HEALTHGRAIN Final Conference
Enhancing health benefits of cereal foods - results, perspectives, challenges

Exploiting genomics and transgenesis for enhanced health benefits of wheat

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Huw Jones, Peter Shewry Rothamsted research, UK
HEALTHGRAIN, very short history

- ECC Congress in Vienna March 2002: Working group “Nutrition and Health”.
- In June 2002 an expression of interest was made to FP6 as one of the 1156 expressions in the area of food quality and safety: “Exploiting European cereal grains for human health”
- Working program appeared end 2002, with a call for project in this field in 2003.
- HEALTHGRAIN proposal was submitted on 5th February 2004.
- In June 2004 the proposal was selected as one of the 12 others considered for funding.
- The project started 1st June 2005
HEALTHGRAIN IN EUROPE

- **green**: countries with HEALTHGRAIN partners and members of IP, NIN and/or CCP
- **orange**: countries with members of IP, NIN, and/or CCP
Dietary fibre
Oligosaccharides
Phytate
Phytosterols
Ferulic acid
Alkylrecorcinols
Lignans
Tocopherols & -trienols
Folate, choline, betaine
Minerals
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Breeders, food industry, trade, consumer organisations, authorities

Resources

CONSUMER RESEARCH
49 person-months

GRAIN IMPROVEMENT AND BIOTECHNOLOGY TOOLKIT
850 person-months

TECHNOLOGY AND PROCESSING
614 man-months

NUTRITION AND METABOLISM
523 man-months

DISSEMINATION AND TECHNOLOGY TRANSFER
108 man-months

22 nutrition experts
About 10 Consumer communicators
50+ companies
Methods of Analysis

Analytical methods used for analysis of bioactive components have now been published by AACC.

Easy to follow detailed protocols.

Wide range of chemical analyses covered.
Assessing Stability with $g \times e$ experiment

1. 150 wheat lines and 50 other cereals grown in Hungary in 2004-5.

2. 26 wheat and 5 ryes lines selected based on differences in composition grown again in Hungary in 2005-6

3. 26 lines grown on 4 sites in 2006-7 UK, France, Hungary, Poland

Martonvasar, Hungary

Correlation with environmental conditions
Assessment of Heritability
Using data from multi site trials

Variance Key
- Variety
- Environment
- Variety x Environment
- Error
Correlations between bioactive components and weather measurements in wheat

<table>
<thead>
<tr>
<th></th>
<th>Average Temperature</th>
<th>Precipitation Heading to Harvest</th>
<th>Precipitation 3 months before heading</th>
<th>Precipitation 3 months before heading to harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folates</td>
<td>0.690</td>
<td>-0.514</td>
<td>0.182</td>
<td>-0.354</td>
</tr>
<tr>
<td>Sterols</td>
<td>0.551</td>
<td>-0.199</td>
<td>0.421</td>
<td>0.110</td>
</tr>
<tr>
<td>% Stanols</td>
<td>0.870</td>
<td>-0.589</td>
<td>0.013</td>
<td>-0.562</td>
</tr>
<tr>
<td>Toccols</td>
<td>0.563</td>
<td>-0.067</td>
<td>-0.159</td>
<td>-0.175</td>
</tr>
<tr>
<td>Alkylresorcinols</td>
<td>0.140</td>
<td>0.041</td>
<td>-0.552</td>
<td>-0.352</td>
</tr>
<tr>
<td>Bound Phenolic Acids</td>
<td>-0.126</td>
<td>0.181</td>
<td>-0.268</td>
<td>-0.019</td>
</tr>
<tr>
<td>Conjugated Phenolic Acids</td>
<td>0.753</td>
<td>-0.744</td>
<td>0.694</td>
<td>-0.207</td>
</tr>
<tr>
<td>Free Phenolic Acids</td>
<td>0.899</td>
<td>-0.706</td>
<td>0.194</td>
<td>-0.525</td>
</tr>
<tr>
<td>Total phenolic Acids</td>
<td>0.317</td>
<td>-0.250</td>
<td>0.116</td>
<td>-0.153</td>
</tr>
<tr>
<td>Bran Tot-AX</td>
<td>0.060</td>
<td>0.138</td>
<td>-0.407</td>
<td>-0.168</td>
</tr>
<tr>
<td>Bran WE-AX</td>
<td>-0.889</td>
<td>0.737</td>
<td>0.190</td>
<td>0.826</td>
</tr>
<tr>
<td>Flour Tot-AX</td>
<td>-0.516</td>
<td>0.259</td>
<td>0.446</td>
<td>0.559</td>
</tr>
<tr>
<td>Flour WE-AX</td>
<td>-0.868</td>
<td>0.692</td>
<td>0.119</td>
<td>0.733</td>
</tr>
<tr>
<td>Glucan</td>
<td>0.306</td>
<td>-0.684</td>
<td>0.728</td>
<td>-0.127</td>
</tr>
</tbody>
</table>

Bioactive components which have lower heritable traits appear to have stronger negative correlations with precipitation between heading and harvest. WE-AX (bran and flour) shows a strong positive correlation with precipitation.
I. From QTL to genes. A metagenomic approach: Example of dietary fibre
Dietary fibres are cell wall components, mostly Arabinoxylans (AX) in wheat.

- **Arabinoxylans (AX)**
  - Water extractible AX (WE-AX)
  - Water unextractible AX (WU-AX)

**Water extraction**

**Enzymatic or alkaline extraction**

**Soluble AX (WE-AX)**
- High viscosity
- Health effects
  - blood cholesterol
  - blood glucose (type II diabetes)
  - gut cancers

- No effect on health reported

Generally, fibres improve:
- Satiety (obesity)
- Improved intestinal function

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Genetic analysis of Flour WE-viscosity (predictor of WE-AX)
Choice of two contrasted breeding lines to develop doubled haploids

INTER-STATIONS 1999
effectif = 33   \( r^2 = 0.87 \)
moyenne x = 3.1   moyenne y = 2.5

Le Moulon

POPULATION RE99006 x CF99007 (année 2003)

DNAchip hybridization to find differentially expressed ESTs

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Construction of genetic maps with SSR and DArTs markers

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A total of 3 meta-QTL were found from literature and new experiments

A x Re, Ct x CS, R6 x C7

Clip 9.3cM
R2 29.0%

Re x R, Ct x Cs

Clip 6.3cM
R2 33.6%

V x I, R6 x C7

Clip 9.9cM
R2 59.0%

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Metagenomic approach: combining mapping and expression data to find candidate genes

- Affymetrix wheat chip
  - 5383 genes
  - 10- lines
  - 10+ lines
  - 1551 D
  - 3064 D
  - 1028
  - 3832 I
  - 1926
  - 3532 I
  - 6596 genes
  - 73 candidates

- Co-location
  - Riz Chro8
  - Riz Chro6
  - Blé 7A
  - C-7AL1-0.39
  - C-7AS8-0.45
  - 7AS1-0.89-1.00
  - 7AS5-0.59-0.89
  - 7AS17-0.71-0.74
  - 7AS18-0.90-1.00

- Adenylate kinase, chloroplast EC 2.7.4.3 (ATP-AMP transphosphorylase)
- Caffeic acid O-methyltransferase
- Chlamydomonas SD2-RRP14 protein
- Cinnamyl alcohol dehydrogenase
- Glyceraldehyde 3-phosphate dehydrogenase, cytosolic 3 EC 1.2.1.12
- Glyoxalase I
- Hydroxamate co-transporter
- OCLS protein
- OsNAC1-like protein
- Oxalate oxidase-like protein or germin-like protein
- Putative cinnamyl-CoA reductase

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Candidate genes 6B

Asso. Genet 6B  MQTL on chrom 6B  Candidate gene from cereal synteny

Fine mapping allowed identification of flanking markers
II. Proteomics of endosperm and aleurone layer

INRA, Clermont-Ferrand, France
DTU, Copenhagen, Denmark
Proteomics of aleurone layer proteins

343 proteins were identified

These aleurone-specific spots are candidate genes for aleurone development, composition or mechanical properties (e.g. friability)
III. Candidate genes for phytochemicals: example of folates

Folic acid, or its naturally occurring form folate, is considered as a potentially health-protecting compound in the human diet.
Selection of candidate genes: folates

- One enzyme for main steps of folate synthesis (from GTP, chorismate and THF polyglutamate)
Association analysis using HD diversity screen data

HG collection - 156 lines of hexaploid wheat

Phenotyping (flour, bran, whole meal)

Polymorphisms from 12 candidate genes

Genetic Structure (Pritchard et al, 2000)

5 groups used as covariates

Genetic Analysis (Tassel, Buckler et al 2006)

Phénotype

Micronutrient content
Folates
Phenolics
AlkylR
Sterols

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GCH1 Genes

- Identification of the three homoeologous GCH1 genes
- Assignation on 2A
  - 2B
  - 2D

- 2A copy: 1 SNP in a single line
- 2B copy: 1 SNP in a single line
- 2D copy: 13 SNPs

No association with folate content 😞
11 out of 13 associated with trienol (P-value from 0.0042 to 0.0362)😊
DHFS Genes

- 6 copies expected
- 3 copies assigned on 4B, 4D and 5A chromosomes

- 4B copy → no SNP
- 4D copy → 10 SNPs unbalanced
- 5A copy → no SNP

No association with folate content 😞 but association with stanol (P-values = 0.000237) 😊

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Exploitation of Results: markers

Marker assisted selection using linked or gene-derived markers
Example of marker assisted back-crossing

Transfer of high micronutrient alleles from exotic into adapted varieties
Marker-assisted backcrossing (MAB)

- MAB has several advantages over conventional backcrossing:
  - Effective selection of target loci
  - Minimize linkage drag
  - Accelerated recovery of recurrent parent
Markers must be tightly-linked to target loci!

- Ideally markers should be <5 cM from a gene or QTL

\[
1 - r_A = \sim 95\%
\]

\[
1 - 2r_{AB} = \sim 99.5\%
\]

- Using a pair of flanking markers can greatly improve reliability but increases time and cost

- Application to Valoris x Premio and Yumai34 x Premio

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Exploitation of Results: Genes

Biotechnology for modifying gene expression (beyond the range found in natural diversity)

Transgenesis of silencing or over-expression of dietary fibre genes
RNAi silencing of CSL6 gene in transgenic wheat

DNA-delivery via gun
Endosperm-specific silencing in transgenic wheat

**HMW Glutenin promoters**

1Dx5 (-1141 +57) ::GUS gives endosperm-specific expression detectable 12-14 dpa


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### Target Enzyme

<table>
<thead>
<tr>
<th>Plasmid ID</th>
<th>No. GM plants generated</th>
<th>Totals per target enzyme</th>
<th>Status</th>
<th>Seed ready Date</th>
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<tbody>
<tr>
<td><strong>Putative Glucan Synthase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>pCSLD4 RNAi</td>
<td>8</td>
<td>35</td>
<td>Complete</td>
<td>July '07</td>
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<tr>
<td>pCSLF6 RNAi</td>
<td>13</td>
<td></td>
<td>Complete</td>
<td>July '07</td>
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<tr>
<td>pCSLD4 RNAi + pCSLF6 RNAi</td>
<td>8 (7*)</td>
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<td>Complete</td>
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<tr>
<td>pAHC1ESwifthfulF6</td>
<td>6</td>
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<td>Oct '09</td>
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<td><strong>Putative Arabinosyl Transferase</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>pGT61_1</td>
<td>12 (8*)</td>
<td>108</td>
<td>Ongoing</td>
<td>Aug '08</td>
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<td>pGT61+ pHMWAt13</td>
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<td></td>
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<td>pRNAi#55p113</td>
<td>21 (11*)</td>
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<td>pHMWGT61-2RNAi</td>
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<td><strong>Putative Xylan Synthase</strong></td>
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<tr>
<td>pHMWGT43RNAi</td>
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<td>53</td>
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<td>pHMWAt13</td>
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<td>pHMWGT47RNAi</td>
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<td>Dec '08</td>
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<td><strong>Putative Feryloyl Transferase</strong></td>
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<tr>
<td>pHMW164RNAi</td>
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<td>57</td>
<td>Complete</td>
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<td>pHMW172RNAi</td>
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<tr>
<td>pHMWFT3RNAi</td>
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<td>Feb '10</td>
</tr>
<tr>
<td>pUbi164RNAi</td>
<td>25 (21*)</td>
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<td>Complete</td>
<td>July '09</td>
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<td><strong>UDP-Glucose dehydrogenase</strong></td>
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<td></td>
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<tr>
<td>pAHC-UDPG-D</td>
<td>22 (21*)</td>
<td>55</td>
<td>Complete</td>
<td>Feb '09</td>
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<td>pUDPG-antisense</td>
<td>5 (4*)</td>
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<td>Complete</td>
<td>March 09</td>
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</tbody>
</table>

See Huw Jones poster for more results

**308 T0 transgenic wheat lines**

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OUTPUTS AND CONCLUSIONS

1. Identification of new sources of genetic diversity for fibre and bioactive compounds (WP2.1 Z Bedö, J Ward)

2. Identify markers for QTLs for WE-AX fibres: marker assisted transfer of favourable alleles (Valoris, Yumai?) into breeding germplasm

3. Functional validation of candidate genes for WEAX fibres

4. Map-based cloning of major QTL for WEAX fibre: Perfect gene-derived markers

5. Markers developed in candidate genes for folates may be helpful for improving… bioactive compounds in connected pathways (pholics, tocols…)

6. Proteomics can help targeting genes for aleurone development/fragility/ expression

7. Transgenesis for manipulating gene expression: improving the biosynthetic pathway of dietary fibre

8. All presentations available on http://www.healthgrain.eu/pub/Final_conference-presentations.php

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Contributions to this work


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