca, Marksman, and Cordiale, whereas a lower ratio was determined for Istabraq.

Baking quality was evaluated by determination of dough mixing properties, loaf volume, crumb colour, and structure. The bread making qualities of all six cultivars were improved by high nitrogen levels, but were also affected by different sites and years. Istabraq consistently made very poor baking quality flour, while Hereward

showed stable good bread baking quality flour. Cordiale and Marksman made medium baking quality flour. Current work within the project is directed at relating transcript abundance differences to differences in flour properties between varieties and in response to nitrogen.

Proteomic analysis of mature wheat kernels of transgenic bread wheat expressing PGIP infected *Fusarium graminearum*

R. D'ovidio[1], P. Laino[1], M. Janni[1], E. Botticella[1], M.S. Dicarli[2], E. Benvenuto[2], K. Lilley[3], D. Lafiandra[1], and S. Masci[1]

[1]Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia, University of Tuscia, Viterbo, Italy; [2]ENEA, Dipartimento BIOTEC, Sezione Genetica e Genomica Vegetale, C.R. Casaccia, Rome, Italy; [3]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom; [4]Present address: CRA-GPG Genomic Research Centre, Fiorenzuola d'Arda (PC), Italy.

Fusarium head blight (FHB), caused mainly by the fungus *Fusarium graminearum*, is a widespread and devastating disease of wheat. This disease effects both productivity and the qualitative properties of wheat flour because of mycotoxins accumulation on wheat kernels. To evaluate the impact of FHB on the wheat flour, we have compared total starch content and the proteome of the mature wheat kernels derived from *F. graminearum*-infected spikes and control bread wheat plants. The same analysis was also performed on mature seeds of control and *F. graminearum*-infected transgenic bread wheat plants expressing a Poly-Galacturonase Inhibiting Protein (PGIP) conferring partial resistance to FHB.

Control and transgenic lines showed comparable values in spikelet number and seed yield, measured as thousand kernel weight (TKW). No significant differences in TKW were observed between *F. graminearum* infected and non-infected transgenic plants. In contrast, significant differences in TKW were observed between infected and non-infected control plants, with the infected control plants showing a reduced seed yield.

Proteins with a metabolic role were analyzed by DIGE, while gluten proteins (gliadins and glutenin subunits) were analysed by one-dimensional SDS-PAGE and A-PAGE. DIGE investigation of metabolic proteins did not identify any component differentially accumulated between the infected and non-infected control plants, nor between infected and non-infected transgenic plants, and nor between infected and non-infected transgenic and control plants.

Similarly, SDS-PAGE and A-PAGE analysis did not identify any considerably qualitative or quantitative difference in the storage proteins (gliadins and glutenin subunits) of transgenic and control seeds. Differently to the results obtained with the proteomic analyses, total starch content showed a significant difference between infected and non-infected control plants, with the infected control plants showing a reduced total starch content. Similarly, a significant difference was observed between infected transgenic and control plants, with the transgenic plants showing a higher total starch content.These results indicate that a moderate infection of FHB do not cause significant changes in the wheat seed proteome but reduces total seed starch content.

Daily changes in the phosphoproteome of the Dinoflagellate *Lingulodinium*

Bolin Liu[1], Samuel Chun-Lap Lo[2], Daniel P. Matton[1], B. Franz Lang[3], and David Morse[1]

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The dinoflagellate *Lingulodinium* has a large number of daily rhythms, many of which have no biochemical correlates. We examined the possibility that changes in protein phosphorylation may mediate some of the rhythmic changes by comparing proteins prepared from midday (LD6) and midnight (LD18) cultures. We used two different methods, one a 2D gel protocol in which phosphoproteins were identified after staining with ProQ Diamond, and the other an LC-MS/MS identification of tryptic phosphopeptides that had been purified by TiO2 chromatography. Two differentially phosphorylated proteins, a light harvesting complex protein and Rad24, were identified using the 2D gel protocol. Six differentially phosphorylated proteins, a polyketide synthase, an uncharacterized transporter, a LIM (actin binding) domain and three RNA binding domain proteins, were identified using the phosphopeptide enrichment protocol. We conclude that changes in protein phosphorylation may underlie some of the rhythmic behavior of *Lingulodinium*.

Towards understanding genetic basis of chapatti (Indian flat bread) making quality

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In India wheat is mainly consumed in the form of flat bread i.e. chapatti. Parameters that define good chapatti are: appealing colour, fully puffed, soft and pliable texture, sweetish taste, pleasing flavour and a good shelf-life. In addition the dough should be non-sticky with high water absorption. Genetic basis underlying chapatti making quality is poorly understood. In an attempt to understand molecular basis of chapatti making quality, differential gene expression analysis of good (C306 and LOK1) and poor chapatti making varieties (Sonalika and WH291) was carried out using Affymetrix GeneChip® Wheat Genome Array. Transcriptomics studies at different developmental stages indicated several up-regulated and down-regulated predicted/unpredicted genes. Among the earlier reported genes affecting wheat processing quality, three (alpha-gliadin, gamma-gliadin and peroxidase) were several folds up-regulated in good chapatti varieties compared to the poor ones. Whereas granule bound starch synthase 1(GBSS1) was several folds down-regulated in good chapatti varieties. Genomic variation of GBSS1 involved in amylose synthesis was studied in several wheat cultivars. Allelic variation of GBSS1 in Indian cultivars indicated that GBSS A1 and GBSS D1 genes were non-polymorphic and present in all the cultivars studied. GBSS B1 gene was polymorphic based on presence/absence alleles. Absence of GBSS B1 gene was co-related with good chapatti making quality. Among starch pasting characteristics, breakdown in viscosity was found to be co-related with good chapatti making quality. Validation of relationship of GBSS1 and RVA-breakdown in viscosity to Chapatti making quality by using backcross derived RIL population is in progress. Marker assisted breeding for GBSS B1 gene is also in progress.

An asparagine residue affects LMW-GS maturation processing

E. Egidi[1], M. Janni[1,2], F. Sestili[1], A. Ceriotti[3], R.D'Ovidio[1], D. Lafiandra[1], D.D. Kasarda[4], W.H. Vensel[4], and S. Masci[1]

[1]DAFNE, Tuscia University, Viterbo, Italy; [2]IGV, CNR, Bar, Italy; [3]IBBA, CNR, Milan, Italy; [4]USDA, ARS, WRRC, Albany, CA, USA.

The low molecular weight glutenin subunits (LMW-GS) are represented by many different proteins, some with a general structure typical of LMW-GS and others that are structurally gliadins—but functionally glutenin subunits, because of the presence of odd cysteine residues which make the gliadin-like proteins capable of forming intermolecular disulfide bonds. Among the typical LMW-GS, the most common are classically known as LMW-m and LMW-s types, according to the first amino acid of the mature sequence, which differs mainly as a consequence of there being three extra amino acids at the N-terminus of LMW-m types. The nucleotide sequences of their encoding genes are in fact practically identical, so that it was not possible to ascertain the exact N-terminal amino acid sequence on the basis of the nucleotide sequence. In 1998, Masci *et al.*, hypothesized that the presence of an asparagine residue in position 23 of the coding sequence might account for the processing of the N-terminal end of the LMW-s sequence, likely due to the cleavage at the asparagine residue by an asparagine peptidase that would thus generate the observed N-terminus of the LMW-s type. This was also hypothesized for ω-gliadins. When the asparagine residue is replaced by a threonine residue, this should instead generate LMW-m types. Because direct correspondence between a specific LMW-GS type and its coding gene was demonstrated only for a LMW-s type, but not for any LMW-m type, we performed site direct mutagenesis of the LMW-s gene previously isolated, in such a way as to introduce a threonine instead of an asparagine, and the opposite (asparagine instead of threonine) on a supposed LMW-m type gene. We then produced transgenic durum wheat lines, transformed with the mutated versions of the LMW-m and LMW-s genes, along with the *wild type* counterpart of the LMW-m type gene, in which two different tags were added at the C-termini (His- and FLAG-tags) in order to facilitate recognition and purification of the heterologous polypeptides.

Western Blot analyses on 2D gels and proteomic comparisons between the transgenic and untransformed lines enabled identification of the transgenic polypeptides. MS-MS analyses and N-terminal amino acid sequences indicated that the processing occurs according to our predictions, namely the presence of the asparagine residue at position 23 of the coding sequence accounts for the processing as a LMW-s type, whereas the presence of the threonine determines the formation of a LMW-m type.

Studying the intracellular trafficking and tissue distribution of gluten proteins by epitope-tagging

Paola Tosi[1], Jackie Freeman[1], Cristina Gritsch[1], Caroline Sparks[1], Huw D. Jones[1], Wakako Funatsuki[2], Katsumasa Niwa[3], Eleonora Egidi[4], Aldo Ceriotti[5], Stefania Masci[4], and Peter R. Shewry[1]

[1]Rothamsted Res., UK; [2]Natl. Agr. Res. Ctr Hokkaido Region (NARCH), Japan; [3]Tokyo Agr.Univ., Japan; [4]University of Tuscia Viterbo, Italy; [5]IBBA-CNR, Milan, Italy.

Wheat is one of the most important food crops in the world and its success is largely due to the unusual functional properties of its flour which allow for the formation of viscoelastic dough that can be processed into bread, noodles, pasta and many other food products. Dough viscoelasticity is controlled by the gluten proteins, consisting of the monomeric gliadins and polymeric glutenins, and representing the most abundant proteins of the wheat grain endosperm. The glutenin polymers consist of two types of subunit, called low molecular weight (LMW) and high molecular weight (HMW) subunits of glutenin and there is a well documented correlation between the proportion of glutenin polymers with high molecular mass and dough strength. The size distribution of the glutenin polymers is determined both by allelic variation in the composition of gluten proteins, and particularly of HMW glutenin subunits, and by their assembly into polymers, which in turn depends on the specific locations of gluten proteins within the endosperm and their segregation during deposition.

It is now widely accepted that two trafficking pathways operate in the wheat endosperm, leading to two mechanisms of protein body formation: one requiring translocation via the ER and Golgi and deposition in the vacuole, and the other direct aggregation and deposition in the ER. It has also been suggested that individual proteins may follow one or both routes and that the two trafficking pathways may differ in their importance during grain development, with accumulation within the ER becoming more prominent at later stages.

Therefore, while all gluten proteins are synthesised on polyribosomes attached to the ER, their pathway of deposition would depend on the properties of the proteins themselves (particularly their insolubility in aqueous solvents), on the developmental stage of the endosperm, and, possibly, on the impacts of environmental conditions on the physiological states of the different cell organelles.

Our understanding of the dynamics of trafficking and deposition of specific gluten protein types and of the mechanism of wheat protein body formation has been limited so far by practical difficulties in developing monospecific antibodies for gluten proteins and by the inability to unequivocally identify the origins of membranes in microscopy sections, which in turn have limited our ability to relate specific molecular features of gluten proteins with their mechanisms of trafficking and our ability to discriminate protein bodies of ER or vacuolar origin *in planta.* Both technical limitations can be overcome by using epitope tagging. To this aim, we have produced a

set of transgenic wheat lines expressing *C*-terminal epitope tagged forms of gluten proteins (HMW glutenin subunit, LMW glutenin subunit and γ-gliadin) whose location in the cells of the developing grain can be determined using highly specific, commercial antibodies against the tags. More recently a second set of transgenic bread wheat lines expressing tagged proteins as markers for specific compartments of the secretory pathway has also been developed; these include lines expressing fluorescent and /or epitope tagged forms of markers for the ER membrane, Golgi membrane and the tonoplast. Crossing of these two sets of transgenic lines will provide a powerful system in which to study the dynamics of trafficking and deposition of the different gluten protein types and the mechanism of wheat protein body formation.

Trafficking pathway of wheat storage proteins in transgenic rice endosperm

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Wheat is the most important crop in Europe, being used both for food processing and livestock feed. Its success is due partly to its adaptability, giving high yields over a range of environments. However, its success as a food crop is also due to the unique properties of the grain storage proteins. These form the gluten fraction which confers the unique viscoelastic properties which allows wheat dough to be processed to form bread, noodles, pasta and many other processed foods. In order to exploit all of these possibilities, it is necessary to understand the pathways and mechanisms which determine the synthesis, processing, trafficking and deposition of storage components in the developing grain.

The trafficking of proteins in the endoplasmic reticulum (ER) is a topic of considerable interest since this organelle serves as an entry point for proteins destined for other organelles, as well as for the ER itself.

Rice differs from wheat in that two major types of storage proteins (prolamins and glutelins) are synthesized in its endosperm tissue and deposited into distinct protein bodies (PBs) of different origin. The prolamins aggregate inside the ER lumen aided by the chaperone BiP and form type-I protein bodies, while glutelins are translated on separate subdomains of the ER and transported via the Golgi apparatus and vesicles into type-II protein bodies of vacuolar origin. This clear segregation of the rice storage proteins into two populations of protein body means that it is an ideal system to study the trafficking of wheat proteins.

We have therefore used the transgenic rice lines to study the pattern and pathway of deposition of the wheat HMW subunit *1Dx5* within the rice endosperm, using specific antibodies to determine whether it is deposited in the same or different protein bodies to the rice storage proteins (prolamin and glutelin), and whether it is located in the same or separate phases within these. The protein distribution and the expression pattern of HMW-Dx5 subunits in different development stages of transgenic rice endosperm were also studied using tissue printing, light-and electron microscopy.

Sections from transgenic rice endosperm were incubated in specific primary antibodies to wheat and rice proteins, used either alone or in combinations. After several rinses of the sections, the binding of primary antibodies were revealed by incubation with secondary antibodies conjugated to a fluorescent label (Alexa 488, or/and Alexa 568), using a Zeiss Confocal Laser Scanning Microscope.

The HMW-GS specific antibodies confirmed the expression of the 1 *Dx5* glutenin protein in the rice endosperm. It was also observed that the *HMW- Dx5* glutenin subunit was deposited particularly in inner layers in the rice endosperm, while the rice

storage proteins deposited mainly in the sub-aleurone layer.

The trafficking pathway of wheat gluten subunit protein was followed by immunogold labelling of ultrathin sections using TEM. Double labelling was also carried out by incubating the sections with combinations of primary antibodies to rice glutelin and prolamin and with secondary antibodies conjugated to gold particles of different sizes.

A unique feature of plants is that they are able to store proteins in the ER in addition to other endomembrane compartments, and the deposition of such storage proteins provides important sources for both human and animal nutrition. Thus, increasing our knowledge of the mechanisms required for the targeting of storage proteins to this organelle will ultimately allow the modification of critical steps, leading to improvement of cereals as a protein source as well as increasing crop yield and productivity.

Virus-induced gene-silencing in wheat spikes and grains and its application in functional analysis of HMW-GS-encoding genes

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Virus-induced gene silencing (VIGS) has been widely used to knockdown genes in seedling plants, but its potential application in reproductive tissues has not been fully explored. In wheat, the Barley stripe mosaic virus (BSMV)-based vector has been used for effective gene silence in wheat seedlings, but conditions for gene silence in wheat spikes and grains have not been evaluated. Genes expressed in spikes or grains are likely important to agronomic traits such as yield, quality and pathogen resistance. In this study, we first demonstrated the feasibility of using BSMV for gene silence in wheat spikes or grains with the phytoene desaturase (PDS) marker gene. We then determined the optimal inoculation location, stage, and other conditions for efficient silence of PDS in wheat spikes or grains. Finally, we used the established BSMV-VIGS system to silence endosperm genes encoding HMW-GSs both singly and all the family. Our results for the first time demonstrated that BSMV-VIGS system is a useful tool for the functional analysis of genes expressed in wheat spikes or grains.

Gluten protein structures: variation in wheat grain and at various applications

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For a number of applications, including bread-making, pasta making, and bio-based plastics production, gluten protein polymerization and structures are of the highest importance in determining the end-use properties. Therefore, we have in a number of studies focused on these characteristics. The results from our studies show that protein composition and polymerization behavior in mature wheat grain is largely dependent on circumstances during the wheat plant development. Environmental factors contribute to a similar extent as does the genetic background of the wheat evaluated and there is also interaction between the two factors. Most important is nitrogen availability during various phases of wheat development and the genetically determined plant development rhythm, although extent of importance of nitrogen availability and plant development rythm is also influenced by the temperature during the plant development time. The genetic variation in protein composition and polymerization has also been found to be enormously wide when using wheat of a broad genetic origin. Furthermore, while mixing dough from wheat with varying composition in terms of polymers in the mature grain, a genetic component was also found in the polymerization of the proteins during mixing. Also, both protein structures and polymerization in mature grain and during mixing was found to influence the properties of the baked bread. Therefore, both these characters are important for bread-making quality. In production of bio-based plastics from wheat gluten, the rearrangements of the structures and polymers of the gluten proteins are increased even further as compared to during bread-making. In one of our most successful wheat gluten materials, we found a changed protein polymer structure from a high content of unordered amide groups, and/or α-helix conformations, towards a structure where β-sheet structures of both intermolecular and extended type were common. An even larger change of the protein structures towards higher amount of extended β-sheet structures led to outstanding characteristics of tensile strength, low protein extraction values, very low diffusion of allergy causing proteins from the material and low oxygen permeability in the material. Further, a hexagonal closed packed structure was found in this material, that has not been reported previously in any bio-based material but which is common in some metals and synthetic polymers. Thus, the understanding of how proteins are aggregating and how to combine different gluten proteins into the most suitable polymers is extremely important both in production of bread with the best baking performance and to form tailored materials from gluten.

A model for the transcriptional regulation of low molecular weight glutenin genes in wheat

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Transcriptional regulation of LMW glutenin genes were investigated *in-silico*, using publicly available gene sequences and expression data. Genes were grouped into different LMW glutenin types and their promoter profiles were determined using cis-acting regulatory elements databases and published results. Based on the promoter sequences and expression characteristic LMW glutenin genes might be transcribed following two different mechanisms. A group of genes, mostly belonging to m-type LMW glutenin genes follow a normally distributed expression pattern, starting to express at around the tenth day after anthesys. Expression reaches its maximum at around 21DPA and the amount of transcripts continuously decreases while reaching the full mature status. Most of the s- and i-type genes show a continuously increasing expression pattern and show a stable level of transcription also after 21DPA.

Differences observed in their expression could be related to the differences found in their promoter sequences. Altogether 24 variants of 12 storage protein gene specific promoter motifs have been identified, mainly belonging to DOF, bZIP and Myb type transcription factor recognition sites.The various cis-acting elements belong to some conserved non-coding regulatory regions (CREs) and might act in two different ways. There are regions, in which regulatory elements, such as the GCN4 motifs found in the -300 element region, could serve as key factors in tissue-specific expression. Some other elements, like the complete endosperm boxes in the -600 region or the individual prolamin box variants might modulate the level of expression. Based on the relatively small distance between different cis-acting promoter motifs possible *DOF-bZIP*, *DOF-Myb*, *bZIP-Myb* interactions can be identified. Polymorphisms in the number and combination of different cis-acting elements and the transcription factors bound to them can be of crucial importance in the diverse levels of production of single LMW glutenin gene types. The results presented here will serve as good bases to better understand the mechanisms involved in wheat storage protein expression and facilitate molecular breeding.

Molecular mechanisms of HMW glutenin subunits from 1S^{l} genome positively affecting wheat breadmaking quality

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A wheat cultivar "Chinese Spring" chromosome substitution line (CS-1S^{l}/1B), in which the 1B chromosome was substituted by 1S^{l} from *Aegilops longissima* was created and found to possess superior dough and breadmaking quality. Phytogenetic analysis based on the HMW-GS genes revealed a close relationship between 1S^{1} and 1B genomes. The molecular mechanism of its super quality conformation is studied in the aspects of high molecular glutenin genes, protein accumulation patterns, glutenin polymeric proteins, protein bodies, starch granules, and PDI and PDI-like protein expressions. Results showed that the introduced HMW-GS *1Slx2.3** and *1Sly16** in the substitution line were large in size with a longer repetitive domain, a higher level of mRNA expressions during grain development, and more HMW-GS accumulation in the mature grain. A higher abundance of PDI and PDI-like proteins was observed which possess a known function of assisting disulfide bond formation. Larger HMW-GS deposited protein bodies were also found in the substitution line. The CS substitution line is expected to be highly valuable in wheat quality improvement since it can be used to cross with the cultivar harbours the superior *Dx5* subunit to develop much better quality bread wheat.

Isolation and characterization of key factors involved in glutelin trafficking in rice endosperm cells

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Rice seeds accumulate large quantities of storage proteins, which mainly serve as nitrogen sources during germination and seedling growth. Unlike most cereals, which generally reserve alcohol-soluble prolamin as storage proteins, rice seeds accumulate glutelins, and these can account for as much as 80% of the total endosperm proteins. Glutelins are synthesized as 57kD-precursors at the rough endoplasmic reticulum (RER). These precursors are transported to protein body II (PBII), a type of protein storage vacuole (PSV), either within dense vesicles (DV) budded from the Golgi apparatus (Krishnan *et al.* 1986), or within ER-derived precursor-accumulating (PAC)-like compartments (Takahashi *et al.* 2005). The glutelin precursors are then cleaved into mature storage proteins, each containing a single acidic (37-39kD) and a single basic subunit (19-20kD) (Wang *et al.* 2009). If any step in these processes is defective, endosperm cells would be expected to accumulate 57kD-proglutelins; such rice mutants are termed 57H mutants, and they are useful for analyzing the molecular mechanisms that control the transport and processing of seed storage proteins in rice. From 2001, large scale screening of 57H mutants was carried out in our lab. Twenty one 57H mutants were obtained till now. Through map-based cloning strategy, five genes have been cloned and characterized, including *OsVPE1*, *OsPDIL1-1*, *GPA1*/*OsRab5a (Glutelin Precursor Accumulation1)*, *GPA2*, and *GPA3*. These genes function at different site of the glutelin trafficking pathway. *OsVPE1* functions at the very downstream of the pathway, which cleaves proglutelin into acidic and basic subunits in PSV/PBII (Wang *et al.* 2010). *OsPDIL1-1* plays its role as a chaperon protein in the ER (Han *et al.* 2010). *GPA1/OsRab5a* (Wang *et al.* 2010), GPA2 and GPA3 localize mainly in the prevacuolar compartment (PVC) and control post-Golgi trafficking (Wan *et al.* unpublished data). Our findings will provide novel insights into the glutelin trafficking pathway, which will be helpful in rice protein quality improvement.

Transferring research findings into industry application

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One objective of wheat quality research is to solve problems for the milling, baking and food processing industries, and ultimately to benefit consumers. A second objective is to provide these same industries and consumers new and innovative products and ingredients. In this regard, research information flows both directions – up and down the wheat research-processor-consumer chain. This presentation will focus on two such examples. The first deals with refining an end-use quality model for soft wheat, the second involve the development of a 'new' type of wheat – soft kernel durum. In the U.S. and elsewhere many 'soft' wheat products include low moisture cookies and crackers. These products may be prepared from 'lean' formulas with little more than wheat flour, water, salt and leavening, to 'rich' formulas with high ratios of sugar and sometimes fat. For most cookies, gluten development is undesirable as it imparts a tough or hard ('flinty') product texture. However, in many instances, the low moisture content of cookie dough and the high formula sugar content prevent gluten development. Conversely, crackers, most notably 'saltines', typically require some level of gluten development and are prepared from a fermented dough which is sheeted, laminated and docked (Morris and Rose 1996). These doughs may contain a considerable amount of hard wheat flour to attain the necessary level of gluten strength. A key feature of cookies and crackers is their very low moisture content and relatively long shelf life (stability). Consequently, one aspect of baking is to drive out dough water down to less than about 4% moisture. This requirement places great importance on dough water relations, especially damaged starch and arabinoxylans. Damaged starch results mostly from the milling process and can be controlled genetically through the deployment of the 'soft' *Puroindoline* genes (*Pina-D1a/Pinb-D1a*). Among soft wheat varieties and germplasm, the 'carbonate solvent retention capacity test' (carbonate SRC) is effective in estimating this parameter. Arabinoxylans (AX), often sub-divided into total and water-extractable fractions, appear to be more complexly inherited, with an approximately twofold range among Pacific Northwest soft (Finnie *et al.* 2006) and hard (Li *et al*. 2009) wheat varieties. Direct measurement of AX may be accomplished via phloroglucinol colorimetric (Kiszonas et al. 2012) or gas chromatography-flame ionization detection (Courtin *et al*. 2000). Similar to before, the sucrose SRC test is targeted at estimating the effect of AX to overall water absorption of flours. The last point to be made here is that there is a marked premium associated with the hard 'bread' wheat classes of wheat in the U.S. and elsewhere. For example, local wheat prices in eastern Washington at the time of this writing were $248/mt soft white vs. $320/mt for 'Dark Northern Spring' (14% protein). Obviously, bakers are willing (forced?) to pay for gluten strength, and differences in 'strength' are implicit features of the U.S. Classes. This generalized landscape is driving a trend to increase gluten strength in U.S. soft wheat. In the western U.S., club wheat is bred to have very weak gluten and thus this sub-class provides an excellent option for "blending down" gluten strength in soft

white. The second example involves the development of soft kernel durum wheat. We successfully transferred through *ph1b*-mediated recombination the *Hardness* locus, including *Pina-D1a/Pinb-D1a*, into Langdon durum, and subsequently crossed the soft kernel trait into the Italian cultivar Svevo (Morris *et al.* 2011). Why did we do it? 1) because we could, and 2) to examine the effect that kernel texture has on durum milling, pasta and bread processing. To-date, we have found no detrimental effects of soft kernel, only neutral-to-positive influences on milling, baking and pasta processing.

Wheat quality improvement in China, progress and prospects

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Genetic improvement of wheat quality in China was initiated in 1980s and significant progress has been achieved in four aspects during the last ten years. (1) Standardized laboratory testing and evaluation methods have been established for traditional Chinese products such as noodles and steamed bread, and key factors responsible for noodle quality have been identified. (2) Comparative genomic approach was employed to understand the allelic variations of key loci associated with products color, and more than 40 functional markers were developed and validated. They include PPO genes responsible for polyphenol oxidase activity, LOX genes responsible for lipoxynase activity, and PSY and ZDS genes associated with yellow pigment content. Furthermore, DNA sequencing and semi-quantitative RT-PCR were used to characterize the expression of *Ppo-A1*, which was regulated by alternative splicing of pre-mRNAs. (3) Genomics and proteomics approaches were employed to develop new methods for rapid identification of HMW-GS and LMW-GS, and LMW-GS gene molecular marker system were developed and all LMW-GS genes in Chinese core collection were determined; this provided new insight into understanding the gene structure. Functional markers for LMW-GS at *Glu-A3* and *Glu-B3* loci were developed and widely used, making it possible to use the LMW-GS in wheat breeding program. MALDI-TOF MS was used to rapidly identify HMW-GS and LMW-GS. (4) Conventional quality testing and molecular markers were used to characterize major Chinese wheat varieties for processing and nutritional traits. Improved varieties with excellent pan bread making quality such as Jinan 17, Jishi 02-1, with noodle quality such as Jimai 19, and dual purpose quality wheat for pan bread and noodle such as Yumai 34 and Jimai 20 were released and sown in large area. Completion of wheat genomic sequencing will definitely speed up the gene cloning and molecular marker development, and more efforts are still needed to understand the traditional Chinese products. Development of new varieties in combination of high yielding potential, excellent quality, and broad adaptation is still challenging, and it is expected that more markers and new technology will be used in breeding program.

Transformation of common wheat (*Triticum aestivum* L.) with *avenin-like b* gene improves functional properties

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Wheat avenin-like b protein is a new type of seed storage proteins each containing 18 or19 cysteine residues which could possibly contribute to the processing properties of wheat via inter-chain disulphide bonds formation. In order to clarify exact role of avenin-like b protein in wheat processing quality, the avenin-like b gene was amplified from the genome of wheat and then was cloned into vector pLRPT to construct eukaryotic expression vector pLRPT-avel under the control of *1Dx5* promoter that expresses specifically in the endosperm. The eukaryotic expression vector pLRPT-avel was transformed into wheat (*Triticum aestivum* L. cv Zhengmai 9023) by particle bombardment. After analysis for the presence and expression of transgene by PCR, Southern blotting, SDS-PAGE and Western blotting in two successive generations (T2 and T3), two transgenic wheat lines overexpressing avenin-like b proteins were obtained for biochemical and functional characterization of wheat flour by SE-HPLC and mixograph. The results of mixograph analysis showed that the increased content of avenin-like b protein in transgenic lines resulted in a significant increase in dough strength measured as increased PR, BWPR, BW at MT, Mid-Timex width and Mid-tail width. Analysis of the gluten proteins extracted from flour demonstrated that this was associated with increased proportion of large polymeric proteins measured as increased the ratio of %F1, %F1/%F2 and %UPP and decreased the ratio of (%F3 + %F4)/%F1. The results of the present study should help to understand the influence and the mechanism of avenin-like b proteins on the processing quality of wheat.

Update on low-molecular-weight glutenin subunit identification

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Allelic variation for the low-molecular-weight glutenin subunits (LMW-GS) is a major determinant of differences in dough viscoelastic properties observed between cultivars of both bread wheat and durum wheat. Technical difficulties in allelic identification due to the complexity of the protein profile produced by each cultivar and the use of different nomenclature systems in different laboratories has historically interfered with information exchange between research groups, a situation exacerbated by the vast number of possible profiles found in different cultivars due to the multi-allelic nature of the principal loci encoding LMW-GSs (*Glu-A3*, *Glu-B3* and *Glu-D3*). These difficulties prompted us to form an international collaborative group aimed at unifying criteria across laboratories, and the current contribution summarises progress made by this group to date and seeks to address remaining challenges. As an initial step, a worldwide collection of cultivars were analysed in different laboratories for their LMW-GS allelic composition (Ikeda *et al.* 2009), where each laboratory applied its own particular range of techniques. The results were compared in order to understand the basis of existing differences in interpretation. For example, apparently minor differences in the conditions employed during one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (1-DE, SDS-PAGE) resulted in separations that were sufficiently different as to result in contrasting conclusions regarding the allelic composition of the cultivars. Three further techniques were applied in order to refine the analyses: two-dimensional gel electrophoresis (2-DE, IEF × SDS-PAGE), matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and polymerase chain reaction (PCR) using allele specific primers. For example, in the collection of cultivars analysed, all alleles observed at the *Glu-A3* locus could be distinguished by 2-DE or PCR, whereas there were specific cases where particular alleles could not be distinguished by 1-DE or

MALDI-TOF-MS. At the *Glu-B3* locus, some alleles could be clearly identified by all four methods, whereas for others, only 2-DE was effective. At the *Glu-D3* locus, allelic identification was at times problematic for 1-DE, 2-DE and PCR; one of the marker bands detected by SDS-PAGE was not a LMW-GS, but a gliadin that contaminated the glutenin fraction; some of the alleles at this locus were only detected by SNPs in PCR; plus it was found that MALDI-TOF-MS has considerable potential for allelic identification at this locus. Hence in general it was shown that the four methods can be regarded as complementary techniques that together provide powerful tools for allelic identification. As an aid to allelic identification across laboratories, a standard set of cultivars was defined to represent all allelic variants in the collection analysed, seed of which has been made openly available from CIMMYT and INRA Clermont-Ferrand (Liu *et al.* 2010), and the results of this study have been coordinated with those presented in the wheat gene catalogue and its supplements (McIntosh *et al.* 2011), in order to ensure consistency in the availability of information to the wheat community. Progress will also be described in allelic identification in cultivars not included in the collection described above, and in the discrimination of alleles between bread wheat and durum wheat. We should also like to propose an International Gluten Research Group as a part of the G20 Wheat Initiative (previously the International Research Initiative for Wheat Improvement), aimed at exchanging and sharing valuable wheat cultivars, unifying the nomenclature of gluten alleles and the standard methods to examine gluten proteins, studying the usefulness of particular gluten compositions for quality under various environmental conditions, and developing wheat cultivars with specific grain quality attributes under different environmental conditions, including heat and drought stresses.

Low-molecular-weight glutenin subunit gene family in micro core collection of Chinese wheat germplasm

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Low-molecular-weight glutenin subunits (LMW-GS) are essential in determining the processing quality of wheat flour. These proteins are encoded by a complex multi-gene family. Although the members of this gene family were identified from several wheat varieties, the allelic variation and composition of LMW-GS genes in bread wheat are not well understood. In the present study, using the LMW-GS gene molecular marker system and the full-length gene cloning method developed previously, we provide a comprehensively molecular genetic analysis of LMW-GS genes in a representative population—the micro-core collection (MCC) of Chinese wheat germplasm. Generally, more than 15 LMW-GS genes were identified from individual MCC accessions, of which 4-6 genes were located at the *Glu-A3* locus, 3-5 genes at the *Glu-B3* locus, and eight genes at the *Glu-D3* locus. LMW-GS genes at the *Glu-A3* locus showed the highest diversity, and then the *Glu-B3* genes, while the *Glu-D3* genes were extremely conserved among MCC accessions. At the *Glu-A3* locus, two m-type genes and 2-4 i-type genes were detected and > 5 allelic variants for each gene were identified in MCC accessions. The i-types genes were tightly linked forming 12 unique haplotypes and the m-type genes were also generally coupled with i-type haplotypes. At the *Glu-B3* locus, 2-3 m-type genes and 1-3 s-type genes were identified. These s-type genes cosegregated and formed two different groups of haplotypes. At the *Glu-D3* locus, one s-type gene and seven m-type genes were detected, and these genes were universal in MCC accessions. The expression and sequence analysis showed that about 10 active genes were present in each accession, i.e., 1-3 active genes at the *Glu-A3* locus, 2-4 at *Glu-B3*, and six at *Glu-D3*. Based on the sequence identity and the location of eight cysteine residues, all the LMW-GS genes were divided into six groups. All the i-type genes formed a single group and were only present at the *Glu-A3* locus. And s-type genes located at *Glu-B3* and *Glu-D3* loci composed a unique group. The m-type genes showing high diversity were classified into four groups and detected in all *Glu-3* loci. Based on the characterization of LMW-GS genes in MCC, we successfully elucidated the complex gene family in common wheat. Here, we constructed the relationship between DNA fragments and their full-length genes, and developed an updated LMW-GS gene molecular marker system. By using this updated marker system, LMW-GS genes in common wheat could be efficiently dissected, which would greatly contribute to the functional analysis of LMW-GS genes and facilitate the improvement of bread-making quality in wheat molecular breeding programs.

Low molecular weight glutenin subunits and gliadins in 150 double haploid wheat lines derived from a cross between Kariega and Avocet

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In this study a double haploid (DH) population of 150 lines was developed from a F_1 cross between Kariega (a hard red South African bread wheat with excellent quality) and Avocet S (a standard white Australian spring wheat), using the wheat-maize technique. Reverse phase high performance liquid chromatography (RP-HPLC) was used to determine the low molecular weight glutenin subunits (LMW-GS) and the gliadin composition of the parents and the population in order to determine the expression of these protein subunits in the DH derived population. Protein extraction was done according to Wieser *et al.* (1998). A Shimadzu HPLC system was used with a YMC-Pack ODS-A 150 × 4.0 mm inner diameter C_{18} column; column temperature was 50 ℃; injection volume was 50 µl for gliadins and 100 µl for glutenins; quantification was achieved by using a detection wavelength of 210 nm. Absorbance units under the different peaks were calculated according to Wieser *et al.* (1998). Eight distinct LMW-GS peaks were seen in both Kariega and Avocet, and the DH lines were found to be different combinations of the parental peaks. Twelve distinct gliadin peaks were seen in Avocet and 14 in Kariega. Once again the DH lines were different combinations of the parental peaks. Association was found within the LMW-GS and gliadins, indicating that they are genetically linked and expressed as pairs. There was also an association between specific gliadins and LMW-GS indicating that they could be coded by the same genes.

Comparison of glutenin subunit composition among Australian and North American wheat classes

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Glutenin subunit composition is one of the most important determinants for wheat end-use properties. We studied the glutenin composition among Australian and North American wheat classes {Australian Standard White (ASW), Australian Prime Hard (APH), No.1 Canada Western (1CW), US Dark Northern Spring (DNS), US Hard Red Winter (HRW) and US Western White (WW) exported to Japan in 2009. We analyzed individual 60 seeds using protein and DNA analyses. For *Glu-1* locus, most of seeds composing 1CW, DNS and HRW had *Glu-D1d*, which increases gluten strength, and about 20% of those composing ASW, PH and WW had this allele. Most of WW seeds had a null allele (*Glu-A1c*) or lacked the one of two *Glu-B1* bands (*Glu-B1a* and *Glu-B1bl*), which decreases gluten strength. For *Glu-3* locus, most of 1CW and DNS, PH and WW seeds had *Glu-B3h*, but most HRW seeds had *Glu-B3b* or *Glu-B3g*, which increases gluten strength. We also found a new *Glu-A3* allele in HRW and WW, and a new *Glu-B3* allele in WW. The glutenin composition of these classes might correspond to their end-product use. The relationships among their glutenin subunit composition, seed protein content and end-product use will be discussed.

High frequency of abnormal high and low molecular weight glutenin alleles in Chinese wheat landraces of the Yangtze-River region

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High and low molecular weight glutenin subunits (HWM-GS and LWM-GS) are most important components of wheat (*Triticum aestivum* L.) storage proteins, which play an extremely crucial role in the determination of end-use quality of common wheat. A total of 485 common landraces of bread wheat were collected from the Yangtze-River region of China. Their HMW-GS and LWM-GS subunit compositions were analyzed by Matrix-assisted laser desorption/ionization time-of-flight Mass Spectrometry (MALDI-TOF-MS). Among all landraces tested, 453 were homogeneous for HMW-GS, 32 were heterogeneous, and 37 contained abnormal subunits. A total of 22 alleles were detected, including 3 at *Glu-A1*, 13 at *Glu-B1* and 6 at *Glu-D1*, respectively. Higher variations occurred at the *Glu-B1* locus compared with *Glu-A1* and *Glu-D1*. *Glu-A1c* (74.0%), *Glu-B1b* (40.4%), *Glu-D1a* (84.9%) appeared to be the most frequent alleles at *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. Two alleles ("*null*" and 1) at the *Glu-A1* locus, three allele compositions (7+8, 7^{OE}+8, 7+9) at the *Glu-B1* locus, and two (2+12 and 5+10) at the *Glu-D1* locus appeared to be the common types in the 485 landraces. Sixteen new alleles represented by abnormal subunits were identified at the *Glu-B1* and the *Glu-D1* locus.

About the low molecular weight glutenin subunits (LWM-GS), we have only identified 17 alleles at three loci: *Glu-A3*, *Glu-B3* and *Glu-B3*, resulting in 64 different allele combinations. Out of 17 alleles detected at all the *Glu-3* loci, four belonged to *Glu-A3*, eight to *Glu-B3* and five to *Glu-D3* locus. MALDI-TOF-MS indicated *Glu-A3a/c* was present in 68.0% of the landraces, *Glu-A3d* in 4.6%, *Glu-A3e* in 0.6%, and *Glu-A3f* in 26.8%. Eight types of alleles were identified at the *Glu-B3* locus: *Glu-B3a* (5.4%), *Glu-B3b* (0.8%), *Glu-B3c* (18.6%), *Glu-B3d* (3.5%), *Glu-B3h* (31.2%), *Glu-B3i* (4.0%), *Glu-B3j* (20.5%) and *Glu-B3m* (16.1%). Five types of *Glu-D3* alleles were detected: *Glu-D3a* (1.5%), *Glu-D3b* (6.7%), *Glu-D3c* (15.3%), *Glu-D3d* (66.7%) and *Glu-D3f* (10.0%). Three new alleles represented by abnormal subunits were identified at the Glu-A3 and the Glu-B3 locus. More detailed studies are needed in order to match these alleles to previously discover abnormal or novel alleles.

Understanding the effects of HMW-GS and LMW-GS on processing quality using near-isogenic lines and development of functional markers for LMW-GS in wheat

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The HMW-GS and LMW-GS play an important role in wheat processing quality. In the present study, the relationship between glutenin subunits and processing qualities of pan bread, Chinese steamed bread and fresh white Chinese noodle was investigated in Aroona and its near-isogenic lines (NILs) with different HMW-GS and LMW-GS. SDS-sedimentation value (SSV), Farinograph and Extensograph values, and SE-HPLC parameters were measured. For dough strength related traits such as Farinograph stability and energy, 7+9, 17+18, 5+10, *Glu-A3b*, *Glu-A3d*, *Glu-A3f*, *Glu-B3b*, *Glu-B3g* were correlated with superior dough properties. Three *Glu-A1* alleles showed no significant difference on all parameters, except for pan bread total score (PBTS) and water absorption. The NIL with *Glu-A1 null* subunit performed inferior PBTS than other two NILs (1 or 2* subunit), although no significant difference was present among three NILs in SDS-sedimentation values, parameters from Farinograph, Extensograph, and SE-HPLC. Significant differences were found among the *Glu-B1* NILs in most Farinograph and Extensograph parameters, some pan bread quality parameters, SV and %UPP, but no significant difference was in PBTS. *Glu-D1* NILs showed significant difference in most quality parameters. The genotype with 1/7+9/5+10/*Glu-A3c*/*Glu-B3b*/*Glu-D3c* showed the best dough strength. For dough extensibility, a significant difference was found in *Glu-A3* NILs, and *Glu-A3e* was the most inferior allele, while the other five *Glu-A3* alleles had similar effects on Rmax, ST, %UPP and Ext. *Glu-B3g* and *Glu-B3b* was associated with higher quality parameters than the other *Glu-B3* alleles, and *Glu-B3c* NIL performed the worst PBTS, Rmax, ST, SV, %UPP and some other parameters. Five *Glu-D3* alleles produced similar quality parameters except for Farinograph water absorption. The genotype 1/7+9/2+12/*Glu-A3c*/*Glu-B3i*/*Glu-D3c* was correlated with superior dough extensibility. For Chinese steamed bread quality, *Glu-B3a* and *Glu-B3b* showed significantly better skin color. *Glu-B3a* showed higher total score than other allelic variations. The genotype 1/7+9/2+12/ *Glu-A3e*/*Glu-B3b*/*Glu-D3c* had significant effect on stress relaxation and total score. For fresh white Chinese noodle, *Glu-B3a* and *Glu-B3d* showed significantly better smoothness than others, and *Glu-A3b*, *Glu-B3g*, *Glu-B3h* exhibited better whiteness than others. The genotype 1/7+9/2+12/*Glu-A3c*/*Glu-B3d*/*Glu-D3c* showed significantly positive effect on viscoelasticity of noodle.

Estimating dough properties and end-product quality from flour composition

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One of the most important task of cereal science is to relate end-product quality to genes involved in determining certain attributes of quality. It requires a good understanding of the complex nature of quality that can lead to the proper measurements of these attributes. Most of our knowledge about the 'genetics of quality' derives from two different experimental approaches : i) direct measurements of quality traits on samples with systematically altered chemical composition; ii) relating quality and chemical composition/genetics of large sample populations using statistical methods. An overview on the recent achievements of these two approaches will be given illustrating the adventages/limitations of small- and micro scale methodology and *in vitro* reconstitution/incorporation methods in basic research and in different applications as well as introducing three prediction procedures for dough properties (Protein Scoring System [PSS]), loaf volume (Protein Quality Index [PQI]) and water absorption. The PSS model relates the individual and interactive contribution of HMW and LMW glutenin alleles to specific dough parameters, predicting the genetic potential of dough strength and extensional properties of dough of a wheat flour with 12% protein content and with the ratios of glutenin to gliadin and HMW to LMW GS of 1.0 and 0.2, respectively. The further developed version of the model is capable to consider the effects of the expression levels of the different storage proteins genes, so the actual dough parameters can be predicted. The input of this model is the allelic composition and the quantitative protein composition (including UPP%), while the output provides a good estimate of the actual dough strength and extensibility of a given sample ($r^2 > 0.85$ and $r^2 > 0.75$, respectively). Further applications of the models are also useful in blending formulation and to combine these data to predict the technology specific bread-making potential [PQI] of the samplesusing a nonlinear model. Results obtained from these models importantly point to the impact of allelic interactions as a major variable. Further genetic and biochemical investigations are needed in the future to satisfactorily explain the molecular aspects of these interactions. A novel approach, intoducing incorporation into rice flour dough can now clearly monitor the direct effects of individual polypeptides on mixing propteries while the same experiments using wheat flour doughs result in the direct plus interactive effects. Because of the large contribution of allele–allele interactions, the different allelic combinations, rather than the individual glutenin alleles needs to be controlled by breeders for selecting wheat with a desired performance.Based on both *in vivo* and *in vitro* studies it seems that the polymeric glutenin and monomeric gliadin proteins have almost identical contribution to water absorption, so this very important quality attribute does not depend on either the

glutenin and gliadin alleles present and or on the glutenin to gliadin ratio. Applying analytical data related to the non-gluten components of the flour, a reasonably good prediction model can be developed by using the soluble protein (albumin plus globulin) and total AX content of the flour, together with starch damage ($r^2 > 0.75$).

MALDI-TOF approach to measure cysteine number of wheat glutenin subunits

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In the current study, we established a fast method to accurately measure the number of cysteine residues in high molecular weight glutenin subunits. An alkylation regent, 4-vinylpyridine (4-vp), was used to treat the proteins during extraction. For every cysteine residue in a protein, such treatment increases its molecular mass value by 105.14 Da, which can be accurately determined by MALDI-TOF equipment. Based on the changes of the molecular mass value after 4-vp treatment, the number of cysteine residue can be reliably determined. We found that this method is also useful in studying non-glutenin proteins such as lupin seed storage proteins. This method is particularly valuable when the number of cysteine residue is of importance.

Reliability of gluten-related small-scale tests to estimate dough visco elasticity and bread loaf volume

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Dough visco elasticity is the main factor defining the bread making quality of wheat. In breeding to improve bread making quality it is necessary to apply selection pressure as early as possible (at least at the early-advanced stages). The Mixograph and the Alveograph are commonly used methods to determine dough mixing properties (development time, DDT and work input, %T), and dough viscoelasticity (deformation energy or dough strength, ALVW, and tenacity/extensibility, ALVPL ratio). Bread loaf volume is the ultimate parameter indicating bread making quality. However, all these method are time consuming and slow, with little use in early stages of selection for quality traits. The present study evaluated three small-scale tests: sodium dodecyl sulfate- sedimentation (SDS-S), lactic acid retention capacity (LARC), and the swelling index of glutenin (SIG), for their value to estimate dough visco elastic properties (DDT; %T; ALVW; ALVPL) and bread loaf volume (LV). A large population (242) of wheat advanced lines was used. SIG and LARC were strongly interrelated (r = 0.92) and both showed larger correlation coefficient than SDS-S with dough strength parameters and bread LV, although SIG showed larger correlation coefficient than LARC to screen for dough strength (R: 0.77 vs. 0.83, respectively) and bread LV (R: 0.54 vs. 0.62, respectively). Testing for LARC is simpler and faster than for SIG. Therefore, the use of either LARC or SIG to screen for dough strength and bread LV depends on how strong should be the selection pressure applied to early advanced wheat germplasm.

New possibilities in micro-scale wheat quality characterisation: micro-gluten determination and starch isolation

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The new enclosure of wheat quality and the support of wheat research activities require more effective and innovative solutions in analytical works. In cases when sample size is limited, development of small-scale dough testing methodology is needed, what is a rather challenging task. Since the development of 2g Mixograph, the first commercially available small-scale dough testing equipment, followed by the appearance of the micro extension instruments and the micro dough inflation procedures, intense research activities were carried out in evaluating different micro scale apparatuses as well as studying the effects of the reduction of sample quantities on the measured parameters. During the last decade, a family of micro-scale instruments has been developed in a long-term cooperation between Hungarian and Australian partners: micro-mill, micro-sieve, a prototype of a Farinograph/ Valorigraph-type micro-Z-arm mixer and a combined macro- and micro- sedimentation tester (Sedicom).

Recently a new type of gluten and starch washer equipment (GluStar, BME-Labintern, Hungary) has been constructed. The novelty of this system is the capability to determine the wet gluten content and isolating the washed out the starch and soluble components of flour from in one experiment. Both macro-and micro- modes are applicable, with the sample requirements of 10 g and 4 g flour, respectively. The modularity of the measuring system ensures the utilization of basic modules (gluten washer, and carbohydrate collector), separately. The gluten washer itself is applicable for measuring the gluten content. This method was validated, the results are well correlated with the generally used standard methods (ISO 21415-2:2006, ICC-Standard No.106/2).

The equipment was involve in different research studies, screening large sample populations of Hungarian and Australian wheat lines providing satisfactory reproducibility in wet gluten content and providing starch samples for further chemical and functional tests.

Some examples for the application the new equipment are given in this presentation including a study of investigating the impact of the AM/AP ratio and the arabinoxylan (AX) content on the rheological properties of samples produced blending bread wheat flours with high-amylose and waxy wheat flours.

Advances in quality property improvement and study of winter wheat in China

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Wheat, being a third grain crop in China, plays a significant role in the agriculture and food industry. In China today, economic and social development have greatly affected the living standard of consumers due toindustrialization and urbanization of the food industry which has developed rapidly. In addition, the food industry requires specialization and large scale (variety and quality) production of food products with a high standard level. Studying and proffering solution to this problem pose a challenge for the sustainable development of wheat production and the Chinese food industry.

This paper has investigated into the quality properties, especially protein properties, extended varieties in production areas, and reviewed existing literature in order to analyze the history, research advantages and recent problems of wheat quality breeding in mainly wheat production zone the and Huai Valleys Winter Wheat Zone, in China. The requirements of wheat quality for sustainable development of wheat production chain and Chinese food industry have been discussed. The recommendations proposed in this paper will be useful for departments of wheat production, storage and food industry as well as agricultural scientists.

Oxidation of gluten peptide

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Celiac disease, or gluten intolerance, is a small intestinal enteropathy caused by the ingestion of proline and glutamine rich protein from wheat, rye and barley. A 33-mer peptide from α_2-gliadin 56-88, with amino acid sequence LQLQPFPQPQLPYPQPQL PYPQPQLPYPQPQPF, was used as a model peptide for celiac disease study. For its high proline content (13 of 33 residues), 33-mer is highly resistant to luminal proteases and intestinal brush-border enzymes. The breakdown of 33-mer could reduce its toxicity to celiac disease patients. A prolyl-endopeptidase was reported a potent peptidase therapy for its post proline specificity. However, due to the limitation in enzyme usage, a non-enzymatic approach for detoxifying gluten peptides was studied as an alternative.

This work investigated non-enzymatic oxidation of a synthesized 33-mer peptide. Reactive oxygen species were formed through Fenton-type reactions. Metal catalyzed reactions were compared, as well as several conditions. The hydrolysis products were analysed by size-exclusion and reversed-phase chromatography. Immunological activities of oxidation products were tested by competitive enzyme-linked immunosorbent assay (ELISA), using antibody against pentapeptide QQPFP (R5). Oxidation of 33-mer peptide resulted in molecular changes as well as over 60% decrease in immunological activities. Therefore, this non-enzymatic method is potential in processing of gluten-free products. Wheat gluten oxidation and barley hordein oxidation are under investigation which includes oxidation mechanism, oxidation products and immunological activities of oxidation products of cereal prolamin proteins.

Study on starch and noodle quality of wheat *Wx* near-isogenic lines in two different genetic backgrounds

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Two sets of eight near-isogenic lines developed in a red wheat cultivar (Yang 17) and a white wheat line (Yang 01-2) were used to evaluate the effect of waxy gene on amylose content, physicochemical characteristics of starch and quality of dry white Chinese noodle. (1) No obvious morphological differences were observed in the starch granules of waxy and non-waxy wheat starch in two backgrounds. Wheat starches contained two types of starch granules, type A-granule (>10 um in diameter) and type B-granule (<10 um in diameter). Waxy and non-waxy wheat starch granules exhibited bimodal size distributions range of 0.4-40 um, the first peak at≈2.5um and the second peak at ≈19 um. There were no significant differences between eight genotypes in the percentage of type A-granule in volume, surface area and numbers. (2) The amylose content of waxy wheat line was the lowest in both two sets of eight near-isogenic lines .The double null lines showed lower amylose content than single null lines and wild-type lines. The reduction effect of amylose content due to these *null* alleles were *Wx-B1 >Wx-D 1 >Wx-A1*. (3) On DSC parameters, the gelatinization transition temperatures (onset, peak, and completion) and enthalpy of gelatinization of waxy starches were significantly higher than those of non-waxy starches.

Retrogradation % of the waxy starches were significantly lower than that of the other genotypes after the gelatinized samples stored at 4 ℃ for 3 days and 7 days，there were no differences between eight genotypes in retrogradation % at 14 days. (4) Compared with recurrent parents, the waxy wheat performed bad noodle quality, while partial waxy wheat performed well on color, firmness and elasticity. The noodle of the genotype *aaBBDD* in Yang 17 background and the genotype *AAbbDD* in Yang 01-2 background showed the highest total sensory score, and both higher than the control Xuehua flour. Hardness, springiness, cohesiveness, chewiness, resilience of TPA parameters tested by texture analyzer showed positive correlation with appearance, firmness of noodles evaluated by sensory at 5% or 1% level. The total noodle score showed positive correlation with TPA parameters except springiness, indicating the sensory evaluation could be substituted by texture analyzer.

Characteristics and evaluation parameters associated with cooking quality of Chinese fresh noodle

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Noodle cooking quality played an important role in assessing processing quality of Chinese fresh noodle. Forty-six Chinese wheat cultivars and advanced lines from the Northern Plain and the Yellow and Huai River Valleys Winter Wheat Regions were used to determine the relationship between wheat quality characters and evaluation parameters of Chinese fresh noodle cooking quality including total organic matter (TOM), cooking losses, water sorption, and noodle stickiness. The results indicated that large variations were observed in milling quality, dough rheology characteristics, starch properties, and noodle cooking quality parameters including TOM value, cooking losses, and noodle stickiness. Extensogram energy and maximum resistance contributed negatively to TOM value, with correlation coefficients -0.66 ($p < 0.01$) and -0.56 ($p < 0.01$), respectively. Correlation coefficients between Farinogram stability, Extensogram energy and maximum resistance and cooked noodle weights at optimal cooking (6 min) and overcooking (10 min) ranged from -0.55 to -0.63 ($p < 0.01$). Farinogram mixing tolerance index was significantly and positively related to cooked noodle weights at 6min and 10min, with correlation coefficients 0.67 ($p < 0.01$) and 0.69 ($p < 0.01$), respectively. Starch pasting temperature was significantly and positively correlated with cooked noodle weights at 10 min ($r = 0.60$, $p < 0.01$). This suggested that increased flour protein content and dough gluten strength contributed positively to noodle cooking quality, flour protein property was the major factor in determining noodle cooking quality, and noodle cooking quality was also affected slightly by starch pasting parameters. TOM value was significantly and positively correlated with cooked noodle weights at 6 min and 10 min, with correlation coefficients 0.66 ($p < 0.01$) and 0.69 ($p < 0.01$), respectively. Correlation coefficient between cooked noodle weight at 6 min and 10 min cooking time was 0.86 ($p < 0.01$). Therefore, it was recommended that cooked noodle weight at 10 min could be an important parameter for evaluation of noodle cooking quality. The cooked noodle weight at 10 min (10 g fresh noodle) should be no bigger than 21.0 g for good noodle cooking quality in Chinese wheat samples.

Starch biosynthesis in rice grains: natural variation and genetic improvement

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Starch, the reserve carbohydrate in cereal grains, makes up approximately 80-90% of the dry weight of the mature cereal grain, and contains two distinct polymers, amylose and amylopectin. The ratio of amylose and amylopectin as well as their fine structure varies with the botanical source of starch and affects its end use quality. Rice, rich in starch, is used as a major diet for more than half of the world's population. In Asia, over 2000 million people obtain 60% to 70% of their daily calories from rice and its processed products. Compared with other crops, rice starch contains tiny starch granules with a narrow size distribution, making rice starch ideally suited as a cosmetic dusting power, a textile stiffening agent, and a fat mimetic in foods.

The grain quality in rice was greatly affected by starch composition and structure. Therefore, the starch biosynthesis might play a crucial role in the formation of rice quality, especially the cooking and eating quality. In our studies, the natural variation of eighteen genes involved in starch synthesis, as well as the diversity of the grain starch quality, was carefully investigated among many representative germplasms. The effects of the variation on grain quality were firstly studied through association analysis, and then confirmed by using near-isogenic lines and/or transformants. In this presentation, the genetic variation of these starch synthesis related genes (SSRGs) and their effects on grain quality will be presented. The strategies and application of molecular techniques for quality improvement in rice will be also presented and discussed.

Bio-fortification for high micronutrients in wheat breeding program in China

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Nutritional problems related to diets of cereal were observed throughout the world, leading to the bio-fortification program known as HavestPlus with the objective to develop high concentrations of micronutrient including Fe, Zn in staple crops such as common wheat through breeding, and HEALTHGRAIN program with the objective to increasie protective roles of phytochemicals such as phenolic compound concentrations in wheat grain against the risk of many chronic diseases, especially those related to metabolic syndrome. Two hundred and sixty-five Chinese wheat cultivars and advanced lines were collected and sown at Anyang experimental station of the Institute of Crop Science, CAAS, in season 2005-2006 to evaluate the genetic variation of major mineral element concentrations in wheat grain, and 24 cultivars were also planted at seven representative locations in seasons 2005-2006 and 2006-2007 to evaluate the effects of genotype, environment, and genotype by environment interaction on mineral element concentrations. The 265 genotypes displayed a large variation for all mineral elements investigated including Fe and Zn, ranging from 28.0 to 65.4 mg kg^{-1} and 21.4 to 58.2 mg kg^{-1} for Fe and Zn. Jimai 26, Henong 326, and Jingdong 8 displayed high Fe and Zn concentrations, and Jimai 26 and Henong 326 also displayed high concentrations of Cu, Mg, K, P, and protein content. Jingdong 8 is the most promising leading cultivar for increasing Fe and Zn concentrations. All mineral element concentrations including Fe and Zn were largely influenced by environment effects. High and significant genotype by environment interaction effects on all mineral element concentrations were also observed, with ratios of genotype by environment to genotype variances all larger than 1.20. Thirty-seven Chinese commercial winter wheat cultivars grown at Anyang station over two seasons were also used to determine phenolic acid concentrations, and the fractions comprising free and bound types were analyzed using HPLC with measurements of individual phenolic acids in each fraction. Most of the parameters were significantly influenced by cultivar, season, and their interaction effects, with cultivar variance being predominant. Wide ranges of concentration among the cultivars were observed. The average concentration of bound type was 661 μg g^{-1} of dm (dry matter), making up 97.5% of the total phenolic acid with ferulic accounting for 70.7% of it, while free type made up only 2.5% of the total phenolic acid with syringic accounted for 44.7% of it. Bound type was the predominant contributions to the total phenolic acid concentration. Cultivars Liangxing 66 and Zhongmai 895 were stable in concentration of phenolic acids across seasons, with high values of free and bound phenolic acids indicating they could be selected as parents in breeding for health beneficial phenolic acid.

Allergen potential of non-prolamin seed proteins in Brachypodium distachyon

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Prolamin proteins are considered as key players in the development of different wheat related health problems, such as celiac disease or different sorts of wheat allergies. Although most of the identification methods and clinical tests are focusing on the identification of these proteins, there are some additional protein families published, causing various sorts of allergic responses. These protein families include members of different enzymes, thioredoxins, expansins, beta-purothionins, heat shock proteins etc. Diversity and number of these proteins as well as their effect compared to prolamin proteins is not clarified so far. The relatively small number of expressed wheat seed proteins found in the protein databases makes it difficult to get a complete overlook of the allergome of the wheat seed. Whole genome sequencing projects carried out on cereals, such as wheat or barley will facilitate to reveal the complexity of genetic effects of cereal allergies. So far, *Brachypodium distachyon* serves as the evolutionary closest sequenced genome for *Triticeae*. To better understand the potential detrimental effect of different seed proteins an *in-silico* analysis has been carried out using published publicly available epitope databases and the *Brachypodium distachyon* proteome. Epitopes not causing pollen allergies, identified from *Pooideae* have been used only in the analyses. Conservation analyses were carried out using 100% sequence homology between epitope sequences and target proteins. Potential seed allergens have been identified using developing *Brachypodium* seed ESTs and rice homologues. Trypsin, chymotrypsin and pepsin were used in *in-silico* digestion experiment and epitopes resistant to enzymatic cleavage were identified. Our results show that about 0.7% of the entire *Brachypodium* proteome possesses one or more allergic epitopes causing either Celiac Disease or wheat allergy or both. Variability in epitope occurrence and antibody response, as well as possible induced diseases was evaluated. The Biological Networks Gene Ontology tool (BiNGO) was used to find protein families with overrepresented Gene Ontology terms, such as nucleic acid binding, transcription factor binding or protein dimerization. Possible toxic effect of these proteins was evaluated using EST library data. Allergic potential of some of these protein families has been characterized in wheat. The results presented here emphasize the importance to study the allergen characteristics of non-prolamin seed proteins and will serve as important basis to identify different allergen protein families in wheat or other member of *Triticeae*. Wild wheat species and primitive wheats can be a potential candidate for replacing the traditional ingredients of certain food products providing safety for people suffering from wheat caused health problem.

Blood pressure lowering peptides from wheat gluten

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High blood pressure or hypertension is an important worldwide health problem because of its high frequency and associated risks of cardiovascular and kidney diseases. Renin-angiotensin system (RAS), which mediates vasoconstriction and extracelluar volume, is the most important system that regulating blood pressure. ACE inhibitors in RAS can lower blood pressure. Hypertension drugs, like captopril, are usually ACE inhibitors. However, long-time take of hypertension drugs causes dry cough and other severe implications. ACE-inhibitory peptides can be obtained from various food protein sources. Applying ACE-inhibitory peptides in food matrix and developing food products containing high content of bioactive peptides are desired. Wheat gluten, a co-product from starch industry, is a potential source for obtaining ACE-inhibitory peptides.

This work studied substrate wheat gluten degradation by enzymatic hydrolysis of thermolysin or/and prolyl endoprotease from *Aspergillus niger* (AN-PEP, Clarex). The hydrolysis condition, like incubation time and substrate to enzyme ratio, was determined by SDS-PAGE, free amino nitrogen content and size-exclusion chromatography. Followed by ultrafiltration with 3kDa cut-off after hydrolysis, the *in vitro* ACE-inhibition activities of hydrolysates was measured, and expressed by IC_{50} value. Furthermore, purification of hydrolysate was carried out by reverse phase liquid chromatography, and coupled with mass spectrometry targeted ACE-inhibitory tripeptides was identified. The optimum condition for thermolysin hydrolysis of wheat gluten was pH 8.0, at 40 ℃ for 4 hours incubation, and for Clarex hydrolysis was pH 4.0, at 70 ℃ for 22 hours. The sample hydrolysed by thermolysin first and then, by Clarex contained 126.6 mg/l free amino nitrogen, and main molecular distribution was under 6.5 kDa. After 3 kDa cut-off ultrafiltration, first thermolysin then Clarex treated hydrolysate showed strongest ACE-inhibition activity, IC_{50} value 0.016 mg protein/ml. According to an external standard, ACE-inhibitory tripeptide Leu-Gln-Pro (LQP) was identified. Targeted liberation of ACE-inhibitory peptides by specific enzymes and isolation by membrane filtration techniques appears a promising approach to produce blood pressure lowering peptides from wheat gluten.

How reliable are the measurements of residual gluten in gluten-free foods?

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People with coeliac disease have to maintain a gluten-free diet, which means excluding wheat, barley and rye prolamin proteins from their diet. Immunological ELISA methods are used to quantify the harmful proteins and to control the purity of gluten-free foods. Prolamin groups of cereals consist of a complex mixture of proteins that vary in their size and amino acid sequences. The diversity among prolamin proteins sets high requirements for their quantification. Modification of gluten proteins further increases the diversity, which questions the reliability of the analysis results.

In this study, the behavior of prolamins in immunological gluten assays and with different prolamin-specific antibodies was examined. Differences between the recognition of antibodies and prolamin subgroups were shown by Western blot studies. The results demonstrate that the currently used gluten analysis assays are not able to accurately quantify barley prolamins. However, we demonstrated with barley prolamin standard that more precise results can be obtained when the standard more closely matches the sample proteins. Further studies were carried out to study, how enzymatic or chemical modification affects measurement of prolamin contents. Competitive and sandwich ELISAs based on R5 antibody were used to detect residual rye prolamins in sourdough systems after enzymatic hydrolysis. The results showed substantial degradation of rye prolamins in an enzyme-active rye-malt sourdough system. The prolamin levels were lowered by 99.5% from the original levels (Loponen *et al.* 2009). Such extensive degradation of rye prolamins suggest the use of sourdough as a part of gluten-free baking. Although, the competitive technique is recommended for samples containing small hydrolyzed peptides, consistent results were obtained with sandwich assay. ELISAs based on R5, A1, G12 and ω-gliadin antibodies were used to measure wheat prolamins after deamidation by acid. According to our results, the analysis methods were not able to accurately quantify wheat gluten after deamidation. Deamidation is used to improve prolamin solubility and ability to form structures such as emulsions and foams. Deamidation, however, changes the protein structure, which has consequences for antibody recognition in gluten analysis. Consequently, deamidated gluten peptides can exist in food products and remain undetected, and thus cause a risk for people with celiac disease. The results demonstrate that current gluten analysis methods cannot accurately quantify prolamins in all food matrices. In future, it is essential to improve gluten analysis methods so that they can reliably be applied to measure residual gluten in gluten-free foods.

Effects of HMW- & LMW-glutenins and grain hardness on size of gluten polymers

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Previous studies on wheat near-isogenic lines have shown that kernel hardness affects molecular mass of storage protein polymers (Lesage *et al.* 2011). Size of glutenins' polymers is known to be involved in rheological properties of dough (strength, elasticity and extensibility). In order to assess effects of both grain hardness and glutenin alleles on storage proteins polymers, genetic diversity of various traits including grain hardness (GH), protein content (PC), glutenin alleles, polymer characteristics: mass (Mw), polydispersity index (Mw/Mn), polymer radius (Rw), and percentages of five protein fractions was investigated in a set of French cultivars grown in six locations in France and two successive years. Polymer characteristics were evaluated by Asymmetrical Flow Field-Flow Fractionation (AFFFF) and percentage of five protein fractions (F1 to F5) was obtained by Size Exclusion-HPLC (SE-HPLC).

Growing locations significantly influenced both PC and Mw, and these two traits were never correlated in each location. Grain hardness had a strong influence on Mw, Mw/Mn and Rw. Variation in GH resulted in an average increase of polymer masses of 21.4% in Soft varieties compared to Hard ones for the two years. Conversely, grain hardness did not influence PC and SE-HPLC fractions. LMW-glutenins were shown to have a major effect on percentage of protein fractions. *Glu-B3* was highly influent on percentages of all protein fractions. Surprinsingly, in addition to grain hardness effects, significant interactions were revealed between GH and glutenin alleles encoded at *Glu-A1*, *-B1*, *-D1*, *-A3*, *-B3*, *-D3* loci. Indeed, glutenin alleles had contrasted effects on polymer characteristics (Mw, Mw/Mn, Rw) according to grain hardness classes. For example, polymer masses were significantly higher in Soft varieties carrying *Glu-A1-2** allele compared to hard ones. In the same way, GH and glutenin alleles interactions strongly influenced percentage of protein fractions. For instance, *Glu-A1-1* subunit was favorable to % F2 fraction in Hard varieties and unfavorable in soft varieties.Allelic variations of puroindolines, genetic basis of softness, were also analyzed in the set of cultivars and their effects on polymers size were tested.

Study on starch quality in the grain of wheat and naked barley

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Starch is an important determinant for end uses of grain of wheat and naked barley and thus great efforts were put into (1) indentifying and characterizing starch granule –bound proteins (SGAPs), (2) developing various mutants with abnormal starch properties to expand the end uses of these grains, (3) understand the mechanism of starch biosynthesis and (4) the application of waxy wheat to wine-making.

A total of nine SGAPs with molecular weight of 57~130 kD were observed in 74 indigenous wheat lines, of which two novel bands were not reported in previous study. Twelve and three lines were identified as *Wx-B1* null and *SGP-B1* (SSII) null mutans, respectively. Sequence analysis indicates *SGP-B1* null mutants were resulted from a novel allelic SSII gene. Additionally, the difference on starch pasting properties of waxy wheat and other wine-making cereal flour was investigated and their starch pasting properties of reconstituted flours through waxy wheat blending with common wheat, sorghum and waxy rice were also analyzed. The results indicated that waxy wheat had certain priority to be used to make wine because it had rapid rate of pasting and less energy for pasting. And then, the trial of making wine using waxy wheat was carried out and the components of the wine were analysed and economic value of waxy wheat used as wine –making source was evaluated.

Naked barley is a staple crop as well as ethnic culture carrier in Qinghai-Tibetan Plateau of China. We have separated 21 different SGAPs with molecular weights more than 45kDa from naked barley using 1D-SDS-PAGE technique and some novel SGAPs were found by combination with 2D-PAGE and LC-MS/MS and ESI-Q-TOF MS/MS. It was found there might be a different mechanism for the production of low amylose naked barley genotypes through studying the sequence characters of the *Waxy* genes and the SGAPs have great effect on starch properties of grains by RILs analysis.

Structural and mechanical properties of compression-molded wheat gluten, gliadin and glutenin enriched films

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Within the future bioeconomy based society, there is need to replace petro-based plastics with renewable materials derived from plant polymers e.g. starch and proteins. Wheat gluten is abundantly produced as a byproduct from the bio-ethanol and starch industry as an inexpensive raw material that can be used in bio-based material industry. The wheat gluten proteins i.e. gliadin and glutenin offer unique and interesting combination of strength and elasticity to be utilized in bio-based material industry. In addition, wheat gluten proteins have good oxygen barrier and film forming properties with high biodegradable potential. The aim of the present study was to examine the structural properties and protein-protein interactions in compression-molded plasticized wheat gluten, gliadin and glutenin enriched films. The differences in structural and mechanical properties of plasticized wheat gluten, gliadin and glutenin enriched films were also evaluated. Size exclusion high performance liquid chromatography (SE-HPLC) and reverse phase high performance liquid chromatography (RP-HPLC) was used to study the structural and polymerization behavior of compression-molded bioplastic films. While tensile testing was used to measure the mechanical properties. Results obtained by SE-HPLC showed that the protein solubility of compression molded wheat gluten, gliadin and glutenin enriched bioplastic films (with 30% glycerol) in SDS extraction buffer was extremely low as compared to raw gluten, gliadin and glutenin enriched proteins. The solubility of proteins remained low even after repeated sonication. The low protein solubility is due to high degree of protein aggregation during compression-molding at high temperature (130 ℃) and pressure (10 MPa). The glutenin enriched proteins seem to be more aggregated and showed strong polymerization compared to the gliadin. The RP-HPLC results indicated solvent-specific behavior of proteins, protein extractability became higher in solvents with reducing agents i.e. DTT and urea due to the disruption of disulphide interchange reactions. The RP-HPLC results also showed that not all the proteins were extracted even when using DTT and 6M urea indicating presence of stronger covalent or other type of bonding other than disulphide cross-linking. The mechanical properties of plasticized wheat gliadin and glutenin enriched films showed that glutenin enriched films are stiffer while the gliadin enriched films showed higher extensibility. This study gives better insight into the polymerization, structural and mechanical properties of bioplastic films prepared from different types of gluten proteins.

Wheat and lupin protein interaction at baking: modifying extractability from lupin-wheat bread

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Mixing of lupin flour to wheat in bread making provides a number of health benefits and has major effects on bread properties including protein extractability. The study investigated wheat and lupin protein interaction as influenced by baking process and modification of protein extractability, which is important for the final quality of bread. MALDI-TOF mass spectrometry and 2 dimensional gel electrophoresis followed by MS/MS peptide sequencing were used to study bread protein matrix and wheat and lupin flour proteins. The results demonstrated that many of the wheat and lupin proteins including high molecular weight glutenins remained unchanged in baking as per their elctrophoretic behavior. However, some of the proteins of wheat and lupin became unextractable from the bread, indicating lupin-wheat protein interaction during baking. For the lupin proteins, most of the α-conglutins could be readily extracted from the lupin-wheat bread even at low salt and non-reducing/non-denaturing extraction conditions. In contrast, most of the β-conglutins lost extractability suggesting that they were cross-linked to the wheat gluten network and trapped into the bread matrix. The higher thermal stability of α-conglutins compared to β-conglutins is speculated to account for this difference.

Poster Presentation

Genomic analysis of the expressed α/β-gliadin gene region in hexaploid wheat

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The wheat genome is particularly large and characterized by repetitive sequences and transposable elements. Therefore, genome sequence information of wheat is still limited. Wheat genome sequence data are powerful for isolating and characterizing important genes. In this study, we analyzed genomic BAC clones in order to characterize the structure and expression of α/β-gliadin multigenes in the hexaploid wheat genome.

BAC clones containing α/β-gliadin genes from the three loci, *Gli-A2*, *Gli-B2*, and *Gli-D2* were screened from a genomic BAC library of *Triticum aestivum* cv. Chinese Spring. Based on their restriction fragment patterns, we selected five BAC clones, namely, two clones for *Gli-A2*, two clones for *Gli-B2*, and one clone for *Gli-D2*, to fully sequence. Approximately 200 kb was sequenced for each locus. We compared total 600 kb of sequences from three homoeo-loci. In total, twelve α/β-gliadin intact genes and four pseudogenes were found. Annotation of these sequences revealed that abundant retrotransposons or other transposons have accumulated around the α/β-gliadin genes.

The pattern of genome segmental duplication within each BAC was revealed by dot-plot analysis. We calculated evolutional time since duplication of each set of α/β-gliadin genes and insertion of retrotransposons based on the number of base substitutions per site. Duplication of all adjacent genes within the same BAC clone took place before or after allotetrapolyplodization, but duplication of certain genes occurred before diploid differentiation of wheat species. Retrotransposons were also inserted before and after the segmental duplication events. Duplication of genome segments containing of α/β-gliadin genes and retrotransposons brought about through unequal crossing-over or saltatory replication, and α/β-gliadin genes for their own were duplicated without any recombination events.

The expression of α/β-gliadin genes annotated from the sequence was estimated by the frequency of ESTs (Kawaura *et al.* 2005). Out of twelve intact α/β-gliadin genes used in this study, nine were expressed, although their patterns of expression were distinct. Since they have similar cis-elements and promoter structures, the mechanisms underlying their distinct gene expression and possible applications are suggested.

Profiling of gliadins from common wheat by using two-dimensional gel electrophoresis

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Gluten contributes significantly to the processing properties of dough. It is composed of gliadin and glutenin, which are seed storage proteins in wheat. Gliadin is a monomeric protein, classified as α/β-, γ-, or ω-gliadin on the basis of amino acid composition and molecular weight. Storage proteins play an important role in influencing the quality of flour, however, they also function as allergens. In paticular, α/β-gliadin is reported to cause celiac disease (CD). Ingestion of α/β-gliadins by CD carrier's, induces inflammation of the small intestine, resulting in malnourishment. Genes encoding α/β-gliadins are organized multigene families at the *Gli-A2*, *Gli-B2*, and *Gli-D2* loci located on the short arm of wheat chromosome 6.

To characterize gliadins, we profiled gliadins from Chinese Spring wheat. We assigned certain spots to the A, B, and D genomes using the nullisomic-tetrasomic lines of chromosome 6. Gliadins were extracted from mature seeds using the NaI method (DuPont *et al.* 2005, Fu *et al.* 1996) and separated by using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). A half of mature wheat seeds excluding the embryos were crushed; each sample consisted of 15 mg of the crushed seeds. Gliadins were extracted in 300 μl of gliadin extract buffer (0.3 M NaI, 7.5% 1-propanol) and centrifuged for 10 min at 15,000 rpm. The supernatants were pooled. SThis step was repeated twice. Next, gliadin was precipitated by adding 4 volumes of NH_4-Ac-MeOH and chilling at -30 ℃ for 4 hours. The mixture was centrifuged for 10 min at 15,000 rpm: the supernatant was discarded. The residue was run on 2D-PAGE. Sample buffer (8.5 M urea, 4% CHAPS, 0.5% isoelectric focusing (IEF) buffer, 25 mM DTT, 16% isopropanol, BPB) was added to the pellets, mixed for 30 min, and centrifuged for 1 min at 15,000 rpm. IEF was performed at pH 6 -9. SDS-PAGE was performed using 15% acrylamide gels at 200V for 5 hours.

We identified 73 gliadin spots in Chinese Spring wheat and assigned 9 spots to the A genome, 7 to the B genome, and 16 to the D genome. Furthermore, we obtained the profiles of gliadins from other bread wheat seeds. The wheat strains showed wide variations in the position of the spots, suggesting the possibility of producing hypoallergenic bread wheat.

Variations of toxic epitopes related to celiac disease in alpha-gliadin genes from C, M, N and U genomes of *Aegilops* species

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Alpha-gliadins are associated with human celiac disease. A total of 23 non-interrupted full open reading frame α-gliadin genes and 19 pseudogenes were cloned and sequenced from C, M, N and U genomes of 4 diploid *Aegilops* species. Sequence comparison of α-gliadin genes from *Aegilops* and *Triticum* species demonstrated an existence of extensive allelic variations in *Gli-2* loci of the 4 *Aegilops* genomes. Some specific structural features were found including the compositions and variations of 2 polyglutamin domains (QI and QII) and 4 T cell stimulatory toxic epitopes. The average numbers of the QI domain in C and N genomes and the QII domain in C, N and U genomes were much higher than those in *Triticum* genomes, and the QI domain in C and N genomes and the QII domain in C, M, N and U genomes displayed greater length variations. Interestingly, the types and numbers of 4 T cell stimulatory toxic epitopes in α-gliadins from the 4 *Aegilops* genomes were significantly less than those from *Triticum* A, B, D and their progenitor genomes. Specific relationships between the structural variations of the two polyglutamine domains and the distributions of 4 T cell stimulatory toxic epitopes were found, resulting in α-gliadin genes from the *Aegilops* and *Triticum* genomes to be classified into three groups. Phylogenetic evolution relationships of the α-gliadin genes showed that the 4 *Aegilops* genomes were closely related and diverged at about 7.23 MYA while the M and U genomes were closer to the D genome than to A and B genomes. These results are potentially valuable for wheat grain quality improvement with lower toxic epitopes.

Towards a more systematic understanding of the structure and function of homoeologous *Glu-3* loci in common wheat

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In common wheat, the complex homoeologous *Glu-3* loci (i.e., *Glu-A3*, *-B3* and *-D3*) contain the genes encoding low molecular weight glutenin subunits (LMW-GSs), which have important influences on the viscoelastic properties of doughs and hence the end use qualities of common wheat grains. Owing to the existence of multiple loci and gene members, it has been difficult to understand the function of individual LMW-GS locus and gene member in end use quality control. This difficulty is further complicated by the lack of structural information on *Glu-A3*, *-B3* and *-D3* genomic regions and the occurrence of extensive allelic variations of the three loci among different wheat genotypes. To contribute to international efforts in studying LMW-GSs in common wheat, we have initiated a series of investigations aiming to achieve a more systematic understanding of the structure and function of homoeologous *Glu-3* loci in Xiaoyan 81, an elite winter wheat variety with superior processing qualities for bread and noodle. The *Glu-A3* and *-B3* alleles of Xiaoyan 81 were found to be *Glu-A3c* and *Glu-B3d*, respectively. However, its *Glu-D3* allele was different from the series of *Glu-D3* alleles that have been characterized previously. Xiaoyan 81 was estimated to have 4, 11 and 2 genes encoding i-, m- or s-type LMW-GSs, respectively, using a PCR protocol designed for investigating the copy number of different types of LMW-GS genes. A set of deletion mutants lacking *Glu-A3*, *-B3* or *-D3* was identified by screening 2400 M2 families created with ion beam mutagenesis. The boundaries of the deleted chromosomal fragments in *Glu-3* loci could be mapped using gene based markers. Aided by these mutants, 3, 3 and 8 LMW-GS gene members have been assigned to the *Glu-A3*, *-B3* and *-D3* loci of Xiaoyan 81, respectively. For future functional analysis, the deletion mutants have been backcrossed to wild type Xiaoyan 81 three times. Apart from the investigations listed above, good progress is also being made in identifying the LMW-GS proteome of Xiaoyan 81 by 2-DE coupled with mass spectrometry. It is hoped that the complementary efforts described above will soon be able to provide new insights into the structure of *Glu-3* loci and the expression and function of LMW-GS genes in common wheat.

Molecular characterization and comparative analysis of α-gliadin genes from *Brachypodium distachyon* and *Triticum* related species

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Four novel full-ORF α-gliadin genes and twenty five pseudogenes containing at least one stop codon were cloned and sequenced from five *Brachypodium distachyon* accessions. Analysis of α-gliadin genes revealed some InDels and a considerable number of SNPs among them. Most of the pseudogenes were resulted from C to T change, leading to the generation of TAG or TAA in-frame stop codon. To compare with both the full-ORF α-gliadin sequences and pseudogenes of *Triticum* and *Triticum* related species, we analyzed their structural characters. According to the analysis of 4 T cell stimulatory toxic epitopes, we found that the species of *Aegilops*, *Triticum and Brachypodium* were ore closely. Phylogenetic analysis demonstrated that the α-gliadin genes of *Triticum* species can be into six groups, and the four novel full-ORF α-gliadin genes from *Brachypodium distachyon* had a high homology with the third group.

Effect of high molecular weight glutenin subunits (HMW-GS) on quality property of wheat kernel protein

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The high molecular weight glutenin subunits (HMW) are coded by genes at three genetically unlinked loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, which occur on chromosomes lA, 1B and lD, respective1y. HMW-GS composition has closely relationship with wheat kernel quality, and it has become the main basis of wheat quality breeding. This paper is to study the effect of the HMW-GSs and their combinations on quality property of kernel protein, include protein content, gluten content, gluten index, sedimentation value, farinograph parameter, extensograph parameter. 80 wheat cultivars and advanced lines were selected from 2009-2010 harvest produced in Guanzhong of Shaanxi province and Northern of Henan province, were used as test materials. The variation characteristic of high-molecular-weight glutenin subunit has been analyzed through the SDS-PAGE electricity. Analyses of variance (ANOVA) were used to investigate the effect of single subunit at *Glu-A1*, *Glu-B1*, *Glu-D1* loci and subunit combinations on quality property of kernel protein. The results of ANOVA showed that the HMW-GS of different locus have significant effects on quality property. For quality property of kernel protein, at *Glu-1* loci, *Glu-D1*＞*Glu-B1*＞*Glu-A1*, at *Glu-A1* locus, 1＞*Null*, at *Glu-B1* loci, 14+15＞7+8＞7+9, at *Glu-D1* locus, 5+10＞2+12＞4+12. For different subunit combinations, wheat cultivars with subunit combination 1/7+8/5+10 or *Null*/7+8/2+12, had higher protein quality property.

Study the interactive effects of the wheat storage proteins in gluten free model system

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The mixing requirement of wheat flour and the rheological properties of the resulting dough largely depend on the composition of gluten proteins. The large variation of storage protein composition of wheat is caused by the significant polymorphism of the 12 prolamin coding loci. Significant information about the effects of individual glutenin subunit proteins on dough properties has been obtained by a specially designed reconstitution study: the alteration of the rheological properties of a so-called "base-flour" dough after supplementation/ incorporation of the glutenin protein of interest provided novel information about the role of these individual proteins. However, the principal limitation of these incorporation studies is that the results of these experiments are largely dependent on the allelic composition of the base flour. The resulting changes in the rheological properties of the wheat dough are partly dependent on the direct contribution of the incorporated protein and also on the protein's interaction with other prolamin type proteins present in the base flour. Rice flour does not contain any wheat prolamin type proteins, and because of this it provides a new approach to investigate the functional properties of wheat proteins. By incorporating these proteins into rice dough, we can investigate the direct effect of the protein on the functional properties of the rice dough in the absence of the interactions with the other prolamin type proteins.

The aim of this work was to use rice and wheat flours in incorporation experiments to determine and compare the effects of purified wheat glutenin subunit proteins individually or in combination on the functional properties of reconstructed rice and wheat doughs.

The incorporation of individual HMW glutenin subunit proteins (*Bx6*, *Bx7*, and *By8*) in different ratios had significant positive effects on the mixing requirements of both rice and wheat doughs. Reconstitution experiments using two *x+y* type HMW-GS pairs together with a bacterially expressed LMW-GS have been also carried out. The largest effects of polymer formation and mixing properties of rice flour dough were observed when *Bx* and *By* subunits were used in a 1:1 ratio and HMW and LMW glutenin subunits in a 1:3 ratio. However, using the same subunit ratios in wheat as the base flour, these synergistic effects were not observed. The observed changes in the functional properties of rice and wheat doughs by incorporation of the wheat subunit type proteins are directly related to modification of the relative amount and the size distribution of the polymeric proteins in them. The variation of wheat and rice flour as base flour provides a possibility to study the effect of a particular protein and determine the individual and interactive effects on two related but different aspects, namely, on polymer formation and on the contribution to the viscoelastic properties of

the dough. This novel information provides a deeper understanding of the structural/functional relationships of wheat glutenin proteins and can be applied to improve wheat quality.

Molecular cloning and characterization of six novel HMW-GS genes from *Aegilops speltoides* and *Aegilops kotischyi*

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In the present work, six novel high molecular weight glutenin subunits (HMW-GS) from *Aegilops speltoides* (SS, 2n = 2x = 14) and *Aegilops kotischyi* (UUSS, 2n = 4x = 28) were identified by SDS-PAGE and designed as *ASy15**, *AKx1**, *AKx3**, *AKx2.3*, *AKy20** and *AKy8**, respectively. Their complete open reading frames (ORFs) were cloned and sequenced by AS-PCR. Sequence comparison demonstrated that these novel genes displayed higher SNP and InDel variations. Particularly, *AKy8** had an extra cysteine residue at position 140 in the central repetitive domain while *AKx2.3* had an unusual long repetitive domain (816aa), which might have positive effects on gluten quality. Phylogenetic analysis showed that *AKx1** and *AKx3** were assigned to 1S genome, *AKx2.3* to 1U genome, and *AKy20** and *Aky8** most likely to 1S genome. The divergence between 6 HMW-GS genes from two *Aegilops* species and those from *Triticum* species occurred about 4.77-23.54 MYA. The authenticity of these isolated endogenous HMW-GS genes was confirmed through heterologous expression in *E. coli* and Western blotting testing.

Molecular characterization and evolution of HMW glutenin subunit genes in *Brachypodium distachyon*

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The small wild grass within *Pooideae* family, *Brachypodium Distachyon* has been emerged as a new model system for the study of temperate cereals. *Brachypodium* has showed to have close relationships to rice, wheat and barley. This makes it an ideal model organism for understanding the genetic and molecular biology of cereal crops. The x- and y-type high molecular weight glutenin subunits (HMW-GS) are highly conserved seed storage proteins in wheat and related species. Here we describe the molecular characterization and evolutionary relationships of HMW glutenin subunit genes from several *Brachypodium distachyon* accessions (2n = 10, 30) such as Bd21 and Bd21-3. SDS-PAGE demonstrated that *Brachypodium* had few HMW-GS compared to Chinese Spring, but one clear band was present in the HMW-GS region in Bd21. To confirm its presence, several pairs of AS-PCR primers based on the conserved regions of wheat HMW-GS genes were designed and used to amplify the genomic DNA of *Brachypodium* accessions. The PCR products obtained were similar to the sizes of wheat HMW-GS genes. After sequencing, a total of 19 HMW-GS genes with nucleotide sequence of around 2100bp were obtained. Sequence multiple alignments showed that all belonged to y-type HMW-GS and demonstrated that those were highly similar structures to HMW glutenin subunits *1Dy12* from bread wheat, but with a stop codon at position 1521 in the ORF. Most of them were pseudogenes with 1-3 in-frame stop codons in the deduced amino acid sequences. However, a true y-type HMW-GS gene with the ORF of 1977bp was cloned from accession Bd21, and Western blot further confirmed its existence in Bd21. Phylogenetic analysis showed a highly conserved *Glu-1* loci present in *Brachypodium*, suggesting its closer phylogenetic evolutionary relationships with *Triticum* and related species. Our results provide new insights into the evolution of HMW glutenin subunits in cereals.

Genetic variation of dough properties independent of high and low molecular weight glutenin subunit genes in wheat

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Allelic differences of glutenin subunit genes are well known to influence bread-making quality, therefore it is important to characterize the alleles of glutenin subunit genes using DNA and protein markers, and to analyze the relationship between allelic differences and dough properties for developing new varieties. We have developed a cultivar 'Haruyokoi', which is a leading variety in Hokkaido, Japan with good bread-making quality, and has been acceptable for end users.

Based on DNA marker analysis and SDS-PAGE, the alleles of glutenin genes of advanced lines and 'Haruyokoi' were identified. The alleles of 'Haruyokoi' were *Glu-A1b, Glu-B1c, Glu-D1d, Glu-A3c, Glu-B3h and Glu-D3a*, and the dough property of 'Haruyokoi' was slightly strong compared with 1CW. On the other hand, one of the advanced lines 'HW5' proved to be the same alleles as 'Haruyokoi'. Although the protein content of 'HW5' was on a level with 'Haruyokoi', the dough property of 'HW5' was not so elastic, and was clearly different compared with that of 'Haruyokoi'. The dough development time determined by mixograph was shorter, and the values of R and R/E determined by extensograph were lower than those of 'Haruyokoi'. In addition, the proportion of the unsoluble polymeric protein (UPP) to the soluble monomeric protein (SMP) determined by SE-HPLC, and SDS-sedimentation volume of 'HW5' were lower than those of 'Haruyoikoi'. These results suggested that the quantity of glutenin polymers of 'HW5' was less than 'Haruyokoi'. In order to analyze factors regulating the differences of dough property between 'HW5' and 'Haruyokoi', 2D-PAGE was carried out focusing on gliadin proteins. Compared between the spots of the two lines, there was some possibility that some gliadin spots were detected only in the flour protein of 'HW5'. In addition, the cell wall of 'HW5' seemed thick through the observation by the electron microscopy. This indicated the arabinoxylan content of 'HW5'might be rich.

In this study, varietical differences controlling dough property and glutenin polymer quantity independent of glutenin genes were observed. The extra gliadin protein and the difference of cell wall thickness may be some of the genetic factors regulating the dough property and polymer content. This suggests that we have to pay attention to other genetic factors different from glutenin genes for selecting lines with high bread-making quality at early generations in our breeding program.

The impact of climate data adjusted to the grain development to explore wheat gluten quality variation

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To invest the effects of climate on the wheat gluten quality we have made climate models adjusted according to the development stage of the grain for field trails in spring and winter wheat grown in different fields from 2005 to 2011. The field trials were located to cover the most important wheat growing area in the south-eastern part of Norway. Major variability was observed from field to field within each year on the important gluten quality characteristics dough resistance. The climate was relatively similar among the fields within each year. However, when the climatic data was aligned according to the grain development there was a major variability from site to site suggesting a time dependent response to the climate condition. The variation in the course of temperature during the grain filling phase was created by: 1) the annual variation, 2) earlier or later sowing of field trials, and 3) the different rates of phenological development of the cultivars. The quality variation within each year was most extreme in 2007 and 2011 where the dough resistance was complete lost in most cultivars at some sites.

Changes of protein components and gluten content in the noodle processing and cooking

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To learn about the change of protein components in the noodle processing and cooking, three kinds of wheat cultivars with largely different qualities were selected. Protein content, protein components content, GMP (glutenin macro-polymer) content, gluten content and gluten index of the flour, dough crumbs, sheets, dried noodles and cooked noodles were investigated. The results showed that protein content had no significant change from wheat flour to cooked noodles. Changes of protein components were different for various wheat cultivars. After dough mixing, the content of globulin and gliadin decreased significantly. During the process from dough crumbs to dried noodles, glutenin content, GMP content, content of wet and dry gluten had no significant change. After cooking, the content of albumin, globulin and gliadin decreased significantly, while the content of glutenin and GMP increased substantially, and the gluten structure disappeared. In conclusion, protein content had no significant change in the noodle processing and cooking, while gluten protein components changed significantly during cooking.

In silico analysis of HMW glutenin's transcriptional regulation in wheat

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The storage proteins of wheat have a crucial role in the development of end-product quality, they allelic composition and expression determine the strength and extensibility of dough made from wheat flour. Prolamins consisting of monomeric gliadins and polymeric high molecular weight (HMW-) and low molecular weight glutenin subunits (LMW-GSs), stabilized through inter- and intra molecular disulfide bonds form a complex protein matrix, the gluten network. The composition and size of this gluten and its physical characteristics resulted in dough strength and extensibility depends on the expressional profile of the different components. The HMW glutenin subunit gene family is encoded by pairs of paralogue x- and y-type genes in a complex loci, denoted *Glu-1*, at the long arm of homologous chromosomes 1A, 1B and 1D. The x-type encodes a higher molecular weight and the y-type a lower molecular weight subunit. The order and the orientation of the genes are strictly conserved. The different loci show significant differences in expression and the y-type present at the *Glu-A1* locus is always silent in hexaploid wheat.

The synthesis and deposition of seed storage proteins is tissue specific, occurs specifically in the endosperm and only at specific times during seed development. In addition, it is influenced by the availability of nitrogen and sulphur in the grain. They expression shows similar profile, indicating a remarkable conservation in the machinery responsible for the storage protein gene expression schedule. Common regulatory *cis*-acting elements and *trans*-acting transcription factors observed in monocot seed storage protein genes have already been described. Comparison of promoter sequences revealed the identity of several *cis*-elements and common trans-activating transcription factors involved in the expression of wheat storage protein genes. *In silico* analysis for LMW glutenins already identified statistical correlations between the gene types' promoter profiles and their transcription rate (Juhász *et al.* 2011). A similar, EST based expression analysis paired with promoter sequence profiling is used to gain a deeper insight into the differing expression rate of HMW GS gene types encoded on the different loci. A possible explanation for the transcription patterns of HMW GS x and y pairs and the three loci will discussed.

Identification of transcription factors for wheat seed storage proteins using pull-down assay

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Seed storage proteins play a globally important role in nutrition. Their quality and composition has a large effect on the end-product of dough-based food. Amongst the crops, wheat is of crucial importance as it is one of the most widely used cereals for human consumption and animal feeding. Prolamins of wheat consist of three main types of storage protein: monomeric gliadins and polymeric high-molecular-weight (HMW-) and low-molecular-weight glutenin subunits (LMW-GSs). These subunits are stabilized through inter- and intra- molecular disulfide bonds to form a complex protein matrix, the gluten network. The composition and size distribution of the gluten matrix and its physical characteristics in dough depends on the genetic background of the individual variety and on the expressional profile of the different components. Thus understanding the mechanisms of gluten genes' expression is crucial to support molecular breeding of wheat.

There are many putative transcription factor genes identified *in silico* by orthology analysis but few has been either confirmed or *de novo* isolated by wet-bench techniques. During this project promoter sequences, about 1000 nucleotides upstream from the first ATG codon, were amplified for all the three types of wheat prolamins using (*T. aestivum*) cv. Chinese Spring. PCR fragments were cloned and the sequences were confirmed. For streptavidin

-beads based pull-down assays short (100-200 bp) fragments of these cloned promoters were amplified with biotinylated primers. Based on the already known sequence motifs the fragments were designed to identify protein-DNA interaction(s) and also to detect possible protein-protein interactions amongst the binding transcription factors. The SDS and MS fingerprints of the proteins were analyzed and the differences and similarities of the profiles will be discussed.

Effect of bug (*Eurygaster spp. ve Aelia spp.*) damaged flours on quality characteristics in cakes, cookies and breads

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Certain heteropterous insects named as *Eurygaster* spp. , *Aelia* spp. and *Nysius huttoni* cause pre-harvest damage to wheat in Europe, Middle East, North Africa and New Zealand. Wheat bugs attack on wheat by piercing it with their mouthparts and injecting their salivary secretions containing proteases. Dough prepared from this flour is very sticky and weak and produces loaves of poor volume and unsatisfactory texture due to gluten degradation. In this study, the effects of high protease activity flour (HPAF) due to *Eurygaster* and/or *Aelia* damage to wheat on rheological properties and quality characteristics of bakery products such as bread, cookie and cake were determined.

HPAF was added at 2.5%, 5%, 10%, 15% (w/w) levels in bread formula. While no significant differences crust colour, a significant reduction in loaf volume was observed with increasing HPAF addition levels. Textural quality (hardness, gumminess, chewiness and stiffness) of breads at high HPAF (10% and 15% w/w) levels was significantly increased, but there was no significant difference on cohesiveness and springiness values. HPAF was added four different levels (25%, 50%, 75%, 100% w/w) in cake formula. The quality of cakes added HPAF was comparable with control cake at all addition levels. Although a slight increase was observed in cake volumes depending on the HPAF addition level, no significant difference between the samples in terms of textural properties such as hardness, cohesiveness, springiness, gumminess, chewiness, adhesiveness, stiffness values and cake crust colour. Cookies were produced with HPAF at 20%, 40%, 60%, 80% and 100% (w/w) levels. No significant difference on colour values was observed, but cookies added HPAF were significantly harder with incsreasing addition level. The spread ratio of cookies was increased, as expected. The results indicated that HPAF had lowering effects on rheological properties and quality characteristics of bread depending on the addition levels. But the effetcs of HPAF on cake and cookie quality were limited.

Molecular cloning and characterization of four i-type LMW glutenin subunit genes from *Triticum zhukovskyi* and *Triticum compactum*

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Four novel low-molecular-weight glutenin subunit (LMW-GS) genes, designated as *TzLMW-i1*, *TzLMW-i2*, *TcLMW-i1* and *TcLMW-i2*, were cloned and characterized from the genomic DNA of *Triticum zhukovskyi* and *Triticum compactum*. The coding regions of *TzLMW-i1*, *TzLMW-i2*, *TcLMW-i1* and *TcLMW-i2* were 759 bp, 837 bp, 756 bp and 1170 bp in length, encoding peptides with 251, 277, 250 and 388 amino acid residues, respectively. Analysis of deduced amino acid sequences showed that the four novel genes were classified as LMW-i types and the comparison results indicated that the four genes had a more similar structure and a higher level of homology with LMW-i-type genes than with the LMW-m and LMW-s type genes. Sequence alignments result showed that *TzLMW-i1*, *TcLMW-i1* and *TcLMW-i2* were found to have all eight cysteines at conserved positions in the C-terminal domain, while *TzLMW-i2* to have an additional cysteine residue in the C-terminal cysteine-rich region. This additional cysteine residue was predicted to to be involveed in the formation of intermolecular disulfide bond. Therefore, *TzLMW-i2* may act as a “chain brancher” and could increase the protein cross-links in the gluten network, leading to the formation of larger glutenin polymers, which contribute to dough elasticity and high bread-making quality. Moreover, *TcLMW-i2* possessed a longer repetitive domain, which was considered to be associated with good quality of wheat. To investigate the evolutionary relationship of the novel genes with other storage proteins, a phylogenetic tree was constructed. The result of phylogeny showed that prolamin genes could be absolutely clustered into five groups, including high-molecular-weight glutenin subunits (HMW-GS), LMW-GS, α/β-, γ- and ω-gliadins, and four LMW-GS genes cloned in the present study was grouped into the LMW-i subgroup.

Quality and endosperm storage protein variation in Slovak registered winter bread wheat (*Triticum aestivum* L.) from 1976 to 2012

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Storage proteins are convenient biochemical markers for identification and registration of wheat cultivars, analysis of their purity. The major seed storage proteins of wheat are glutenin and gliadin. Glutenin is composed of high molecular weight (HMW) subunits and low molecular weight (LMW) subunits. The HMW glutenin subunits are encoded by homoeologous loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, which are located on the long arms of homoeologous group-one chromosomes. It has been demonstrated that good bread-making quality is strongly associated with the presence of specific HMW glutenin subunits. Sixty-three samples of common wheat varieties registered in the List of Registered Varieties from 1976 to 2012 in Slovakia, kept in the collection of the Gene bank Piešťany, were surveyed to determine their high molecular weight glutenin subunits as separated by polyacrylamide gel electrophoresis (SDS-PAGE). The HMW glutenin subunits were classified according to the numbering system of Payne and Lawrence (1983). Gliadin polymorphism was detected by electrophoresis according to the PAGE ISTA methodology. The allelic block *Gli-1B3*, the marker of rye translocation 1BL.1RS, also the marker of poor bread-making quality was detected in 12 genotypes. Fourteen high molecular weight (HMW) - glutenin subunits (GS) were found, three belonged to *Glu-1A*, seven to *Glu-1B* and four to *Glu-1D* locus. The most frequent (38 %) HMW-GS at the *Glu-1A*, *Glu-1B* and *Glu-1D* complex loci were 0, 7+9, 5+10 respectively. The allele most strongly associated with good quality, *Glu-D1* subunits 5+10, was present in 82.6 % of the studied wheat genotypes. The most common banding patterns were *Null* (on chromosome 1A), 7+9 (on chromosome 1B) and 5+10 (on chromosome 1D), respectively. However, low frequent alleles such as 17+18 and 20 were observed. The glutenin-based quality score ranged from 4 to10. For any further improvement of bread-making qualities in wheat cultivars, it will be necessary to replace the *null* allele with alleles 1 or 2* or with any other known alleles that have not been utilized in crop breeding so far.

The diversity of HMW-glutenin alleles in European wheat landraces

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Wheat landraces and primitive cultivars represent interesting biological material from historical, geographical and genetical points of view. Wheat landraces usually comprise much broader intra-specific genetic diversity than modern cultivars. Therefore they are considered a valuable source of useful genes. The study of landraces attracts more attention whereupon molecular markers have been set out to be intensively utilised for this purpose. Seed storage proteins are the first products of gene expression and because of that they are considered to be usable markers for the study of wheat genetic resources. High-molecular-weight (HMW) glutenins are major components of storage proteins in wheat and have a major role in the determining of dough characteristics. The genes controlling the synthesis of glutenins in hexaploid wheat (*Triticum aestivum* L.) are located on the long arms of Group-1 chromosomes (at the loci *Glu-A1, Glu-B1*, and *Glu-D1*). A total of 163 common landraces of bread wheat were collected from Europe. Their high molecular weight glutenin subunit (HMW-GS) composition was analyzed using the traditional SDS-PAGE based allele identification. Genotypes with rare and abnormal subunits were analyzed by Matrix-assisted laser desorption/ionization time-of-flight Mass Spectrometry (MALDI-TOF-MS). Results showed a high level of heterogeneity at all *Glu-1* loci. We found out that 45 of the examined accessions (27.6%) were observed to be homogeneous, while 118 (72, 4%) of them were heterogeneous in protein profiles, possessing at least 2 different protein lanes. The most heterogeneous accession was the Bulgarian genotype No. 11 (Sofia), which contained 13 glutenin phenotypes. We have already identified eighteen *Glu-1* encoded alleles and allelic pairs. All three allelic variants (*null*, 1, and 2*) were detected at the *Glu-A1*, with *null* allele (*Glu-A1c*) being the most frequent (47.7%). Allele 2* (*Glu-A1b*) was present in 8.3% of lines. High polymorphism was observed at the locus *Glu-B1* where alleles 6, 7, 8, 9, 20 and allelic pairs 6+8, 7+8, 7+9, 13+16, 17+18 and 7+15 were detected. Alleles *Glu-B1c* (HMW-glutenin subunits 7+9) and *Glu-B1b* (7+8) were the most frequent (34.1% and 26.2% respectively) among the evaluated lines of wheat landraces. Combinations 13+16 and 17+18 were relatively rare, being found in some landraces which originated from Germany, Switzerland, Slovakia, Austria, Hungary, Poland and Bulgaria. Allelic pair 7+15 was found only in the Swiss landrace Sarrayer 602H. Subunits 17+18 are associated with good bread-making quality. Subunits 6, 7, 8, 9 and 20 were identified with a low occurrence (0.2 -10.5%) of landraces. The allele *Glu-B1an* (subunit 6) was found in three accessions (Kooperatorka, Odesskaya 16 and Saumur d'Automne). Four allelic pairs were identified at the *Glu-D1* locus: 2+12, 3+12, 4+12 and 5+10. In addition, two new alleles at the *Glu-B1* locus were found in one line of the France landrace Saumur d'Automne and in one line of the Swedish landrace Kotte. New alleles at the *Glu-1D* locus were identified in the French landrace Noe.

DNA sequence polymorphism in wheat triticin gene and its relationship with bread-making quality

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Increased urbanization and associated changes in dietary habits have resulted in increasing demand for processed wheat products including bread. The quality of wheat flour for bread making depends on the visco-elastic properties of the dough, which are influenced by quantity and quality of the gluten forming storage proteins of endosperm namely glutenins, gliadins, albumins and triticin. Allelic variations in the HMW and LMW subunits of glutenin have been shown to directly affect wheat properties. On similar grounds, it is expected that the allelic sequence variations in triticin protein will also have an effect on wheat quality but studies regarding triticin have not been conducted in the past. We conducted the present study with a focus to analyze the effect of 'triticin' on both nutritional and technological quality of wheat and to look for variations in the hypervariable region (HVR) of triticin gene in wild wheat. Thirty-eight wild wheat and thirty bread wheat accessions were screened for allelic variations in triticin protein. A set of nine primers were designed from HVR and intron regions of triticin gene sequence of Chinese spring. Good level of polymorphism was observed with the use of primers from HVR region whereas the primers from intronic region produced single monomorphic bands of expected size when checked on bread wheat and wild wheat varieties. PCR product from wild wheat accessions were sequenced and assembled taking 'Chinese spring' triticin gene sequence as a reference sequence and six SNPs were obtained from intronic region of wild wheat accessions. Sequencing of cultivated wheat accessions will be carried out after cloning. The information gained can prove quite valuable for the designing of breeding programme for bread-making quality character.

Genetic dissection reveals effects of wheat high molecular weight glutenin subunits and waxy alleles on dough-mixing properties

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The glutenin and waxy loci of wheat are important determinants of dough quality. This study was conducted to evaluate the effects of high molecular weight glutenin (HMW-GS) and waxy alleles on dough-mixing properties. Molecular mapping was used to investigate these effects on Mixograph properties in a population of 267 (Nuomai1 × Gaocheng8901) recombinant inbred lines (RILs) from three environments in the harvest years 2008, 2009 and 2011. The results indicated the following: 1) the *Glu-A1* and *Glu-D1* loci have greater impacts on Mixograph properties compared to the *Wx-1* loci and the effects of *Glu-D1d* and *Glu-D1h* on dough mixing are better than those of *Glu-D1f* and Glu-*D1new1* in this population; 2) the interactions between the *Glu-1* and *Wx-1* loci affected some traits, especially the midline peak value (MPV), and the lack of *Wx-B1* or *Wx-D1* led to increased MPV for all types of *Glu-1* loci; and 3) thirty quantitative trait loci (QTL) over nine wheat chromosomes were identified with ICIM analysis based on the genetic map of 498 loci. Eight major QTL and sixteen QTL in the *Glu-1* loci from the three environments were found. The major QTL clusters were associated with the *Glu-1* loci, and also were found in two regions on chromosome 3B and one region on chromosome 6A, which is one of the novel chromosome regions influencing the dough-mixing strength. The two QTL for MPV are located around Wx-B1 on chromosome 4A. *QMPT-1D.1*, *QMPI-1D.1* and *Q8MW-1D.1* were stable in different environments and could potentially be used in molecular marker-assisted breeding.

Difference of Zeleny sedimentation and SDS sedimentation of gluten NILs of wheat cultivar Longmai19

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To clarify the effect of Zeleny sedimentation and SDS sedimentation in prediction of wheat quality, Two new types of wheat cultivar Longmai 19 were produced by 6 consecutive backcrosses with biochemical marker-assisted selection, which are different from their parent longmai19 (L-19, contained *Glu-D1a* coding HMW-GS 2+12 and *Glu-A3c*) at two loci: *Glu-D1* coding HMW-GS and/or *Glu-A3* coding LMW-GS. One of them, called L-19-613, contained *Glu-D1d* coding HMW-GS 5+10 and *Glu-A3c*, and L-19-626 contained *Glu-D1d* and *Glu-A3e*. The donor of the *Glu-D1d* genes and the *Glu-A3e* genes is Canadian wheat variety Marquis. L-19 (*Glu-D1a/Glu-A3c*) and L-19-613 (*Glu-D1d/Glu-A3c*) is a set of nearly isogenic lines (NILs) of HMW-GS 2+12 and 5+10, and L-19-613 (*Glu-D1d/Glu-A3c*) and the L-19-626 (*Glu-D1d/Glu-A3e*) is another set of the NILs of LMW-GS *Glu-A3c* and *Glu-A3e*. According to the percentage of the difference of sedimentation between NILs, the effect of Zeleny sedimentation and SDS sedimentation in wheat quality evaluation can be clarified. These NILs were grown in the experimental field of Crop Breeding Institute of Heilongjiang Academy of Agricultural Sciences in 2007~2009, with the three-column contrast arrangement design and eight replicates. The quality data from three years experiments showed that flour protein content variation can be 0.1%~3.3% in three types of Longmai19 NILs in the same year. Glutenin alleles main effects were ranked as follows: *Glu-D1d/Glu-A3c* (L-19-613) > *Glu-D1d/Glu-A3e* (L-19-626) > *Glu-D1a/Glu-A3c* (L-19) for gluten index and Zeleny sedimentation, while *Glu-D1d/Glu-A3c* (L-19-613) > *Glu-D1a/Glu-A3c* (L-19) > *Glu-D1d/Glu-A3e* (L-19-626) for SDS sedimentation. Further studies indicated that the Zeleny sedimentation showed larger differences (ranged from 16% to 28%, and 18.8% in average) than the SDS sedimentation (ranged from 4%~9%, and 5.5% in average) between the NILs of the *Glu-D1a* and *Glu-D1d* encoding HMW-GS 2 +12 and 5 +10, while the SDS sedimentation showed larger differences (ranged from 16%~25%, and 21.3% in average) than Zeleny sedimentation (ranged from 2%~22%, and 12.6% in average) between for the NILs of the *Glu-A3c* and *Glu-A3e* encoding LMW-GS. These results indicate that Zeleny sedimentation and SDS sedimentation reflected different characters of wheat quality, and can not be replaced each other in wheat quality prediction by sedimentation. Both Zeleny sedimentation and SDS sedimentation will be necessary for full evaluation of genetic characteristics of wheat quality.

Application of molecular markers of HMW-GS in wheat cultivar improvement

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Yield and quality improvement are the most important breeding objectives. Application of molecular markers can improve yield and quality simultaneously; however, they are few reports from practical breeding programs. Although HMW-GS only occupy about 10% of the wheat storage proteins, they play a key role in determining bread-making quality, and especaily the composing of HMW-GS has stronger effect on it. All studies indicated that some HMW-GS, such as 5+10, are more important than others in determining bread-making quality. The lower frenquence of good bread-making HMW-GS, for example 2*、7+8、5+10, in Chinese wheat cultivars is one of the most important reasons for poor bead-making quality Therefore, improving gluten quality through HMW-GS will play an important role in Chinese wheat breading.Totally twelve BC_2F_5 breeding lines with high molecular glutein subunits (HMW-GS) 7+8 and 5+10 derived form the cross Yumai 34 / Lunxuan 987[3] by molecular markers assisted selection were used to investigate the performance of yield and quality. The cultivar Yumai 34 with high bread making quality was used as the donor of HMW-GS 7+8 and 5+10, and Lunxuan 987 as the high yield backcross parent. Results showed that nine out of twelve lines with higher value than Lunxuan 987 on major quality parameters, such as dough development time and stability of farinogragh, maximum resistance of extensogragh, mixing time, peak integral and Time × width at 8 minute of mixogragh, loaf volume and total score, ranging from 0.5 to 4.3 minutes, 2.6 to 12.4 minutes, 118.4 to 315.3 BU, 0.7 to 3.5 minutes, 23.6 to 134.9, 6.1 to 9.7, 80 to 165 cm^3, 12.5 to 30 points, respectively. 1BL/1RS translocation had great negative effect on quality, but with exception. The yields of 11 lines increased from 3.3% to 19.5% compared with Lunxuan 987. Among the 11 lines, three lines named CA1063, CA1062 and CA1061, were significant higher than that of Lunxuan 987, and also 4.1%, 3.5%, 1.9% higher than that of check cultivar Zhongmai 175. Eight lines, which did not show significant difference in yield with Zhongmai 175, exhibited better quality compared with Lunxuan 987 and Zhongmai 175. It indicated that yield and quality could be improved at same time by molecular marker assisted selection.

Complex quality characterisation of Hungarian wheat cultivars

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The alterations in the meaning of wheat quality require more complex enclosure and scientific background than before. The knowledge about the relation among gluten proteins, rheological character of dough and the quality of and products gives possibility not only for understand but also for predict the technological behaviour. However the poor correlation in case of some parameters, like water absorption and viscous properties draw the attention again the roles of further quality related wheat components, mainly starch, non-starch carbohydrates and non-gluten proteins. The spread of more healthy cereal products like whole grain products and enrichment in dietary fibre gives an extra importance to understand the detailed effects of different non-gluten components.

This integrated approach was applied in our work. Old and new Hungarian wheat cultivars originated from Agricultural Institute of Hungarian Academy of Sciences (Martonvásár, Hungary) have been characterised covering the qualitative and quantitative analysis of gluten and non-gluten proteins as well as the starchy and non-starchy carbohydrates. The objective of our study was (i) to typify the genetic potential of these lines, (ii) looking for correlations between the results of different conventional, and novel analytical methods, (iii) and get an improved understanding about rheological parameters and biochemical background.

For profiling of gluten proteins and albumin and globulin fractions, lab-on-a-chip (LOC), Bioanalyzer 2100 from Agilent was applied. The possible improvements for sample preparation for this technology were investigated, and the results were compared with corresponding reference methods. Moreover, SE-HPLC was applied to characterise the protein polymer profile by the UPP% (unsoluble protein polymer percentage). The amylase/amylopectin ratio was determined with colorimetric method, and the damage starch was also measured with SDmatic (Chopin Technologies). The quantitative variation of water extractable, and total arabynoxylan content was screened by the GC-FID, with the hydrolysis and derivatisation of sugars. These chemical parameters were related to different rheological measurements, such as MixoLab (Chopin Technologies), RVA (Rapid Visco Analyser, Perten Instruments.), and the automated, micro sized version of Zeleny sedimentation test (Sedicom, BME-Labintern Ltd, Hungary).

Results were evaluated with regard to their different genetic background and protein composition. The chemical parameters were also involved in correlation studies and to develop a mathematical modelling for the estimation of complex wheat quality.

Bread-making qualities of blend between protein-removed rice flour and extra-strong wheat flour

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Demand for food made from domestically produced materials has been increasing in Japan. However, only 14% of wheat that Japanese people consume is domestically produced. As many as 95% of rice is domestically produced, while lately the gross consumption of rice has been reducing in Japan. Almost all (95% <) of rice is consumed as boiled rice, few are utilized as ingredients of wheat flour products such as bread, noodles, and confectionery. It is therefore considered that utilizing of rice flour to make bread is effective to increase production and self-sufficiency rate of Japanese cereals, as bread is mostly consumed flour food in Japan. However, it has been known that bread made of rice/wheat flour show smaller values of specific loaf volumes (SLV) than wheat-flour bread, and that generally the SLVs are low in proportion to rice flour amounts. To explore rice and wheat flour materials suitable for rice/flour bread with good bread-making qualities, SLVs of bread made of blend between protein-removed '*japonica'* rice flour and an extra-strong wheat flour (20:80) were analysed. The SLV values tended to be small in portion to protein contents of rice flour. A panel test demonstrated that bread using rice flour with protein contents less than 0.2% showed high scores and that such bread are possibly meet the market demand. We predict that storage protein of *japonica* rice flour might prevent polymerizations of glutenin in blended flour and eventually reduce the SLVs of bread.

Transfer of HMW-glutenin genes from *Th. elongatum* for improvement of bread making quality

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Cultivated wheat has limited variation due to single point of origin in the Fertile Crescent. Wild species of wheat are important source of genetic variation. After screening of 177 disomic addition lines (DALs) of wheat (*Triticum aestivum*) containing a pair of chromosomes from different wild species, the chromosome 1E addition line of *Agropyron elongatum*, was found to have potential for improvement of wheat bread making quality. This was indicated by increased SDS sedimentation, specific sedimentation, mixograph peak time and SE-HPLC analysis of polymeric proteins. This addition line spontaneously gave rise to a substitution line for chromosome 1D in subsequent generations. 1E(1D) substitution line showed weak dough strength compared to addition line and background line indicating that chromosome 1D is very important for bread making quality. Further 1E-encoded seed storage proteins have potential for improvement of wheat end-product quality only if transferred to specific chromosomes i.e. 1A. 1E (1D) substitution line was crossed with nulli-1A tetra-1D line to create double monosomics for chromosome 1A and 1E. Plants (F_1) were either selfed or crossed with different cultivars (Cham 6, Norin 61, AC Domain, PBW343, Fukusayaka). Seeds (F_2 and F_1) were screened for presence of HMW-GS (present on long arm) and absence of gliadins (present on short arm) from *Th. elongatum.* Genomic *in situ* hybridization (GISH) was carried out to identify translocation line from selected lines. A translocation line carrying long arm of chromosome 1E transferred to short arm of chromosome1A (1EL-1AS translocation line) was identified in F_1 plants in the cross of cultivar Norin 61. Translocation line was then backcrossed three times with Norin 61 and then selfed two times to obtain homozygous translocation line. HMW GS was used as marker for selection at each generation. BC_3F_3 translocation line was generated in the background of cultivar Norin 61(HMW- 2*, 7+8, 2.2+12) by replacing Ex and Ey orthologous HMW-GS genes with *Ax2** and *Ay null* gene of wheat. Processing quality analysis of the line is in progress.

Allelic variations of functional markers for quality traits in Indian bread wheat (*Triticum aestivum* L.) cultivars and their correlation with bread loaf volume

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Direct estimation of quality traits is costly and time consuming as well as requires a large amount of grain. Some wheat quality traits such as high and low molecular weight glutenins, puroindolines and variation in granule-bound starch synthase, absence of IBL.1RS translocation are good predictor of bread making quality. In the present study, forty two spring wheat cultivars from five major wheat cultivating agro-climatic zones of India viz., North Western Plains Zone (NWPZ), North Eastern Plains Zone (NEPZ), North Hill Zone (NHZ) Peninsular Zone (PZ) and Central Zone (CZ) were used to investigate the allelic variations for HMW and LMW glutenin subunits, grain texture, GBSS1 and presence of 1RS.1BL translocation using PCR-based DNA markers. In total, eight allele/ allelic pairs at *Glu-1*, six alleles at *Glu-3* loci, two alleles for puoindolines, and two alleles at *Wx-B1* were identified. Allele or allelic pair *Ax2** (60%) at *Glu-A1*, *Bx17+By18* (30%) at *Glu-B1* and *Dx2+Dy12* (56%) at *Glu-D1*, *Glu-A3b* (83%) at *Glu-A3* loci, *Wx-B1b* (60%) at *Wx-B1* loci and absence of 1RS.1BL translocation (60%) were present most frequently in wheat genotypes tested. The correlation between alleles for above mentioned quality traits and bread loaf volume was also studied.

Biochemical and molecular marker studies of polyphenol oxidase (PPO) genes in Indian wheat cultivars

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Quality parameters, such as wheat's physical properties, flour protein and grain color is correlated with grain PPO activity**.** The enzyme activity of polyphenol oxidase (PPO) in grain has been related to undesirable brown discoloration of wheat based end products such as noodles and pasta, leading to low consumer acceptability. In the present study, 32 Indian wheat genotypes were analyzed for phenol color reaction and total polyphenolic content. Further validation was done using PCR-based DNA markers. Phenol color reaction using 1% phenol was used as a preliminary test .The results revealed that kernel of 18% wheat genotypes showed weak color reaction .The highest total polyphenol content was found in the extract of bread wheat genotype BW-9105 and lowest in durum wheat genotype PDW-291 with 36.7 and 9.95 mg/g GAE of whole grain flour, respectively. Statistically significant differences ($p < 0.05$) were obtained among the tested wheat genotypes for total polyphenolic content.The *Ppo-A1a* allele (high PPO activity) on chromosome 2A has predominant distribution in wheat cultivars with a frequency of 78% and *Ppo-D1b* allele (high PPO activity) on chromosome 2D with a frequency of 62%.

Characterization of wheat LMW glutenin subunits by reversed-phase ultra performance liquid chromatography (RP-UPLC)

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Low molecular weight glutenin subunits (LMW-GS) with molecular weight range 20,000–45,000Da, are the major determinant for unique viscolation characteristics of wheat dough. The *Glu-3* loci encoded LMW-GS display extensive allelic variations in *Triticum* and related species, and their gene copies in a genotype of common wheat are estimated to have 15-30. Thus, more powerful techniques for separating and characterizing LMW-GS are highly important. SDS-PAGE has been the most widely used method for LMW-GS analysis, but its disadvantages appear to be obvious. In this study, a more powerful reversed-phase ultra performance liquid chromatography (RP-UPLC) method for LMW-GS characterization was optimized and developed based on traditional RP-HPLC. The rapid RP-UPLC separation with high resolution and reproduciblility compared to RP-UPLC could be obtained with eluting gradient 21-47% in 30 min at 60^{o}C and 0.6ml/min. With this method, one sample could be completed less than 30 min, and the resolution and efficiency are much higher than RP-HPLC. The optimized RP-UPLC method could be used for rapid cultivar and germplasm discrimination, allelic variation detection, genetic control and expression analysis of LMW-GS. Therefore, RP-UPLC is expected to be useful for desirable LMW subunit and allele screening and identification in wheat quality improvement.

The relationship gluten and gluten Index between alveograph, mixograph and some physical traits in bread wheat (*T.aestium L.*)

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In this study, twentyfive wheat genotips were grown in two years rain-fed conditions in Konya 2009-2010, 2010-2011 growing season at the Centrel Anotolian provinces in Turkey. Samples were analyzed in the laboratory as two replications. Combined Anova and correlation were analyzed in wheat traits results. After wheat genotips of properties were analyzed GGE-biplot statistic program.This methedology uses a biplot to show the factors that are important in genotype evaluation. Combined ANOVA analysis showed that wheat genotypes of traits between differences were significant.

Traits of wheat genotypes were determined as the mean; protein ratio (PRT) % (14.05), Wet Gluten (WGL) % (38.31), Gluten İndex (GIN) (73.7), Alveograph energy (W) (Kjoulx10-4 (232.7), alveogram height/alveogram large (P/L) (0.54), Mixograph Peak Time (PKT) min. (2.93), Mixograph Peak heigh (PKV) % (54.8), Hardness (PSI) (53.7), Zeleny Sedimantation (ZLN) ml (42.5), Kernel weight (KWT) g. (34.2), test weight (HKW) kg.(77.1). It was obtained that the relationships between wet gluten; PRT ($p < 0.01$), KWT ($p < 0.05$), were significant and positive, and GİN ($p < 0.01$), HKT ($p < 0.05$), HRD ($p < 0.05$) significant and negative, Relationship between Gluten index; HKT ($p < 0.05$), ZLN ($p < 0.01$), W ($p < 0.01$), PKT ($p < 0.01$), HRD ($p < 0.05$) were found significant and positve. GGE-Biplot, wheat traits are assesment provide as visual.Traits were distributed in three groups in biplot graph. The groups were found as follow; First group: Genotypes with PCA1 value is greater than zero (WGL, HTL, W, ZLN, GIN); Second group: Genotypes with PCA1 value is smaller than zero (PRT, KTW, PKW), and third group: Genotypes were distributed independently between two groups (P/L, HRD).

Investigation of molecular background of wheat allergy in plant protein

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Wheat is one of those food components that are responsible for a large amount of food hypersensitivity reactions. The most widespread diseases are wheat allergy and celiac disease triggered by certain proteins derived from wheat and a few other cereals, such as barley, rye and oats. 20% of allergic patients are wheat allergic, while the rate of the population affected by celiac disease is about 1%. Clinical symptoms of these disorders are often overlapping (urticaria, atopic dermatitis, nausea, vomiting, diarrhoea, rhinitis, anaphylaxis, etc. however their pathogen mechanisms are absolutely different that makes their differentiation and diagnosis difficult. In case of both disorders the information about the target antigens are incomplete. The main impact is assigned to proteins member of the prolamin family, but there are epitopes in non-prolamin fractions, which are recognized by celiac patients, while other elicit wheat allergy. Aditionally previous studies discussed the difference between the protein composition of spelt and bread wheat and some reported spelt wheat products, producing from European spelt cultivars, resulted in better tolerance if were consumed by wheat allergenic patients. Similarily, products made from a spelt genotype grown in Australia possessing some mutations in a wheat allergy related beta-expansin gene proved to be be less immune-reactive and suitable to consume by patients.

These facts and the increasing number of newly detected non-prolamins as allergens - some of them also can be related to technological properties of wheat flour - signify the needs to characterise wheat seed allergome in a complex way and generate the challenge, how the agriculture and food industry can maintain the quality of wheat and cereals products, while reducing or even better eliminating negative health effects. The aim of this project is to contribute to solve these problems is by improving the appropriate identification and determination of IgE mediated immunogenic proteins in bread wheat and spelta genotypes and furthermore by developing a set of molecular markers for breeding programs to able to identify genotypes which lack some or majority of allergic proteins.

At the first period of our research a comparative analysis was undertaken on selected cultivars. A total of 54 spelt Hungarian breeding lines and samples from European and Australian germplasm collections were included in this research of reviewing their allergen characteristic. A molecular marker developed for a beta-expansin gene, TaEXPB11 was used in a gene-specific PCR method to identify the wild type and the mutant allele variants. Among these genotypes 17 genotypes contained the wild type allele of the beta-expansin genes, the rest of the lines might possess a different allelic variant.

The presence and the amount of proteins containing immunogenic epitopes highly depend on the genetic background of the varieties. Previously all these materials were also analysed for calculation of their genetic distance by AFLP. Two main clusters could be separated, one including those spelt accessions that have common wheat in their pedigree consisting the wild type allele of beta-expansin gene, within the other group members, the pure spelt populations were not contained this allele.

Our results demonstrated that the Hungarian breeding lines didn't contain the allergenic beta-expansin gene and they also linked to pure spelt cultivars, so they can be appropriate for the non-allergenic wheat breeding and representing a new perspective of our breeding programs. Further examinations of spelt genetic resources, covering as broad as possible diversity of the spelt gene pool is essential to its systematic exploitation, such as using in non-allergenic breeding.

Effect of cultivar and roller milling process on the level of phenolic compounds in Italian durum wheat

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Functional bioactive compounds are either naturally occurring compounds in food or substances formed during the processing of foods that may have physiological or biochemical functions when consumed by humans. Among them, phenolic substances have been widely studied for their functional value. They may act in a variety of ways, such as protecting DNA from oxidative damage, deactivating carcinogens, and inhibiting the expression of mutated genes and the activity of enzymes that promote carcinogenesis, as well as promoting detoxification of xenobiotics. Phenolic compounds are a widespread group of substances in the plant kingdom, present in virtually all plant foods, but their dietary intake greatly varies depending on the type and quantity of vegetable foods consumed. Phenolic substances are present also in wheat, with ferulic acid present in the highest amount.Various studies analysed the polyphenol content of soft, medium and hard wheat and showed that it varies greatly according to the grain portion considered, so that refining processes in milling reduce polyphenol contents. Also the effect of environmental factors on the total polyphenol content, such as soil pH, rainfall and temperature, was pointed out, as well as the varietal effect. Finally, polyphenols antioxidant activity is affected by the action of polyphenol oxidase enzyme. Marginal attention has been devoted to the polyphenol content of durum wheat, the essential raw material to produce high quality pasta, one of the basic foods in the Italian diet. The aim of this work has been to evaluate the effect of roller milling process and cultivar on the level of phenolic compounds in durum wheat. A set of 20 cultivars of Italian durum wheat was considered, chosen among the most diffused and cultivated, as well as a set of different commercial milling streams derived from the same grain lot. The relations between phenolics level, PPO activity and dough colour were also investigated.

The determination of the total phenolic content (TPC) of the free phenolic fraction showed levels ranging from 1.28 to 1.94 mg ferulic acid equivalent (FAE)/g wholemeal. These levels were higher than those reported in soft wheat, although the methods used to extract and evaluate the TPC vary considerably among reports, making rather difficult the comparison of data. Moreover, an environmental effect cannot be excluded, since it has been reported that climatic factors associated to Mediterranean conditions such as drought stress and high temperature during grain filling, typical of the area where the samples were grown, lead to higher TPC. In fact, among their physiological functions, these plant metabolites can stabilize cell walls and act as screens against UV radiation. The TPC was significantly correlated to the PPO activity. The brown index (BI) of dough obtained from the cultivars examined ranged from 30.04 to 35.74. No correlation was observed between TPC and BI of

dough, but further investigations would be needed on greater number of samples. The TPC of different roller milling fractions derived from the same grain lot differed significantly, with the highest values in bran and middlings. The decrease in TPC was progressive as the contributor of inner parts of the kernel increased. In conclusion, as in soft wheat, durum wheat bran from selected Italian cultivars can be considered an interesting source of phenolic substances to be used as functional food ingredient as it is or to prepare enriched antioxidant extracts.

Effect of bug (*Eurygaster* spp. ve *Aelia* spp.) damaged flours on formation of acrylamide and hydroxmethylfurfural (HMF) in cakes, cookies and breads

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Grain pests like Pentatomid insects named as wheat bugs (*Eurygaster spp., Aelia spp.* and *Nysius huttoni*) or "suni bug" (*Eurygaster spp.*) have detrimental effects on grains, especially Gramineae, in some Mediterranien, Middle Eastern, East European, Near Eastern countries and New Zealand. While the insects absorb their nutrients during their nymph and adult periods, they leave their digestive secretions in the grain before harvest[1]. The proteolitic enzymes in their salivary secretion result processing problems in dough and low-quality end product. It has been shown that damage of wheat bugs causes high protease activity in flour then hydrolyzing of gluten and releasing of some amino acids due to increasing of solubility[2,3]. Acrylamide and hydroxymethylfurfural (HMF) are mainly formed through Maillard Reaction and dehydration of certain sugars. They can be regarded as the most important heat-induced contaminants occurring in bread and bakery products. Formation of acrylamide in food, free amino acids, especially asparagine and reducing sugars are important precursors. In this study, the effects of high protease activity flour damaged by *Eurygaster* and/or *Aelia* on formation of acrylamide and HMF in bakery products such as bread, cake and cookie were determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Variation of free amino acids during incubation (0, 30, 45, 60 and 120 min) was also determined by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS).

High protease activity flour due to *Eurygaster* and/or *Aelia* damage was added at 2.5%, 5%, 10%, 15% (w/w) and 25%, 50%, 75%, 100% (w/w) levels in bread and cake formulations, respectively. Cookies were produced with high protease activity flour at 20%, 40%, 60%, 80%, 100% (w/w) addition levels. No variations were observed on acrylamide and HMF contents of both bread and cake samples. Although no change was observed HMF content of the cookies, formation of acrylamide was increased up to 30% (100 µg/kg) at the highest addition level (100%) as compared to those of control (77.06 µg/kg). Amount of asparagine was increased by approximately 26%. These results suggested that bug damaged flour causes an increase potential health risk due to acrylamide formation in cookie.

DNA sequence variations of four *Wx* alleles generating polymorphic Wx-A1 protein or decreased amylose content in wheat

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Waxy (*Wx*) gene encodes the granule-bound starch synthase (also called Wx protein) responsible for amylose synthesis of cereal grain starch. Because amylose content affects food products from wheat flour, it will be important to find and investigate *Wx* alleles affecting amylose content in wheat (*Triticum aestivum* L.). In this study, four *Wx-A1* alleles of wheat, i.e. *Wx-A1c*, *-A1e*, *-A1i* and *-A1j* producing polymorphic Wx protein were studied at DNA sequence level. It has been suggested that apparent amylose content by the alleles increases in the order of *Wx-A1e* (= waxy phenotype) < *-A1i* < *-A1c* < *-A1j* (= amylose level of wild genotype *Wx-A1a*, about 20%). DNA sequencing these alleles detected SNPs and insertion/deletion variations, of which a particular SNP causing amino acid change in *Wx-A1e* and *-A1c* was almost identified as the factor responsible for decreased amylose. On the other hand, a transposable-like element of 376 bp present in the 3'-UTR of *Wx-A1i* most possibly lowered the level of Wx protein and amylose. The fourth allele *Wx-A1j* possessed four SNPs, of which two SNPs altered amino acids of the *Wx-A1j* protein and should cause the protein polymorphism. Based on the DNA sequence determination, functional codominant markers for *Wx-A1c*, *-A1e* and *-A1i* will be developed.

Phase transitions of pea starch over a wide range of water content

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Starch gelatinization is defined as the "collapse of molecular orders within the starch granule with the solubilisation of starch polymer molecules" (1, 2). The changes that starch undergoes during gelatinization are major determinants of its functional properties for food processing and digestion. Although the phase transition of starch has been studied extensively by differential scanning calorimetry (DSC), the exact nature of multiple DSC endothermic transitions of starch-water systems is still not well understood (2-4). In this study, the phase transitions of pea starch over a wide range of water content (from 34.0 to 97.2 wt %) were investigated by DSC. To the best of our knowledge, this is the first study to address the mechanism of thermal transitions of starch granules over such a wide range of water content. Swelling of starch granules increased progressively with increasing water: starch ratio up to 20:1. The main endotherm G broadened progressively with increasing water content up to 94.5 wt % (water: starch ratio 15:1), above which it became too broad to define. The corresponding peak and conclusion temperatures and enthalpy change increased with increasing water content. Scanning electron microscopy (SEM) imaging of dried starch samples after DSC heating showed that at a water: starch ratio of 2:1, starch granules only swelled partially with discernable granular contours. The above results answer the question why at water: starch ratios of 2:1, there are still considerable residual crystallinity and lamellar structure at the end of the so-called complete gelatinization transition. The gradual transition from a sharply defined to broad endotherm G reflects the changes from very limited swelling to maximum swelling of starch granules (mainly amylopectin molecules) and partial dissolution of starch polymers (mainly amylose molecules).

Hemp fiber reinforced wheat gluten, glutenin and gliadin based plastics; evaluation of the mechanical properties and biodegradability

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In this study, biocomposites has been made by using wheat gluten (WG) and its major protein fractions, gliadin and glutenin, reinforced with industrial hemp fiber. Use of natural fibers in the composites not only improves mechanical properties but also makes materials that are potentially biodegradable. The aim of the study was to evaluate the mechanical properties and biodegradability of the composites. Protein powder of WG, gliadin and glutenin was vibration infiltrated into hemp fiber mats with a protein to hemp fiber ratio of 50%. Vibration infiltrated mats of protein and hemp fiber were processed by compression molding the materials at 110 ℃, 120 ℃, and 130 ℃ at 400bar for 15 minutes, without use of plasticizers or solvents. The maximum stress, strain at maximum stress and E-modulus were evaluated by tensile testing. In addition, biodegradability of the composites was examined by using standard ASTM-D5988-03 for the degradation of the composites in soil. Tensile properties of the composites showed significant increase in values with increase in pressing temperature from 110 ℃ to 130 ℃. Gliadin reinforced hemp fiber composites pressed at 130 ℃, showed higher values of E-Modulus (1868 MPa) as compared to WG (1469 MPa) and glutenin (1372 MPa). WG and gliadin composites pressed at 130 ℃ showed similar behavior in the case of maximum stress values, 34.87 MPa and 38.06 MPa respectively, whereas glutenin showed the lowest value at 21.06 MPa. The results show that the polymerization of the proteins increase with an increase in temperature, making a better cross linked network. The overall results show that the WG and gliadins possess better extensibility compared to glutenin. The preliminary results for biodegradability show that the materials are biodegradable. This study concludes that higher temperature (130 ℃) and the use of hemp fibers in WG protein based plastics improves the tensile properties in materials manufactured without using plasticizers or solvents, or even water.

Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in wheat (*Triticum aestivum* L.)

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Dietary micronutrient deficiencies affect hundreds of millions people worldwide, such as the lack of vitamin A, which are prevalent in developing countries, leading to significant economic losses. Carotenoids in food can function as provitamin A in humans, but wheat cultivars generally have low carotenoid contents. In order to improve the nutritional value of wheat, we used genetics engineering to increase the carotenoid contents in wheat endosperm. Overexpression of the bacterial genes *crtB* (a phytoene synthase) driven by a endosperm-specific *1DX5* promoter and *crtI* (functiona as desaturase for the four desaturation steps of the carotenoid pathway catalysed by phytoene desaturase and δ-carotene desaturase in plants) under the constitutive *CaMV 35S* promoter control in wheat variety Bobwhite, resulted in obvious increase of the carotenoid content in the endosperms of transgenic wheat lines visually showing a light yellow color. Due to the variability of total carotenoid contents in different transgenic lines, we used Southern hybridization and quantitative real-time RT-PCR technologies to analysis the transgene copy number and expression of key genes involved in carotenoid biosynthesis. We found that transgene copy numbers did not correlate well with carotenoid content. The expression of transgenes and endogenous carotenoid synthesis related genes were investigated in wild-type and transgenic wheat lines. No significant changes in expression levels of these genes were observed in leaf, but the expression of the endogenous lycopene cyclases in seeds were highly increased in transgenic lines compared with wildtype. The results suggest that overexpression of bacterial *crtB* and *crtI* genes in transgenic lines increase lycopene content, which in turn may upregulate lycopene cyclases through a feedforwards control, and finally lead to the accumulation of carotenoid in transgenic wheat endosperms.

QTL mapping of starch granule size using recombinant inbred lines in common wheat

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Starch, a major component of wheat endosperm, accounts for 65-75% of the dry weight in the mature wheat grain and is known to be a crucial trait related to wheat quality. Wheat mature endosperm generally contains A- and B-type starch granules, thus showing a bimodal granule size distribution. A-type granules are bigger (>10 μm) and disc- or lenticular-shaped, whereas B-type granules are smaller (<10 μm) and spherical or angular. A and B starch granules have different chemical and structural properties, which leads to different functions and effects on flour. Therefore, A:B granule ratio in wheat (*Triticum aestivum* L.) endosperm affects the edible quality and processing quality.

In the present study, quantitative trait locus (QTL) of starch granule size in common wheat were analyzed using 240 recombinant inbred lines (RIL) derived from PH82 × Neixiang188 cross, grown in Anyang, Henan Province during two cropping seasons. We determined the volume percent of A-type granule, B-type granule and A:B ratio. Five QTLs were detected across two environments using composite interval mapping. One QTL for A granule on 1BL between the markers *Xwmc44* and *wmc719* was identified in 2006, explaining 3.8% of phenotypic variations, with increased effects coming from Neixiang188. Three QTLs for B granule were mapped on chromosomes 2BL, 3A and 3BL, accounting for 5.9, 4.4 and 7.2% of phenotypic variations, respectively, with increased effects coming from PH82-2. One QTL for A: B was detected on chromosome 6BS in 2006 and 2011, explaining approximately 3.6% of phenotypic variations, with increased effects coming from Neixiang188, indicating a stable effect across environments. This study provides useful information for the improvement of wheat quality targeting for starch properties.

Allelic variation of seed dormancy (*Sdr*) gene located on chromosome 2B and development of functional marker in common wheat

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Wheat pre-harvest sprouting (PHS) is the germination of grain on the plant before harvesting, resulting in a reduced grain quality and lower harvestable grain yield. PHS is affected by multi-factors, among which seed dormancy is the most important factor. *Sdr* is a seed dormancy-specific regulator during seed maturation, and it is in the central regulatory network of *VP1* and *DOG1* pathway. The objective of this study was to clone the *Sdr* genes for seed dormancy and develop ideal functional markers (FM) for marker-assisted selection for PHS.

The wheat *TaSdr-B1* gene sequence of 1503 bp on chromosome 2B was cloned using comparative genomics, including a 255-bp 3' untranslated region (UTR), a 267-bp 5'-UTR and a 981-bp open reading frame (ORF), encoding a peptide of 327 amino acids. No variation was detected among coding sequence (CDS) of *TaSdr-B1*. But, there was a G/A SNP in -11 site before the initation codon TAG between PHS susceptible and resistant wheat varieties. A CAPS marker was developed for wheat pre-harvest sprouting based on this allelic variation. A PCR fragment of 826 bp was firstly amplified using the primer pair sdr-90 and sdr-96, then the PCR product was cut by restriction endonuclease *Pml*I. A specific 652-bp band was obtained in the PHS resistant varieties, whereas a 826-bp band was present in the PHS susceptible varieties because of the absence of enzyme site, and these two alleles were designated as *TaSdr-B1a* and *TaSdr-B1b*, respectively.

Two sets of Chinese wheat varieties were used for the validation of the CAPS marker. A set of 118 Chinese wheat varieties was planted in Beijing and Zhengzhou during 2000-2001 and 2001-2002 cropping seasons. The other set of 84 Chinese wheat varieties were planted in Anyang, Henan province during 2005-2006 and 2006-2007 cropping seasons. The results indicated that the average of germination index (GI) was 24.8% and 38.1% for the genotypes *TaSdr-B1a* and *TaSdr-B1b* in the 118 Chinese wheat varieties and it was 28.2% and 39.4% for the genotypes *TaSdr-B1a* and *TaSdr-B1b* in the 84 Chinese wheat varieties. The analysis of variance using the Statistical Analysis System (SAS V8.1) showed that the allelic variants of *TaSdr-B1* were significantly associated with PHS in Chinese wheat varieties. Compared with the varieties with *TaSdr-B1b*, those with *TaSdr-B1a* had a significantly higher PHS tolerance.

Effect of alkaline treatment on functionality and *in vitro* digestibility of pea starch granules: the role of amylose molecules

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Alkaline treatment by agents such as sodium hydroxide is used widely to enhance food quality characteristics of colour, flavour and texture, and also used in the isolation of pure starch granules with well-defined physical properties. However, the mechanism of alkaline treatment on starch structure and properties is not well understood. This report shows that treatment of native pea starch granules under conditions commonly described in the literature (0.1 M NaOH for15 days at 35 ℃) results in relatively small structural changes, which bring about large consequential changes in functional properties and *in vitro* digestibility. Alkaline treatment caused a loss of about 10% of total starch and decreased amylose content from 35 to 28%. These changes were accompanied by only small decreases in relative crystallinity and double helix content, indicating that the majority of molecular and crystalline structure is retained after alkaline treatment. Swelling power was also largely unaffected, but the endothermic transitions, pasting and *in vitro* digestion of pea starch were altered significantly. Alkaline treatment resulted in the broadening of the endothermic transition, and decreased pasting viscosities (peak and final) and set back by about 25% and 40%, respectively. *In vitro* digestibility of the granules by α-amylase was greatly increased, with rapidly digested starch increasing from 4 to 16% and resistant starch decreasing from 66 to 50%. Based on the results, we propose that amylose molecules contribute in different ways to the internal organisation of pea starch granules. While most amylose molecules are in a state of low organisation in the granule core and in amorphous regions, there are likely to be some amylose molecules that are radially interspersed in the amylopectin clusters (5), which act as the stabilizing and reinforcing structural features between amylopectin clusters. Removal of these reinforcing amylose molecules could weaken the structure of the granules, resulting in significant changes to functional properties.

Gluten and starch in doughs: new methods and evaluations with Brabender® instruments

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Gluten and starch in food do determinate the performance of dough making, proofing, frying, baking, steaming or cooking properties. The knowledge of the physico- and chemical properties of these two mayor ingredients is therefore a must for a constant product quality. The way how to test and evaluate is the base of the C.W. Brabender® 3 phase system.

There are many methods used for the determination of the properties of these two ingredients. AACC, ICC and ISO show a wide range of instruments, and methods.

However, for Gluten, standard and new technologies, like Farinograph® (dough mixing), Extensograph® (dough stretching), Glutograph® (sheer-recovery of dough or gluten) or GlutoPeak® (new, fast technique for flours or Vital gluten) are available for getting fast clear data and specifications. The GlutoPeak® is a new technique, which helps to determinate the properties with just 10g or less sample size. The principle is a high sheer based technique under standard conditions in which a gluten network is very fast washed out and after this directly fast destroyed again. Test time is short, 1-10 min. Strong gluten flours have short test times, while wafer flours as other extreme do show long times. This enables the GlutoPeak® to help in the Brabender® 3 phase system to make right decisions for breeders, millers, bakers, steam bread or producers of bake mixes.

The starch properties on the other side are important as well. The informations about the kind of starch swelling and starch gelling, absorption of water and enzymatic modifications in flour mixes is of mayor interest. These informations are given by the Amylograph®, in which you can see during a test, like in a "video", the starch viscosity changes during the heating process. This enables you to make right decisions about a possible use of the flour, or possible enzyme addition or changing of the baking process for example.

The presentation shows the improved principle of the Brabender® 3 phase system, it ś daily "how to use" in the field and the way, how to make right decisions with the instruments data provided to save money and time.

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