HEALTHGRAIN Final Conference

Enhancing health benefits of cereal foods - results, perspectives, challenges

Exploiting genomics and transgenesis for enhanced health benefits of wheat

Gilles Charmet INRA France
Huw Jones, Peter Shewry Rothamsted research, UK

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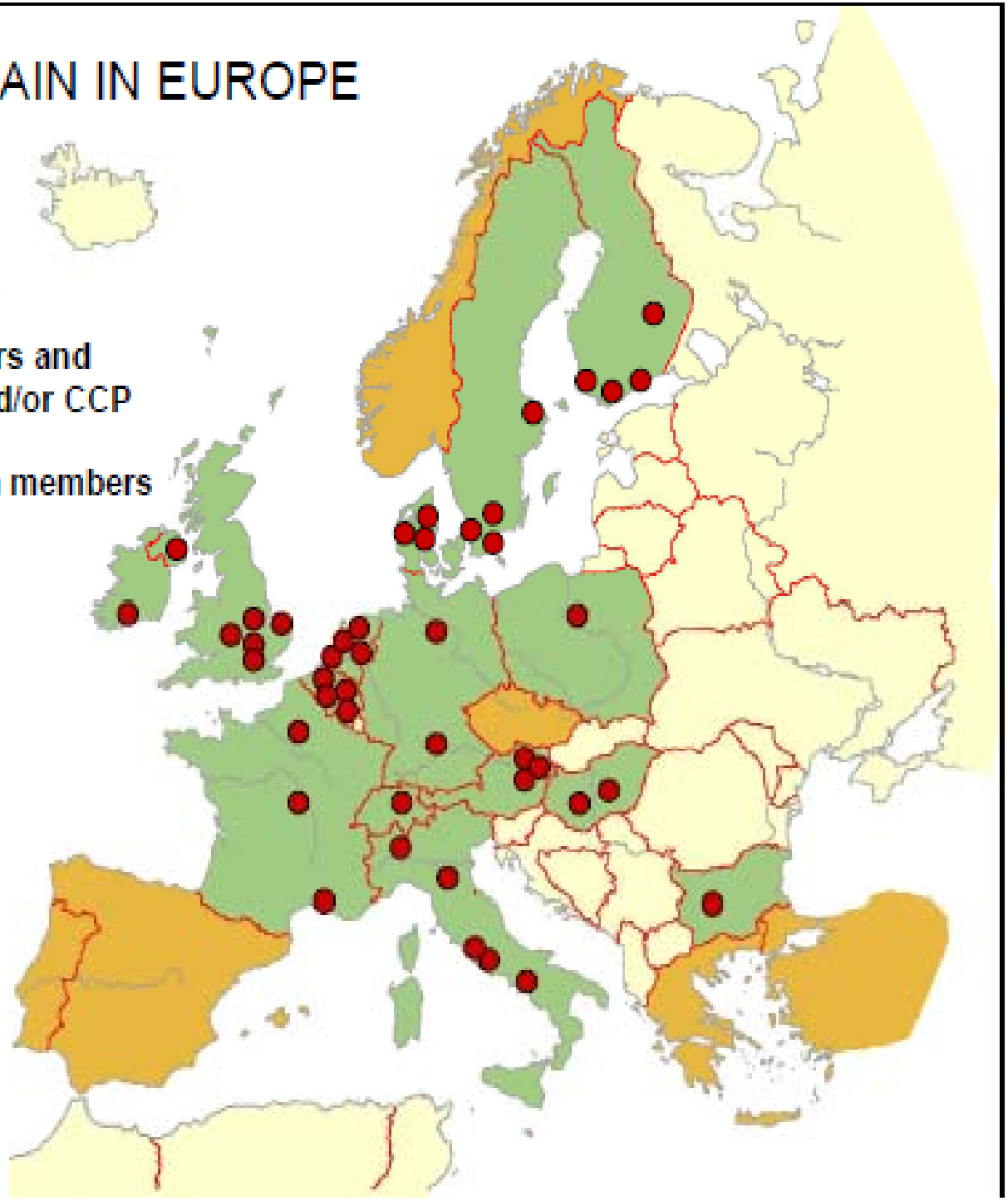
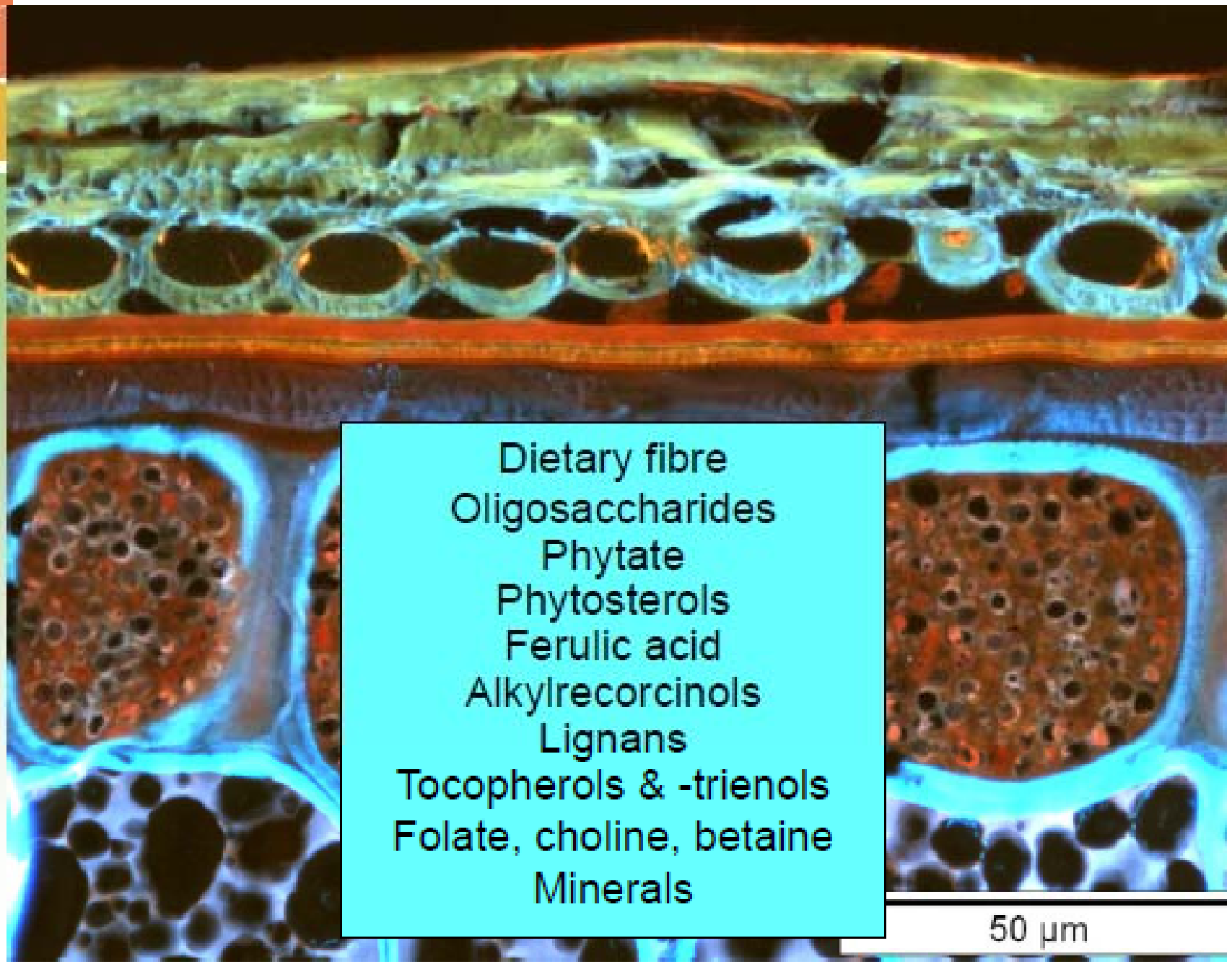
HEALTHGRAIN, very short history

- 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

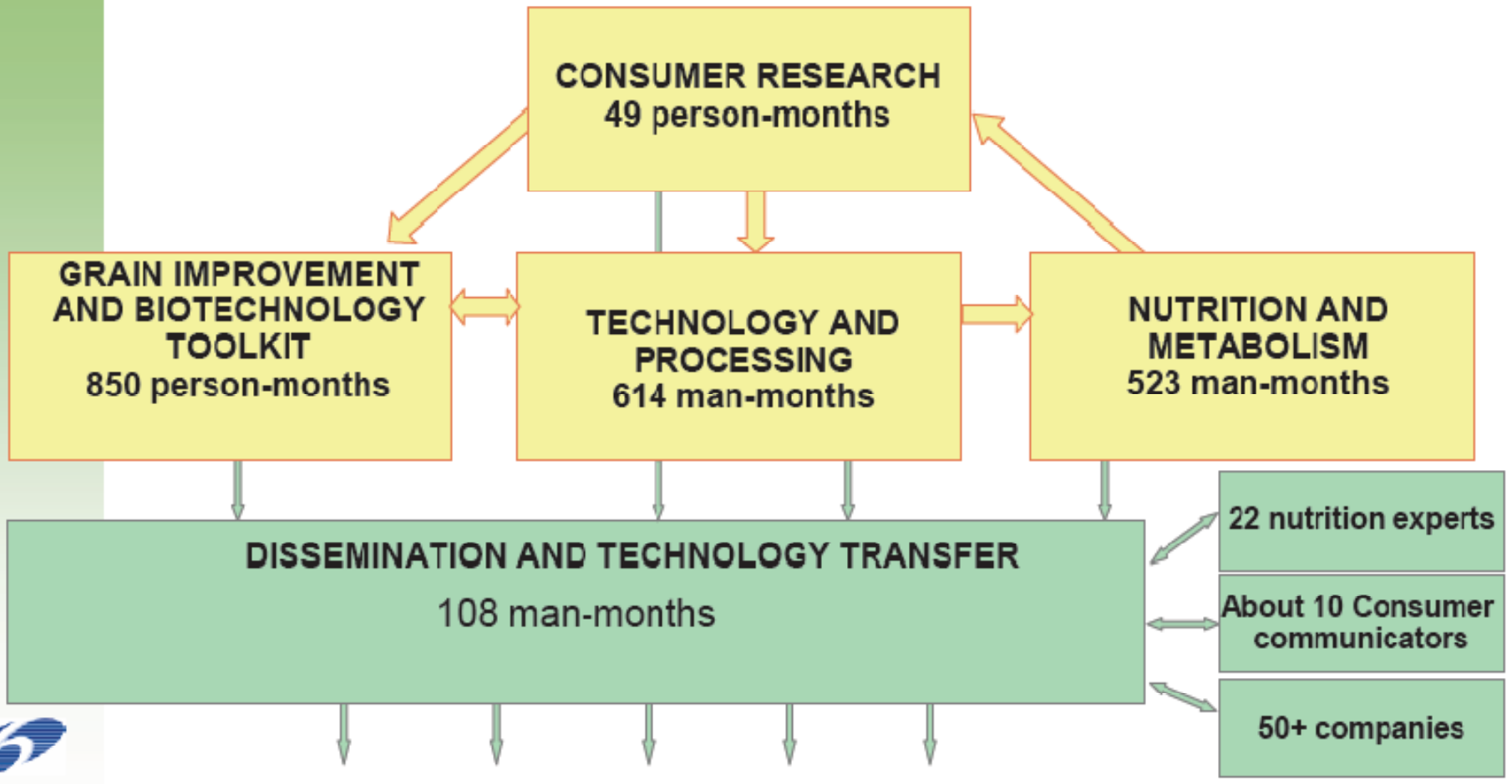
- green: countries with HEALTHGRAIN partners and members of IP, NIN and/or CCP
- orange: countries with members of IP, NIN, and/or CCP




Dietary fibre
Oligosaccharides
Phytate
Phytosterols
Ferulic acid
Alkylrecorcinols
Lignans
Tocopherols & -trienols
Folate, choline, betaine
Minerals
50 µm

HEALTH·GRAIN FOOD-CT-2005-514008

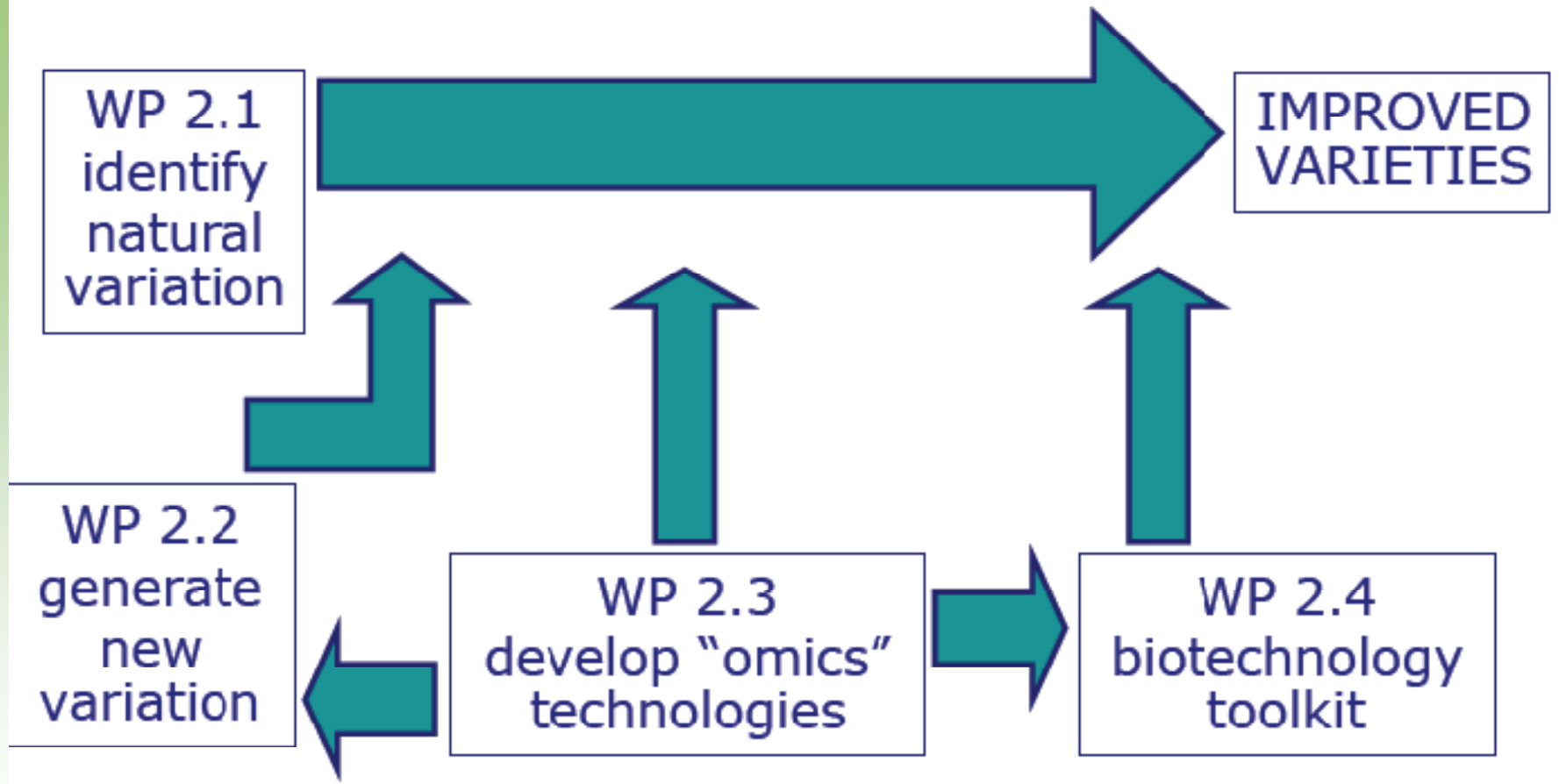
Resources



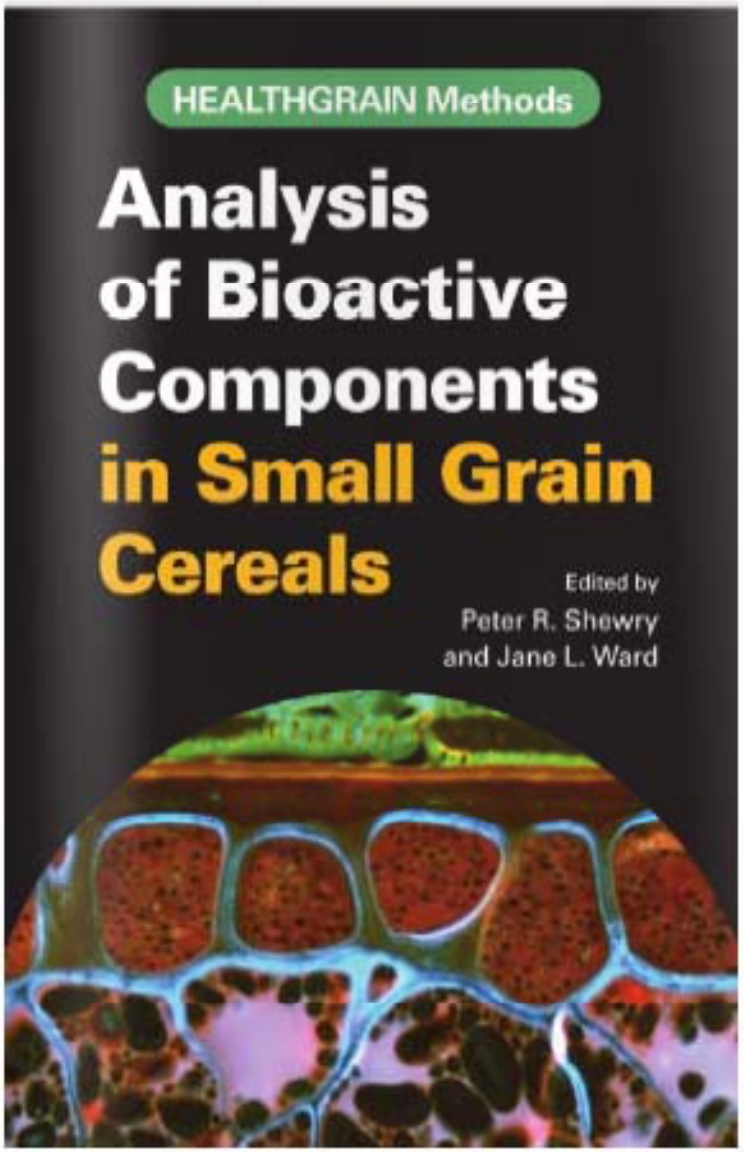
HEALTH·GRAIN MODULE 2

CROP IMPROVEMENT AND BIOTECHNOLOGY TOOL KIT

Leader: Peter Shewry, Rothamsted Research, UK



Methods of Analysis

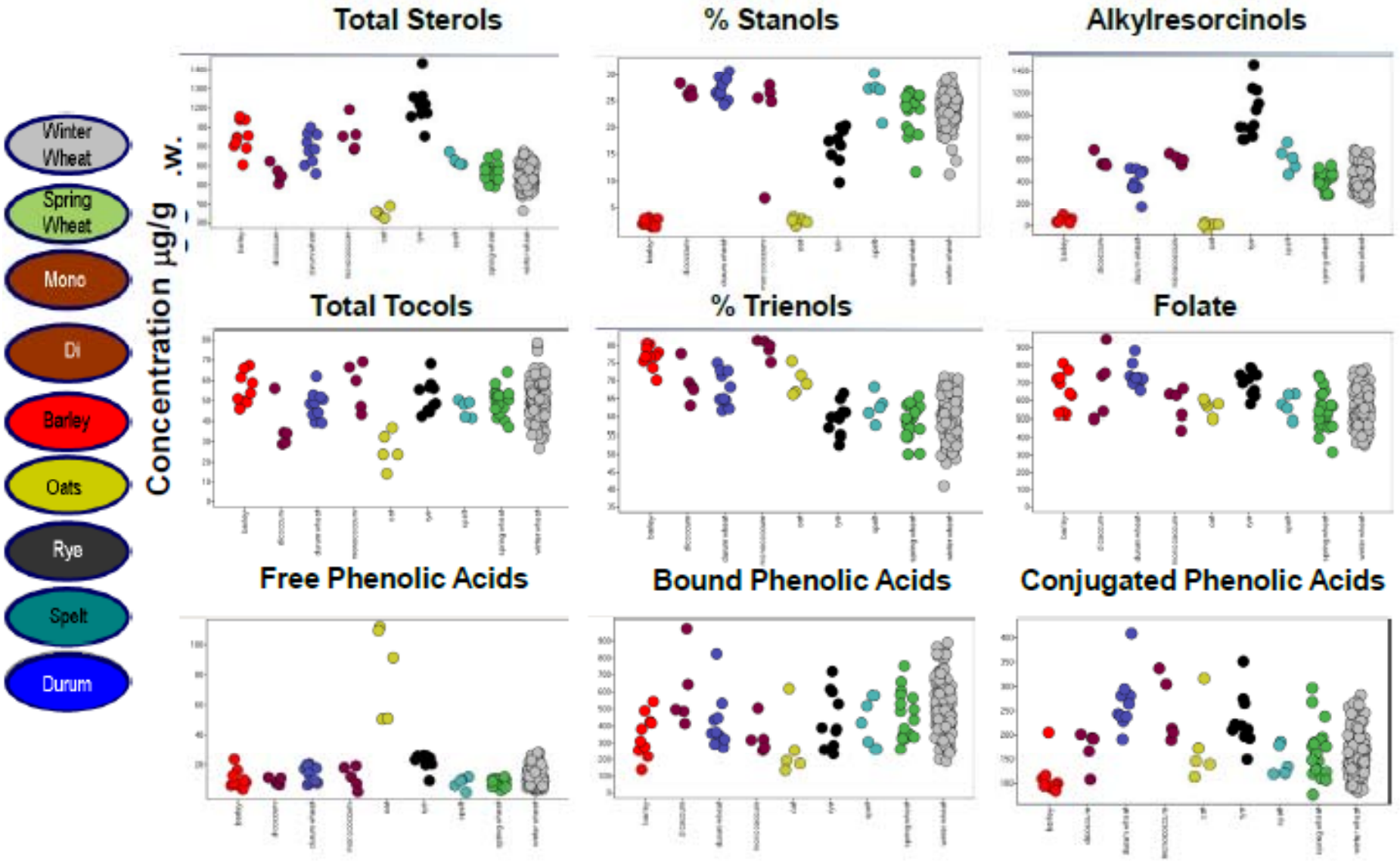


Analytical methods used for analysis of bioactive components have now been published by AACC

Easy to follow detailed protocols

Wide range of chemical analyses covered

Phytochemical Data from 200 line Diversity Screen – Comparison of Cereals



Assessing Stability with g x e experiment

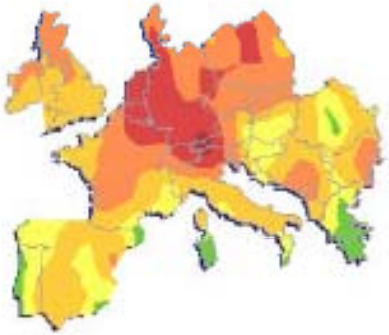
Martonvasar, Hungary

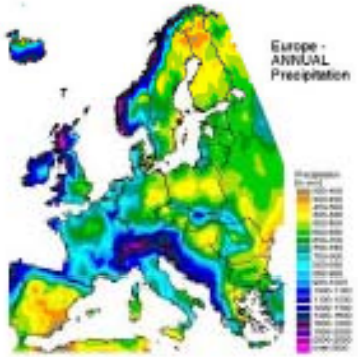
1.150 wheat lines and 50 other cereals grown in Hungary in 2004-5.

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

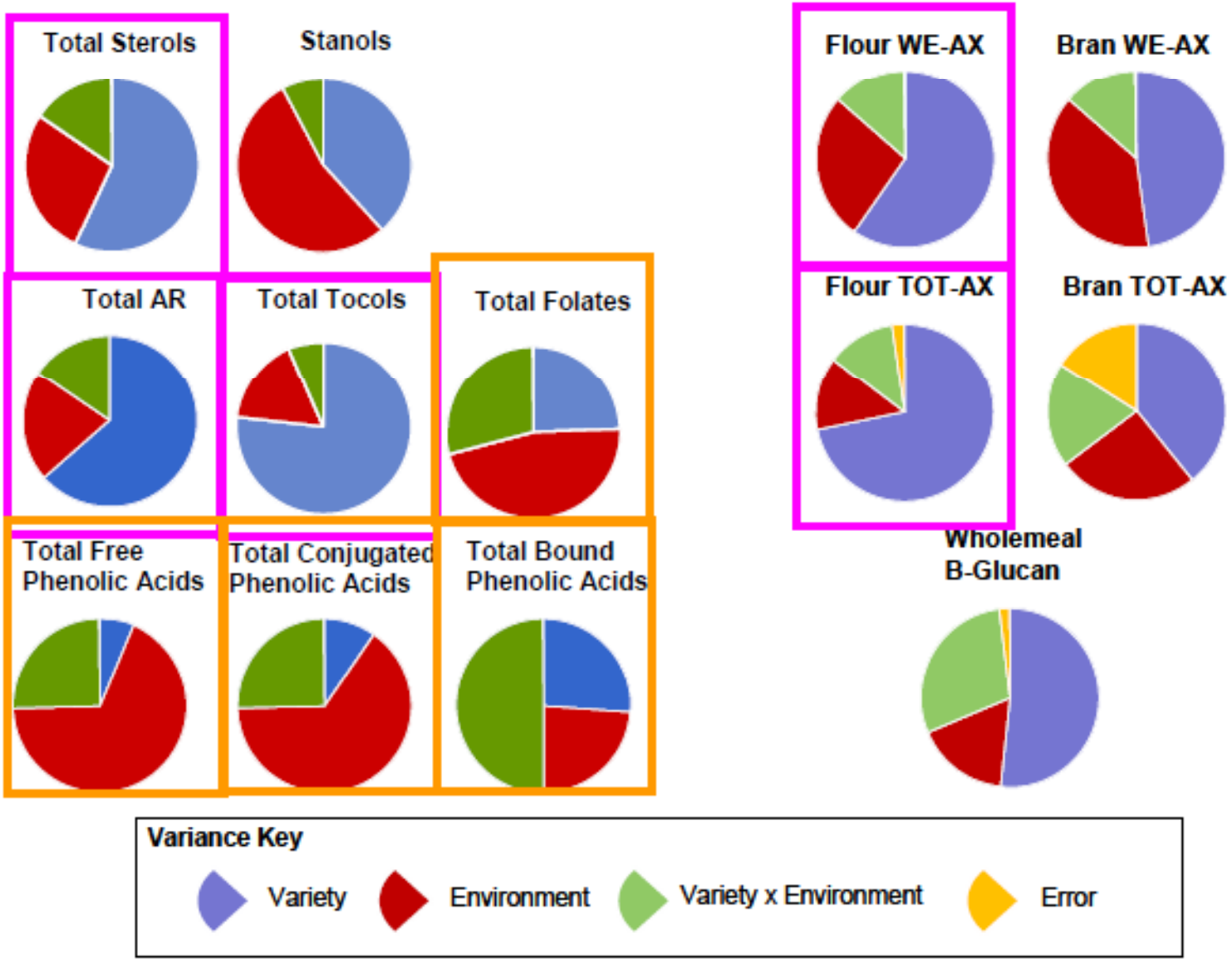
3. 26 lines grown on 4 sites in 2006-7 UK, France, Hungary, Poland

Correlation with environmental conditions



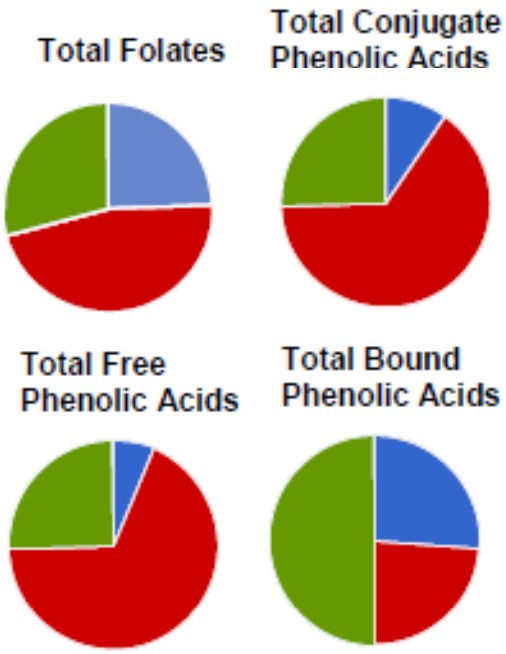


Assessment of Heritability
Using data from multi site trials



Correlations between bioactive components and weather measurements in wheat

	Average Temperature	Precipitation Heading to Harvest	Precipitation 3 months before heading	Precipitation 3 months before heading to harvest
	R	R	R	R
Folates	**0.690**	**-0.514**	0.182	-0.354
Sterols	**0.551**	-0.199	**0.421**	0.110
% Stanols	**0.870**	**-0.589**	0.013	**-0.562**
Tocols	**0.563**	-0.067	-0.159	-0.175
Alkylresorcinols	0.140	0.041	**-0.552**	-0.352
Bound Phenolic Acids	-0.126	0.181	-0.268	-0.019
Conjugated Phenolic Acids	**0.753**	**-0.744**	**0.694**	-0.207
Free Phenolic Acids	**0.899**	**-0.706**	0.194	**-0.525**
Total phenolic Acids	0.317	-0.250	0.116	-0.153
Bran Tot-AX	0.060	0.138	**-0.407**	-0.158
Bran WE-AX	**-0.889**	**0.737**	0.190	**0.826**
Flour Tot-AX	**-0.516**	0.259	**0.446**	**0.559**
Flour WE-AX	**-0.868**	**0.692**	0.119	**0.733**
Glucan	0.306	**-0.684**	**0.728**	-0.127



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

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Dietary fibres are cell wall components, mostly Arabinoxylans (AX) in wheat

Arabinoxylans (AX)

Water extraction

Water extractible AX (**WE-AX**)

Water unextractible (**WU-AX**)

Enzymatic or alkaline extraction

Soluble AX (WE-AX)

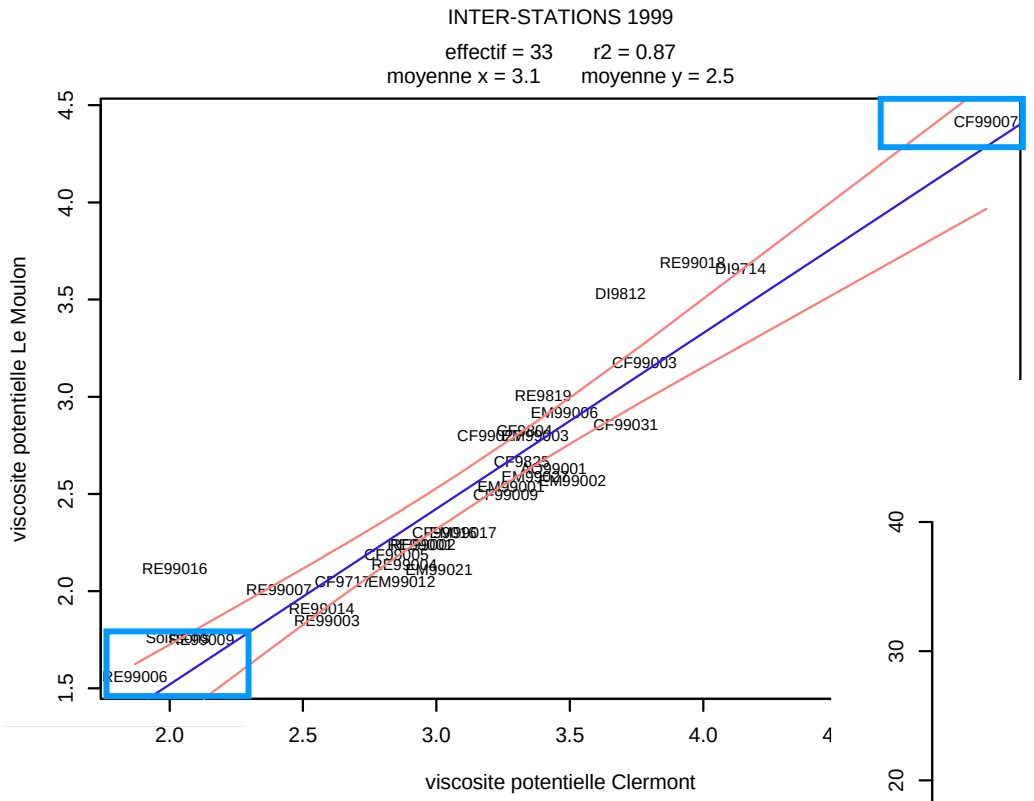
- High viscosity
- Health effects
  - blood cholesterol
  - blood glucose (type II diabete)
  - gut cancers

- No effect on health reported

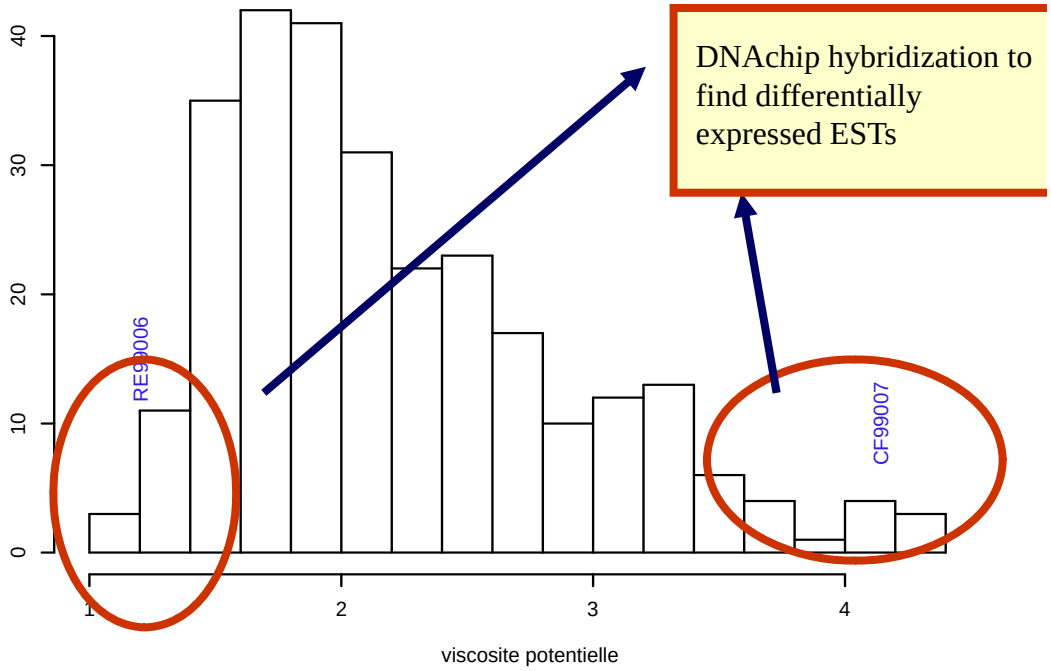
Generally, fibres improve :

- Satiety (obesity)
- improved intestinal function

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



POPULATION RE99006 x CF99007 (annee 2003)



DNAchip hybridization to find differentially expressed ESTs

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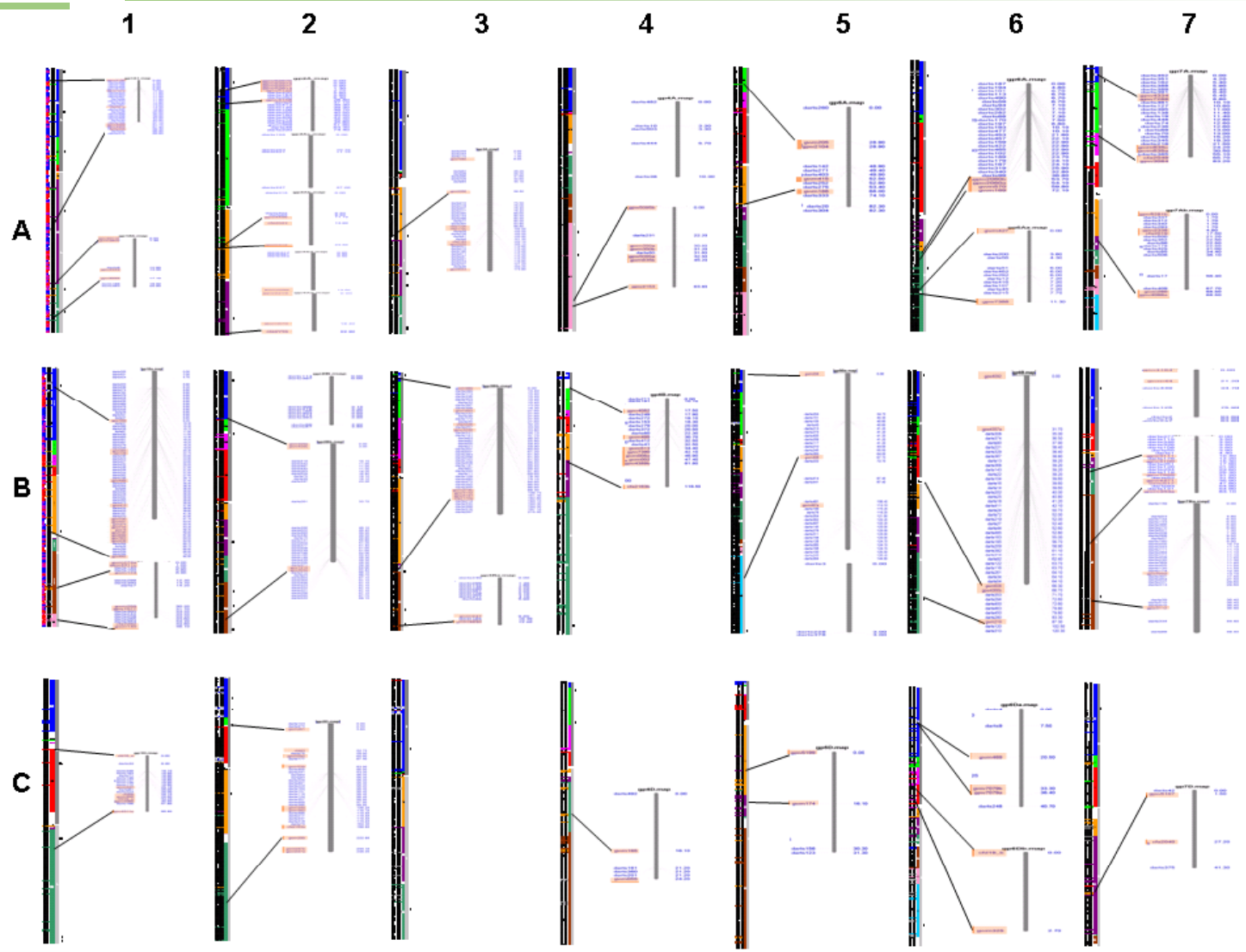
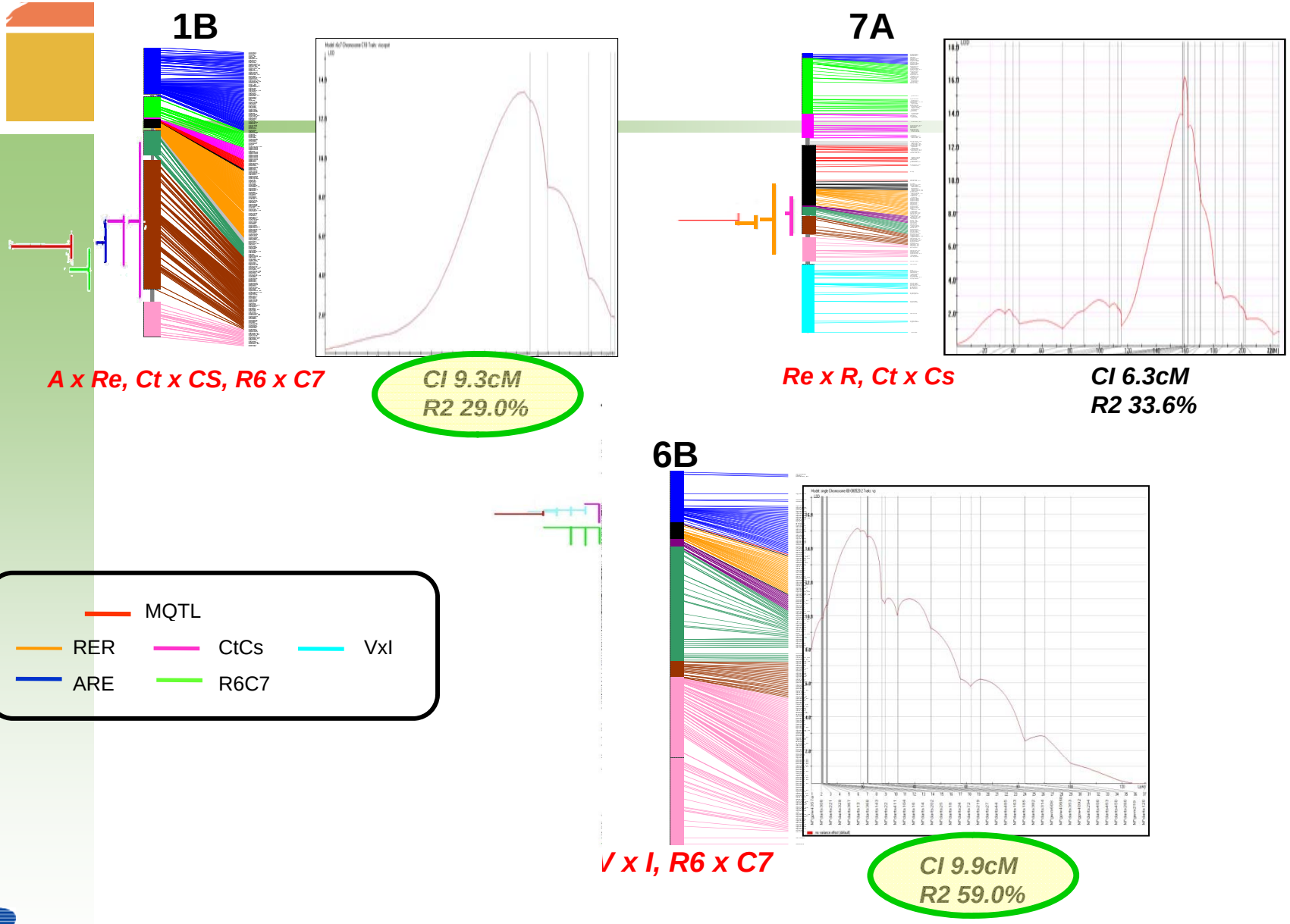
Construction of genetic maps with SSR and DArTs markers



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



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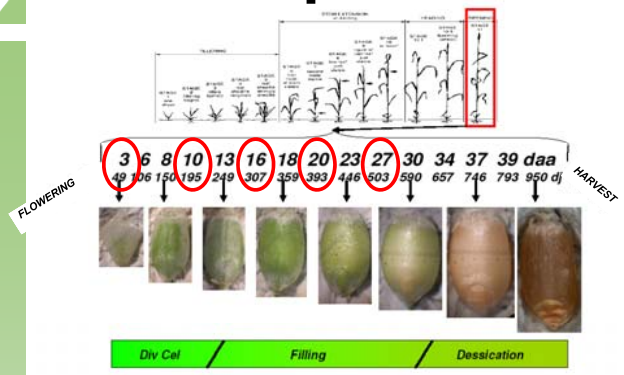
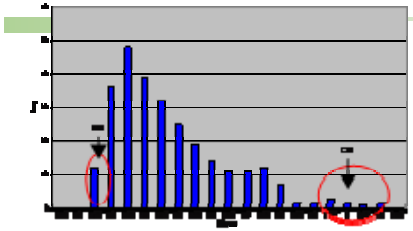
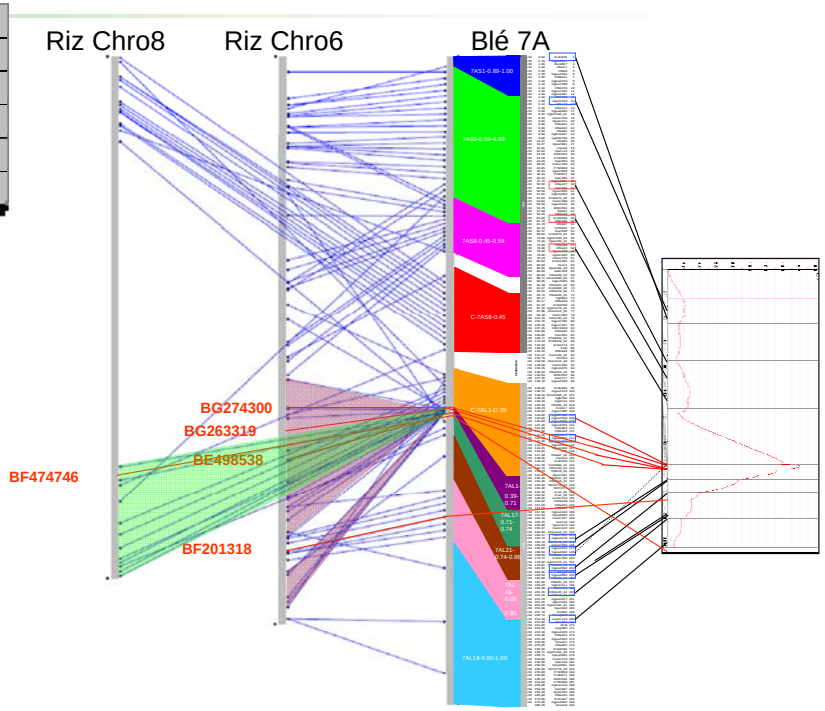
Metagenomic approach: combining mapping and expression data to find candidate genes



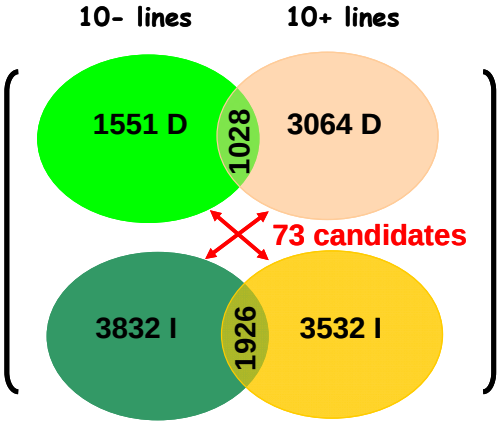


Co-location



Affymetrix wheat chip

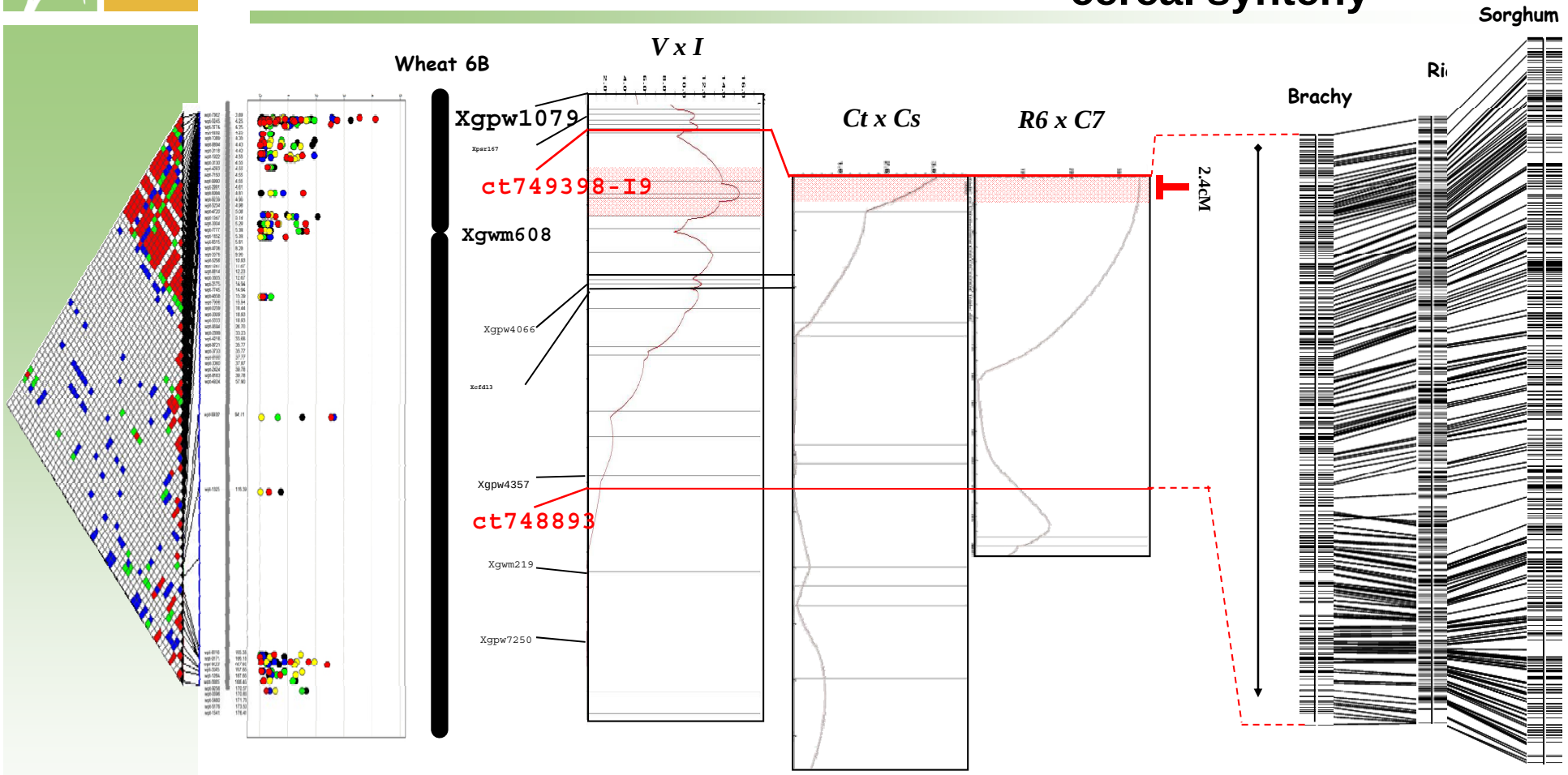
5383 genes



6596 genes

Adenylate kinase, chloroplast EC 2.7.4.3) (ATP-AMPtransphosphorylase).	
Caffeic acid O-methyltransferase.	**RNAi**
Chimeric SDH2-RPS14 protein.	
Cinnamyl alcohol dehydrogenase.	**OverExp**
Glyceraldehyde 3-phosphate dehydrogenase, cytosolic 3 EC 1.2.1.12).	
Glyoxalase I.	**OverExp**
H+)/hexose cotransporter.	
OCL5 protein.	
OsNAC6-like protein.	**RNAi**
Oxalate oxidase-like protein or germin-like protein.	
Putative cinnamoyl-CoA reductase.	**OverExp**

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



Fine mapping allowed identification of flanking markers

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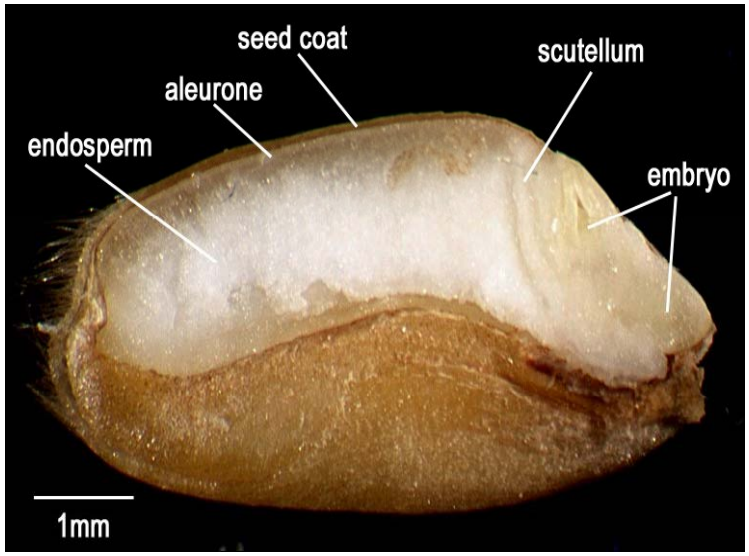
II. Proteomics of endosperm and aleurone layer



INRA, Clermont-Ferrand, France
DTU, Copenhagen, Denmark

DTU

INRA

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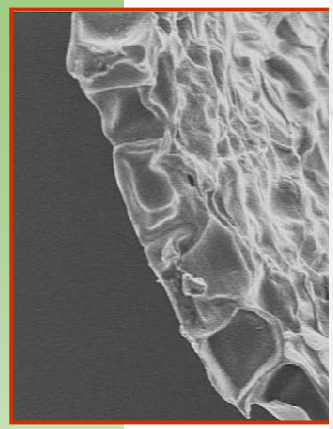
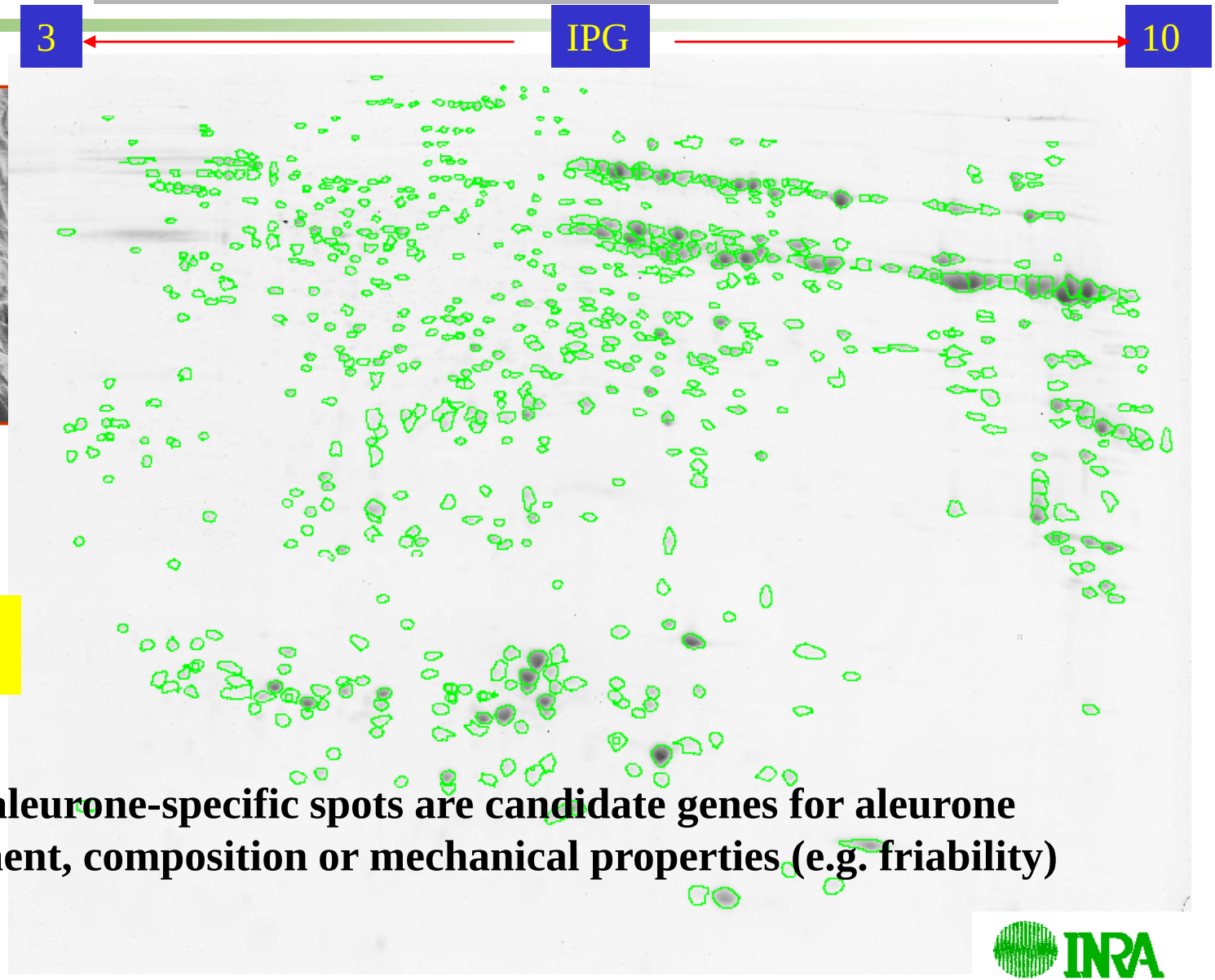
Proteomics of aleurone layer proteins





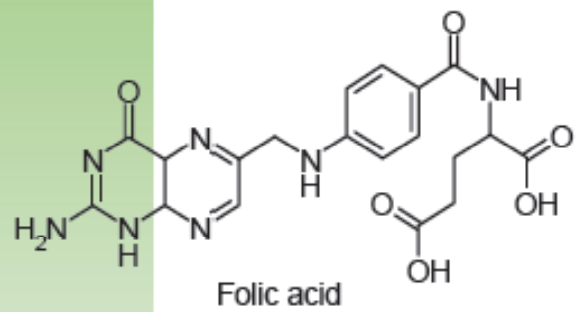
343 proteins were identified

These aleurone-specific spots are candidate genes for aleurone development, composition or mechanical properties (e.g. friability)

DTU

INRA

III. Candidate genes for phytochemicals: example of folates



Folic acid

Folic acid, or its naturally occurring form folate, is considered as a potentially health-protecting compound in the human diet.

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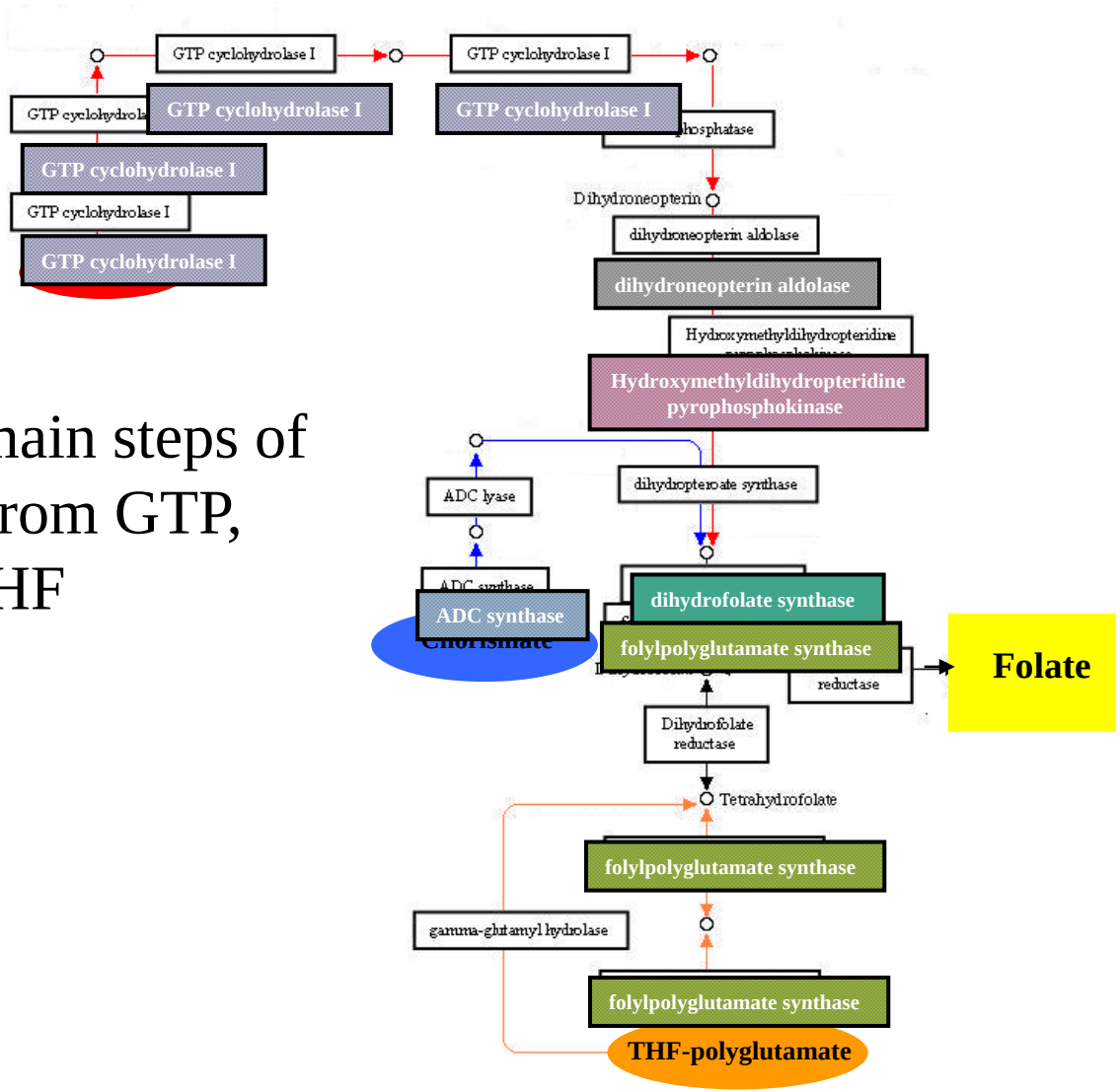
Selection of candidate genes: folates

- One enzyme for main steps of folate synthesis (from GTP, chorismate and THF polyglutamate)



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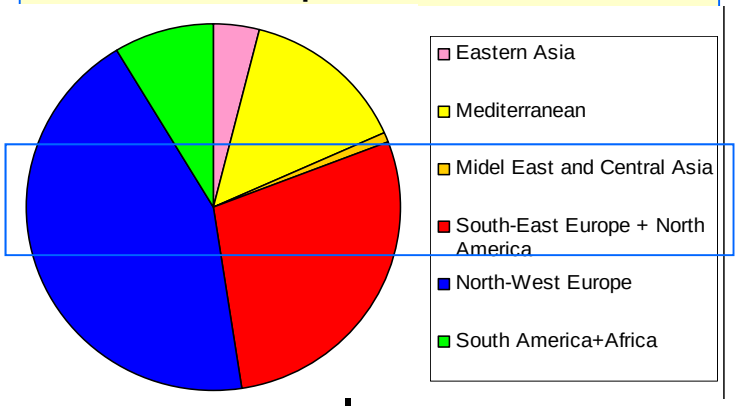
Association analysis using HD diversity screen data

HG collection - 156 lines of hexaploid wheat



Phenotyping (flour, bran, whole meal)

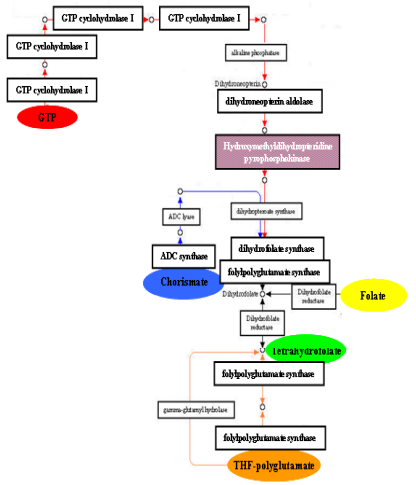
Micronutriment content
Folates
Phenolics
AlkylR
Sterols
...

Phénotype

Genetic Structure (Pritchard et al, 2000)

5 groups used as covariates

Polymorphisms from 12 candidate genes



DHPA, GCH1, DHFS, HPPK, ADCS, FPGS

Génotype

Genetic Analysis (Tassel, Buckler et al 2006)

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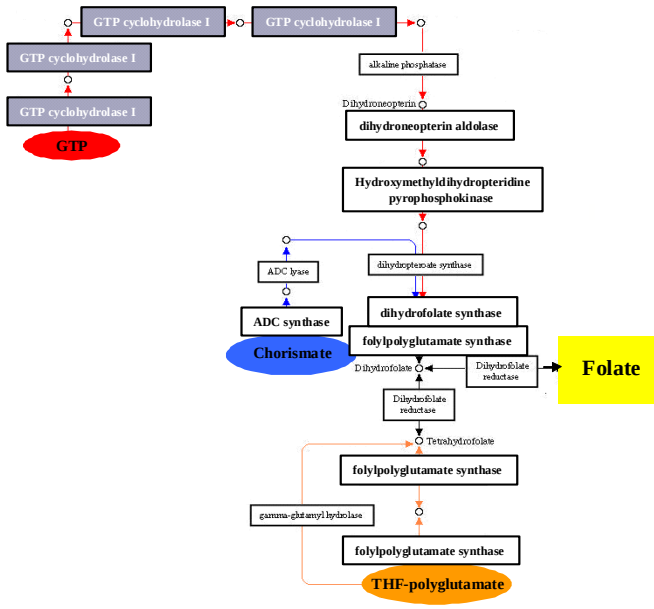
GCH1 Genes



- 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) ☺

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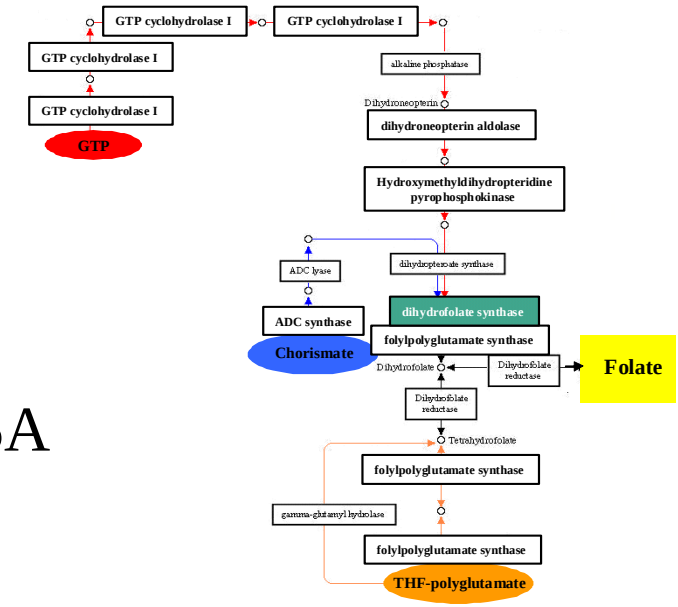
DHFS Genes



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

- 4B copy → no SNP
- 4D copy → 10 SNPs unbalanced
  → 1 SNP → No association with folate content ☹ but association with stanol (P-values = 0,000237) ☺
- 5A copy → no SNP

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Exploitation of Results: markers

Marker assisted selection using linked or gene-derived markers

Example of marker assisted back-crossing

Tranfer of high micronutrient alleles from exotic into adapted varieties

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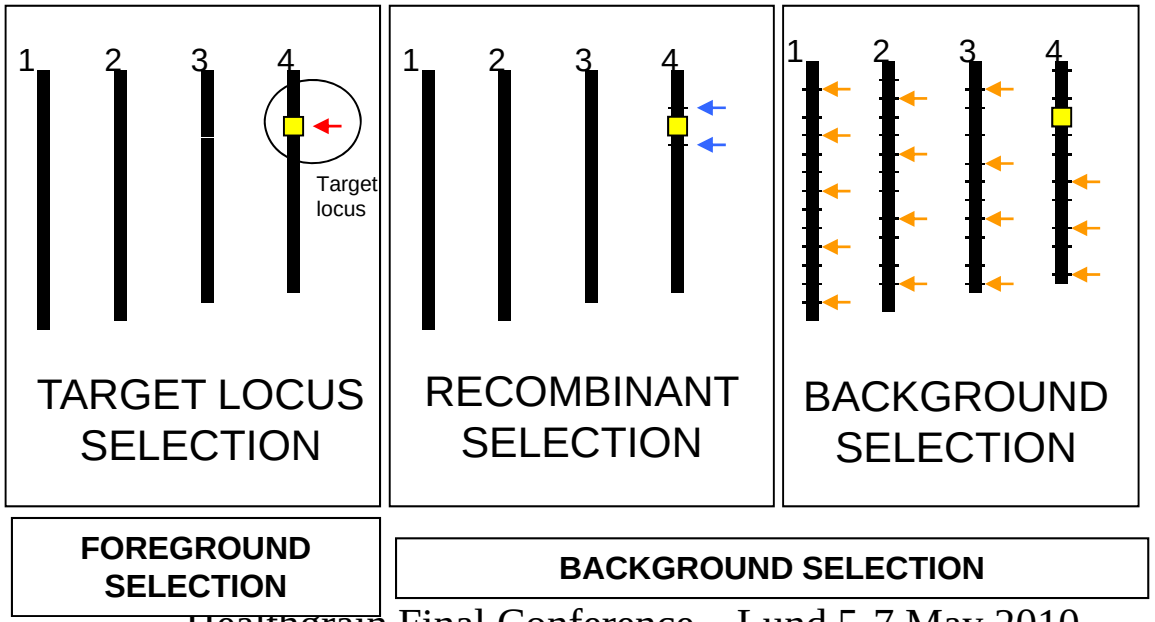
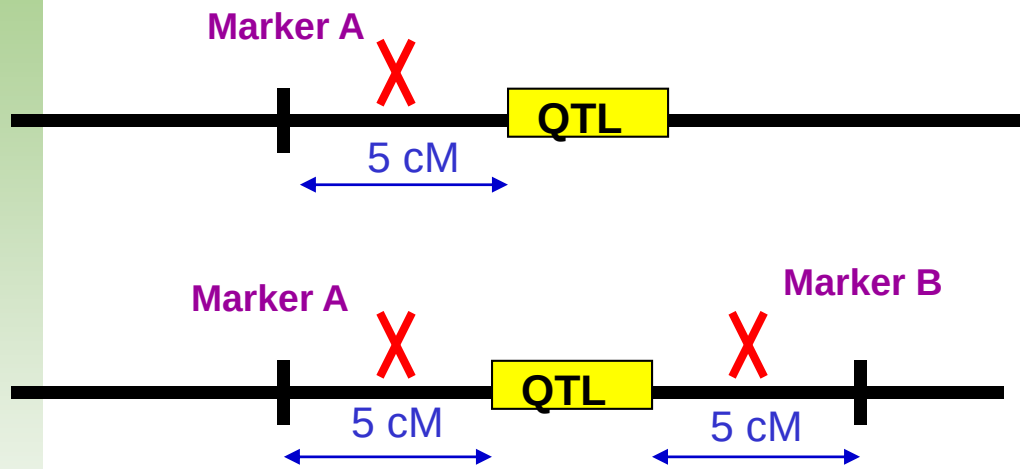
Marker-assisted backcrossing (MAB)

- **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**



RELIABILITY FOR SELECTION

Using marker A only:

$1 - r_A$ = ~**95%**

Using markers A and B:

$1 - 2\, r_A r_B$ = ~**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

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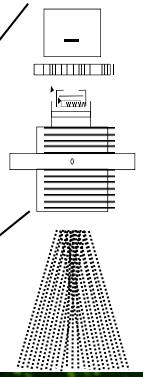
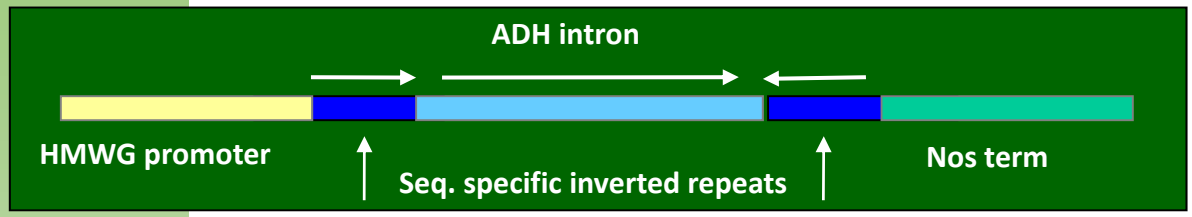
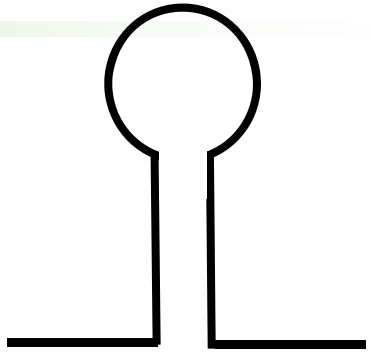
RNAi silencing of CSL6 gene in transgenic wheat

DNA-delivery via gun



Endosperm-specific silencing in transgenic wheat

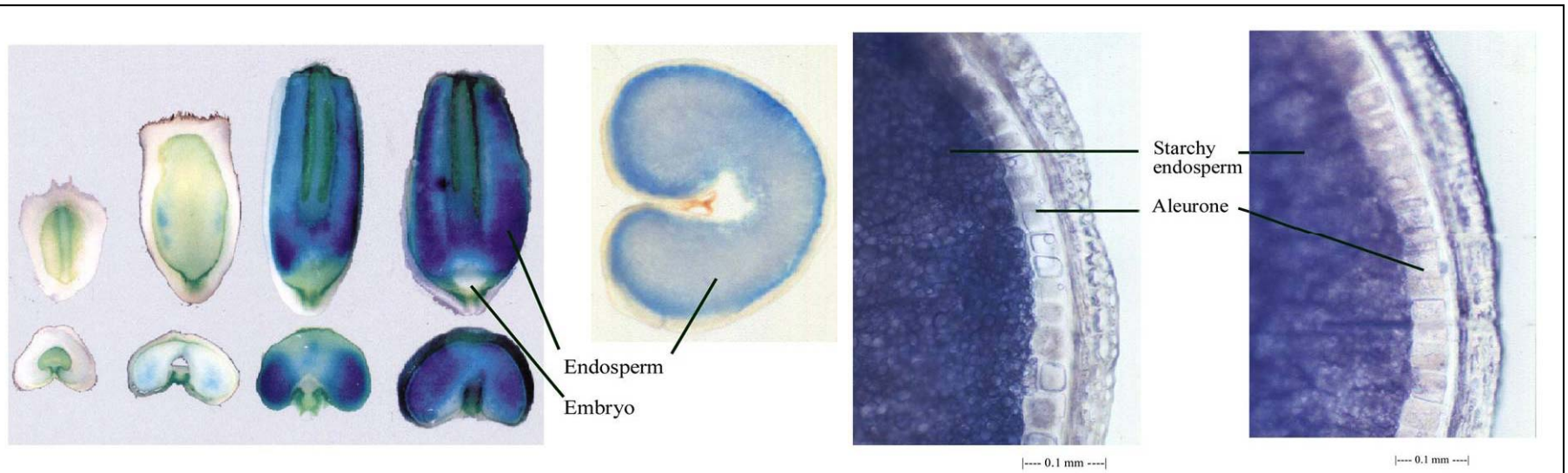




Hair-pin ds RNA structure

HMW Glutenin promoters

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



Lamacchia *et al.* (2001) *Journal of Experimental Botany* 52:243-250.

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ROTHAMSTED RESEARCH

308 T0 transgenic wheat lines

See Huw Jones poster for more results

Target Enzyme	Plasmid ID	No. GM plants generated	Totals per target enzyme	Status	Seed ready Date
Putative Glucan Synthase	**pCSLD4 RNAi**	**8**	**35**	**Complete**	**July '07**
	pCSLF6 RNAi	**13**		**Complete**	**July '07**
	pCSLD4 RNAi + pCSLF6 RNAi	**8 (7*)**		**Complete**	**July '07**
	pAHC1ESwithfullF6	**6**		**Complete**	**Oct '09**
Putative Arabinosyl Transferase	**pGT61_1**	**12 (8*)**	**108**	**Ongoing**	**Aug '08**
	pGT61+ pHMWAt13	**6**		**Complete**	**July '08**
	pRNAi#55p113	**21 (11*)**		**Complete**	**July '08**
	pGT61_1	**8**		**Complete**	**May '09**
	pRNAi#13p113 (LS. INRA)	**4***		**Complete**	**May '09**
	pRNAi#63p113 (LS. INRA)	**9 (6*)**		**Complete**	**July '08**
	pHMWGT61-2RNAi	**48***		**Complete**	**Oct '09**
Putative Xylan Synthase	**pHMWGT43RNAi**	**15 (12*)**	**53**	**Complete**	**Feb '09**
	pHMWGT43-2 O/E	**4**		**On-going**	**April 10**
	pHMWAt13	**20 (15*)**		**Complete**	**Nov '07**
	pHMWGT47RNAi	**14 (8*)**		**On-going**	**Dec '08**
Putative Feryloyl Transferase	**pHMW164RNAi**	**14 (12*)**	**57**	**Complete**	**Nov '09**
	pHMW172RNAi	**10 (8*)**		**On-going**	**Feb '10**
	pHMWFT3RNAi	**8***		**On-going**	**Feb '10**
	pUbi164RNAi	**25 (21*)**		**Complete**	**July '09**
UDP-Glucose dehydrogenase	**pAHC-UDPG-D**	**22 (21*)**	**55**	**Complete**	**Feb '09**
	pUDPG-RNAi	**28 (27*)**		**Complete**	**April 09**
	pUDPG-antisense	**5 (4*)**		**Complete**	**March 09**

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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 developped in candidate genes for folates may be helpful for improving… bioactive compounds in connected pathways (pholics, tocols…)**
6. **Proteomics can help targetting 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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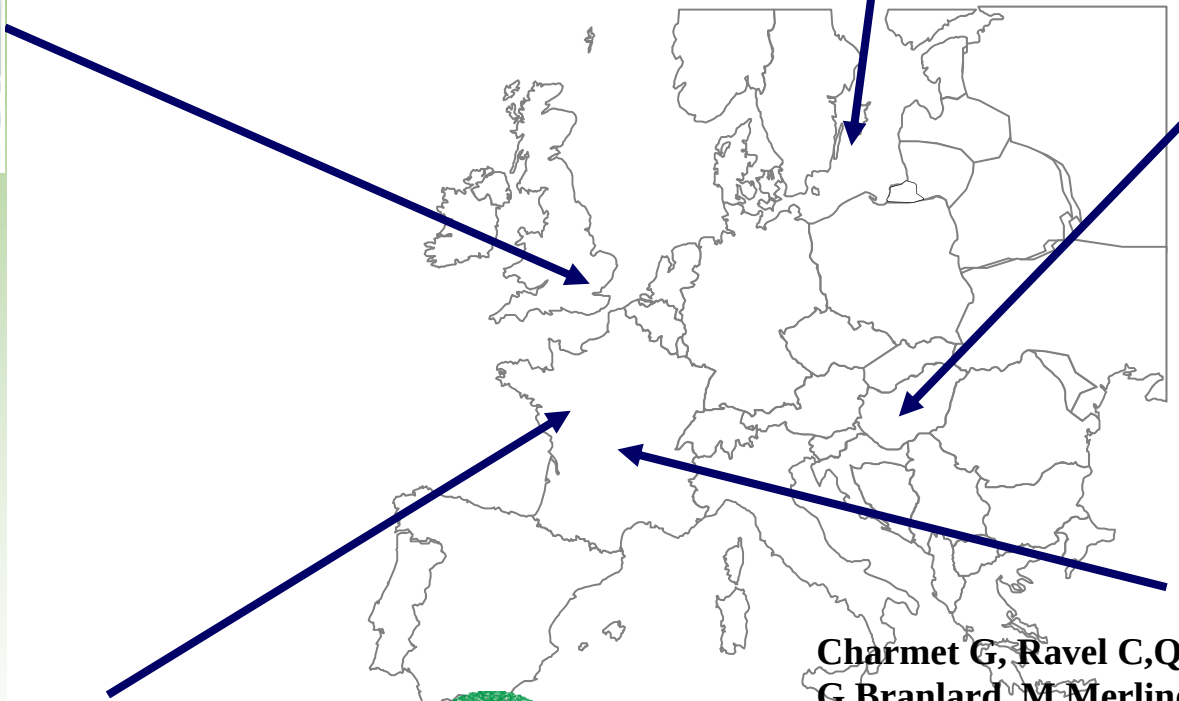
Contributions to this work

EPC, BioCentrum-DTU
Sabrina Laugesen, Kenji Maeda,
Birgit C. Bønsager, Birte Svensson

Peter Roepstorff
Xumin Zhang

Nemeth C, Freeman J, Jones H, Mitchell RAC, J Ward,
M Wilkinson, Shewry PR, Rothamsted Research,
Harpenden, UK



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