

# 2006 GLUTEN WORKSHOP

September 14-16, 2006  
San Francisco, CA





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## **ORGANIZING COMMITTEE**

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Dr. Frances M. Dupont  
Professor Khalil Khan  
Professor George L. Lookhart  
Professor Finlay MachRitchie  
Dr. Ron L. Madl  
Dr. Ody Maningat  
Professor Perry K.W. Ng

# PROGRAM SCHEDULE

*All sessions take place at the Renaissance Parc 55 Hotel, 4<sup>th</sup> floor*

## Thursday, September 14

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3:00 – 5:30 p.m.	Registration	Atrium
3:00 – 5:30 p.m.	Poster Set-up	Atrium
5:30 – 8:00 p.m.	Welcome Reception <i>George Lookhart and Perry Ng</i>	Atrium
5:30 – 8:00 p.m.	Poster Viewing	Atrium

## Friday, September 15

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8:00 – 10:00 a.m.	Registration	Atrium
8:00 – 8:45 a.m.	Breakfast	Parc Ballroom II - III
8:45 – 10:00 a.m.	Session 1 – Biotechnology and Genetics <i>Co-Chairs: Ann Blechl and Peter Shewry</i>	Parc Ballroom II - III
10:00 – 10:30 a.m.	Break	
10:30 – 12:00 p.m.	Session 1 ( <i>continued</i> )	Parc Ballroom II - III
12:00 – 1:30 p.m.	Lunch	Atrium
1:30 – 3:00 p.m.	Session 2 – Structure Characterization and Functional Relationships Among Gluten Monomers and Polymers <i>Co-Chairs: Finlay MacRitchie and Herbert Wieser</i>	Parc Ballroom II - III
3:00 – 3:30 p.m.	Break	
3:30 – 5:00 p.m.	Session 2 ( <i>continued</i> )	Parc Ballroom II - III
5:00 – 7:00 p.m.	Break	
7:00 – 9:30 p.m.	Banquet <i>MC – Frances DuPont</i>	Barcelona

## Saturday, September 16

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8:45 – 10:00 a.m.	Session 3 – Rheology: Application and Prediction of Gluten Properties <i>Co-Chairs: Steve Mulvaney and Clyde Don</i>	Parc Ballroom II - III
10:00 – 10:30 a.m.	Break	
10:30 – 12:00 p.m.	Session 3 ( <i>continued</i> )	Parc Ballroom II - III
12:00 – 1:30 p.m.	Lunch	Atrium
1:30 – 3:00 p.m.	Session 4: Health and Nutritional Aspects of Gluten Properties <i>Co-Chairs: Michael Tilley, Scott Bean, and Rob Hamer</i>	Parc Ballroom II - III
3:00 – 3:30 p.m.	Break	
3:30 – 5:00 p.m.	Session 5: Proteomics and Transcriptomics <i>Co-Chairs: Frances DuPont and Gerard Branlard</i>	Parc Ballroom II - III
5:00 – 5:30 p.m.	Closing Remarks and General Discussion <i>Jerry Bietz</i>	Parc Ballroom II - III
5:30 p.m.	Remove Posters	Atrium

**SESSION 1: BIOTECHNOLOGY AND GENETICS  
METHODOLOGY, GENETICS, ENVIRONMENT AND GLUTEN QUALITY**

*Co-Chairs: Ann Blechl and Peter Shewry*

**Oral Presentations:**

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1. (75) Mapping Approach Relating Quantitative Trait Loci to Dough Rheology in an Australian Doubled Haploid Population. G. Mann, S. Diffey, L. Rampling, Z. Nath, I. Kutty, P.E. Leyne, F. Azanza, K.J. Quail, B. Cullis and A. Smith.
2. (50) Allelic Variation of Low-Molecular Weight Glutenin Subunits and its Functional Importance. T.M. Ikeda, M. Yanaka and K. Takata.
3. (23) Development and Application of Fast Immunological Selection Methods for High Molecular Weight Glutenin Subunits in Wheat Breeding. H. Gruber and B. Killermann.
4. (38) Structure, Evolution, and Expression of the Wheat Prolamine Loci. O. Anderson and Y.Q. Gu.
5. (88) A Simple Integration Pattern Observed by Transformation with 1Dx5 Gene Cassettes in Wheat. Q. Yao, L. Cong, G.Y. He, J.L. Chang, K.X. Li, G.X. Yang and P.R. Shewry.
6. (41) Transgenic Wheats with Elevated Levels of Dx5 and/or Dy10 Glutenin Subunits: Agronomic, Biochemical and End-use Quality Properties. A.E. Blechl, J.W. Lin, S. Nguyen, F.M. Dupont, W.H. Vensel, R. Chan, O.D. Anderson, P. Bregitzer and D. Fielder.
7. (29) Characterization of Wheat with Strongly Reduced  $\alpha$ -Gliadin Content. H. Wieser, P. Koehler, A. Folck and D. Becker.
8. (18) Using Epitope Tagging to Explore the Trafficking, Location and Functional Properties of Wheat Gluten Proteins. P.R. Shewry, J. Freeman, H.D. Jones, C. Sparks, C. Gritsch, W. Funatsuki, K. Niwa, A. Huttly, J.A. Napier and R. D'Ovidio.
9. (2) Effects of Mineral Nutrition and Temperature on Accumulation of Gluten Proteins is Related to Their Content of Cys and Met. F. M. Dupont, W.J. Hurkman, W.H. Vensel, R. Chan, C.K. Tanaka and S.B. Altenbach.

**Poster Presentations**

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1. (16) Frequencies of Gluten-Protein Alleles in a Worldwide Collection of Over 4,600 Wheat Genotypes. F. Bekes, C.W. Wrigley, S. Uthayakumaran, C.R. Cavanagh, I.L. Batey and W. Bushuk.
2. (34) Polymorphism of High-Molecular-Weight Glutenin Subunit in Tibetan Wheat (*Triticum aestivum* ssp. *tibetanum* Shao). Y. Liu, C.E. Zhang, J.R. Zhang, K.X. Li, M.J. Chen, G.X. Yang, P.R. Shewry, and G. He.
3. (35) Chromosome Location of Genes Controlling High Molecular Weight (HMW) Glutenin Locus in Species Related to Wheat and their Effect on Bread Making Quality. M. Garg, H. Tanaka and H. Tsujimoto.
4. (4) Effects of Genotype and Environment on HMW-GS Expression and Its Relationship with Steamed Bun and Bread-Baking Quality. Z. Deng, J. Tian, R. Hu, Y. Zhang, Y. Wang and G. Sun.
5. (5) The Quality Implication of Changing Glutenin Alleles in a Century of Australian Wheat Breeding. G.B. Cornish.
6. (36) Physico-Chemical and Molecular Analysis of Bread Making Quality Traits in Indian Hexaploid Wheat. M. Elangovan, R. Rai, B.B. Dholakia, M.S. Oak, R. Tirawi, R.A. Gupta, M.D. Lagu, S.A. Tamhankar, V.S. Rao and M.S. Roder.
7. (76) A Glutenin Composition Necessary to the Extra-strong Dough. Y. Maruyama-Funatsuki, K. Takata, T. Tabiki, M. Ito, Z. Nishio, H. Funatsuki, H. and H. Yamauchi.
8. (30) Inhibition of  $\alpha$ -Gliadins in Hexaploid Bread Wheat. D. Becker, A. Folck, P. Pnies, H. Lorz and H. Wieser.
9. (89) Over-expression of Transgenes 1Dx5 and 1Ax1 in Elite Chinese Varieties of Wheat (*Triticum aestivum* L). Y.S. Wang, P.R. Shewry and G.Y. He.

**SESSION 1: BIOTECHNOLOGY AND GENETICS**  
**METHODOLOGY, GENETICS, ENVIRONMENT AND GLUTEN QUALITY**

*Co-Chairs: Ann Blechl and Peter Shewry*

10. (93) Integration and Expression of Gluten Strength and Grain Hardness Genes by Crossing Transgenic Plants with Elite Wheat Varieties. J.R. Zhang, K.X. Li, G.X. Yang, L.T. Luo, H.D. Jones, P.R. Shewry and G.Y. He.
11. (54) Functionality of Glutenin Subunits Produced by Transgenic Yeast. R. Kieffer, H. Wieser, I. Bauer, R. Hoffman and F. Meuser.
12. (87) Functional Studies of Wheat Storage Proteins in Model System. M. Oszvald, S. Tomoskozi, L. Tamas and F. Bekes.
13. (51) Overexpression of a Defence Gene Effective in Limiting Fungal Infection Does Not Alter the Expression of Gluten Components. M. Janni, J.W. Lin, A. Blechl, S. Masci and R. D'Ovidio.
14. (65) Identifying Transcriptional Networks That Determine Carbon Flux into Starch in Developing Wheat Caryopsis. B. Stamova, D. Laudencia-Chincuanco, F. You, D. Beckles and O.D. Anderson.
15. (66) Transcriptional Profiling of Caryopsis Development by cDNA Microarray Analysis. D. Laudencia-Chincuanco, B. Stamova, F. You, G. Lazo, D. Beckles and O.D. Anderson.
16. (94) Biochemical Composition and Transcript Profile of Wild and Cultivated Wheat Endosperms. D. Beckles.
17. (99) Transcription of the *Glu-1Bx* HMW Glutenin Subunit Gene During Grain Filling in Several Wheat Cultivars. M. Gárdonyi, P. Sz cs, J. Bányai, Z. Bed and L. Tamás.
18. (100) Considerations About The Effect of Incorporation of Two Rare LMW-GS in Durum Wheat in Comparison to Bread Wheat Doughs. P. Ferrante, M.C. Gianibelli, O. Larroque, R. D'Ovidio, D. Lafiandra, S. Masci.
19. (44) Differential Processing of Low Molecular Weight Glutenin Subunits Met- and Ser types at Their N-Terminal End. P. Ferrante, R. D'Ovidio, D. Lafiandra, A. Ceriotti, W.H. Vensel, D.D. Kasarda and S. Masci.
20. (60) Characterisation of B- and C-type Low Molecular Weight Glutenin Subunits in Durum Wheat. B. Margiotta, M. Urbano, G. Colaprico, V. Mucilli, R. Saletti, S. Foti and D. Lafiandra.
21. (61) Characterization of Expressed and Unexpressed Y-Type Genes in Diploid and Polyploid Wheat. F. Sestili, C. Mattei, R. D'Ovidio and D. Lafiandra.
22. (62) Effect of D-Genome Associated Gluten Proteins on Durum Wheat Quality. D. Lafiandra, B. Margiotta, M. Urbano, G. Colaprico, M.G. D'Egidio, R. Carozza and C. Ceoloni.
23. (85) Proteins Alteration in Triticum Durum by Eurygaster and Aelia Insects Species. L. Salis, C. Alvarez and E. Gordun.
24. (6) Lipid Selectivity of Puroindolines and the Relationship to Endosperm Hardness. L.A. Clifton, R.J. Green and R.A. Frazier.
25. (42) Interaction of Thioredoxin h with Gluten Proteins. R. Cazalis.
26. (8) Novel Puroindoline B Alleles in Aegilops Species. M.J. Chen, T. Fang, J.L. Chang, L.T. Luo, K.X. Li, G.X. Yang, M. Wilkinson, P. Tosi, P.R. Shewry and G. He.
27. (73) The Molecular Evolution and Genome Sequence of Grain Hardness Genes in Genera of *Triticeae dumont*. L. Luo, M. Chen, J. Wang, G. Yang, K. Li, J. Chang, P.R. Shewry and G. He.
28. (22) Interaction of the Starch Granule Surface and Associated Proteins. A. Bako, M. Gardonyi and L. Tamas.
29. (72) *Puroindoline a* Enhancing the Resistance of Leaf Rust Disease in Transgenic Durum Wheat. L.T. Luo, J.R. Zhang, Y. Li, P. Shewry and G.Y. He.
30. (67) Survey of *Brachypodium distachyon* Species as a Possible Model System for Wheat. G.R. Lazo, D. Laudencia-Chincuanco, Y.Q. Gu, J. Vogel and O.D. Anderson.

## SESSION 2: STRUCTURE CHARACTERIZATION AND FUNCTIONAL RELATIONSHIPS AMONG GLUTEN MONOMERS AND POLYMERS

*Co-Chairs: Finlay MacRitchie and Herbert Wieser*

### Oral Presentations:

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1. (15) On-the-spot Analysis of All Gluten Polypeptides by Lab-on-a-chip Capillary Electrophoresis. S. Uthayakumaran, I.L. Batey and C.W. Wrigley.
2. (26) Solid State Spectroscopies for Assessing the Structural Features of Gluten Proteins in Wheat Flour and Semolina. S. Iametti, F. Bonomi, N.A. Pagani and E. Ragg.
3. (79) Comparison of Analytical Methods for Breadmaking Quality Prediction in a Genotype by Environment Study: SE-HPLC versus Spectrophotometric Measurement of HMW Glutenin. H.A. Naeem and H.D. Sapirstein.
4. (58) Non-aqueous Fractionation of wheat Flour – Method to Prepare Native Gluten Proteins. P. Koehler and S. Hartmann.
5. (64) Redox Agents Impact Gliadin-glutenin Cross-linking during Hydrothermal Treatment. B. Lagrain, K. Brijs and J.A. Delcour.
6. (77) Tyrosine Cross-Linking of Wheat Gluten Proteins and Its Functional Importance. A.R. Mateos, S.J. Millar, D.G. Bhandari and R.A. Frazier.
7. (74) Analysis of the First Steps of Prolamins Assembly and Polymerization during Wheat Grain Development. C. Mangavel, L. Dubreil, C. Loussert, J. Barbot and Y. Popineau.
8. (97) The Origin of Glutenin Particles. R.J. Hamer and T.W.J.M. Van Herpen.
9. (46) Wheat Gluten-based Biomaterials: Composites and Nanocomposites. S. Guilbert, E. Gastaldi, H. Angelier, P. Menut, T. Kuanopparat and N. Gontard.

### Poster Presentations:

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1. (80) Ultra-fast Separation of Wheat Glutenin Subunits by Reversed-Phase HPLC using a Superficially Porous Silica Support Column. H.A. Naeem and H.D. Sapirstein.
2. (68) A Rapid Spectrophotometric Assay for Measuring Functional Protein in Wheat. O.M. Lukow, J. Suchy, D. Brown, R.M. Depauw, S.Fox, G. Humphreys and S. Woods.
3. (70) Study of Glutenin Functionality using a Transgenic Wheat System. O.M. Lukow, J. Suchy, S. Uthayakumaran, M. Jordan and S. Cloutier.
4. (69) Effect of Low Molecular Weight Glutenin Subunit composition of Wheat on Dough Properties. O.M. Lukow, J. Suchy, K. Adam and G. Humphreys.
5. (1) Elucidating the Role of Low Molecular Weight Glutenin Subunits in determining Wheat Quality. J. Lambourne, D. Bhandari, R.A. Frazier, P. Tosi and P.R. Shewry.
6. (90) Differentiation of Allelic Variations of the HMW Glutenin Subunits of Wheat Flours by Use of Mixing Parameters and Polymeric Protein Content. H. Akdogan, M. Tilley, S.R. Bean and R. Graybosch.
7. (92) Probing Protein-Lipid Interactions in Gluten-Acetic Acid Fractionation Approach. T.H. McCann, I.L. Batey and L. Day.
8. (27) Distribution of Protein Composition in Bread Wheat Flour Mill Streams and Relationship to Breadmaking Quality. Y.G. Wang, K. Khan, G. Hareland and G. Nygard.
9. (55) Influence of Sulfur Fertilization on the Technological Properties of Wheat Flour. P. Koehler, H. Wieser and S.V. Tucher.
10. (59) Studies on the Degradation of Gluten Proteins During Germination of Wheat. P. Koehler, H. Wieser and G. Hartmann.
11. (43) Relationships of Glutenin Macropolymer Quantity and Properties to Strength and Composition of Gluten Proteins for Diverse Durum Wheat Genotypes. N.M. Edwards, R.J. Hamer and J. Dexter.

## SESSION 2: STRUCTURE CHARACTERIZATION AND FUNCTIONAL RELATIONSHIPS AMONG GLUTEN MONOMERS AND POLYMERS

*Co-Chairs: Finlay MacRitchie and Herbert Wieser*

12. (3) Effect of Seeding Time on Gluten Strength and Protein Composition of Italian Durum Wheat Cultivars Harvested in Sardinia in 2004 and 2005. S. Fois, L. Schlichting, B. Marchylo, J. Dexter, R. Motzo and F. Giunta.
13. (52) Quality and Protein Characterization of Triticale Lines with the Pair of Subunits 5+10 and 2+12. R. Jonnala and F. MacRitchie.
14. (19) Potentials and Method Improvements of Capillary Zone Electrophoresis for Use in Spelt Breeding Programs. T. Schober and S.R. Bean.
15. (91) Puroindoline Synthesis in Developing Seeds of Common Wheat Cultivars with Contrasting Grain Texture Characteristics. L. Gazza, F. Taddei, N.E. Pogna, S. Conti and P.K.W. Ng.
16. (17) Conferring Gluten-like Properties on Soy Proteins to Improve Soy-Wheat Bread Quality. S. Uthayakumaran, E. Maforimbo, G. Skurray and C.W. Wrigley.

## SESSION 3: RHEOLOGY: APPLICATION AND PREDICTION OF GLUTEN QUALITIES AND PROPERTIES

*Co-Chairs: Steve Mulvaney and Clyde Don*

### Oral Presentations:

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1. (31) Status of Global Wheat Quality Test Methods. R. Chinnaswamy, L.D. Freese, W.C. Burden, T.D. Norden and D.B. Funk.
2. (78) Elastic Recovery and Plastic Flow in "5 + 10" Glutens. S.J. Mulvaney, P. Rayas-Duarte, R. Chinnaswamy, B. Allvin and D. Zhou.
3. (33) Gluten, GMP, Glutenin Particles, Models and Practical Reality "Connecting Science and Practical Reality". C. Don and P. Weegels.
4. (12) Fourteen Years Strain Hardening as an Indicator of Bread-Baking Performance, Questions Still to be Solved. T. Van Vliet and R.J. Hamer.
5. (40) On the Mechanism of Gluten Network Development in Flour-Water Batter Doughs. F. Auger, A. Redl, J. Lefebvre and M.H. Morel.
6. (9) Rheological Properties of Low-Hydrated Starch Gluten Blends Affected by Their Quality and Quantity. H. Chanvrier and S. Uthayakumaran.
7. (83) Rheology of Gluten Film Around Gas Cells in Bread Making. B.S. Sroan and F. MacRitchie.
8. (81) Determination of Wheat and Breadmaking Quality with Small-scale Methods – An Overall Comparison Study. S. Tomoskozi, M. Nadosi, K. Ercsey, R. Haraszi, F. Bekes and A. Salgo.
9. (28) Changes in HMW-GS Composition of German Wheat Varieties 1994 – 2005 and Impact on Breadmaking Quality. B. Killermann and G. Zimmermann.
10. (95) The Impact of Nutrition on the Metabolome, Protein Composition and End-Use Quality of Wheat. D.D. Godfrey, P.R. Shewry and M.J. Hawkesford.

### Poster Presentations:

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1. (7) Rheological Properties of Doughs and Breadmaking Qualities of Several Wheat Cultivars Grown in Japan. P.V. Hung, T. Maeda and N. Morita.
2. (13) Effects of Oxygen Ozonation Process On Bread Dough Quality and Protein Composition. F. Violleau, O. Surel, M. Dubois, A.G. Despres, C. Coste and D. Kleiber.
3. (37) Measuring the Rheology of Grain Hardness. R. Haraszi and R.S. Anderssen.
4. (39) Contribution of Glutenin Alleles to Dough Rheological Parameters. M. Appelbee and G.B. Cornish.
5. (49) Study on the Quality Change of Wheat Flour During Storage in Controlled Condition. H. Sun, W.L. Jiang, X.H. Tian, J.Y. Lin and J.Y. Ling.

6. (56) Influence of the Fatty Acid on the Baking Activity of Phosphatidylcholine. B. Fischer and P. Koehler.
7. (57) Modification of Gluten by Emulsifiers and Effects on Dough Stabilization. R. Kieffer and H. Wieser.
8. (98) Use of the Reconstitution Method to Elucidate the Role of Gluten Proteins in Controlling Durum Semolina Dough Properties and Pasta Quality. M.J. Sissons, H.N. Soh, N. Egan, M.C. Gianibelli and M.A. Turner.

#### SESSION 4: HEALTH AND NUTRITIONAL ASPECTS OF GLUTEN PROTEINS

*Co-Chairs: Michael Tilley, Scott Bean and Clyde Don*

##### Oral Presentations:

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1. (21) Post-anthesis Fertilizer Influences Expression of Genes Encoding Allergenic Proteins. S.B. Altenbach and K. Kothari.
2. (25) Alpha-gliadin Genes from the A, B, and D Genomes of Bread Wheat Contain Different Sets of Celiac Disease Epitopes. T.W. Van Herpen, S.V. Goryunova, J. Van Der Shoot, M. Mitreva, E. Salentijn, O. Vorst, M. Schenk, P.A. Van Veelen, F. Koning and L. Van Soest.
3. (96) The Dog as a Model for Assessing Food Allergens in Wheat. B.B. Buchanan.
4. (71) Problems in Detecting Prolamin Contaminants in Oat-based Foods by Commercial ELISA Kits. P.M. Kanerva and T.S. Sontag-Strohm.
5. (53) Degradation of Celiac Toxic Peptides by Cereal Proteases. G. Hartmann, P. Koehler and H. Wieser.

##### Poster Presentations:

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1. (45) In Durum Wheat in Comparison to Bread Wheat Doughs. P. Ferrante, M.C. Gianibelli, O. Larroque, R. D'Ovidio, D. Lafiandra and S. Masci.
2. (86) Development of Gluten-Free Bread. O. Polenghi, R. Kuktaite and V. Cerne.

#### SESSION 5: PROTEOMICS AND TRANSCRIPTOMICS

*Co-Chairs: Frances DuPont and Gerard Branlard*

##### Oral Presentations:

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1. (14) Mass Spectrometry Based Identifications of LMW Glutenin Subunits. W. Vensel, F. DuPont, R. Chan and W. Hurkman.
2. (84) Comparative Transcriptional and Proteomic Profiling of Bread Wheat cv. Bobwhite and Its Derived Transgenic Line Over-Expressing a LMW-GS Gene. F. Scossa, D. Laudencia-Chincuanco, O.D. Anderson, W.H. Vensel, D.D. Kasarda, D. Lafiandra, R. D'Ovidio and S. Masci.
3. (101) Wheat Proteomics in the HEALTHGRAIN Project. S. Laugesen, B. Laubin, M. Merlino, G. Branlard and B. Svensson.
4. (10) Proteomics Studies on Wheat Developmental and Mature Kernel. G. Branlard, E. Bancel, E. Allain, C. Girousse, M. Merlino, B. Laubin, I. Nadaud, C. Debiton, G. Bronner and P. Martre.

# ABSTRACTS

## Oral and Poster Presentations

1

### **Elucidating the Role of Low Molecular Weight Glutenin Subunits in Determining Wheat Quality**

J. J. LAMBOURNE<sup>1</sup>, D. G. BHANDARI<sup>2</sup>, R. A. FRAZIER<sup>3</sup>, P. TOSI<sup>1</sup>,  
and P. R. SHEWRY<sup>1</sup>

<sup>1</sup> Rothamsted Experimental Station (RRes), Harpenden, Hertfordshire, AL5 4SE, UK

<sup>2</sup> School of Food Biosciences, The University of Reading, Berkshire, RG6 6AP, UK

<sup>3</sup> Campden and Chorleywood (CCFRA), Gloucestershire, GL55 6LD, UK

The low molecular weight glutenin subunits (LMW-GS) of gluten are considered to have an important impact on grain processing quality. They are major components of the glutenin polymers, accounting for about 80 percent of the total fraction. However, differences in the distribution of “unpaired” cysteine residues in different subunits means that they may have both positive and negative effects on dough strength, by acting as chain extenders and chain terminators, respectively. Despite the availability of many amino acid sequences little is known about the structures of individual subunits, as they are difficult to prepare in sufficient quantities for characterisation. We are therefore using an *E. coli* expression system to express whole LMW subunits and parts of subunits corresponding to specific structural domains, including subunits corresponding to the major structural types. These proteins and domains are being studied in detail to determine their conformations and properties, focusing on the ability of the repetitive domains to form regular secondary structures such as poly-L-proline II helix and beta-reverse turns. The results are expected to provide insights into the roles of the different types of subunit in determining the structure and properties of the glutenin polymers.

2

### **Effects of Mineral Nutrition and Temperature on Accumulation of Gluten Proteins is Related to Their Content of Cys and Met**

F.M. DUPONT, W. J. HURKMAN, W. H. VENSEL, R. CHAN,  
C. K. TANAKA and S. B. ALTENBACH

USDA Agricultural Research Service, Western Regional Research Center, Albany, CA 94710

Excess nitrogen and/or high temperature had effects somewhat similar to sulfur deficiency on flour protein composition. Grain was produced under temperature controlled conditions with average day and nighttime temperatures of 24°C and 17°C (cool), or 37°C and 28°C (hot), from anthesis until maturity. Pots were irrigated with or without NPK (20:20:20) fertilization from anthesis until maturity. Flour proteins were fractionated into NaI-soluble/methanol-insoluble gliadins; NaI-soluble/methanol soluble albumins, and NaI-insoluble glutenins. Amounts of HMW-GS, LMW-GS,  $\omega$ -gliadins, and  $\alpha + \gamma$ -gliadins were determined by RP-HPLC. Despite the extremes of temperature and fertilization, flour from three of the four treatments had nearly identical protein compositions. Flour produced under cool conditions with NPK and flour produced under hot conditions with or without NPK had greater proportions of S-poor protein types, whereas, flour produced under cool conditions without NPK was enriched in the S-rich protein types. NPK had little effect under the hot conditions. Although S-poor proteins also increase with S-deficiency, application of S after anthesis did not alter the results, and flour S-content did not indicate S-deficiency. Protein accumulation was followed throughout grain fill by 2DE of KCl-insoluble gluten proteins from endosperm. Under cool conditions, accumulation rates were increased by addition of NPK, with the biggest increases for the S-poor  $\omega$ -gliadins and HMW-GS. Under the hot conditions, accumulation rates in terms of thermal degree days were low, similar to those under cool conditions without NPK. However, S-poor proteins accumulated at sufficiently higher rates than S-rich proteins that the final flour protein composition was very similar to that produced with NPK under cool conditions. We propose that high temperature or ample N during grain fill favored the production of S-poor gluten proteins. Although three of the four regimens produced high protein flour with similar protein composition and high values for SDS-sedimentation and loaf volume, mixing tolerance was poor for the flours produced under the high temperature regimens.

**Effect of Seeding Time on Gluten Strength and Protein Composition of Italian Durum Wheat Cultivars Harvested in Sardinia in 2004 and 2005**

SIMONETTA FOIS<sup>1</sup>, LINDA SCHLICHTING<sup>2</sup>, BRIAN MARCHYLO<sup>2</sup>, JAMES DEXTER<sup>2</sup>, ROSELLA MOTZO<sup>1</sup> and FRANCESCO GIUNTA<sup>1</sup>

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Sowing time for durum wheat in Sardinia occurs over a period of up to two months depending on availability of moisture. Depending on sowing time and cultivar choice, anthesis occurs from mid-to-late April to late May, resulting in variable growing conditions during grain filling, which may well impact quality. In this project we looked at the impact of sowing time on the strength and composition of gluten protein and processing quality of six Italian durum wheat cultivars grown in replicated field plots in Sardinia and harvested in 2004 and 2005 (Svevo, Creso, Trinakria, Trigo Murru, Cappelli and Ichnusa in descending order of strength). The cultivars differed in morphology, duration of phenological phases, year of release and in traits related to yield and grain quality. Crops were grown without water limitation but, due to differences in sowing time, grain filling occurred under different thermal conditions. Moreover, in 2005 spring temperatures were higher than in 2004, and hence responsible for greater thermal stress. In 2004, the crop sown in February exhibited stronger gluten for all cultivars compared to the crop sown in November; sowing time had no consistent impact on protein content. In 2005, the crop sown in March had weaker gluten and an average of near 3% higher protein content than the crop sown in January. Reversed-phase high-performance liquid chromatography (RP-HPLC) confirmed a direct relationship between gluten strength and the magnitude of the ratio of protein soluble in propanol to protein sequentially extracted in dithiothreitol and propanol, both within and among the various cultivars. The texture of spaghetti dried at 70°C prepared from mature grain was primarily influenced by protein content. Characterization of protein by RP-HPLC for several of the cultivars at intervals throughout kernel development demonstrated the strong impact of growing conditions on the relative rates of accumulation of gluten protein.

**Effects of Genotype and Environment on HMW-GS Expression and Its Relationship with Steamed Bun and Bread-Baking Quality**

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Three winter wheat cultivars from eight different locations with 3 replications were used to study the effects of genotypes, environments and their interactions on high molecular weight glutenin subunits (HMW-GS) expression and its relationship with steamed-bun and bread-baking quality. The results indicated that although some subunits were affected by environments, genotypes played an important role for X-type HMW-GS, Y-type HMW-GS, Glu-1D location and Glu-1B location. Total HMW-GS amounts were affected by the interactions between genotype and environment. Individual HMW-GS content of the same genotype was affected by environment in different degrees. Yantai location was superior to other locations for many HMW-GS expression amounts. The same kind of HMW-GS from different genotypes appeared different in the same environments. Correlation analysis suggested that the HMW-GS expression amount positively correlated with bread volume, and negatively with bread peak firmness. Effects of individual and total HMW-GS on steamed bun quality were different from that on bread quality, and effects of total HMW-GS on bread volume were better than that of individual HMW-GS. Bread quality was determined first by HMW-GS types, second by the HMW-GS expression amount; while steamed bread quality was mainly affected by HMW-GS expression amounts.

**The Quality Implications of Changing Glutenin Alleles in a Century of Australian Wheat Breeding**

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High molecular weight (HMW) and low molecular weight (LMW) glutenin alleles in wheat are coded by the genes Glu-1 and Glu-3, on the long and short arm of chromosome 1 respectively. The glutenin alleles have a major influence on the rheological properties of dough and hence are a major determinant of end-product quality. In the period 1901-2001, 244 wheat varieties have been released by Australian wheat breeding programs. By looking at the changing frequency of glutenin alleles it is

possible to observe concurrent shifts in wheat quality. In the past the major driving force for new wheat varieties has been to improve the agronomic yield and the disease resistance. However this emphasis is changing to selecting varieties for quality traits on the basis of their genetic composition. By following the movement of glutenin alleles in and out of the gene pool it is possible to determine the source of alleles, which have produced improved quality varieties. In the past some of the quality gains have been fortuitous. With the increased knowledge of the effects of various glutenin alleles, it is possible to make much faster gains in quality by increasing the selection pressure on alleles associated with improved quality.

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### **Lipid Selectivity of Puroindolines and the Relationship to Endosperm Hardness**

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Puroindolines are lipid-binding proteins that consist of two isoforms, Pin-a and Pin-b, both possessing a tryptophan-rich domain; this domain consists of 5 Trp residues in Pin-a and is truncated to 3 Trp residues in Pin-b. When expressed in their native form, the puroindolines confer soft texture to the wheat endosperm; however, the expression of Pin-b mutants or the non-expression of either Pin-a or Pin-b are linked to hard endosperm texture. It is thought that the biochemical mechanism of endosperm hardness may be related to the interaction of the puroindolines with wheat lipids, and we have therefore investigated the interactions of puroindolines with negative (dipalmitoylphosphatidyl-dl-glycerol, DPPG) and zwitterionic (L- -dipalmitoylphosphatidylcholine, DPPC) phospholipid films at the air/water interface using the combination of external reflectance FTIR spectroscopy, neutron reflectivity and surface pressure measurements. Wild-type Pin-a and Pin-b isolated from soft wheat both showed specificity for negative DPPG films, with Pin-a having the highest affinity, presumably due to its larger Trp domain. Investigation of the interactions of Pin-b mutants isolated from hard wheats and containing the single amino acid substitutions Gly to Ser or Trp to Arg, revealed a loss in lipid selectivity in comparison to wild-type Pin-b. The role that this loss in selectivity may play in endosperm hardness will be discussed.

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### **Rheological Properties of Doughs and Breadmaking Qualities of Several Wheat Cultivars Grown in Japan**

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The rheological properties of doughs of several wheat flours grown in Japan and their breadmaking quality were estimated. The doughs made from one strong (Kitanokaori, 11.4% protein) and two extra-strong (Glenlea and Blenlea, 15.6 and 15.8% protein, respectively) wheat flours had high water absorption, development and stability times as well as good resistance to extension. In contrast, the soft (Norin 61 and Nebarigoshi) and semi-strong (Haruibiki, Haruyutaka and Hokushin) wheat flours, which had low protein contents (7.2 – 10%), showed lower water absorption, development and stability times of doughs during mixing. Among them, Hokushin, Norin 61 and Haruibuki had better dough strength than Haruibuki and Haruyutaka. Especially, Haruyutaka (9.7% protein) had the lowest resistance to extension because of the effect of endo-protease activity during the pre-harvest sprouting damage. Appearances of doughs observed by scanning electron microscopy (SEM) showed that Norin 61, Haruibuki, Hokushin and Kitanokaori had tight structure with mostly starch granules buried in gluten matrix similar to Cameria (a commercial wheat flour) dough. In contrast, Nebarigoshi, Haruyutaka, Bluesky and Glenlea had low extensibility of gluten matrix. The dough properties of these wheat flours did not correlate to their breadmaking qualities. Specific volumes of Hokushin and Kitanokaori breads were similar to Cameria bread, whereas Haruibuki, Haruyutaka, Bluesky and Glenlea showed the lower specific volumes of breads. These results suggest that the growing environments and harvest conditions largely affect the quality of wheat flours. The Japanese climate is not generally suitable for the cultivation of bread wheat. Therefore, the breadmaking qualities of Glenlea and Bluesky, the extra-strong red spring wheats, decreased considerably when they were cultivated in Japan.

### **Novel Puroindoline B Alleles in Aegilops Species**

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Grain hardness is a major determinant of the milling and processing quality of wheat. Current theories suggest that hardness is determined by the degree of adhesion between various components of the starchy endosperm cells of the mature wheat grain, notably between starch granules and matrix (gluten) proteins but also between proteins and cell walls. Furthermore, it has been proposed that one group of proteins, called puroindolines (PINs, including puroindoline a and puroindoline b), play a specific role by acting as “non-stick” proteins on the starch granule surface, resulting in grain softness. In hexaploid wheat, puroindolines are encoded by genes located at the Ha (hardness) locus. Genomic DNA from eight hexaploid, tetraploid and diploid species of Aegilops with the C, D, S, M and U genomes was amplified with specific PCR primers to identify sequences encoding puroindoline b, with *Ae. tauschii* (DD) and the hexaploid wheat (*T. aestivum*) (AABBDD) cv Hiline being used as controls. Eight new allelic forms of Pin b were identified, including the two forms with mutations within the tryptophan motif. In all species studied, the expression of Pin b in grain endosperm was verified by RT-PCR. Southern blot analysis revealed the presence of multiple identified genes in some Aegilops species, which implies that Aegilops provide an extensive reservoir of genetic resources for cultivated wheat quality improvement by wide crossing.

### **Rheological Properties of Low-hydrated Starch-gluten Blends Affected by Their Quality and Quantity**

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The rheological properties of low-hydrated wheat flour (<40% moisture content) in the molten state are influenced by the changes its components undergo during processing. In order to investigate the quantitative and qualitative effects of the individual flour components on rheological properties, model blends of wheat starch and wheat gluten with different starch/gluten ratios were studied. The effects of gluten and starch quality were also investigated by using different gluten types and by modifying the amylose content of starch. The shear viscosity of the blends was determined by capillary rheometry under controlled conditions (35% moisture content, 140°C). The changes undergone by wheat gluten under these conditions were analysed by HPLC (determining the proportion of unextractable polymeric proteins) and by Lab-on-a-Chip (analysing the protein composition). This study indicated that in low hydrated products in the molten state, shear viscosity was affected by the structure of the blends as observed by fluorescence microscopy and by the molecular changes occurring during processing.

### **Proteomics Studies on Wheat Developmental and Mature Kernel**

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Thanks to progress in 2-dimensional electrophoresis (2DE) and to mass spectrometry, the proteomics approach is a tool, which offers biochemists and geneticists opportunities to investigate the diversity and function of thousands of proteins that are simultaneously expressed in the different cell organites and kernel compartments. The composition of the wheat kernel is the result of the expression of numerous enzymes involved in the accumulation of storage proteins and starch. Using the proteomics approach, the analysis of the enzymes expressed in the kernel will provide the basis for future genetic progress aimed at improving the stability of kernel composition and end use quality. To illustrate the usefulness of the proteomics tool, we report on different experiments carried out at INRA, Clermont Ferrand, France, on developmental or mature wheat kernel: (1) Proteomic analysis every 50 °C day (cumulative day average temperature) during kernel formation from the ovule stage to 15 days after fertilization allowed many proteins to be tracked during cell and tissue formation; (2) Differential proteomics used during grain development of three cultivars submitted to heat treatment or not (control) enabled us to study the effect of heat treatment on the kinetics of synthesis of some storage proteins, and allowed many up- and down-regulated enzymes to be

revealed; (3) The thousands of albumins and globulins expressed either in endosperm or in the aleurone layer and the numerous storage proteins revealed in 2DE were mapped using chromosome deletion lines of the cv Chinese Spring. Current progress in study 3 is reported. Although the wheat genome is far from being completely sequenced, the identification of wheat proteins by MALDI-TOF and MS/MS mass spectrometry will benefit from data from plant species whose genome has already been sequenced. To facilitate exchanges between laboratories involved in the transcriptomics and proteomics in the framework of the IGROW project, a data base of wheat proteome could be set up to facilitate genetic progress particularly with respect to wheat gluten quality.

11 – Withdrawn from conference

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**Fourteen Years Strain Hardening as an Indicator of Bread-Baking Performance,  
Questions Still to Be Solved**

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It has generally been accepted that strain hardening of bread dough is an important indicator for its bread baking quality. At the end of the proving stage and during baking the lamellae between two gas cells will be often so thin that they can not be considered anymore as a homogeneous dough film, but should be considered as predominantly gluten films containing large starch granules. Hence, it is the gluten that provides the dough with its strain hardening properties. With respect to the use of strain hardening as a quality indicator of gluten there are still several aspects that are not yet satisfactorily solved. Remaining problems are related to what is the most relevant indicator of strain hardening and how to measure it, as well as a model allowing understanding of strain hardening in terms of composition and structure of the hydrated gluten network. In the original paper of 1992, in which strain hardening was proposed as an important property of dough, it was assumed that biaxial extension is the dominant way of deformation with respect to gas cell coalescence during proofing and baking. However, dough deformation during baking involves both uniaxial and biaxial extension and strain hardening in uniaxial and biaxial extension are not directly correlated. Regarding strain hardening-structure relationships two main types of models are presently advocated. The first type is based on models developed for describing the relation between the mechanical behaviour of flexible synthetic polymer systems and the molecular structure of these polymers. The basic hypothesis behind these continuum models is that the physical properties of such models do not depend on scale down to molecular scale. The other class of models, so-called multiscale models are basically a hierarchy of sub-models, which describe the mechanical behaviour at different spatial scales in such a way that the sub-models are interconnected. The hyper-aggregation model of Hamer and Van Vliet is an example of such a model. The pros and cons of the different type of models will be discussed in relation to observed data for strain hardening.

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**Effects of Oxygreen® Ozonation Process On Bread Dough Quality and Protein Composition**

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Oxygreen® ozonation process for wheat grain is very efficient to modify technological properties of wheat flour. A central composite design (3 levels; 3 factors) has been realized to evaluate the influence of operational conditions (humidification rate, ozone pressure and ozone concentration in the inlet flow) on technological properties and protein composition. Dough alveographic data (P, L, P/L, W) and protein composition (SDS-unextractable polymeric protein/ SDS-extractable polymeric protein (UPP/UEP)) have been followed on Paulic bread wheat (French miller's brand). The results were compared with a standard treated without ozone. Dough kneaded from flour of ozonated wheat grain present W between 282.8 10<sup>-4</sup> and 226.7 10<sup>-4</sup> J, P from 86 to 105 mm and L from 1.01 to 1.86 mm. These modification leads to P/L ranging from 1.02 to 1.74 for a nominal value around 1. An increase of W and P and a decrease of L were observed after ozone treatment. Ozonation of wheat grain caused an increase of dough resistance strength and a decrease of extensibility if compared to the control. Nevertheless ozonation treatment had to be moderated. In fact, detailed analysis of response surface curve indicates that an increase of ozone concentration and pressure in the reactor have a negative impact on dough strength. Protein composition has also been studied. UPP/UEP was analysed in flour. For treatment performed with low ozone pressure and concentration, these parameters were higher (ranging from 0.49 to 0.65) than for the control (0.49) whereas they were lower (ranging from 0.41 to 0.49) after a treatment at high ozone pressure and concentration. Our study shows that the Oxygreen® wheat ozone treatment leads to different flours with higher force and tenacity and lower extensibility than control. Precise analysis of response surfaces and protein composition indicate that there is a competition between a polymerization and a de-polymerization phenomenon probably due to protein oxidation by ozone.

### **Mass Spectrometry Based Identifications of LMW Glutenin Subunits**

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Tandem mass spectrometry (MS/MS) is routinely used to identify wheat endosperm proteins. In this method, peptide fragmentation patterns generated by MS/MS are identified using a 'search engine' to compare the spectra to those generated in silico from protein sequence databases. Trypsin is a commonly employed enzyme as it produces peptides that give good fragmentation patterns in the mass spectrometer. Gliadins and glutenins, however, are difficult to distinguish because they have a small number of cleavable tryptic sites and have very similar sequences, including repetitive motifs rich in proline and glutamine. Often glutenins and gliadins yield only one or two tryptic peptides that provide enough significant information for identification by MS/MS analysis. Confounding this problem, different search engines have different sensitivities and provide slightly different results. To address these problems we used enzymes with cleavage specificities different from trypsin and analyzed the results using three different search engines. This approach increased the number of peptides generated and detected. All glutenin proteins tested with trypsin gave identifiable fragments by ESI-MS/MS. However, trypsin yielded fewer fragments than chymotrypsin while thermolysin produced fragments that allowed identification of the highly similar LMW-GS. Using a post MS/MS search tool, to combine data from different search engines, increased the number of peptides detected thus increasing the percent 'coverage' and confidence in the identification. The improved MS analysis, using two or three enzymes and multiple search engines, increased the confidence of MS/MS identifications of the glutenins and should similarly improve the identification of the gliadins.

### **On-the-spot Analysis of All Gluten Polypeptides by Lab-on-a-chip Capillary Electrophoresis**

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Dramatic improvements in speed and efficiency for gluten polypeptide analysis are provided by micro-fluidics capillary electrophoresis. Size-based fractionation of the full complement of flour polypeptides takes about 50 seconds. Results are portrayed either as elution profiles or as simulated gel-electrophoresis patterns. The system has been used to provide distinction among national sets of varieties of wheat and barley. It has been shown to be applicable to many other grain species. Other practical applications include the detection of defects in wheat grain. In breeding studies, the method has assisted in correcting errors of mis-labeling and it has been used to analyze the glutenin-subunit composition of progeny at an early stage of the selection process. In the latter situation, the quantitative facility has been important for distinguishing the forms of HMW-subunit 7, namely, its normal or over-expressed forms. Quantitation of individual subunits offers the opportunity of predicting their individual contributions to dough quality. Importantly, the versatility of the equipment has been demonstrated in the recent Australian harvest by using it at many grain-receival sites for varietal identification "in real time".

### **Frequencies of Gluten-protein Alleles in a Worldwide Collection of over 4,600 Wheat Genotypes**

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A catalog of gluten-protein alleles has been assembled for thousands of wheat genotypes from around the world. The list was originally planned to be an appendix to the new book "Gliadin and Glutenin: The Unique Balance of Wheat Quality", edited by Wrigley, Bekes and Bushuk, and published by AACC International. However, the list grew so large that a web site ([www.aaccnet.org](http://www.aaccnet.org)) was the only possible means of dissemination. The catalog includes three lists, namely, gliadins, HMW glutenin subunits and LMW subunits. The list for HMW subunits is the longest, involving over 4,600 genotypes, mainly named varieties. Country of origin is provided for each entry, plus a reference to the source of the information – usually a publication or a personal communication. Analysis of the catalogue is providing answers to various questions. What alleles have been used over time by breeders in specific countries? How broad has been the spread of alleles? What correspondences are seen between alleles for gliadins and for LMW subunits? Corrections and additions to the catalog are invited.

### **Conferring Gluten-like Properties on Soy Proteins to Improve Soy-wheat Bread Quality**

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Soy-wheat bread is nutritionally desirable to address protein malnutrition in developing countries. However, high levels of soy flour in wheat dough harm bread-making properties. A simple heat-treatment process was developed for soy to improve its contribution to soy-wheat composite bread, even at up to 50% soy content. The heat-treated soy flour provided greatly improved dough and baking quality in soy-wheat doughs, compared to composite doughs made with raw soy flour. The size distribution of the proteins in the soy-wheat composite doughs was determined to elucidate the changes produced by the soy treatment. Analysis of the composite doughs by size-exclusion high performance liquid chromatography indicated that the composite doughs made with raw soy flour had lower levels of unextractable polymeric protein compared to doughs made with the heat-treated soy. Heat treatment of soy appears to have conferred gluten-like properties on the soy flour, thereby increasing molecular-weight distribution and improving its contribution to bread-making properties.

### **Using Epitope Tagging to Explore the Trafficking, Location and Functional Properties of Wheat Gluten Proteins**

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Previous studies indicate that wheat gluten proteins may differ in their spatial distribution within the starchy endosperm and in their pathways of trafficking and deposition within the endosperm cells, depending on the properties of the proteins and the stage of development of the tissue. We are therefore constructing a series of transgenic lines of wheat to explore these phenomena and their impact on gluten functionality. We are using three different epitope tags as fusions to individual gluten proteins ( $\gamma$ -gliadins, LMW subunit, HMW subunit) and two different promoters to drive expression of the proteins either in the sub-aleurone and outer cells or in the central cells of the starchy endosperm. These tags allow the proteins to be detected in tissue sections, cells and gluten using highly specific antibodies. Crosses made between lines expressing proteins with different tags will also allow up to three proteins to be followed independently within the same cells. Results from this study will be discussed including data from the expression of tagged LMW subunits in bread and pasta wheats.

### **Potentials and Method Improvements of Capillary Zone Electrophoresis for Use in Spelt Breeding Programs.**

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Capillary zone electrophoresis (CZE) in acidic buffer systems is capable of separating cereal storage proteins based on similar separation principles as classical acidic polyacrylamide gel electrophoresis. However, it is faster, its resolution is distinctly higher and data evaluation is much simpler. Applying a 100 mM sodium phosphate buffer system pH 2.5 containing hydroxypropyl methylcellulose (HPMC), and using a 60 cm capillary, CZE was successfully used in a spelt breeding program. Several examples are given: mislabeled samples could be identified, although the differences in the patterns were very small. Relatedness between different spelt cultivars could be shown. However, it was not possible to clearly differentiate between pure spelts and wheat-spelt crosses. Crossing spelt with modern wheat may be, but is not necessarily, reflected in the gliadin pattern. This latter finding is in agreement with several studies, showing that one single protein class (LMW glutenin subunits, gliadins) did not always reflect purity of spelt. The acidic phosphate buffer system was compared to an isoelectric buffer system composed of 50 mM iminodiacetic acid (IDA), HPMC and acetonitrile, using a short (27 cm) capillary. It was found that the IDA system provided more than 10 times faster separations with almost the same resolution as the sodium phosphate system. It is concluded that CZE, especially with the IDA buffer, is a fast and powerful tool in spelt breeding programs to avoid mislabeling, and gain insight into the relatedness of new lines with unknown pedigrees.

### **Post-anthesis Fertilizer Influences Expression of Genes Encoding Allergenic Proteins**

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Omega-gliadins comprise between 5-10% of wheat flour protein and have been implicated in food allergies. In particular, omega-gliadins encoded by the 1B chromosome have been associated with wheat-dependent exercise-induced anaphylaxis (WDEIA) and urticaria in adults and immediate-type wheat allergies in children. Because the omega-gliadins consist almost entirely of repetitive sequences, the genes encoding these proteins have proved difficult to clone. Therefore, the complement of omega-gliadin genes expressed in the US spring wheat 'Butte 86' was surveyed by examining expressed sequence tags (ESTs) available from the TIGR Wheat Gene Index (<http://www.tigr.org/>). Fifteen ESTs from 'Butte 86' fell into 5 gene assemblies. Three of the assemblies encode proteins with PQQFP as the predominant repetitive motif and are similar to proteins encoded on chromosomes 1A and 1D. In one of these, the substitution of a T with a G in the DNA sequence results in the replacement of a phenylalanine residue with a cysteine and suggests that the protein may be incorporated into the glutenin polymer. The other two gene assemblies encode proteins with FPQQQ and QQIPQQ repeats and are similar to omega gliadins encoded on chromosome 1B. Primers specific for the two types of omega-gliadin genes were designed and quantitative real time RT-PCR was used to investigate the accumulation of transcripts in developing wheat grains produced with or without post-anthesis fertilizer supplied as NPK 20-20-20. Transcripts for both types of omega-gliadin proteins were detected at 8 days post-anthesis (DPA). The levels of transcript changed little between 8 and 32 DPA in the absence of NPK. When plants were supplied with NPK, transcript levels increased gradually between 8 and 32 DPA so that levels of omega-gliadin transcripts were significantly higher in grains that received NPK than in those that did not. Transcripts for a gamma-gliadin showed a different profile, with the greatest amounts of transcripts during mid-stages of grain development. In contrast to omega-gliadins, both the timing and levels of gamma-gliadin transcripts changed little in response to NPK. The data suggest that the application of nitrogen to improve grain protein content may also lead to increased levels of one type of allergenic protein.

### **Interaction of the Starch Granule Surface and Associated Proteins**

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It is widely accepted that the strength of adhesion between starch granules and endosperm matrix determines grain hardness (texture) of wheat. Soft, hard and durum grains fracture differently during milling. Therefore, grain hardness restricts potential utilization of the different cultivars. The nature of the starch granule – protein matrix interaction is not clear. There is an unbroken linkage between grain hardness and the composition of protein on the granule surface. A similar pattern was observed for starch lipids. In soft wheat, the friabilin protein fraction is abundant on the surface of starch granules and adhesion between granules and matrix is largely impaired. The mechanism underlying the strong adhesion observed in hard and durum wheats, where friabilin is scarce or absent, is unknown. The major components of friabilin are puroindoline a and b. Both are able to bind lipids. Wheat cultivars carrying mutations in any of the puroindolines have hard texture and both proteins are absent in 'very hard' durum wheat. Purified friabilin was shown to bind to wheat starch granules *in vitro*. In this work, we studied starch binding properties of several wheat endosperm proteins. Proteins were extracted from flour by Triton X-114 phase partitioning and from starch granules by isopropanol-salt. For binding experiments, three different types of starch were used (prepared from soft, hard and durum wheat). Several components of the crude extracts bound to the granules specifically. Besides friabilin, proteins with 35-40 kDa Mol. weight are also able to bind to the granules. An antiserum raised against wheat storage protein hybridized to the two major bands in Western blot analysis. These results were confirmed using prolamins extracted by 70% ethanol. Specific binding of  $M_r$  approx. 33 kDa protein was observed. To determine whether starch surface lipids play a role in starch granule – protein interaction, starch was treated with 9:1 isopropanol-water. The results indicated that binding of non-friabilin components is lipid independent, whereas isopropanol treated starch bound significantly less friabilin. Studying the starch binding properties of the approx. 35 kDa proteins may improve our understanding of granule-matrix adhesion.

**Development and Application of Fast Immunological Selection Methods for High Molecular Weight Glutenin Subunits in Wheat Breeding**

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For the screening of wheat breeding material fast and reliable methods are desired to determine the allelic composition of the high molecular weight glutenin subunits (HMW-GS). Our research is concentrated on the development of monoclonal antibodies (mAbs) specific for single HMW-GS and their incorporation in an enzyme-linked immunosorbent assay (ELISA) format. As the HMW-GS show a very high degree of homology in their amino acid sequence we decided to develop mAbs on the basis of peptides representing unique sequence sections of the glutenins of interest. Synthetic peptide conjugates were used as immunogens for the development of mAbs in mice hybridoma cell lines. For the production of antibody specific for HMW-GS 1Dx5, a cysteine directed modification of the peptide immunogen was necessary to generate a structural epitope big enough to be recognized specifically by the antibody. As a result of our research we present two mAbs, Antipep592, which is highly specific for HMW-GS 1Ax1 and Antipep4414, which is specific for the cysteine modified variant of HMW-GS 1Dx5. Both antibodies were introduced in an indirect ELISA. The Glu-A1-Assay using mAb Antipep592 allows discrimination of the Glu-A1a allele from the Glu-A1c allele while the Glu-D1-Assay using mAb Antipep4414 makes it possible to distinguish the Glu-D1a and the Glu-D1d allele. The reliability of the two assays was verified in doubled haploid lines and varieties with defined HMW-GS composition. In summary the Glu-A1-Assay and the Glu-D1-Assay allowed fast and easy high throughput screening for the presence of the most important HMW-GS 1Ax1 (allele Glu-A1a) and 1Dx5 (allele Glu-D1d) in breeding material.

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**Alpha-gliadin Genes from the A, B, and D Genomes of Bread Wheat Contain Different Sets of Celiac Disease Epitopes**

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Bread wheat (*Triticum aestivum*) is an important staple food. However, in 0.5 to 1% of the general population wheat gluten proteins cause celiac disease (CD). More specifically, discrete sequences of  $\alpha$ -gliadins have been identified as T cell stimulatory epitopes in CD patients through binding to HLA-DQ2/8. In this study we wanted to explore the distribution of four of these sequences in the  $\alpha$ -gliadin gene family. To this end we obtained 230 distinct  $\alpha$ -gliadin gene sequences from several diploid wheat species representing the ancestral A, B, and D genomes of the hexaploid bread wheat. The  $\alpha$ -gliadin sequences could be distinguished according to the genome of origin on the basis of sequence similarity and of the differences in the presence of the four T cell stimulatory epitopes. By sequence similarity,  $\alpha$ -gliadins from the public database of hexaploid *T. aestivum* could be assigned directly to chromosome 6A, 6B, or 6D. The differences in epitope composition resulted mainly from point mutations. These substitutions appeared to be genome specific. Our analysis determines the relative contribution of  $\alpha$ -gliadin genes from the three genomes with regard to the occurrence of four CD epitopes in gluten. Such a systematic analysis of all known epitopes in gliadins and glutenins will lead to better understanding of the differences in toxicity among wheat varieties. On the basis of this insight, we have designed a strategy to generate a less toxic wheat variety that may be tolerated by CD patients.

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**Solid State Spectroscopies for Assessing the Structural Features of Gluten Proteins in Wheat Flour and Semolina**

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Solid-state spectroscopy represents a useful tool for studies on structural reporters, also when dealing with insoluble proteins or the semi-solid systems often found in foods. We used solid-state fluorescence (also known as front-face fluorescence) and various NMR techniques to assess some structural features of proteins in wheat flours and semolina, since these approaches allow one to perform direct structural measurements without requiring previous protein solubilization in conditions that strongly modify the structure of proteins (and their interactions with other food components) in the original material. We first addressed

the structural changes – as detected by tryptophan fluorescence - ensuing from protein solvation in semolina samples known to have different pasta-making ability. Solvation also was found to affect overall protein surface hydrophobicity, as detected by using the fluorescent hydrophobic probe, 1,8 aniline naphthalene sulphonate (ANS). Solvation-related changes were also compared with data on water distribution between phases (i.e., starch, protein, and liquid) obtained by wide-line and solid-state NMR. By titrating flours - mixed with an identical amount of water to a dough-like consistency - with increasing concentrations of ANS, it was possible to evaluate the correlation between protein structural features and the technological performance of individual samples. To test the usefulness of the structural information obtained from these approaches, they were also applied to characterize doughs at different levels of mechanical stress, prepared either from wheat flour or from semolina. In subsequent studies, these solid-state spectroscopic approaches were also applied to the characterization of non-gluten doughs obtained from a number of pseudo-cereals, as well as in mixtures including non-cereal ingredients used in the preparation of gluten-free foods. This work was supported from grants from MiUR, Rome, Italy (PRIN-2005077017), and from the Regional Government of Lombardy (Progetto Metadistretti SAGRADE).

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### **Distribution of Protein Composition in Bread Wheat Flour Mill Streams and Relationship to Breadmaking Quality**

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Wheat protein quantity and composition are important parameters for wheat baking quality. The objective of this study was to use fractionation techniques to separate the proteins of flour mill streams into various protein fractions, to examine the distribution of these protein fractions, and to establish a relationship between protein composition and breadmaking quality. Nine break streams, nine reduction streams and three patent flours, obtained from three samples of Nekota (a hard red winter wheat), were used in this study. A solution of 0.3 M NaI + 7.5% 1-propanol was used to separate flour protein into monomeric and polymeric proteins. The protein fractions, including gliadin, albumin+globulin, HMW-GS and LMW-GS, were precipitated with 0.1 M NH<sub>4</sub>Ac-MeOH or acetone. The fractions were statistically analyzed for their distribution in the mill streams. The quantities of total flour protein and protein fractions in flour were significantly different among mill streams. The ratio of polymeric to monomeric proteins in break streams was significantly greater than in reduction streams. The relationship between protein composition and breadmaking quality showed that the quantities of total flour protein, albumin and globulin, HMW-GS and LMW-GS in flour were significantly and positively correlated with loaf volume. The ratio of HMW-GS to LMW-GS had little association with loaf volume. The gliadin content in total flour protein was negatively and significantly correlated with loaf volume. These results indicated that the quantity and composition of protein among the mill streams was different, and this resulted in differences in breadmaking quality.

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### **Changes in HMW-GS Composition of German Wheat Varieties 1994 – 2005 and Impact on Breadmaking Quality**

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We have recently hypothesised that the glutenin particles as isolated from wheat flour and observed using Confocal Scanning Laser Microscopy (CSLM) originate from Protein Bodies in immature wheat endosperm. In this study we report our experiments to challenge this hypothesis. Glutenin particles were isolated from mature wheat (cv Cadenza) using a detergent solution and ultracentrifugation. Protein bodies were isolated from immature (15 DAF) wheat of the same variety and purified using Percoll density centrifugation. Both glutenin particles and protein bodies were analysed at the molecular level (protein composition), oligomer level (size distribution after sonication), and microscopic level (CSLM with specific stains for protein and free sulfhydryl groups). The results demonstrate similarities between Protein Bodies and glutenin particles at all levels, but also distinct differences. In addition, we simulated the extraction of GMP with a detergent (in this case SDS or Triton X-100) with pre-labeled Protein Bodies. This allowed us to directly observe effects on solubility of proteins and proteins with free SH groups. Our results lend further support to the proposed relation between Protein Bodies and glutenin particles.

### **Characterization of Wheat with Strongly Reduced $\alpha$ -Gliadin Content**

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An RNA interference technology was applied to silence  $\alpha$ -gliadin genes of wheat cv. 'Florida'. A transgenic line with strongly reduced  $\alpha$ -gliadin content was grown in a greenhouse. Mature kernels were harvested, milled into white flour and compared with the wildtype flour by the determination of flour protein composition and technological properties. The crude protein content of both flours was nearly identical. A modified Osborne fractionation and RP-HPLC analysis revealed that the loss of  $\alpha$ -gliadins was compensated by the increase of albumins, globulins,  $\omega$ -,  $\gamma$ -gliadins and HMW glutenin subunits accompanied by a significant decrease of the ratio of gliadins to glutenins. Rheological dough and gluten properties and baking performance were determined by extension and baking tests on a micro-scale. Dough resistance and extensibility of the transgenic line were similar to those of the wildtype dough, whereas gluten strength of the transgenic line increased drastically. Bread volume of the transgenic line was slightly lower and the crumb had a good appearance with regular pores showing that  $\alpha$ -gliadins are not necessary for the baking quality of wheat.

### **Inhibition of $\alpha$ -Gliadins in Hexaploid Bread Wheat**

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Wheat storage proteins are the base for the rheological properties of wheat flour and its superior quality in breadmaking. In susceptible patients some of these proteins, most likely gliadins, are causing coeliac disease, an inflammatory condition of the gastrointestinal tract. The gliadins belong to the major seed storage proteins of wheat and determine the viscosity of the dough from wheat flour. The gliadins are made up of different subtypes ( $\alpha$ ,  $\gamma$ ,  $\omega$ ) each encoded by multiple genes. In this project, we try to knock down the  $\alpha$ -gliadin expression in wheat. A 313 bp target sequence which was highly homologous in known DNA sequences of  $\alpha$ -gliadins, was selected, isolated from wheat cv. Florida and cloned into three different RNAi transformation vectors in sense and antisense orientation. The vectors were transformed into the scutellar tissue of immature cv. Florida embryos using the biolistic transformation method. More than 180 independent transgenic lines were selected on kanamycin containing media and after kanamycin spraying. The transgenic lines were further characterized by Southern analysis for the integration of RNAi constructs and by RT-PCR for the expression. Their phenotype and fertility were the same as those of the wildtype. Kernels of numerous transgenic lines were analyzed for their  $\alpha$ -gliadin content by a combined extraction/HPLC procedure. The results demonstrated that wheat lines were created with strongly reduced or completely silenced  $\alpha$ -gliadins.

### **Status of Global Wheat Quality Test Methods**

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The Grain Inspection, Packers and Stockyards Administration (GIPSA), a congressionally mandated U.S. agency, sets and upholds grain standards and establishes methods to facilitate marketing of U.S. grain, including wheat. The U.S. wheat classes and grades are intended to provide common terms for the fair marketing of wheat and to generally reflect the physical and end-use qualities. Industry and wheat quality laboratories use several methods to assess the mixing properties, water absorption, dough physical properties and wet gluten content of single wheat cultivars and blends of wheats. These tests include, but are not limited to, the Farinograph, Glutomatic, Alveograph, Extensograph, Mixograph, and Mixolab methods. Even though these wheat quality testing methods have been standardized and harmonized in the past by collaborative studies through professional organizations such as the AACC International and ICC, potential testing method variations have been identified by GIPSA as a major concern. A recent GIPSA examination of wheat intrinsic quality test methods brings out three important test method needs; 1) increased standardization of popular methods, 2) identification of the most relevant test methods for accessing wheat quality, and 3) the development of new rapid test methods. As a first step in meeting these market needs, GIPSA intends to raise market awareness of the issue of test method variation and to engage AACC International and ICC for increased global

standardization of important wheat quality test methods. With large diverse growing regions, many popular wheat cultivars, and matured blending techniques, the U.S. wheat industry is poised to provide consistent and high quality wheat based on current, well-founded, and standardized functional test methods. The goal of the GIPSA wheat functional quality assessment efforts is to help make U.S. wheat the global choice for consistent high value product.

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**Gluten, GMP, Glutenin Particles, Models and Practical Reality  
“Connecting Science and Practical Reality”**

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Since the discovery of gluten it is clear that this fraction holds the secrets to understanding wheat end-use quality. From the 1950s till the end of the 20<sup>th</sup> century, efforts to unravel the structure-function relationships were based on studies of a key fraction: glutenin. Insolubility in even the most denaturing solvent was the recurring theme, it led to fractions as: GMP, UPP and AUC. The harsh conditions make these fractions perhaps questionable as a good start-point for studying ‘native structure – function relations’ of these proteins. This is still a question of debate. However, practical useful correlations with quality have been revealed. A direct cause and effect relationship or a mechanistic explanation cannot be derived from these correlations. Recent advances in glutenin particles and glutenin rheology have been of some help, but the models explaining the functionality of glutenin still are models. We should find a balance between philosophising on correct procedures and models, and the practical reality of the industrial world. The gaps between these two realities will be addressed in the paper and examples from both the scientific realm and industrial practice will be used.

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**Polymorphism of High-Molecular-Weight Glutenin Subunit in  
Tibetan Wheat (*Triticum aestivum* ssp. *tibetanum* Shao)**

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A collection of accessions of Tibetan wheat (*Triticum aestivum* ssp. *tibetanum* Shao) was analysed by SDS-PAGE for the variants of high molecular weight glutenin subunits encoded by the Glu-1 loci. Forty-three out of 50 accessions were homogeneous for subunit composition while seven were heterogeneous with two different alleles being present at either one or two of the Glu-1 loci. Ten Glu-1 alleles were detected, two at Glu-A1, four at Glu-B1 and four at Glu-D1. The 1A null, 1Bx7+ 1By8 and 1Dx2+ 1Dy12 alleles were the most common alleles for each of the Glu-1 loci with frequencies of 96%, 80.4% and 94.9%, respectively. Furthermore, two new subunits were identified at the Glu-B1 locus, which are provisionally designated as 1By8\*\* and 1Bx7\*\*, forming two novel allelic combinations (1Bx7+1By8\*\* and 1Bx7\*\*+1By8). All together, seven combinations of HMW subunit alleles were found among these accessions with 1A null, 1Bx7+ 1By8, 1Dx2+ 1Dy12 being the most common with a frequency of 68.4%. The collection analyzed showed a high degree of variation in HMW subunit alleles and could provide a useful source of variation for wheat improvement.

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**Chromosome Location of Genes Controlling High Molecular Weight (HMW) Glutenin Locus  
in Species Related to Wheat and their Effect on Bread Making Quality**

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TACBOW (Tottori Alien Chromosome Bank of Wheat, Japan) was established in Tottori University to intensively accumulate alien chromosome preservation accessions, examine the chromosome and provide researchers with appropriate materials. From these accessions, alien addition lines were studied for High Molecular Weight (HMW) glutenin subunit profile, to determine the presence/location of HMW glutenin locus, on individual chromosome of wild species. HMW glutenin locus in wheat and wheat related species was found to be present at chromosome, similar to homeologous group 1 of *Triticum aestivum*. In lines whose

chromosomes were not yet identified, HMW glutenin profile helped in identifying homeologous group 1 chromosome addition line. Some of these lines with HMW glutenin locus from wild species were studied for sodium dodecyl sedimentation value, to determine their effect on quality. A few of them showed increased gluten content. More lines will be screened this season. While studying HMW glutenin profile of addition lines, several discrepancies from the normal pattern were observed, ranging from appearance of new bands, disappearance of regular bands or change in migration rate of regular bands. Fluorescent genomic in-situ hybridization (FGISH) was performed to find out the reason behind the discrepancies. Some lines had lost additional chromosomes; some had lost 1D chromosome, including other chromosome modifications. Involvement of more than one recipient parent in the generation of some of these addition lines was responsible for change in migration rate. But in some lines FGISH was not able to explain the unexpected banding pattern. More attempts are being made to understand behavior of HMW glutenin subunits in addition lines, as addition lines are unstable, and tend to lose alien chromosomes. But continuous selection for presence of alien chromosomes, leads to changes in wheat chromosomes, which is needed to be understood clearly, before experimenting with such useful materials.

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### **Physico-Chemical and Molecular Analysis of Bread Making Quality Traits in Indian Hexaploid Wheat**

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The current trend of wheat research in the country has been to improve the quality of wheat for the end use product. A mapping population of 105 recombinant inbred lines (RILs) was generated from a cross between HI977 (good BMQ) and HD2329 (poor BMQ). It was grown at three different agroclimatic regions (Pune- central peninsular zone; Karnal & Kota- North plain zone) for two consecutive years. Data were collected for loaf volume and of 4 mixogram parameters namely, midline peak value (MPV), midline peak time (MPT), midline peak integral (MPI) and midline curve tail value (MCTV). All the four mixogram parameters showed positive correlation with loaf volume, which is the main BMQ parameter. Correlations among mixogram parameters revealed positive correlation of MPT with MPI, MCTV and negative correlation with MPV. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) analysis on selected RILs comprising two groups of good and poor loaf volume revealed the genotypic and the environmental effect on differential accumulation of the gliadin proteins. High accumulation of omega, alpha and beta gliadin, with low level of gamma gliadin was observed for Kota compared to Karnal and Pune locations. For parental survey, 814 SSR and 100 ISSR primers were used, from which 205 SSR and 4 ISSR were employed on the whole population. Single marker analysis, followed by interval mapping with QGENE, identified 10 QTLs for MPT, one for MPV, five for MPI and two for MCTV at Pune location. Data are being generated for the remaining treatments. These studies will help in identifying QTLs influencing BMQ, their interaction with other traits, as well as different environments.

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### **Measuring the Rheology of Grain Hardness**

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It is well-known that grain hardness is an important predictive phenotype of various cereal grain properties. For wheat, it is a useful predictor for dough rheology behaviour and end-product quality. However, current measures such as particle size index (PSI) and SKCS hardness index (HI), on which such predictions are based, only see the cumulative effect of the strengths of the various botanical layers in cereal kernels. In order to improve the predictability of grain hardness, there is a need to directly measure and assess the rheological response of the various botanical layers in cereal kernels. In this respect, recent research has shown, for wheat, that the individual crush response profiles (iCRPs), which record the response of individual wheat kernels to their crushing on a Perten Instrument's SKCS 4100 device, contain such detailed rheological information when a suitable number is averaged to give an averaged CRP (aCRP). This talk will discuss the measurement of iCRPs and their averaging to generate aCRPs, the rheological interpretation of an aCRP in terms of the strength of the internal botanical layers in wheat kernels and the utilization of this technology in the construction of enhanced, rheological based, phenotype predictors of wheat properties including that for blended wheats.

### **Structure, Evolution, and Expression of the Wheat Prolamine Loci**

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The application of molecular biology and genomics tools to the study of the wheat prolamine loci has provided significant information on the higher order structure of these loci, the details of evolution of these multi-gene families, the identification of the number of transcriptional active genes, and the relative rates of transcription of different members of the same multigene family. The HMW-glutenin locus has been studied in the most depth of any portion of the wheat genome, with complete sequencing of this region from nine genomes to give the most detailed picture yet of any portion of a Triticeae genome. The larger gliadin loci have been studied to a lesser depth, but give information on the structure and evolution of large multigene families, the large number of incomplete gene copies in such families, and suggestions on the modes of gene copy number variation. EST databases can be mined to provide information on the structure of the active members of prolamine multigene families, and the relative levels of transcription for different members of these families within specific germplasm and among different germplasms. Results on all levels indicate these gene families are more complex in structure and functionality than previously presumed, and show features thus far unique to the Triticeae compared to other well-studied plants.

### **Contribution of Glutenin Alleles to Dough Rheological Parameters**

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Glutenin, the main protein component responsible for the genotypic variation associated with end-use quality among different wheat varieties, comprises high-molecular-weight glutenin subunits (HMW-GS) and low-molecular weight glutenin subunits (LMW-GS) which are encoded at the Glu-1 and Glu-3 loci of group 1 chromosomes, respectively. Extensive allelic variation exists at each of the Glu-1 and Glu-3 loci resulting in numerous possible combinations of HMW-GS and LMW-GS being present in bread wheat. To study the effects and interactions of different HMW- and LMW-GS on dough rheological parameters, a set of near isogenic substitution lines of the Australian cultivar, Aroona have been produced. The isolines consist of single, double and triple allelic substitutions at the Glu-1 and Glu-3 loci providing numerous HMW-GS and LMW-GS combinations in a common background for evaluation. Field trials, subsequent dough rheology tests and subsequent statistical analysis using the REML algorithm, largely confirmed what had already been established for HMW-GS but this work also serves to increase our understanding of glutenin alleles by determining the effects of various LMW-GS. The results presented here describe the relationship between glutenin alleles and some of the functional properties of wheat flour dough and show that alleles which exert positive additive effects can be accumulated to improve wheat quality as it relates to bread making potential.

### **On the Mechanism of Gluten Network Development in Flour-Water Batter Doughs**

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It is now well documented, by laser confocal microscopy analyses, that during the bread dough mixing, uneven protein lumps evolve into a continuous network of protein strands. But mechanism of gluten network development is still unclear and difficult to study as, in the range of breadmaking hydration, gluten network development is brief. In order to better characterize this dynamic event, the dough development rate was delayed by increasing the water content. Mixing was performed on a planetary mixer coupled with a Brabender lab-station, which allows continuous torque recording. The mixer was calibrated in order to express torque value in terms of apparent viscosity (in Pa.s). Effects of mixing speed and hydration level on the development of flour-water batter doughs were studied. At high water content, mixing curves show a delayed torque rise, with the occurrence of a lag phase. Lag phase duration increases as dough viscosity drops in relation with water dilution. For a given water content, time required to reach the maximal torque is related to the square of the mixing speed, and not to the specific mechanical energy nor to the number of blade revolutions. The evolution of the protein phase distribution during the mixing was assessed by adding a protein staining agent directly into the dough. After a few minutes of mixing, gluten is shown to exist mainly as floc structures. Floc structures exist during all the lag phase and the evolution of flocs into protein strands is related to the torque

rise. The present study suggests that, in the case of slack doughs, collisional energy might be an important factor in the development of a percolating gluten network.

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**Transgenic Wheats with Elevated Levels of Dx5 and/or Dy10 Glutenin Subunits:  
Agronomic, Biochemical and End-use Quality Properties**

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In order to study the effects of independently increasing the levels of high-molecular-weight glutenin subunits Dx5 and Dy10, we added copies of their genes to wheat by genetic transformation. Among 30 lines produced, six exhibited transgene-mediated co-suppression and eight showed the presence of extra bands of unpredicted sizes in SDS-PAGE. Several of the extra bands were isolated from gels and characterized by mass spectrometry. All but one were variants of glutenin subunit Dx5, evidently altered in size during the transformation process by an unknown mechanism. Thirteen lines with increases in Dx5 and/or Dy10 were further characterized. Increases in subunits Dx5 or Dy10 ranged from 2.3 to 3.5-fold or 2.8 to 5.4-fold, respectively, their levels in the non-transformed parent. The amount of polymeric protein increased more with increases in Dx5 than with increases in Dy10. In the 2-gram mixograph, doughs from the transgenic lines had longer mix times and improved tolerance compared to those from the non-transformed parent. However, doughs with more than 2.6-times the parental Dx5 levels could not be mixed in this instrument, while doughs with 5.4 the parental Dy10 levels were mixable if sufficient time was allowed. Agronomic characteristics of these lines were assessed in two years of field trials. Most were indistinguishable from their non-transformed parent, but two exhibited reductions in yield and height. These experiments show that mixing properties can be changed by adding genes to increase the levels of Dx5 and/or Dy10 and that such changes can be made without altering yield. Dx5 and Dy10 contributed to functionality in qualitatively and quantitatively different ways and their effects on mixing were at least partially additive. Some transformation events showed unintended properties including low yield, reductions in synthesis of native HMW-GS, and altered transgene-encoded protein products. The latter results underscore the importance of extensive characterization of transgenic wheat lines.

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**Interaction of Thioredoxin h with Gluten Proteins**

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In cereals type-h thioredoxins (Trx h) are abundant both in developing and germinating grains. In vivo interactions of Trx h and storage molecules have been described especially after imbibition. Little is known about interactions during seed developing phase and to what extent the nature and concentration of these small cysteine-rich proteins affect gluten properties. In order to advance in this field, in vitro interaction between Trx h and recombinant high molecular weight glutenin subunit, as well as glutenin subunits extracted from wheat seed, were studied. Three Trx h isoforms found in wheat seed (*Triticum aestivum* cv. Soissons) and stated Trxh1, Trxh2 and Trxh3, were used in these experiments. Trxh1 and Trxh3 deduced proteins show a high identity among them and with other Trx h previously described from wheat, and contain exclusively the two cysteine residues forming part of the active site. In contrast, Trxh2 shows a lower level of identity and contains an additional cysteine residue. Electrophoretic and chromatographic analysis show that these Trx h, either in their native forms or mutated ones at the active site, do not exhibit the same kind of interaction with the targets proteins. The different characteristics of the three Trxh suggested that each of these thioredoxins, or at least Trxh2, might have a different function since they are differentially expressed in wheat seeds. Furthermore, care must be taken with the reaction conditions, since these proteins may undergo substantial unfolding that leads to exposure of SH and S-S groups. A model of interaction of Trx h with high molecular weight glutenin subunit is discussed.

**Relationships of Glutenin Macropolymer Quantity and Properties to Strength and Composition of Gluten Proteins for Diverse Durum Wheat Genotypes**

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Eighteen durum wheat genotypes of various origins (Argentina, Australia, Canada, France, Germany, Italy, Spain and USA) were grown in two locations in western Canada in 2001. Three genotypes contained the weak gluten marker  $\gamma$ -gliadin 42, associated with low-molecular weight (LMW) glutenin subunit (GS) pattern LMW-1, and the remainder contained the strong gluten marker  $\gamma$ -gliadin 45, associated with GS pattern LMW-2. Among the LMW-2 genotypes, six high-molecular weight (HMW) GS patterns (6+8, 7+8, 7+16, 14+15, 20 and 2\*, 20) were identified. All LMW-1 genotypes exhibited weak dough properties as expected, whereas LMW-2 genotypes ranged from slightly stronger to very strong, with HMW 20 genotypes being consistently weaker than other HMW groupings. The amount of glutenin macropolymer (GMP) and its stiffness ( $G'$  plateau value) were strongly positively correlated ( $P < 0.001$ ) to unextractable polymeric protein (UPP) content, and to gluten strength and physical dough properties as measured by gluten index, alveograph and mixograph. The ratio of HMW GS to LMW GS in semolina was negatively correlated ( $P < 0.001$ ) to GMP content, UPP content and strength, indicating that as the relative proportion of LMW-GS increased dough strength increased, in contrast to common wheat where increased proportion of HMW-GS is associated with strength. A more detailed analysis of factors influencing the amount of UPP or GMP formed revealed strong effects of glutenin subunit composition. The LMW-1 genotypes were not able to form substantial quantities of this highly aggregated fraction. The same holds for those lines that contained HMW GS 20 only. GMP plateau  $G'$  values of the strongest durum wheat genotypes were less than 30 Pa, considerably less than values previously reported for common wheat. These results confirm the relationship between durum wheat gluten strength and the content and properties of GMP, while demonstrating the different basis for the association between gluten protein composition and gluten strength between durum wheat and common wheat.

**Differential Processing of Low Molecular Weight Glutenin Subunits  
Met- and Ser types at Their N-Terminal End**

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The gluten polymeric fraction is composed by high and low molecular weight glutenin subunits (HMW-GS and LMW-GS, respectively). Whereas functional and structural properties of HMW-GS have been extensively investigated, LMW-GS are much less characterized due to their greater number and heterogeneity. On the basis of amino acid sequences, LMW-GS have been classified into two main groups, namely LMW-s (or LMW-Ser) and LMW-m (or LMW-Met) types. LMW-s are the most common and their amino acid sequences start with SHIPGL-, conversely LMW-m show more various sequences represented by METSHIPGL-, METSRIPGL-, METSCIPGL-. It has been hypothesized that the substitution of threonine at position 23 of the immature polypeptide by an asparagine residue could determine a differential processing at the N-terminal end of LMW-s type sequences that might generate the cleavage of the peptide MEN by an asparaginyl endoprotease. In order to investigate the correctness of this hypothesis, we have expressed two LMW-GS in *Nicotiana benthamiana* by using an episomal vector based on PVX (Potato Virus X). The two LMW-GS expressed are represented by wild types LMW-m and LMW-s, along with the mutated forms at position 23. In particular, in the LMW-m type, the threonine at position 23 was substituted by an asparagine and in the LMW-s type the asparagine was conversely replaced by a threonine. Preliminary results give indication that the N-terminal amino acid sequence of the mutated LMW-m type (T23N) might be SCISGLERWQ- whereas the sequence of the wild type LMW-m is METSCISGLE-, thus supporting the hypothesis that the presence of an asparagine in position 23 causes a differential processing at the N-terminal end of the mature polypeptide. This work is still in progress and we are currently purifying the wild type and mutated LMW-s type in order to determine their N-terminal amino acid sequence.

### **In Durum Wheat In Comparison to Bread Wheat Doughs**

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The effects of incorporation of an i-type low molecular weight glutenin subunit (LMW-i) and of a modified  $\gamma$ -gliadin showing an additional cysteine residue, on 2 gr Mixograph parameters of durum (biotypes 42 and 45 of the Italian cv. Lira) and bread wheat (cv. Kukri) doughs were studied. The incorporation of the modified  $\gamma$ -gliadin in bread wheat flour resulted in a significant decrease in dough strength, represented by the decreases in Mixing Time (MT) and Peak Resistance (PR), but at the same time it produced an increase in dough stability, as evidenced by the decreased resistance to break down (RBD). The incorporation of the LMW-i type in bread wheat flour produced slight or null effects on dough mixing requirements. The weakening effect exerted by the modified  $\gamma$ -gliadin is probably related to the presence of the extra cysteine located at the beginning of the repetitive domain that makes the  $\gamma$ -gliadin behave as a chain terminator of the glutenin polymers. The LMW-i behaves probably as a chain extender of the glutenin polymers but its incorporation into bread doughs do not produce a strengthening effect probably because of the very strong nature of the flour used. The incorporation of both LMW-i type and of the modified  $\gamma$ -gliadin in durum wheat semolina produced a significant decrease in the overall dough strength, especially in Lira biotype 45 doughs. RP-HPLC, SE-HPLC and 2D gels analyses performed on gliadins and glutenins extracted from control and reconstituted doughs, showed that the two polypeptides were in the polymeric fraction. The data obtained in durum wheat are controversial and suggest that other factors need to be taken into consideration in order to explain the differences observed between durum and bread wheat. Some remarkable differences between durum wheat semolina and bread wheat flour, consisting of a different polymer organization, a different degree of starch damage and particle size, could be responsible for the dissimilar effects exerted by the two polypeptides in bread and durum wheat doughs.

### **Wheat Gluten-based Biomaterials: Composites and Nanocomposites**

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Wheat gluten is an attractive agropolymer to be used in forming bioplastics because of its high availability and its good material-forming properties. Wheat gluten based materials are biodegradable and exhibit interesting barrier properties in high relative humidity conditions. The main drawbacks are their high water sensitivity and low mechanical properties. Improvement of materials water resistance, mechanical and transport properties through formulation of blends or composites (gluten/other biopolymers such as PLA as well as gluten/paper or gluten/fibers) is presented and discussed according to microstructure evolution. Wheat gluten-based nanocomposites (gluten/montmorillonite) have been produced either by both wet process (casting) and dry process (thermomolding). The degree of exfoliation of nanoclays in the wheat gluten matrix was controlled using wide angles X-ray scattering and transmission electron microscopy. Contact angles analysis, water uptake experiments and water vapour sorption measurements have shown that the presence of montmorillonite led to a significant reduction in the water sensitivity of gluten-based materials. This effect was attributed to a different structuring of wheat gluten protein network in the presence of layered silicates. Significant changes in permeability towards water vapour and aroma compounds were observed for filler contents higher than 5 wt%. No effect on O<sub>2</sub> and CO<sub>2</sub> permeability was observed. Finally, a slight improvement in tensile properties was obtained, for filler contents as low as 2 wt%. Functional properties (especially optical, barrier and mechanical) of these composites materials are often specific and unique, and some potential application for agricultural, pharmaceutical and medical industry are discussed.

47 & 48 – Withdrawn from conference.

### **Study on the Quality Change of Wheat Flour During Storage in Controlled Condition**

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Fourteen wheat flour samples with different gluten strength were used to study the quality changes during storage in a controlled environment where the temperature was 38C and the relative humidity was 70%. It showed that after 2 weeks the activity of  $\alpha$ -amylase decreased sharply and could hardly be tested by falling number method. Meanwhile, dough rheology properties tested by Farinograph, Alveograph and RVA methods also changed during storage. Water absorption of gluten decreased while the Farinograph water absorption increased. Chopin Alveograph values changed obviously, the maximum overpressure (P) rose up and the average abscissa at rupture L lowed down so that the curve configuration ratio (P/L) increased. The peak and final viscosity, setback and hold ability obtained from the RVA test rose up, and the steamed bread quality deteriorated with storage. The quality change caused by storage was connected with gluten strength. The stronger the gluten was, the more the change happened. Further research will be done to detect the relationship between the gluten strength and the quality deterioration.

### **Allelic Variation of Low-Molecular Weight Glutenin Subunits and Its Functional Importance**

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Allelic variation of low-molecular-weight glutenin subunits (LMW-GSs) encoded by Glu-3 loci was studied by two-dimensional gel electrophoresis and DNA sequence analyses. Glu-A3d encoded two major subunits with two minor subunits encoded by at least two genes, instead of one subunit encoded by one gene in other Glu-A3 alleles except Glu-A3e null allele. Since we found that Glu-A3d allele exerted a positive effect on gluten strength, more LMW-GSs might be related gluten properties. On the other hand, Glu-B3b (commonly found in Australian wheats) and Glu-B3g (involved in Canadian extra-strong hard wheats) that increased gluten strength encoded one major and two minor subunits as Glu-B3h (commonly found in Canadian wheats). The structural differences among these subunits should be involved in their effect on the gluten property. We will discuss the relationship between the allelic variation of LMW-GSs and its functional importance.

### **Overexpression of a Defence Gene Effective in Limiting Fungal Infection Does Not Alter the Expression of Gluten Components**

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A number of approaches have been used to produce wheat lines with increased resistance to plant pathogens. The strategy we have used relies on the ability to inhibit the pathogen's capabilities to degrade the plant cell wall. Polygalacturonase-inhibiting proteins (PGIPs) are plant defence glycoproteins associated with the cell wall of both monocot and dicot species. They interact with fungal endopolygalacturonases (PGs) and modulate their activity, favouring the accumulation of oligogalacturonides active as elicitors of plant defence responses. To assess the effectiveness of these proteins in protecting wheat from those fungal pathogens producing PGs during pathogenesis, we have produced a number of transgenic wheat lines constitutively expressing a bean PGIP (PvPGIP2) under control of the maize Ubiquitin1 promoter. Three transgenic lines over-expressing PvPGIP2 showed a significantly reduced symptom progression through leaves and spikes following infection with *Bipolaris sorokiniana*. Because of the importance of gluten components for the technological properties of wheat flour, we analyzed the effect of the transgene on the expression of gluten proteins. We used two-dimensional electrophoretic techniques to perform a proteomic comparison between the wild type cultivar, the line expressing the transgenic protein, and the corresponding 'null' genotype that had lost the transgene by segregation. Results show that the lines expressing the transgene do not exhibit any significant alteration of the gluten components. These results will be compared with those obtained in transgenic lines overexpressing other transgenes.

**Quality and Protein Characterization of Triticale Lines  
with the pair of subunits 5+10 and 2+12**

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Genetic lines that differ at one or more loci coding for proteins can be successfully used to elucidate the relationships between protein composition and functionality relationships. A set of fifteen near-isogenic triticale lines has been characterized by protein composition (SDS-PAGE, SE-HPLC) and quality measurements (Mixograph, Baking tests). This allowed the effects on quality of different loci coding for gluten proteins to be evaluated using a common genetic background. These new triticale lines were developed using the triticale Rhino possessing 1RL.1DL or 1AL.1DL translocations produced by A. Lukaszewsky and contain genes corresponding to either the pair 5+10 or 2+12. The triticale lines, Rigel, Trim and GDS7 were used in the translocation. The main variables that were found to affect quality were the number and type of HMW-GS, the relative amounts of glutenins and gliadins and the presence of chain terminator proteins. The mixograph studies support the SE-HPLC data in which, higher UPP correlates with higher peak development time.

**Degradation of Celiac Toxic Peptides by Cereal Proteases**

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Celiac disease (CD) is an inflammatory disease of the upper small intestine and caused by glutamine and proline rich peptides derived from digested gluten. Such peptides are insufficiently degraded by gastrointestinal enzymes and activate the intestinal immune system of CD patients. Cereal proteases are known to degrade storage proteins during the germination of the seed. Therefore, they were tested for their capability to detoxify gluten peptides by extensive fragmentation. Kernels of wheat, rye and barley were germinated up to seven days, freeze-dried and milled into flour and bran. Generally, the proteolytic activity was significantly higher in the bran compared with flour; wheat and rye brans were more active than barley bran. A highly active protease fraction was extracted from rye bran and incubated with CD toxic peptides; their degradation was followed by RP-HPLC and mass spectrometry. The results demonstrated that the peptides were cleaved into non-toxic small peptides and amino acids very quickly. The protease fraction consisted of endo- and exopeptidases; they were active within a pH-range from 3.0 to 9.0 with optima at 4.0 and 6.5, and at temperatures up to 60 °C. Proteases can easily be enriched by ammonium sulfate precipitation. In comparison with bacterial and fungal proteases described in literature, proteases of germinated wheat and rye appear to be more effective and much cheaper.

**Functionality of Glutenin Subunits Produced by Transgenic Yeast**

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The aim of our part of the German research project "Development of wheat, rye and barley proteins without celiac toxicity and their use for the production of food" was to characterize the techno-functional properties of HMW subunits Dx5 and Dy10 as well as of LMW subunit LMWa3 produced by transgenic yeast. Corresponding wheat genes of these subunits were transformed and expressed in the yeast *Saccharomyces cerevisiae*. Glutenin subunits were isolated from propagated yeast by different extraction and precipitation steps. The resulting preparations were characterized by HPLC quantitation, N-terminal sequence analysis and mass spectrometry. The different preparations contained 7.8 to 46.7 % of single subunits or mixtures of them. The functionality of the preparations was studied by extension tests after addition of gliadins and by micro-baking tests after addition to gluten-free flour. Dough stabilization started at a concentration of 1.2 % of subunit Dx5, the addition of 4 % resulted in a very good bread shape and crumb. Subunits Dy10 and LMWa3 generated a more viscous dough resembling dough from rye flour. The results showed that the rheological and baking tests were appropriate for monitoring the techno-functional properties of the recombinant proteins. They confirmed that the glutenin subunits produced by transgenic yeast had functional properties like the native proteins from wheat. The preparations can be used to produce gliadin-free bakery products, with properties similar to those made of native wheat flour.

### **Influence of Sulfur Fertilization on the Technological Properties of Wheat Flour**

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The supply of wheat plants with sulfur (S) during growth is important for the synthesis of gluten proteins and consequently, for the technological properties of flour. The aim of the present study was to investigate the influence of differential S-fertilization on the rheological properties and baking performance of wheat dough. Wheat cv. 'Star' was cultivated in pots and fertilized with six different levels of S (0 - 150 mg per pot). Flours of the six wheat samples were analyzed for the N- and S-content and the quantitative composition of gluten protein types. Dough properties were studied by extension tests, stress rheometry and baking tests on a micro-scale. The S-content of the flours ranged from 0.066 to 0.158 % and the N/S ratio from 32 to 17. S-poor proteins such as  $\omega$ -gliadins increased and S-rich proteins such as  $\gamma$ -gliadins and LMW glutenin subunits were reduced by S-deficiency. Flours with a low S-content showed poor technological properties such as short dough development time, low extension area, high dynamic viscosity and low bread volume. In conclusion, high quality wheat flour should have an S-content of at least 0.150 % and an N/S ratio lower than 17.

### **Influence of the Fatty Acid on the Baking Activity of Phosphatidylcholine**

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Due to common structural elements phospholipids act as emulsifiers and influence the baking performance of wheat doughs. Therefore, those polar lipids, e.g. lecithin, which can be isolated on an industrial scale from plant sources, are used in improvers for breadmaking. The baking activity of whole lecithin is well known, however, very little information is available about the effect of the fatty acid present in phospholipids. Therefore, the aim of this study was to determine the influence of the fatty acid in phosphatidyl choline on the baking performance. A homologous series of phosphatidyl cholines with fatty acid chain lengths ranging from 6:0 to 20:0, 18:1, and 18:2 was synthesized by reacting glycerophosphatidyl choline with the respective fatty acid anhydrides in the molten state. The synthetic phosphatidyl cholines were purified and characterized by chromatography, <sup>13</sup>C and <sup>1</sup>H NMR as well as by mass spectrometry. The techno-functional properties were determined by means of a micro-scale baking test and by micro-extension tests with 10 g of flour. Within the homologous series the baking performance was best for dicaprynylphosphatidyl choline, which caused an increase of the loaf volume by 55 %. Longer C-chains (12:0 to 20:0) and double bonds within the chains (18:1 and 18:2) had a less positive effect on the loaf volume (increase by up to 40 %). For phosphatidyl cholines with short-chain fatty acids an optimal concentration of 0.2 % based on flour weight was found, whereas higher concentrations were required for compounds with longer C-chains. Comparative studies with lysophosphatidyl cholines showed, that longer C-chains were required to get the best baking performance as compared to phosphatidyl choline. Additional rheological tests confirmed the differences between individual compounds and compound classes.

### **Modification of Gluten by Emulsifiers and Effects on Dough Stabilisation**

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Gluten was isolated from doughs containing different types and concentrations of emulsifiers used in bakery. The properties of gluten and dough were monitored by microscopy and by rheological methods, and the protein composition of gluten was determined by an extraction/HPLC method. The chemical composition of gluten was strongly affected by the type and the concentration of the emulsifier. From this, it could be concluded that also the gluten network in dough was changed by the addition of emulsifiers. This was confirmed by microscopy and rheological methods. A strong effect of the emulsifiers on the elastic properties of dough, gluten and bread crumb was found. The results allow facilitating the choice of the appropriate type and concentration of emulsifier for a specific product quality.

### Non-aqueous Fractionation of Wheat Flour – A Method to Prepare Native Gluten Proteins

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Since the functional properties of wheat flour are not maintained during fractionation in aqueous solvents, an approach using non-aqueous solvents was made. The separation of protein and starch was based on differences in their densities. Therefore, ball milled flour was suspended in a mixture of inert solvents (toluene/tetrachloroethylene) with a density of 1.47 g/cm<sup>3</sup> and centrifuged. Due to its higher density the starch fraction was obtained as sediment whereas the protein fraction formed a layer on the surface of the solvent because of its lower density. The protein fraction was purified in a solvent mixture with a density of 1.355 g/cm<sup>3</sup> yielding a middle fraction (sediment) and the purified protein fraction (upper layer), which was then defatted with toluene (0.87 g/cm<sup>3</sup>). The influence of ball milling under air or in the sedimentation solvent on the yield, the purity and the functional properties of the protein fraction was studied. Wet milling was superior to dry milling. The maximum yield was 7.8 % of protein fraction with a protein content of 87.4 %. Protein composition, concentration of thiol groups and gel chromatographic pattern were similar to flour. Hydration, mixing, and baking experiments with the protein fraction using reconstituted flour revealed differences to recombinates containing gluten isolated with water. The properties of the protein fraction became similar to those of gluten only after appropriate mixing. However, the milling conditions also strongly affected the functional properties.

### Studies on the Degradation of Gluten Proteins During Germination of Wheat

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Although the degradation of gluten proteins during germination has repeatedly been described in literature, no systematic studies on the degradation of single protein fractions and types under the same conditions of germination are available. Therefore, the aim of the present study was to investigate the enzymatic degradation of wheat gluten proteins depending on temperature and duration of germination. Wheat kernels were germinated for up to seven days at four different temperatures (15, 20, 25, 30 °C). Samples of different stages of germination were taken and analyzed for their quantitative protein composition using a combined extraction/HPLC method. During germination the amount of protein decreased as a function of time. Glutenins were degraded faster and to a higher extent as compared to gliadins. These differences in the degradation of gliadins and glutenins might be of great interest particularly with regard to using germinated wheat kernels for breadmaking. Within each fraction protein types were affected to a different extent. Differences were also observed in the degree of degradation as a function of temperature. For example, the ω-gliadins were degraded faster at 20 °C and the α-gliadins faster at 30 °C.

### Characterisation of B- and C-type Low Molecular Weight Glutenin Subunits in Durum Wheat

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Glutenin polymers are formed by high (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). The latter group of subunits have been less characterised compared to the former due to their great number and heterogeneity. LMW-GS have been subdivided into B, C and D groups on the basis of their biochemical differences, though only subunits included in the B group are considered typical LMW-GS. In fact, it has been demonstrated that C and D groups correspond to mutated gliadins in which a different organisation of their cysteine residues has enabled them to form intermolecular disulphide bonds. Typical LMW-GS have been classified into three major groups, namely LMW-s, LMW-m and LMW-i types according to their first amino acid residue. The latter group of subunits has been found to have a few peculiarities compared to the first two, such as a different distribution of cysteine residues (which could affect capability of these subunits in glutenin polymer formation and dough quality characteristic) and encoding genes associated only to the chromosome 1A. In order to gain more information on the LMW-GS we have used a durum wheat line carrying a 1BL.1RS translocation, in which the short arm of the chromosome 1B is replaced by the short arm of the chromosome 1R of rye. This line was obtained using the durum wheat cultivar Cando, in which the translocation is present, crossed and back-crossed three times with the Italian durum wheat cultivar Svevo. Comparative electrophoretic, chromatographic and mass spectrometry analyses carried out on LMW-GS prepared from the durum wheat cultivar Svevo and line carrying the 1BL.1RS translocation have provided further information on these complex groups of proteins.

**Characterization of Expressed and Unexpressed Y-Type  
Genes in Diploid and Polyploid Wheat**

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The Ay subunit is always absent in hexaploid (AABBDD) and tetraploid (AABB) cultivated wheats, however, the molecular mechanisms responsible for the lack of expression of this gene vary between cultivars. The Ay gene of the bread wheat cv Chinese Spring is disrupted by an insertion of a Wis retroelement within the repetitive domain, whereas that of the cv Cheyenne contains an in-frame stop codon. Moreover, the analysis of the Ay gene in durum wheat cv Langdon revealed the presence of the Wis retroelement at the corresponding position of cv Chinese Spring. In an attempt to verify whether the Wis elements present in durum and bread wheat originated from the diploid ancestor or arose at polyploid level, we analyzed a number of A genome related diploid wheats (*T. urartu*, *T. monococcum* ssp *monococcum* and *T. monococcum* ssp *boeoticum*) accessions. The analyses confirmed that silencing of the Ay gene occurs also in the diploid progenitors and in all cases the analyzed accessions do not contain the Wis element but in frame stop codons within the repetitive domain of the gene, suggesting that the Wis retroelement has been incorporated into the Ay gene in polyploid wheats during or after their formation. Moreover, the complete sequencing of expressed *T. urartu* and *T. boeoticum* Ay genes have shown a high sequence similarity with the silent Ay genes from cultivated wheats, including the absence of a cysteine residue, near the C-terminal part of the repetitive domain, that is normally present in the orthologous By and Dy subunits. Since this cysteine has been shown to act as a chain brancher, implications for breadmaking properties are discussed.

**Effect of D-Genome Associated Gluten Proteins on Durum Wheat Quality**

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Glutenin and gliadin genes present at the Glu-D1 and Gli-D1/Glu-D3 loci have been transferred to durum wheat through chromosome engineering or using spontaneous translocations existing in bread wheat. In particular, segments containing genes corresponding to the pairs of high-molecular-weight glutenin subunits (HMW-GS) 5+10 or 2+12, normally present at the Glu-D1 locus in bread wheat, have been introgressed into chromosome 1A of different durum wheat cultivars, replacing the null allele present at the Glu-A1 locus. Using the same approach on the bread wheat cultivar Perzivan, carrying a translocation involving the short arm of chromosomes 1A and 1D, the two alleles present at Gli-D1/Glu-D3 loci in most bread wheat cultivars, encoding gliadin and low molecular weight glutenin subunits (LMW-GS), have also been introduced into durum wheat. Effect of the presence of D-genome related proteins in durum wheat on quality characteristics have been assessed and will be described.

**Redox Agents Impact Gliadin-glutenin Cross-linking During Hydrothermal Treatment**

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Heat treatment of wheat gluten proteins and the resulting changes in rheological properties are of considerable importance for the characteristics of baked products. Heat causes gliadin to link to glutenin by disulfide bonding and increases the size of gluten protein aggregates. The aim of this study was to increase the insights in gluten protein polymerization during heating by use of oxidizing (potassium iodate and bromate), reducing (gluthathione, dithiothreitol) and thiol blocking (N-ethylmaleimide) agents. The Rapid Visco Analyser (RVA) was used to apply temperature profiles to gluten-water suspensions and to monitor RVA viscosity changes. Additives were added at different points during hydrothermal treatment. SDS-extractabilities and molecular weight distributions of the proteins were analysed with SE-HPLC. Changes in specific gliadin and glutenin fractions were determined with RP-HPLC. N-ethylmaleimide and oxidizing agents decreased RVA viscosity at 95°C probably because they reduced the level of thiol groups available for polymerization reactions. While potassium iodate and potassium bromate under such conditions primarily increased gliadin extractabilities, N-ethylmaleimide increased both gliadin and glutenin extractabilities. The decreased RVA viscosity upon addition of N-ethylmaleimide or oxidants was largely reversed by addition

of reducing agents. We suggest that redox additives affect the capacity of gluten proteins to associate during heating through sulfhydryl-disulfide exchange reactions by impacting the level of free thiol groups which can initiate the polymerization reactions and by affecting the flexibility of glutenin chains. The relevance of the present findings for breadmaking systems will be discussed.

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**Identifying Transcriptional Networks That Determine Carbon Flux  
Into Starch in Developing Wheat Caryopsis**

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The development of the cereal caryopsis is a complex process orchestrated by changes in the expression of global networks of genes. Carbohydrate metabolism is the major metabolic activity occurring in the developing seed and starch is the major storage compound and has immense importance for food and feed purposes. We used a global approach by exploiting cDNA microarrays to better understand the transcriptional networks determining the flux of carbon into starch in developing wheat caryopses. We focused on 6 time-points, between 3 to 35 days post-anthesis (DPA) of the developing caryopsis, when massive genetic reprogramming reflective of the physiological, biochemical and ultra-structural changes occur. Our goal was to focus on genes involved in the starch biosynthesis pathway and glycolysis and identify genes differentially and coordinately expressed during seed development; to formulate hypotheses for the possible involvement of genes with unknown function which show synergistic relationships with known starch metabolism genes, as well as to look at the expression of regulatory factors and their possible involvement in starch biosynthesis. Biochemical analysis of sugar composition and amylose/amylopectin content, major determinants of carbon partitioning into starch in the seed, and starch granule distribution through the caryopsis development allowed us to correlate biochemical and phenotypic data with gene expression profiles on the array. The simultaneous examination of the transcript levels of a series of starch biosynthesis genes revealed multiple temporal expression patterns, suggesting the relative importance of the different isoforms throughout the caryopsis development. In this report we present an integrated view on the carbon partitioning into starch in developing wheat caryopsis by examining the expression patterns of glycolysis and starch biosynthesis metabolic and regulatory genes, and sugar and amylose content and their synergistic patterns with not-well annotated genes.

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**Transcriptional Profiling of Caryopsis Development by cDNA Microarray Analysis**

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Wheat (*Triticum aestivum* L.) grain, and mainly its endosperm, is of immense nutritional significance for mankind. It stores nitrogen and carbon primarily in the form of storage proteins and starch, respectively. Their unique compositions make wheat endosperm properties indispensable for the production of a wide range of food products. Transcriptional profiling of a large subset of genes provides insights into the global changes in gene networks that occur during, and often underpin, different biological processes in plants. We studied changes in transcript abundance that occurred during wheat caryopsis development using cDNA microarrays. The wheat cDNA array had 10,800 features, representing 7,833 tentatively unique genes, 75% (5,818) of which had been mapped to the wheat genome with aneuploid wheat deletion lines. We focused on six time-points between 3 to 35 days post-anthesis (DPA) when protein and starch content are known to increase dramatically. We used Mixed Model Analysis of Variance (ANOVA) to determine which genes were differentially expressed during the caryopsis development. Our analysis showed that 2,237 unique genes were differentially expressed ( $p \leq 0.01$ ). Most transcriptional changes occurred between 3 DPA to 14 DPA, and more strikingly, between 7 DPA to 14 DPA. Genes involved in the production and accumulation of storage protein and starch were generally coordinately expressed for each of the product pathways. Clustering of the differentially expressed transcripts allowed the identification of genes that were co-expressed during the grain development, as well as helped annotate "transcript expression" for the yet uncharacterized genes.

### **Survey of *Brachypodium distachyon* Species as a Possible Model System for Wheat**

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The plant *Brachypodium distachyon* is a candidate to serve as a model for wheat genetic studies. This plant has a small genome (~360 Mb) in comparison to that of bread wheat (~16,000 Mb), it has a short life cycle (2 months) and a small stature. For this plant to present itself as a model organism, many features need to be developed or investigated; presented are some of the attributes. Methods for this plant to be transformed by microprojectile bombardment or *Agrobacterium tumefaciens* have been reported (Christiansen et al., 2005; Vogel et al., 2006a). A preliminary survey of 20,000 expressed sequence tags (ESTs) derived from five *Brachypodium* cDNA libraries have been produced to initiate an understanding of the genes expressed in this species (Vogel et al., 2006b). In addition, two large-insert genome BAC libraries have been constructed representing over 20X genome coverage (Huo et al., 2006). From the EST sequencing, the presence of many close matches to sequences derived from wheat have been identified. In the sequencing of *Brachypodium* ESTs derived from spike tissue, examples of candidates related to the storage proteins of wheat have been identified. Likewise, protein profiles derived from seed tissue demonstrate some interesting comparisons to those of wheat. Sample sequencing of BAC ends from the large-insert libraries reveals a low percentage of repetitive DNA elements. Many of the above mentioned factors appear to point to a manageable genome toward aiding the exploration of genome structure, organization, and function of it and related species, including that of wheat.

### **A Rapid Spectrophotometric Assay for Measuring Functional Protein in Wheat**

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The development of premium wheat cultivars requires screening of large and diverse sets of early generation wheat breeding material for the desired quality traits. The objective of this study was to develop a rapid and an inexpensive protein quality test. A procedure was developed to extract and measure the concentration of monomeric-rich, glutenin-rich and total soluble protein using a UV-spectrophotometer reading at wavelength 280 nm. Each protein fraction was also quantified by RP-HPLC with a UV detector at 280 nm. A positive high correlation ( $r = 0.94$ ) was obtained between the concentration of glutenin-rich protein fraction measured by RP-HPLC and by UV-spectrophotometer. The concentration of glutenin measured by the UV-spectrophotometer correlated highly ( $r = 0.82-0.91$ ) to farinograph and mixograph dough strength parameters. This simple and rapid spectrophotometric assay was used to screen a large population of early generation (F4) wheat breeding lines. The results of selection were compared to traditional quality tests typically carried out in early generation screening. Wheats with overall weak dough strength were eliminated using an algorithm that took into account the concentration and the proportion of gliadin and glutenin protein in flour or whole meal. The results were comparable to lines eliminated based on dough quality parameters. The UV-spectrophotometric procedure for functional protein determination presented here is easy to perform and inexpensive and has the potential for automation.

### **Effect of Low Molecular Weight Glutenin Subunit Composition of Wheat on Dough Properties**

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To evaluate the influence of the low molecular weight glutenin subunit (LMW-GS) composition on dough properties and the amount of functional glutenin, a set of double haploid wheat lines with all eight possible LMW-GS composition combinations from the cross of semi-dwarf Glenlea by AC Domain was evaluated. Significant increases in the dough strength and baking quality were attributed to the Glu-B3 LMW-GS alleles. A significant increase in the mixograph dough development time, mixograph energy to peak, farinograph dough development time and farinograph stability was observed in lines with the LMW-GS allele 'g' compared to lines with the allele 'h'. Greater bread loaf volume was produced in wheat lines with the allele 'h'. Lines with the LMW-GS allele 'c', coded by chromosome Glu-D3, increased dough strength as determined by the mixograph and farinograph, but decreased bread loaf volume compared to the allele 'a'. Examination of the protein composition of the lines with different LMW-GS composition showed that the effect of different LMW-GS on dough quality was associated with the proportion of the glutenin in flour rather than the total flour protein content.

### **Study of Glutenin Functionality Using a Transgenic Wheat System**

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Wheat dough functionality is predominantly influenced by two storage protein classes: gliadins (single chain polypeptides) and glutenins (multiple chain polypeptides). Glutenins, composed of high molecular weight (HMW-GS) and low molecular weight (LMW-GS) glutenin subunits, strongly influence the strength and elasticity of dough. To investigate the contribution of glutenin to dough functionality, a transgenic hexaploid wheat that was null at all three Glu-1 loci was developed. Glutenin-rich fractions (consisting of a mixture of HMW-GS and LMW-GS and gliadin) from three Canadian wheat cultivars of different classes were incorporated into the transgenic base flour using a reduction/oxidation process. The composition of the glutenin fractions was verified using SDS-PAGE and RP-HPLC methods. The functionality of the glutenin fractions in the transgenic flour was evaluated using the 2-g mixograph and micro-extensograph. The transgenic wheat had very poor dough mixing and extension properties despite relatively high protein content. Incorporation of glutenin-rich fractions in the proportions found in the donor flour, resulted in significant changes in dough properties of the null line that were dependent on the donor glutenin fraction.

### **Problems in Detecting Prolamin Contaminants in Oat-based Foods by Commercial ELISA Kits**

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Clinical trials have shown that coeliac patients can tolerate oats. Oat-based products add variety to the coeliac diet and increase the amount of the important fibres. The problem is that oats can be contaminated with other cereals. This contamination can occur in the field, but it can also happen during transportation, storage, milling or food-processing. Thus a reliable method to verify the purity of oats and oat products is necessary. In this study, immunoblotting and ELISA methods were used to determine the prolamin contents of pure oats and oat samples contaminated with barley. Omega-gliadin antibody, which recognizes the heat-stable fraction of wheat prolamins, and R5-antibody which is able to recognize the five amino acid peptide, QQFPF, were used. Immunoblotting showed that both antibodies cross-reacted with oat prolamins, though the reaction was so weak that they could not be quantified by ELISA methods based on these antibodies. ELISA methods failed to measure contaminating barley prolamins accurately from the oat samples. With R5-antibody the results obtained in this study were too high and with omega-gliadin antibody the results were too low when compared to the calculated barley prolamin content. These results showed that the barley prolamin content remains difficult to quantify by the commercially available ELISA methods. There is a risk of serious over-estimating on the concentrations of contaminating prolamins when analysing gluten-free foods. Further studies are in progress to solve the quantification problem of barley contaminations in oat samples and oat-based foods as well as in other foods intended for the coeliac diet.

### **Puroindoline a Enhancing the Resistance of Leaf Rust Disease in Transgenic Durum Wheat**

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Antimicrobial peptides play an important role in the immune systems of animals and plants by the inhibiting the pathogen infection and growth. The puroindoline a protein expressed by pin a gene located at 5D controlling the grain hardness in *Triticum durum* has in vitro antimicrobial properties in fungi and gram positive and gram negative bacteria. Wheat leaf rust caused by *Puccinia triticina* is considered to be one of the most important fungal diseases for common wheat with the genetic constitutions of AABBDD genomes. Durum wheat, with AABB genomes, does not contain the puroindoline (pina) gene located at D genome and has no disease-resistance obtained from the puroindoline gene products. Transgenic Durum wheat varieties Luna and Venusia, transformed with pina gene driven by the ubiquitin promoter by particle bombardment, expressed pina constitutively. Crude protein extracts, containing PIN A protein from leaf and grain in the transgenic durum, reduced in vitro the growth of *P. triticina* in the potato dextrose agar medium. After the transgenic and control plants were sprayed with the *P. triticina*, the control plants died while the transgenic lived. PIN A protein effectively inhibits in vitro the growth of fungal hyphae and the transgenic durum wheat grows well in the Hubei Province, Central China, where the Durum wheat varieties Luna and Venusia have poor yield due to their disease sensitivity.

### **The Molecular Evolution and Genome Sequence of Grain Hardness Genes in Genera of Triticeae dumort**

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The molecular evolutionary relationship among grain hardness genes of coding regions was studied by using different genera in the Triticeae dumort. The PCR technique was used to amplify genes of puroindoline a (pina), puroindoline b (pinb) and grain softness protein (gsp) of several species of Aegilops L., Triticum L and Avena sativa, and neighbor-joining method was applied to construct the phylogenetic trees of pina/pina-like, pinb/pinb-like and gsp genes downloaded from GenBank. The alternative topologies of tree reconstructions were supported by the maximum parsimony method in MEGA3.1 softness package. The results indicated that the evolution of grain hardness genes of coding regions was very conservative and they began to split off after these species diverged, which were presented in those phylogenetic tree. The evolution relations of three genes among different genera were decided by different genomes instead of by different genera. From these tree reconstructions, it is inferred that the ancestor of A genome from Triticum is the S genome from Aegilops L. However, the evolution relationship of hardness genes in the genera of Hordeum L is closely related and independent from other genera in the Triticeae dumort. The coding sequences of the three hardness genes from different genera were highly conserved. Only one point mutation was present in the tryptophan-rich domains of puroindoline a protein (PINA) or puroindoline b (PINB), which is of vital importance in retaining the function of these proteins. A similar structural domain, tryptophan-rich domain, was found in all grain hardness proteins (GSP) in this study, while the content of tryptophan of GSP is less than that of PINA and PINB in the domain.

### **Analysis of the First Steps of Prolamins Assembly and Polymerization During Wheat Grain Development**

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In the mature wheat endosperm, prolamins are involved in a matrix that surrounds starch granules. This matrix is the origin of the gluten network, in which prolamins are associated together both through non-covalent and covalent bonds (disulfide bonds), and which confers to the dough its viscoelastic properties. Though a lot of studies have been done on gluten network, it is not completely characterized yet, because of the complexity of the structure and because the very large size and insolubility properties of the prolamin polymers limit the possible approaches. It has been previously shown that these polymers are formed progressively during wheat grain maturation, but the studies mainly focused on the desiccation step of the wheat grain (i.e. the latest steps of polymers formation). To have a better understanding of the formation of prolamins assembly in the grain, we focused on the first steps of this process, that are supposed to occur very early after prolamins biosynthesis, i.e. when these proteins are stored in protein bodies. These organelles were studied both in the endosperm cells and in isolated fractions at various development stages. Wheat protein bodies were characterized by Western blot and microscopy techniques (TEM and CLSM), using monoclonal and polyclonal antibodies (specific of different prolamins types). We showed that different prolamins types were colocalized in the same organelles, thus suggesting that some oligomerization had still occurred. Prolamins polymers in these protein bodies were characterized by size exclusion chromatography after sequential extraction. Therefore, wheat protein bodies isolation allows a detailed study of the first steps of prolamins polymerization during grain development.

### **Mapping Approach Relating Quantitative Trait Loci to Dough Rheology in an Australian Doubled Haploid Population**

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Traditionally, the dough rheological traits measured by various methodologies have been used to predict the end product quality of wheat. In this study our aim was to locate molecular markers linked to quantitative trait loci (QTL) for grain composition, dough rheology and end-product traits in a set of 160 doubled haploid lines derived from the cross between Australian cvs.

Kukri x Janz. Protein expression levels, dough mixing and extensional rheological properties of the DH lines were assessed from two different environments, Narrabri and Hillston. Based on a genetic map of 350 loci, novel and significant QTLs relating to grain composition, dough rheology and end-product traits will be discussed.

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#### **A Glutenin Composition Necessary to the Extra-strong Dough**

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Blended flour consisting of middle-soft wheat flour and extra-strong flour is a big hope in Japan where more than 2 million tons of strong flour is used but only 20,000 – 30,000 tons of strong flour is produced. It is known that the compositions of high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight (LMW) GSs are important factors for strong dough property of wheat. We have found that LMW-GSs KS2/KS3a and HMW-GS 5+10 (encoded by Glu-D1d) of a winter wheat line 'KS831957' had interaction effects on extra-strong property. KS2 and KS3a are highly similar molecules to GL1 and GL2 of Canadian western extra-strong wheat cultivar 'Glenlea', respectively. KS2/KS3a and GL1/GL2 are considered to be derived from Glu-B3g allele because of the similarity of the resolution patterns in 2D-PAGE with standard varieties. We developed a DNA marker linked to the Glu-B3g allele and have examined the contribution of the combination of Glu-D1d and Glu-B3g to the extra-strong dough using RILs and DH lines.

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#### **Tyrosine Cross-linking of Wheat Gluten Proteins and Its Functional Importance**

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To establish its significance during commercial breadmaking, dityrosine formation was quantified in flours and doughs of six commercial wheat types at various stages of the Chorleywood Bread Process. Dityrosine was formed mainly during mixing and baking, at the levels of nmol/g dry weight. Good breadmaking flours tended to exhibit higher dityrosine content in the final bread than low quality ones, but no relationship was found for dityrosine content expressed as a proportion of flour protein content. This indicates that the latter was still a dominant factor in determining baking performance. There was no correlation between gluten yield of the six wheat types and their typical dityrosine concentrations, suggesting that dityrosine cross-links were not a determinant factor for gluten formation. Ascorbic acid was found to inhibit dityrosine formation during mixing and proving, and to have no significant effect on dityrosine level in the final bread. Hydrogen peroxide promoted dityrosine formation, which suggests that a radical mechanism involving endogenous peroxidases might be responsible for dityrosine formation during breadmaking. The existence of other types of covalent interactions involving tyrosine residues in wheat gluten will be discussed.

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#### **Elastic Recovery and Plastic Flow in "5+10" Glutens**

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It is often said that what makes wheat unique is the viscoelasticity of the hydrated gluten proteins. But, words like "viscous", "elastic" and "plastic" apply to many types of natural and synthetic materials that have very different chemistry. So, what exactly makes gluten unique? In the 1930's it was becoming apparent that certain polymeric materials had an unusual capacity to recover their shape after large extensions. This material property came to be called "high elasticity" and non-rubber materials that showed high elasticity were called "rubber-like". It is this power to recover from large extension that separates high elastic materials from other extensible soft plastic solids. The retractive force that develops in gluten during large extension occurs simultaneously with plastic flow around room temperature and normal (~ 65-70%) water content of gluten. We have observed that the elastic and plastic properties of glutens with similar "good" HMW-GS vary, even though they have similar values for

wet gluten content and gluten index. Although not a completely new idea, we feel that development of a rapid, objective test method, which can separate elastic recovery and plastic deformation of gluten in a logical way, is a useful strategy to characterize the unique viscoelastic properties of gluten. Such a test would complement and extend current methods used to determine wet gluten content and could provide for more objective comparison of the rheological “quality” of glutes. Development and assessment of the usefulness of such a rheological test will be based on glutes obtained from well-characterized wheat cultivars. The resulting combined rheological and chemical property database could also provide new insights into the types of structures that exist in whole gluten.

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**Comparison of Analytical Methods for Breadmaking Quality Prediction in a Genotype by Environment Study: SE-HPLC versus Spectrophotometric Measurement of HMW Glutenin**

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Two analytical methods were compared for determination of wheat protein quality for breadmaking: 1) SE-HPLC of protein extracted in SDS-phosphate buffer with sonication, and 2) UV spectrophotometry of extracts of insoluble (HMW) glutenin. Material for study comprised six hard spring wheat genotypes grown in replicated field plots at seven site years in western Canada. Flour protein was extracted with SDS-phosphate buffer, pH 6.9, with sonication to determine total and unextractable polymeric protein (TPP and UPP, respectively) using SE-HPLC. In the second method, protein was sequentially extracted using 50% 1-propanol without and with 0.1% DTT to ultimately extract HMW glutenin which was quantified by UV absorbance. Standard methods were used to characterize the technological quality of the samples including farinograph, mixograph, extensigraph, and breadmaking. HMW glutenin determined by spectrophotometry was highly correlated ( $r = 0.95$ ) to both TPP and UPP. Consequently, both small-scale methods generated similarly strong relationships for quality prediction: mixograph band width ( $r=0.87$ ); extensigraph  $R_{max}$  ( $r=0.76$ ); farinograph dough development time ( $r=0.84$ ); bread loaf volume ( $r=0.83$ ). Accordingly, predicting breadmaking quality by the spectrophotometric method was comparable to that of the sonication/SE-HPLC procedure. The advantages of spectrophotometric determination of HMW glutenin include its simplicity, substantially lower cost, higher sample throughput, and lower solvent waste.

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**Ultra-Fast Separation of Wheat Glutenin Subunits by Reversed-Phase HPLC using a Superficially Porous Silica Support Column**

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A relatively new silica support for RP-HPLC was evaluated for the separation of wheat gluten proteins. The product named “Poroshell” by the manufacturer consists of a solid core and a porous coat instead of solid silica spheres used in conventional RP-HPLC column packings. This architecture favours rapid mass transfer thus facilitating faster separation of biomolecules. The main objective of this study was to evaluate the quality of separations of glutenin subunits (GS), as well as to optimize conditions to produce the fastest possible run times without sacrificing resolution using a Poroshell 300SB-C8 12.5 x 2.1 mm column. Two different bread wheat genotypes were used for optimisation of separation conditions and six more genotypes possessing different subunit combinations were used for further evaluation. Glutenins were extracted with 0.08M Tris-HCl buffer (pH 7.5) containing 50% 1-propanol under reducing conditions after pre-extraction of soluble proteins with 50% 1-propanol. Different flow rates, acetonitrile (ACN) gradients, and column temperatures were tested. The best resolution was obtained in ~13 min using a 23-44% ACN gradient and a flow rate of 0.7 mL/min at 65° C. Quantitative results were highly repeatable even after several hundred injections. Highly satisfactory separation of HMW-GS and quantification of ratios of HMW- to LMW-GS was achieved in less than 4.5 min per sample. Results indicated the usefulness of the ultra-fast technique for wheat cultivar development activities and for rapid prediction of dough strength and baking quality.

**Determination of Wheat and Breadmaking Quality With  
Small-Scale Methods – An Overall Comparison Study**

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Flour quality assessments are based on different methods and partly on different flour or dough properties in various regions of the world. For example mixograph is widely used in the USA and Australia, while the application of Farinograph-type dough mixer is preferred in the European countries. However, the sedimentation value is a widely used parameter for wheat qualification all over the world. On fields where the amount of sample available for testing is very limited, the use of micro-scale methods is desirable, but it means further challenge in the standardization procedure of wheat quality evaluation methods. The micro-mixograph was the first available small-scale instruments on the market. In the last years, a whole micro-scale instrument family was developed at our Department with strong cooperation with Australian (CRC, Newport Scientific, Sydney) and Hungarian (Metefém Ltd, LAB-INTERN Ltd., Budapest) partners. The members of this family are the micro-mill, sieving machine and micro-Z-arm mixer. Recently, this family was extended with a new type of Zeleny-tester. This instrument is an automated version of the traditional equipment, applicable also for macro (standard)- and micro-scale measurements. The development phases and the validation processes of micro-scale methods are summarized in some previous papers.

In this work, all of the available micro-scale methods and rheological properties such as water absorption, mixing parameters obtained both mixograph and Z-arm mixer, extensibility and micro-Zeleny number, were compared and statistically evaluated. The most important finding is that similar relationships can be obtained between different micro-parameters and normal size procedures. This result shows that the down-sizing effects are limited in these measurements. The other finding is, that a relatively simple quality parameters, like sedimentation value gives strong correlations with some classical parameters, like water absorption or peak resistance. Our results confirm the applicability of the micro-tests and appliances also for research work and routine analysis. Additionally, these findings provide a better understanding of relationships in properties measured with different methods and the physical chemistry or molecular background of the parameters. Finally, the established general relationships are applicable for simplifying the qualification procedures.

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**Rheology of Gluten Film Around Gas Cells in Bread Making**

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The primary gluten film (apart from secondary liquid lamellae) around gas cells, determines the extent to which gas cells can expand biaxially during fermentation and baking without undergoing coalescence, thus affecting bread volume. Rheology of the gluten film is important to determine gas cell stability. The gluten film must be sufficiently extensible to respond to gas pressure but also have sufficient strength to resist collapse. Strain hardening has been shown to be a necessary rheological property delaying coalescence of gas cells. This rheological behavior of the gluten film during biaxial expansion depends upon the ratio of polymeric to monomeric proteins and on the molecular weight distribution of the polymeric fraction. The phenomenon of strain hardening appears to depend on the proportion and molecular weight distribution of the polymeric fraction having molecular weight greater than a critical level.

**Comparative Transcriptional and Proteomic Profiling of Bread Wheat cv. Bobwhite and its  
Derived Transgenic Line Over-Expressing a LMW-GS Gene**

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Recent efforts to increase the quantity of specific wheat gluten proteins, directly correlated with the quality of end-use products, have focused on the introduction of additional gene copies by means of genetic engineering. We have thus produced and characterized a transgenic bread wheat line over-expressing a LMW glutenin subunit. In order to define the consequences of transgene(s) insertion/expression and the effects of genetic transformation on the global endosperm gene expression, we have carried out a comparative proteomic and transcriptional profiling between the seeds of the transgenic line with its non-transformed counterpart. Microarray analysis showed that, during the seed development, 542 unigenes were significantly differentially expressed. Those genes, for which a reliable annotation was available, have been classified according to their putative functional category, to provide an overview of the genome responses to genetic transformation and transgene(s) expression. Most of the differentially expressed genes encode various classes of storage proteins, as well as putative transcription/translation-related proteins or proteins involved in plant defence responses. By expression pattern matching, during the process of seed maturation, a number of genes strongly correlated to the expression of the transgenes were identified. Transcript abundances of several seed storage-related genes was also confirmed at the protein level, both in developing and mature seeds, with quantitative proteomic analyses of the corresponding encoded subunits. Further confirmation of microarray data is currently under way, at the protein level, using a combination of N-terminal sequencing and MS analyses.

**Proteins Alteration in Triticum Durum by Eurygaster and Aelia Insects Species**

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The sunn pest has a large distribution in some areas of Europe, Asia y North Africa. Insects belonging to genus Eurygaster and Aelia attack the wheat grains changing protein quality. The effect of insect feeding activity has been studied mainly in bread wheat (*Triticum aestivum*) but not in other *Triticum* species. This study reports about the *Triticum durum* gliadins and glutenins degradation by bug effect (*Eurygaster* or *Aelia* species), in seven different wheat varieties originally from the island of Sardinia (Italy), using a high-performance capillary electrophoresis separation proteins method. For every variety the damaged grains were separated from healthy grains and milled separately to obtain integral flour. Quality gliadins and glutenins of healthy flour, blend healthy (70%) /damaged (30%) flour has been tested at different incubation times (0, 1 and 3 hour) and temperatures (4°C and 45°C). The results obtained showed that sunn pest has a great influence in *Triticum durum* proteins quality lost; in particular, the glutenins are severe and quickly breakdown, irrespectively to temperature incubation (4°C or 45°C). Therefore, degradation of glutenins is due not only to the action of proteases enzymes, but also due to other salivary agent residues, yet to be determined.

**Development of Gluten-Free Bread**

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Requirements for gluten-free cereals to obtain viscoelastic dough properties for non gluten products were examined. A specific combination of ingredients, such as flours, starches, vegetable proteins and water was used. Additives, including vegetable fibre and thickeners, were employed in order to improve the bread-making performance. Selection of gluten-free raw materials (flours and starches) was investigated and compared to wheat flour. Rheological behaviour of flour and starches was studied by analyses of the starch profile (Micro-visco-amilograph, Brabender) and the dough (Mixolab, Chopin). Gluten-free flours and starches showed different viscoelastic properties compared to wheat flour. Even with the use of additives, the final gluten-free formula showed different viscoelastic properties of the dough compared to wheat flour dough. Despite that, gluten-free bread baking tests resulted in a product with a good taste, profile and sensory characteristics similar to wheat bread. Sensorial tests of gluten-free bread were carried out according to the DIN procedures (Deutsche Institute für Normung) by a trained panel. Sensorial characteristics of the product were evaluated with the high quality score. Changes of bread texture during its shelf

life were studied. Analyses of the bread texture profile were examined using TA XT2i (Stable Micro Systems). The softness of the bread crumb showed acceptable characteristics during 4 months of evaluating period.

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### **Functional Studies of Wheat Storage Proteins in Model System**

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The structure and properties of the storage proteins of wheat and rice - the two most important cereals for human consumption - are extremely different. While the rice proteins are mostly globulins, the major seed storage proteins in wheat are prolamins, which are able to form gluten by their hydration, providing unique rheological properties for the wheat based products. Most of the knowledge about the functional properties of gluten proteins is derived from indirect correlative or studies, or from direct reconstitutive experiments. In the first case, populations of samples with different chemical compositions are characterized and then compared using statistical methods. In case of reconstitution studies the chemical composition of a base flour is altered systematically to study the effects on functional properties. One of the limitations of the 'base flour' method is that the supplemented constituents obviously interact with the components of the flour. An ideal solution to avoid this problem would be to use base flour - such as rice flour - not containing any wheat flour components. Supplementing rice flour components with wheat storage proteins - either by in vitro methods or by in vivo transformation - could improve our basic understanding about the possibilities of improving/altering the functional properties of rice/wheat flour. In this study, wheat gluten or its components, such as gliadin and glutenin rich fractions, have been obtained using both in vivo and in vitro studies. Rice plants were transformed with HMW Dx5 -GS by biolistic method as well as different amounts of prolamins were added/incorporated into the rice flour. A prototype micro-farinograph was used to successfully monitor the impact of these alterations in chemical composition on the rheology.

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### **A Simple Integration Pattern Observed by Transformation with 1Dx5 Gene Cassettes in Wheat**

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Wheat is one of the important crops for improvement of agronomic characteristics via genetic manipulation. In the current investigation, particle bombardment was used to transfer linear gene cassettes lacking vector backbone sequences encoding the high molecular weight (HMW) glutenin subunit 1Dx5 gene into immature embryos of two wheat cultivars. Gene cassettes were isolated from the plasmid pHMW1Dx5, corresponding to promoters, open reading frames and terminators. Two other gene cassettes were purified from the plasmid pAHC25, contained the scorable and selective marker genes gus and bar, and were co-transferred along with a 1Dx5 gene cassette. PCR analysis confirmed the presence of gene cassettes in the host genomes of transgenic plants; 6 from common wheat of cv Bobwhite and 9 from durum wheat of cv Luna. Histochemical assays showed that the gus and bar gene cassettes were integrated and segregated in a single locus in the T1 generation. SDS-PAGE analyses indicated that expression of the additional 1Dx5 gene cassette led to increased accumulation of HMW glutenin subunit and the expressions of endogenous HMW subunits have been effected. Transgene cassettes were inherited in the T1 generation as a dominant trait abiding Mendelian segregation ratios and the result of southern blot analysis for the transgene showed transgene integrated with low copy number (1-2 hybridized bands) in a single locus. These data suggested that stable transgenic wheat were recovered with simple integration patterns using 1Dx5 gene cassettes.

**Over-expression of Transgenes *IDx5* and *IAx1* in Elite Chinese Varieties of Wheat (*Triticum aestivum* L)**

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Crosses between transgenic lines of wheat produced in the UK and elite Chinese wheat varieties have been carried out in order to determine the impact of the transgenes on grain quality. The transgenic wheat lines B72-8-11b and B102-1-2 were generated by particle bombardment and express the HMW subunit *IDx5* and *IAx1* transgenes, respectively. The elite Chinese varieties are Chuan 89-107, Emai 12 and Emai 18, which have the HMW subunit constitutions 1Bx7+ 1By8, 1Dx2+ 1Dy12; 1Bx7+ 1By9, 1Dx2+ 1Dy12 and 1Ax1, 1Bx7+1By8, 1Dx2+ 1Dy12, respectively. The parents and the F<sub>1</sub> to F<sub>5</sub> generations of crosses with the transgenic wheat as the male parent were analyzed for HMW subunit composition by SDS-PAGE. This allowed the selection of F<sub>4</sub> and F<sub>5</sub> lines with the *IDx5* and *IAx1* transgenes expressed in the presence of the endogenous HMW subunits: 1Bx7+ 1By8, 1Dx2+ 1Dy12; 1Bx7+ 1By9, 1Dx2+ 1Dy12; 1Ax1, 1Bx7+1By8, 1Dx2+ 1Dy12; 1Bx7+ 1By8; 1Bx7+ 1By9; 1Dx2+ 1Dy12. The potential applications of these lines in wheat improvement are being explored.

**Differentiation of Allelic Variations of the HMW Glutenin Subunits of Wheat Flours by Use of Mixing Parameters and Polymeric Protein Content**

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The mixing parameters and polymeric proteins (PP) of two different wheat cultivars, Centurk and OK102, each with four different lines of differing HMW-GS composition were analyzed. The mixing parameters from a 10-g mixograph were utilized to discriminate among different cultivars as well as different HMW-GS by using multivariate statistical analysis techniques. Stepwise discriminant analysis was employed in order to identify significant mixing parameters at P<0.0001 level. The selected variables, mixing tolerance, peak mixing time, and peak height (torque), were then subjected to Principle Component Analysis (PCA). The score plots of the first two principal components (PC 1 and PC 2) indicated a clustering in samples: Centurk with 7+8 and 7+9 at the Glu-B1 and 5+10 at the Glu-D1 loci; Centurk with 7+8 and 7+9 at the Glu-B1 and 2+12 at the Glu-D1 loci; OK102 with 6+8 and 7+9 at the Glu-B1 and 5+10 at the Glu-D1 loci; OK102 with 6+8 and 7+9 at the Glu-B1 and 3+12 at the Glu-D1 loci. Samples belonging to different cultivars (Centurk and OK102) were successfully grouped using the same score plots. Furthermore, polymeric proteins in samples consistently correlated well with mixing tolerance and peak mixing time. While IPP (insoluble polymeric proteins) presented a positive relationship with mixing tolerance and peak time, SPP (soluble polymeric proteins) showed a negative correlation with the same parameters. Overall, SPP was a better identifier in terms of grouping Glu-D1 subunits and it contributed to higher correlation coefficients than IPP. This method and information could be beneficial in developing analysis tools in early selection of lines for quality traits in wheat breeding programs.

**Puroindoline Synthesis in Developing Seeds of Common Wheat Cultivars with Contrasting Grain Texture Characteristics**

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Gluten viscoelastic properties were found to be affected by puroindolines a (pinA) and b (pinB), two basic isoforms encoded by the Pina-D1 and Pinb-D1 loci controlling grain hardness in common wheat. Puroindoline synthesis in developing seeds was studied in common wheat cultivars with contrasting grain texture characteristics, as determined by the SKCS method. Qualitative and quantitative differences in the transcription rate of puroindoline genes were revealed by RT-PCR and RACE on mRNAs. Moreover, the amounts of pinA and pinB on the surface of starch granules were determined by densitometry scanning of A-PAGE fractionations. Quantitative RT-PCR analyses revealed high amounts of mRNAs transcribed from both puroindoline loci in extra-soft (mean SKCS values = 12±5) and soft (mean SKCS values = 23±1) cultivars as compared with medium-soft (mean SKCS values = 42±1) or hard (mean SKCS values = 74±1) cultivars. Extra-soft and soft cultivars also showed over-

expression of the Gsp-D1 gene, which is tightly linked to Pina-D1 on chromosome 5DS. Sequencing of PCR amplification products revealed wild-type alleles Pina-D1a and Pinb-D1a in extra-soft, soft and medium-soft cultivars, and Pina-D1a plus Pinb-D1b in hard cultivars. The promoter microsatellite of Pina-D1 in extra-soft cv. AR 910 was found to contain supernumerary GA dinucleotides, GAGA elements in promoters playing a regulatory feature in gene expression in animals and higher plants. A-PAGE fractionations revealed continuous accumulation of pinA and pinB on starch granules from 7 to 35 days after anthesis. The amounts of both puroindolines on starch granules decreased in the final period of kernel maturation, this phenomenon being remarkable for pinB in hard cultivars. Extra-soft and soft cultivars accumulated high amounts of puroindolines on starch granules as compared with medium-soft or hard cultivars. A highly significant inverse correlation was observed between puroindoline content and SKCS value, whereas no correlation was found between the amount of puroindolines on starch granules and protein content.

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### **Probing Protein-Lipid Interactions in Gluten – Acetic Acid Fractionation Approach**

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Wheat flour contains a small quantity of lipids, about 1.5-2.0% of total flour weight. However, when produced from flour, gluten contains as much as 8% (wt.) lipids. The majority of the lipids in gluten presents as 'bound' form while most of these in flour occurs as 'free' form. The different levels of 'free' and 'bound' lipids between gluten and flour are known to be related to protein-lipid interactions during dough mixing. These interactions have been shown to have a positive effect on dough formation, loaf volume of bread and the texture of short-dough biscuits. The mechanism of the protein-lipid interactions in gluten has not been well understood. An improved knowledge about these interactions in gluten could provide better control in the application of gluten and lipids for different types of bakery products. In this study, acetic acid at various concentrations (0.01 M-0.10 M) was used to selectively separate gluten into monomeric and polymeric protein fractions. Proteins of these fractions were characterized by SE-HPLC and SDS-PAGE. The free and bound forms of lipids in each fraction were extracted and analysed using ELSD-HPLC. The results showed that acetic acid caused a dissociation of lipids with the gluten-protein matrix. With increasing acetic acid concentration, an increased amount of free form lipids was released. At low concentration (0.01 M), the protein fraction extracted contained a relatively high amount of monomeric protein and was associated with higher levels of phospholipids. At high concentrations (0.05 M or 0.1 M), high levels of polymeric proteins and bound form of non-polar lipids and glycolipids were found in the extractable fraction. This indicates a strong association of polymeric proteins with these classes of lipids. The results demonstrated particular interactions of protein groups with different types of lipids in gluten.

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### **Integration and Expression of Gluten Strength and Grain Hardness Genes by Crossing Transgenic Plants with Elite Wheat Varieties**

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Genetic engineering and conventional crossing are complementary methods to improve quality traits in wheat. We have used both methods to combine genes controlling grain hardness and gluten strength to make a novel matrix of breeding line combinations suitable for different end-uses of wheat: hard and strong, hard and medium, hard and weak, soft and strong, soft and medium and soft and weak. We intend to make two sets of these breeding lines; one in tetraploid durum wheat and another in hexaploid bread wheat. Three sets of transgenic wheat lines; two over-expressing high molecular weight glutenin subunit genes (HMW-GS) 1Ax1 and 1Dx5, and one over-expressing the puroindoline a (Pin a) gene have been crossed, either with each other or with both hexaploid and tetraploid elite germplasm. The transgenic 1Ax1 and 1Dx5 lines are available in both hexaploid bread and tetraploid durum wheat. The transgenic Pin a lines are in tetraploid durum wheat only. In one hexaploid genetic background (En1) and two tetraploid backgrounds (Luna and Ofanto) we now have lines expressing 1Dx5, 1Ax1 and Pin a individually and in all possible combinations. Initial analysis of 20 F<sub>1</sub> lines from the first successful crosses indicate that the transgenes are predictably transmitted and expressed. Also, crosses between tetraploid and hexaploid lines have been made and chromosome analysis of hybrid seeds are being carried out. Differentiation of allelic variations of the HMW glutenin subunits of wheat flours by use of mixing parameters and polymeric protein content

### **Biochemical Composition and Transcript Profile of Wild and Cultivated Wheat Endosperms**

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My lab is interested in understanding the genetic control of starch and protein accumulation in wheat endosperm. The relative amounts and the physico-chemical properties of these compounds determine grain nutritive and economic value. Because grain starch and to a lesser extent – grain protein were heavily selected for during domestication and later in breeding programs, we reasoned that the biochemical composition and underlying gene expression profiles in the grain of wild vs cultivated wheat may have diverged significantly because they faced different selection pressures for increased grain yield. To test this hypothesis we examined storage product accumulation and transcript levels in developing endosperm of cultivated *Triticum aestivum* cv. Hi Line (AABBDD) and two wild cultivars - *Aegilops crassa* (DDXX) and *Aegilops tauschii* (DD). Transcriptomics was performed using a custom designed cDNA macroarray with 1150 elements. Our results showed that while there are marked differences in the characteristics of the storage products stored in the endosperm of *T. aestivum*, *Ae. crassa* and *Ae. tauschii*, these differences are not reflected in the relative abundance of transcripts. We were however, able to identify some transcripts that showed species-specific expression. These results of this study will be presented.

### **The Impact of Nutrition on the Metabolome, Protein Composition and End-Use Quality of Wheat**

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Conventional agriculture in the UK is heavily reliant on inorganic fertilisers. This dependence has been reflected in the majority of wheat nutrition research which has focused on the optimisation of yield and quality through inorganic fertiliser use. The effects of organic fertilisers on grain quality have received relatively little attention by comparison. The organic food market in the UK has grown rapidly in recent years but farmers trying to produce organic grain for bread-making purposes struggle to achieve high enough grain protein content. An improved understanding of crop physiology under organic fertiliser conditions might indicate how grain protein quality can be improved. We are utilising samples from the classical Broadbalk experiment at Rothamsted Research, which applies a range of nutrient treatments and cultivation practices to a winter wheat crop. A recent study demonstrated that following anthesis significant transcriptome differences existed between the grain endosperms of wheat grown under organic and inorganic nutrient regimes. These included differences in the expression levels of glutenin and gliadin genes. We have now determined the metabolome, gluten protein composition and functional properties of white flours to determine the impact of these conditions on the composition and end use quality of the mature grain.

### **The Dog as a Model for Assessing Food Allergens in Wheat**

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The need for an animal model to detect and evaluate allergens in foods is increasing as a greater portion of the population suffers from food allergy and as a growing percentage of genetically modified products enters the food chain, raising additional concerns for consumers. Research conducted during the past decade in collaboration with a number of investigators, including O. L. Frick, S. S. Teuber and P.G. Lemaux, has shown the dog to be an excellent model for assessing human allergens in foods. Humans and dogs not only share many of the same food allergies, but the dog also shows clinical symptoms typical of humans—i.e., vomit and diarrhea. Due to its primary dietary position and to its importance as a source of allergens, wheat ranks among the foods that require special scrutiny. Our results have shown the dog to be ideal for studying wheat allergies. Based on skin tests and immunoblots, dogs and humans were found to respond to wheat allergens in a similar manner (gliadins > glutenins > globulins > albumins). A similar conclusion applies to allergens in milk (skin test and feeding challenges) and to allergens in peanuts and tree nuts (skin tests and immunoblots). Our work also suggests that the dog may provide a means to test genetically modified foods for both known and unexpected allergens.

### The Origin of Glutenin Particles

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We have recently hypothesised that the glutenin particles as isolated from wheat flour and observed using Confocal Scanning Laser Microscopy (CSLM) originate from Protein Bodies in immature wheat endosperm. In this study we report our experiments to challenge this hypothesis. Glutenin particles were isolated from mature wheat (cv Cadenza) using a detergent solution and ultracentrifugation. Protein bodies were isolated from immature (15 DAF) wheat of the same variety and purified using Percoll density centrifugation. Both glutenin particles and protein bodies were analysed at the molecular level (protein composition), oligomer level (size distribution after sonication), and microscopic level (CSLM with specific stains for protein and free sulfhydryl groups). The results demonstrate similarities between Protein Bodies and glutenin particles at all levels, but also distinct differences. In addition, we simulated the extraction of GMP with a detergent (in this case SDS or Triton X-100) with prelabelled Protein Bodies. This allowed us to directly observe effects on solubility of proteins and proteins with free SH groups. Our results lend further support to the proposed relation between Protein Bodies and glutenin particles.

### Use of the Reconstitution Method to Elucidate the Role of Gluten Proteins in Controlling Durum Semolina Dough Properties and Pasta Quality

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Durum wheat is the preferred raw material for the production of pasta and early studies have shown a consistent relationship between wheat protein content and composition with pasta quality. Understanding the relationship between protein composition and end use measurement of dough and pasta should allow for improved knowledge on how the final product can be manipulated. An approach to do this is to use addition and reconstitution experiments. Changing the glu/gli by 1.3-1.6 fold increased mixograph peak time but had variable effects on pasta firmness. Reconstituting flour with increasing quantities of LMW-GS caused a reduction in water absorption, weaker dough, depending on the source of the fraction followed by a drop in pasta firmness at high rates of incorporation. HMW-GS improved dough strength by mixograph and extensograph but had no effect on pasta texture. Additional studies on the affect of altering the glutenin composition by using different HMW-GS alleles with a common LMW-GS allele and vice versa using the reconstitution approach, will be discussed.

### Transcription of the *Glu-1Bx* HMW Glutenin Subunit Gene During Grain Filling in Several Wheat Cultivars

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In wheat cultivars over-expressing the Bx7 HMW glutenin subunit the promoter of the *Glu-1Bx* gene contains a 43 bp insertion compared to the *Glu-1Bx* promoters found in Bx7 normal-expressing wheat cultivars. However, it is not known, if this insertion has any effect on the transcription efficiency of the promoter. The transcription of *Glu-1Bx* gene has been studied in wheat cultivars normally expressing Bx7 (Bánkúti 1201 B102 and Chinese Spring) and in wheat cultivars over-expressing Bx7 (Bánkúti 1201 B67 and Glenlea) during the development of the endosperm. Bx7 mRNA was quantified by real-time RT-PCR in seeds harvested between 6 to 30 days after flowering (DAF) from plants grown in growth chamber. The pattern of *Glu-1Bx* gene transcription observed was different between Bx7 normal- and over-expressing cultivars. In case of the Bx7 normal-expressing Bánkúti 1201 line B102 and in Chinese Spring, the normalized level of the Bx7 mRNA reached its maximum around 10-12 DAF then declined. Decrease of the Bx7 mRNA level between 15-30 DAF showed kinetics similar to the chemical decay kinetics with an apparent half-life of 7 days. On the other hand, in the Bx7 over-expressing Bánkúti 1201 line B67 and in Glenlea the Bx7 mRNA level showed continuous increase until 24 DAF. In all cultivars tested the level of the Bx7 mRNA was in the same range at 12 DAF. However, the maximal levels detected in the over-expressing cultivars at 24 DAF were an order

of magnitude higher than maximal levels found in normal-expressing cultivars. It was reported earlier that some Bx7 over-expressing wheat cultivars harbor two copies of the *Glu-1Bx* gene. Copy number of the *Glu-1Bx* gene has been determined by real-time PCR in all cultivars studied for transcription. Possible effects of the *Glu-1Bx* gene duplication and the 43 bp insertion in the *Glu-1Bx* promoter on the temporal transcription pattern will be discussed.

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**Considerations About The Effect of Incorporation of Two Rare LMW-GS  
In Durum Wheat In Comparison to Bread Wheat Doughs**

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The effects of incorporation of an i-type low molecular weight glutenin subunit (LMW-i) and of a modified  $\gamma$ -gliadin showing an additional cysteine residue, on 2 gr Mixograph parameters of durum (biotypes 42 and 45 of the Italian cv. Lira) and bread wheat (cv. Kukri) doughs were studied. The incorporation of the modified  $\gamma$ -gliadin in bread wheat flour resulted in a significant decrease in dough strength, represented by the decreases in Mixing Time (MT) and Peak Resistance (PR), but at the same time it produced an increase in dough stability, as evidenced by the decreased resistance to break down (RBD). The incorporation of the LMW-i type in bread wheat flour produced slight or null effects on dough mixing requirements. The weakening effect exerted by the modified  $\gamma$ -gliadin is probably related to the presence of the extra cysteine located at the beginning of the repetitive domain that makes the  $\gamma$ -gliadin behave as a chain terminator of the glutenin polymers. The LMW-i behaves probably as a chain extender of the glutenin polymers but its incorporation into bread doughs do not produce a strengthening effect probably because of the very strong nature of the flour used. The incorporation of both LMW-i type and of the modified  $\gamma$ -gliadin in durum wheat semolina produced a significant decrease in the overall dough strength, especially in Lira biotype 45 doughs. RP-HPLC, SE-HPLC and 2D gels analyses performed on gliadins and glutenins extracted from control and reconstituted doughs, showed that the two polypeptides were in the polymeric fraction. The data obtained in durum wheat are controversial and suggest that other factors need to be taken into consideration in order to explain the differences observed between durum and bread wheat. Some remarkable differences between durum wheat semolina and bread wheat flour, consisting of a different polymer organization, a different degree of starch damage and particle size, could be responsible for the dissimilar effects exerted by the two polypeptides in bread and durum wheat doughs.

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**Wheat Proteomics in the HEALTHGRAIN Project**

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The project HEALTHGRAIN concerns “Exploiting Bioactivity of European Cereal Grains for Improved Nutrition and Health Benefits”. The proteome analysis within HEALTHGRAIN includes establishing the seed proteome reference map of the wheat cultivar Chinese spring as well as proteome profiling of cultivars selected on the basis of properties relevant for health and nutrition and agronomical performance. The purpose of the proteome mapping is to provide data for integration with genetics analysis and for correlation of protein profiles with specific grain quality properties. In addition individual identified seed proteins will be characterized with regard to spatio-temporal occurrence and post-translational modification. An overview of the current status of the project focusing on albumins and globulins and tissue localization will be given in addition to an outline of future plans.

**NOTES**

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## NOTES

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