PRIFYSGOL ABERYSTWYTH UNIVERSITY

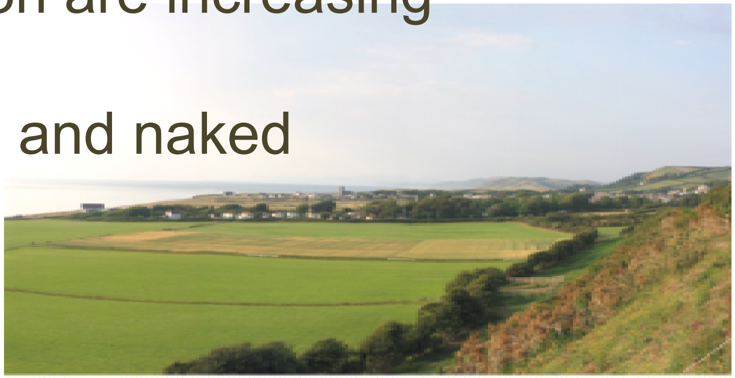
Strategies for improvement of β-glucan content in oats

Catherine Howarth, Tim Langdon, Irene Griffiths, Sandy Cowan, Athole Marshall

Sefydliad y Gwyddorau Biolegol, Amgylcheddol a Gwledig
IBERS ABERYSTWYTH
Institute of Biological, Environmental and Rural Sciences

Background

- 65% of the oats grown in UK are IBERS varieties
- 120,000 ha grown in UK
  - (70% winter and 30% spring)
- 750,000t produced per year
- Husked oats for human consumption are increasing
- Programme: winter, spring, husked and naked
- Naked oats for poultry



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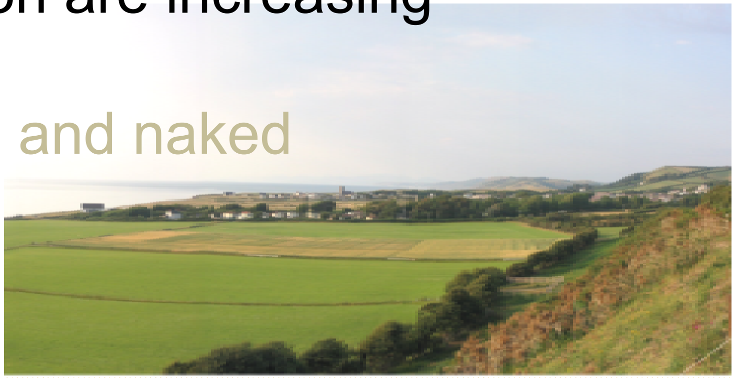

www.QUOATS.org

HARNESSING NEW TECHNOLOGIES FOR SUSTAINABLE OAT PRODUCTION AND UTILISATION

❖The ***Quality Oats*** (QUOATS) project brings together research organisations, levy boards, and industrial partners representing the oat production chain and the end users of the crop.

From breeder to plate, this project aims to harness new technologies to advance the yield, value and functionality of oats.

Research

Levy Boards

Industry

Breeding objectives

Economic competitiveness

- **Yield**
- **Disease resistance**
  - Crown rust
  - Powdery mildew (see poster: Pearson et al)
- **Lodging resistance**
- **Nitrogen use Efficiency** (see poster: Griffiths et al.a)
- **Milling quality**
  - Kernel content
  - Size/shape (see poster: Griffiths et al. b)
  - β-glucan
- **Animal feed** (Cowan et al. paper)
  - Oil (see poster: Cowan et al.)
  - Low lignin

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  - Oil
  - Low lignin

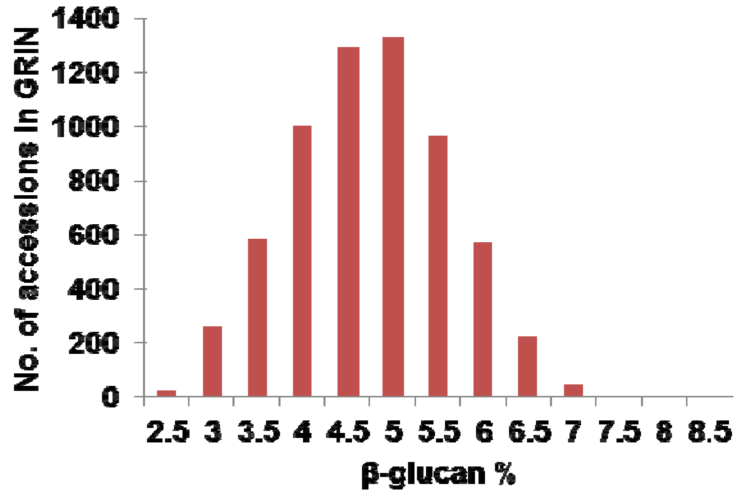
Requirements

- Genetic sources of enhanced β-glucan content
- Novel population development
- High throughput precise phenotyping
- Multi-locational testing
- High throughput marker systems
- Genomic resources eg ESTs, BAC libraries, TILLING, comparative genetics etc.

- **Identification of genetic variation for β- glucan content**
  - Oat varieties world-wide
  - Wild relatives of oats
- **Development of high throughput screening methodology**
  - Modified megazyme method
  - NIR
- **Conventional breeding using high β- glucan genetic sources**
- **Identification of markers closely linked to β- glucan content and use in marker assisted breeding**
- **Identification of genes involved in β- glucan synthesis and their control – comparative genomics**

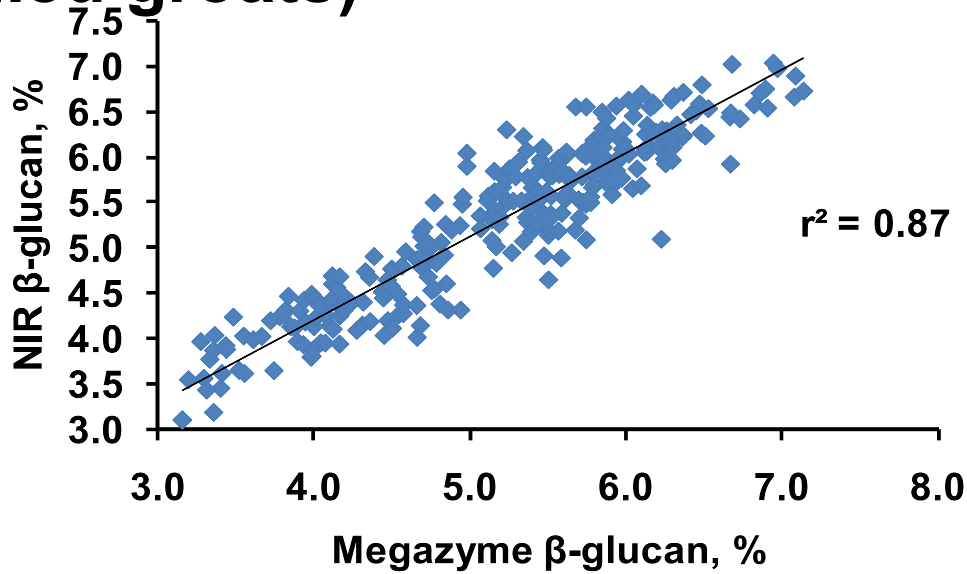
Genetic improvement of β-glucan content

- **High β- glucan sources**
  - CDC-Solfi , SA 99572 Brian Rossnagel, Saskatoon
  - HiFi, ND9508252-9, ND030287 Mike McMullen, North Dakota
  - IA03146-4, IA03146-6, IA03150-5 Jan Luc Jannick, Iowa
- **Novel variation in germplasm collections, landraces and wild species**
  - β- glucan content of accessions in USDA national genetic resources program collection (GRIN)
  - β- glucan content of 109 AFRI-CORE accessions
  - Survey of β- glucan content of wild relatives:
    maximum 11.3%
    minimum 2.2%

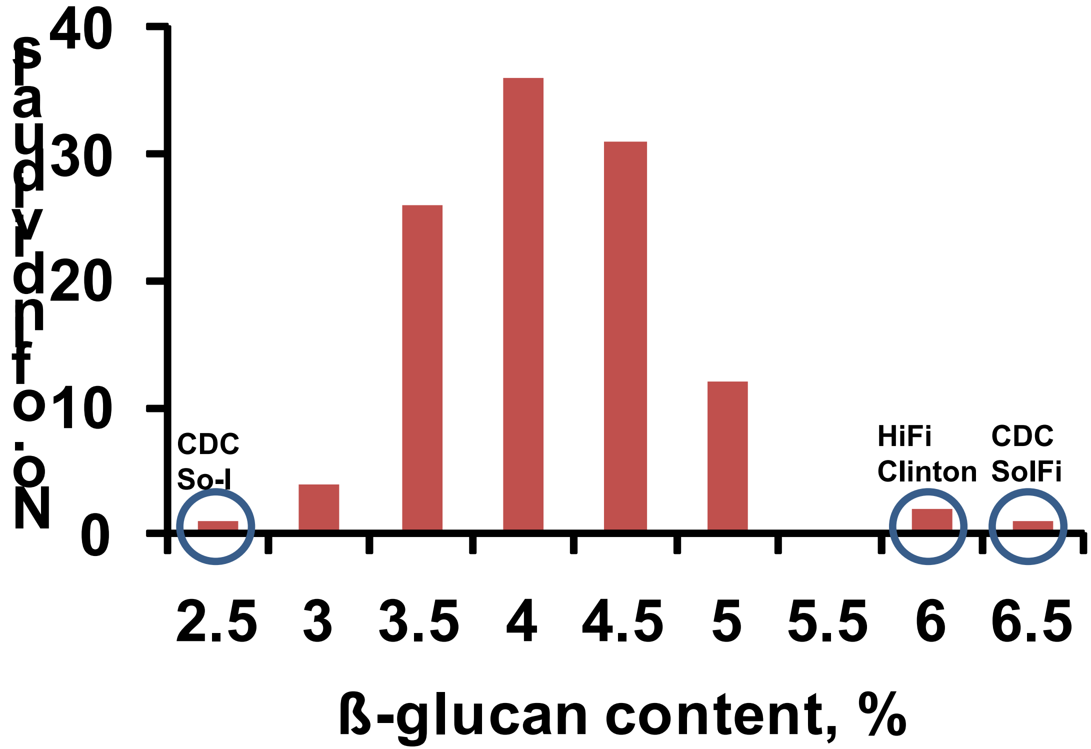


Development of high throughput phenotyping

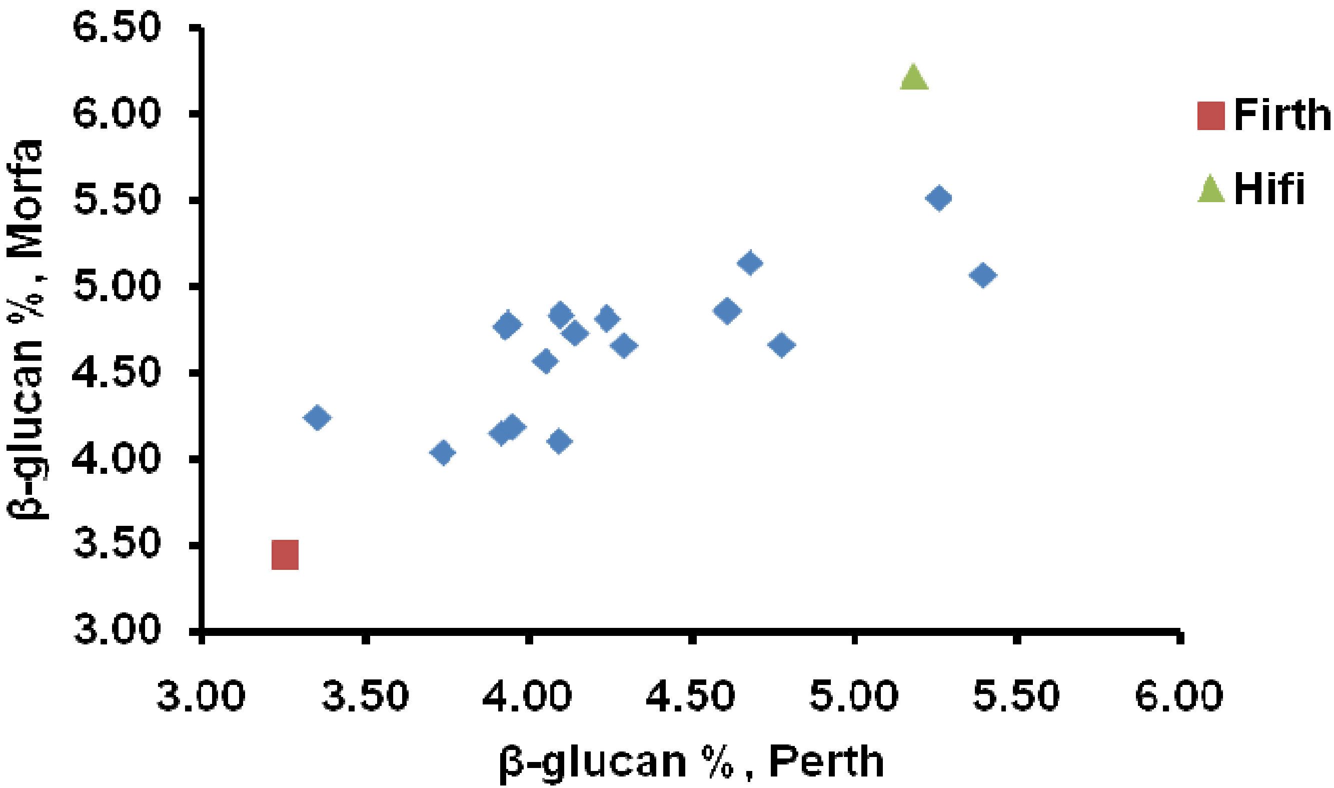
- Modified McCleary (Megazyme) assay for use in microtitre plate
- NIR calibration (de-hulled groats)
- FT-IR
- GC-MS



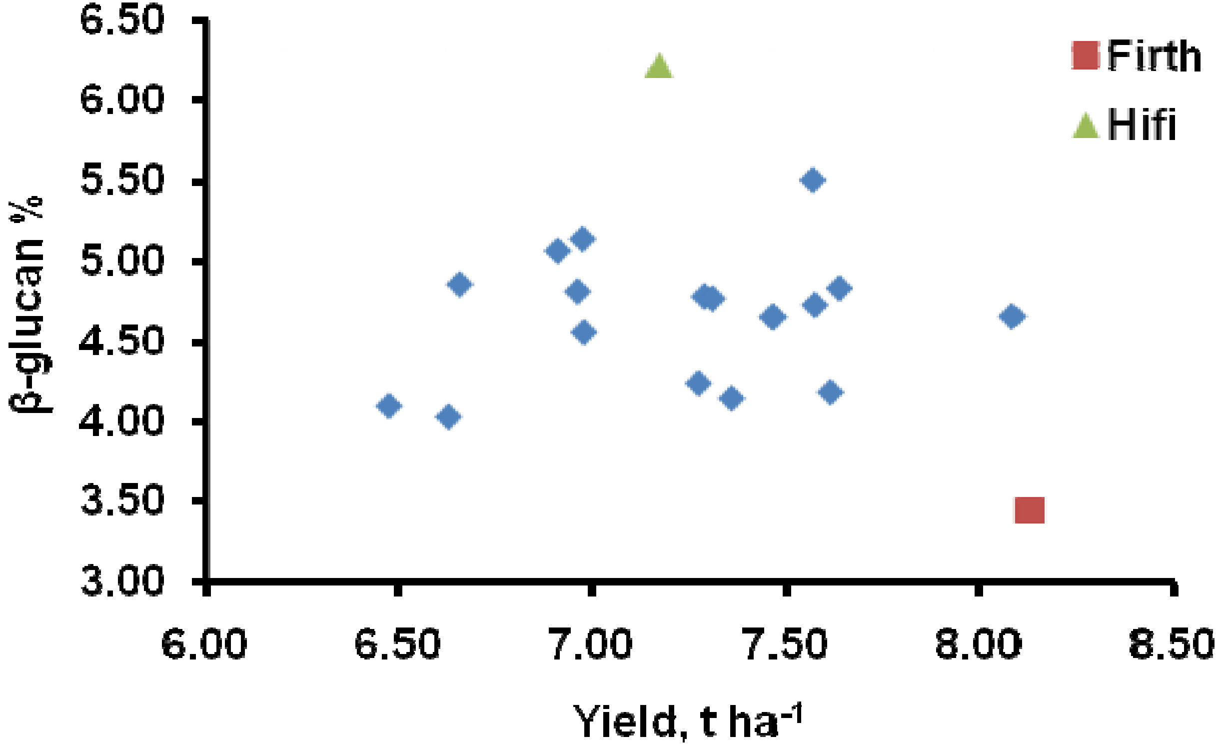
β-glucan content- 2011 Aberystwyth



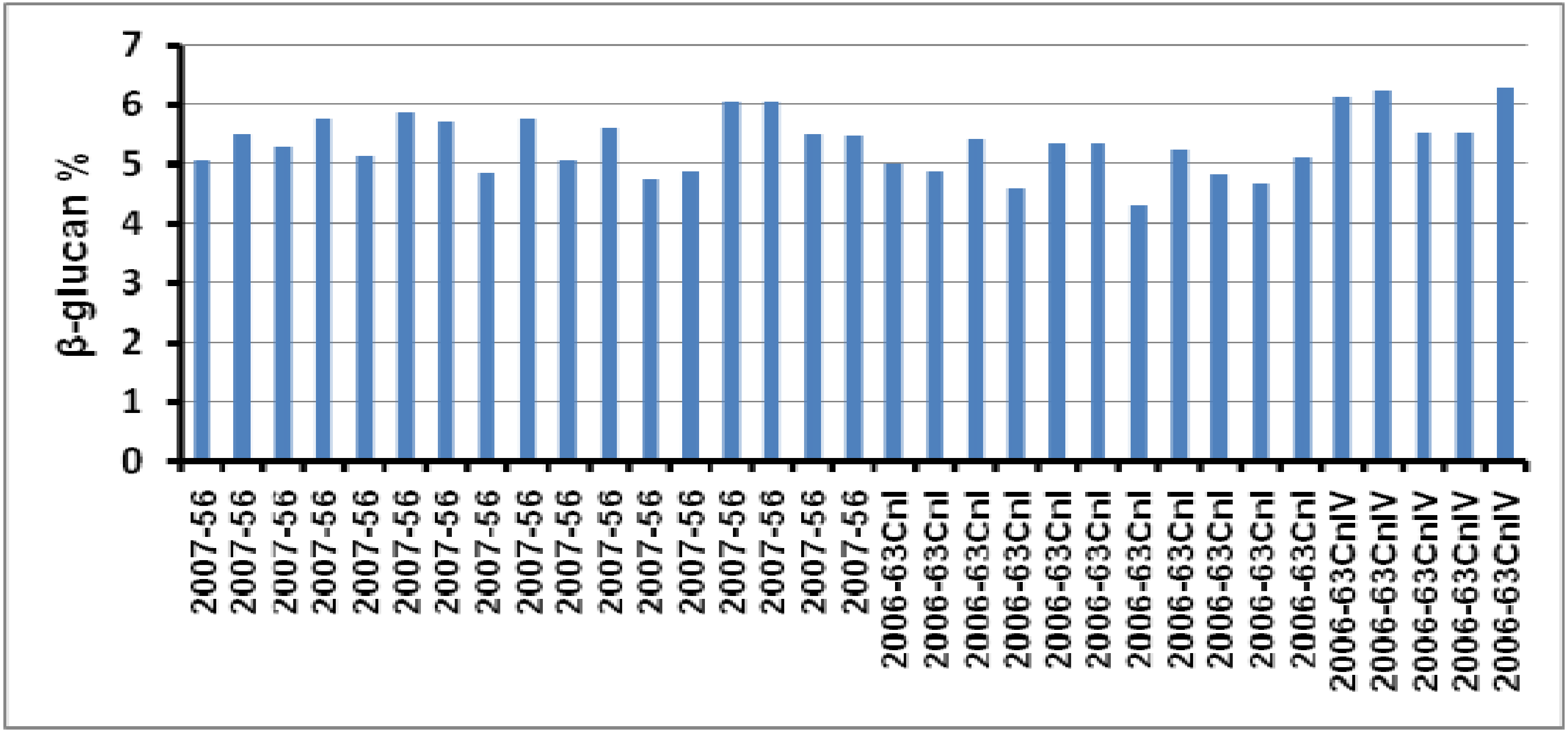
Success in improving β-glucan content in UK spring oats



Yield of β-glucan selections



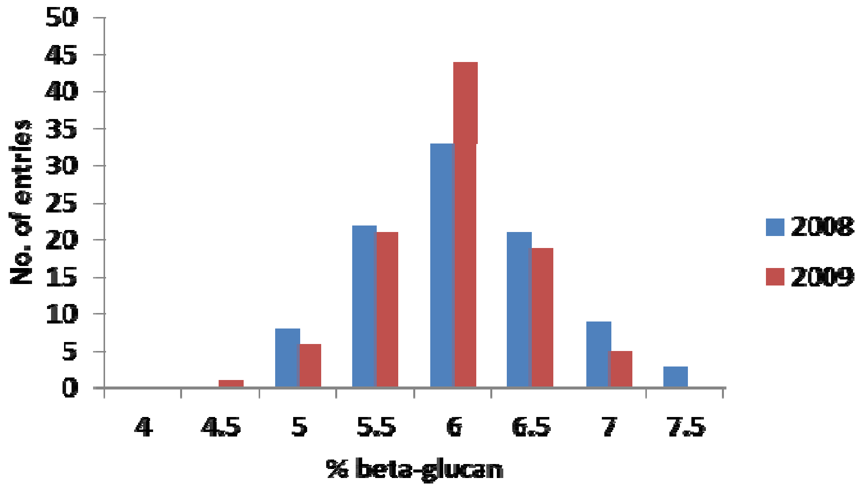
Success in improving β-glucan content in UK winter oats

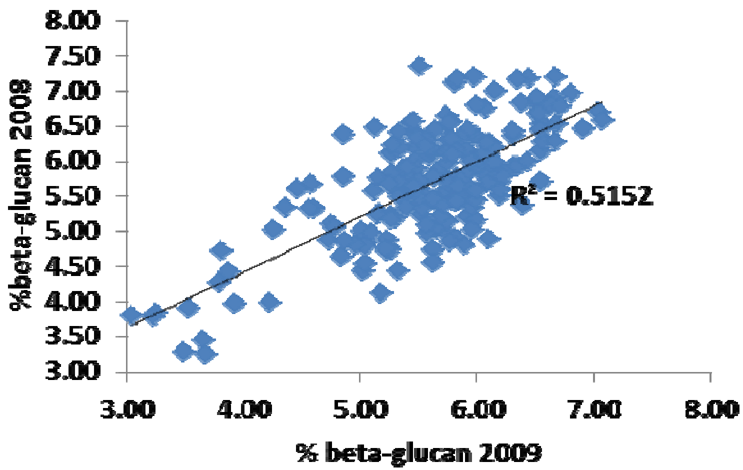


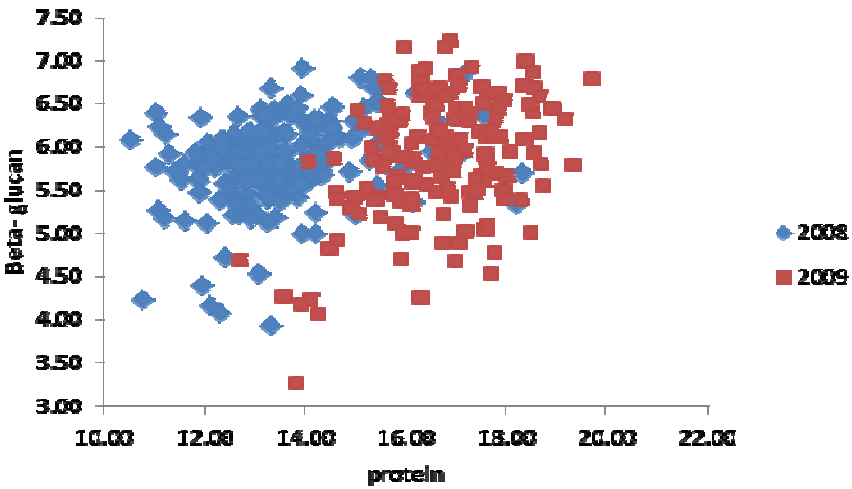
Spring oat mapping population: CDC-Solfi x HiFi

collaboration with Brian Rossnagel, Aeron Beatie Saskatoon

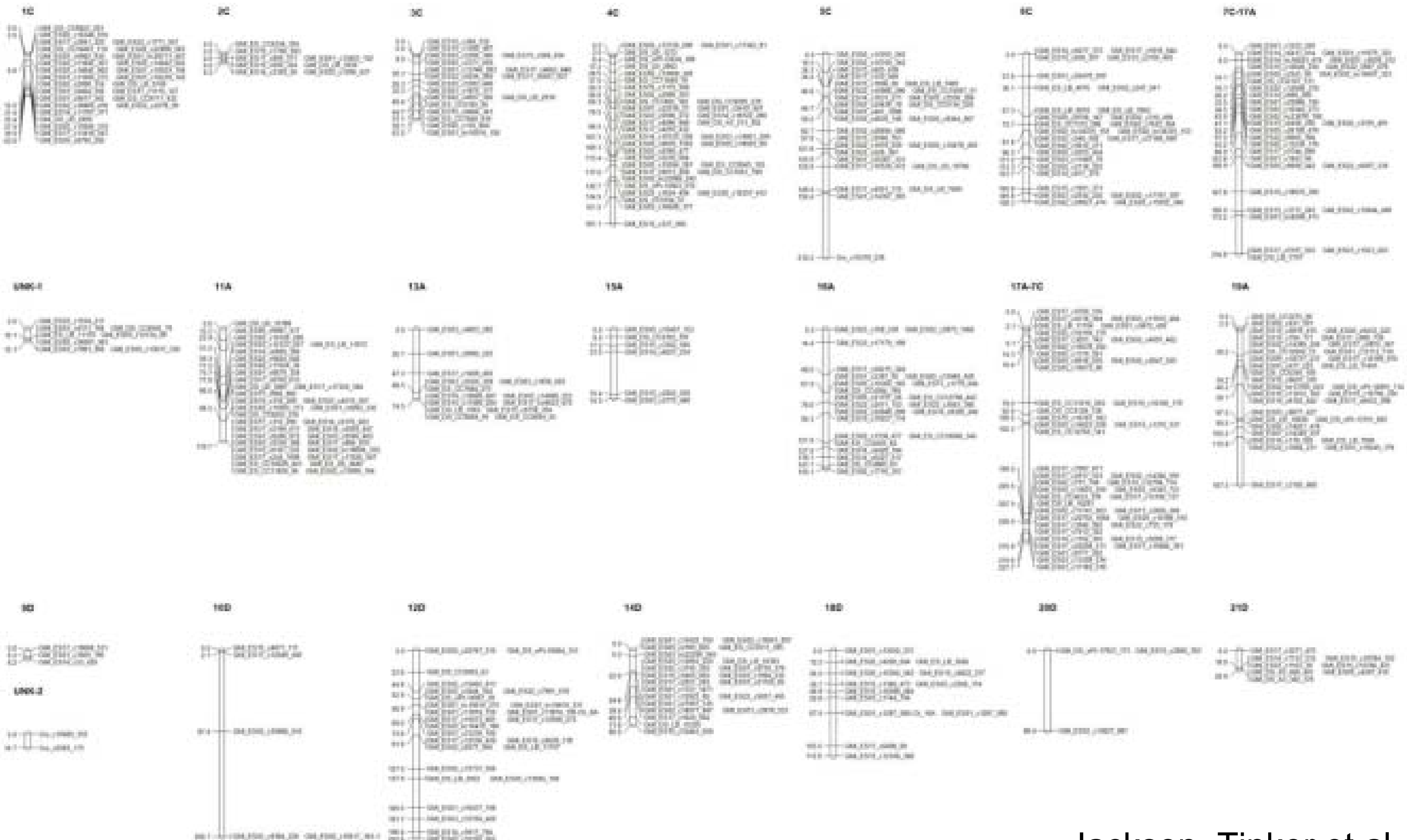
	2008	2009	2011
CDC Sol-Fi	6.02	6.15	6.40
HiFi	6.18	n.d.	6.12



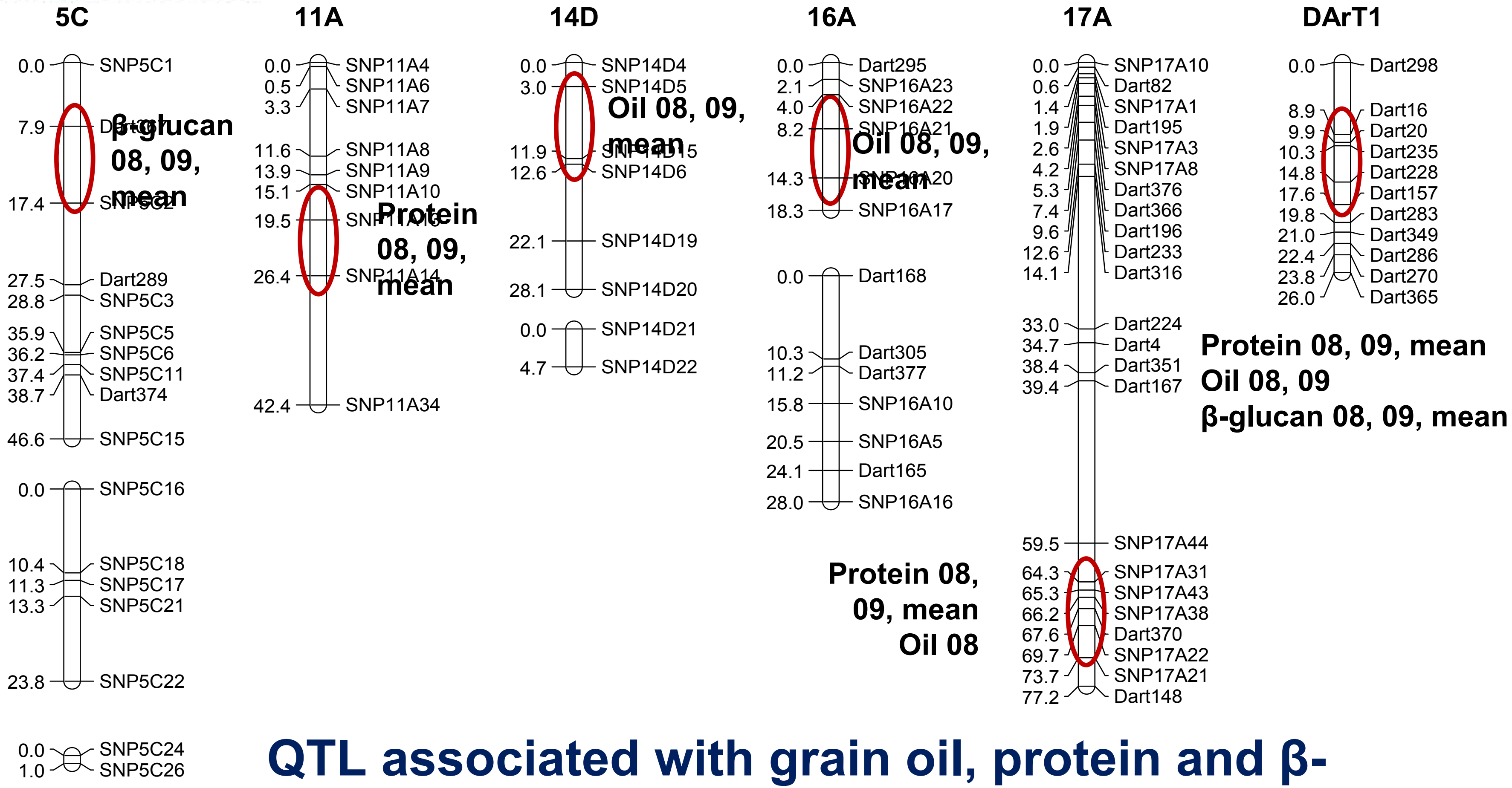




CDC SolFi x HiFi genetic map



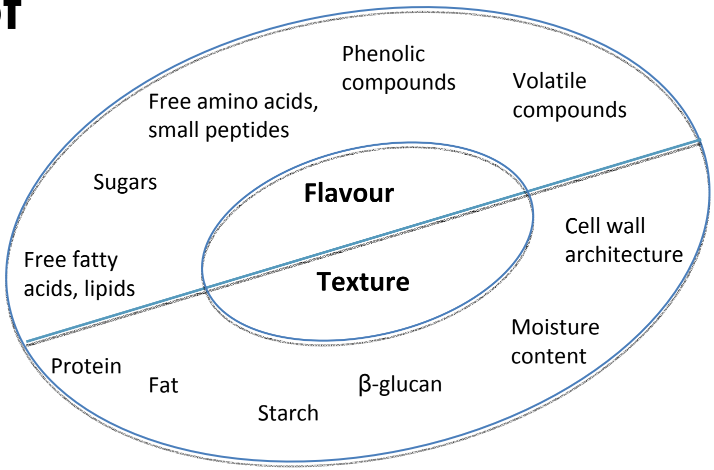
Jackson, Tinker et al



QTL associated with grain oil, protein and β-glucan content in CDC SolFi x HiFi population grown in Aberystwyth

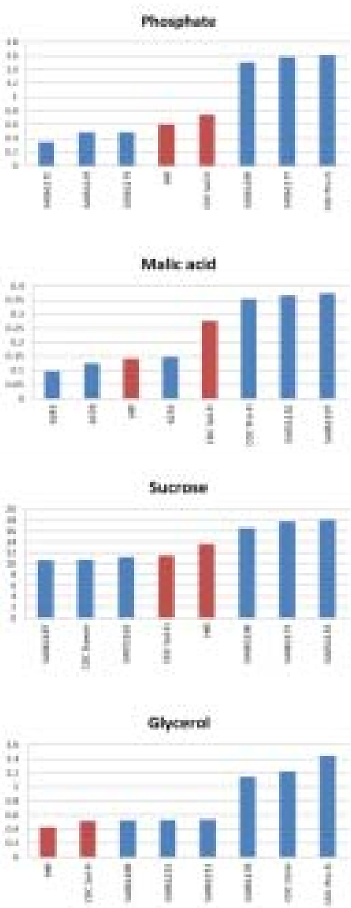
Current work CDC Sol-Fi x HiFi mapping population

- In field again this year
- PhD student, Claudine Cognat (James Hutton Institute) under supervision of Derek Stewart and IBERS scientists is undertaking metabolomic analysis of grain to understand the variability in metabolite content
- Relate metabolomic data to genetic information: develop mQTLs.



Components of oat grain influencing perceived texture and flavour (*Salmenkallio-Marttila et al., 2011*)

Metabolomic analysis



Extraction
Oat (milled) + Internal Standards
In Methanol/Water/Chloroform

Polar Fraction

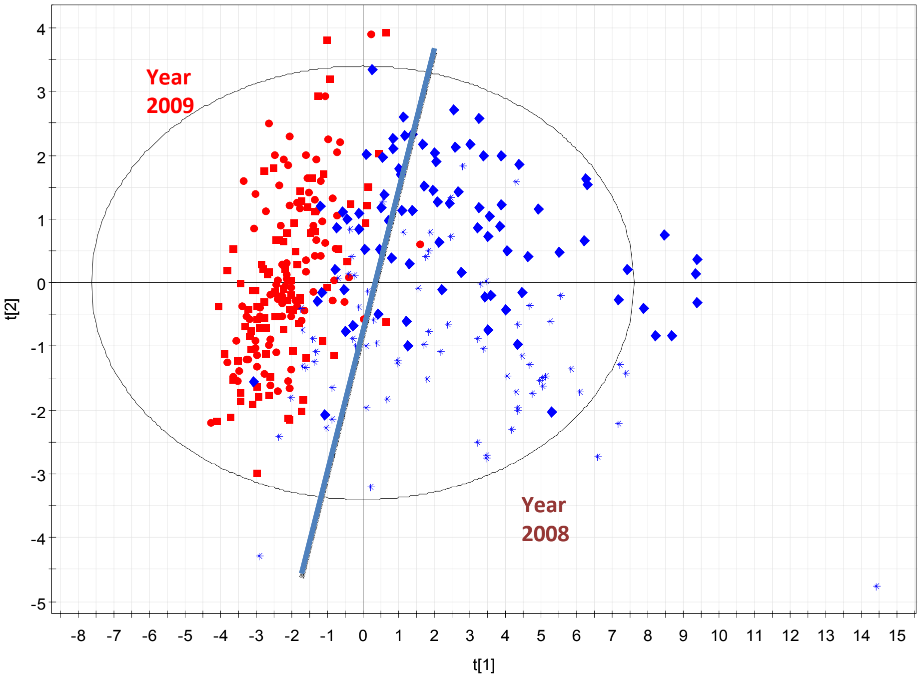
Amino acids, Organic acids, Sugars etc.

Non-Polar Fraction

Fatty Acids, Fatty Alcohols, Alkanes, Sterols.

Analysis by GC-MS

CDC SolFi x HiFi mapping population



PCA (Principal component Analysis) of all polar metabolites from CDC Solfi x HiFi population (milled samples), coloured by year (■:2009; ■: 2009). Components 1 and 2 explain up to 56% and 11% of the variation, respectively.

Based on 41 polar metabolites:

•Separation between year 2008 and 2009, due to higher amounts of all metabolites in year 2008.

•*Hypothesis*: oat samples cultivated at the same location in Wales, but clearly the annual environments were not the same.

Data from Claudine Cognat

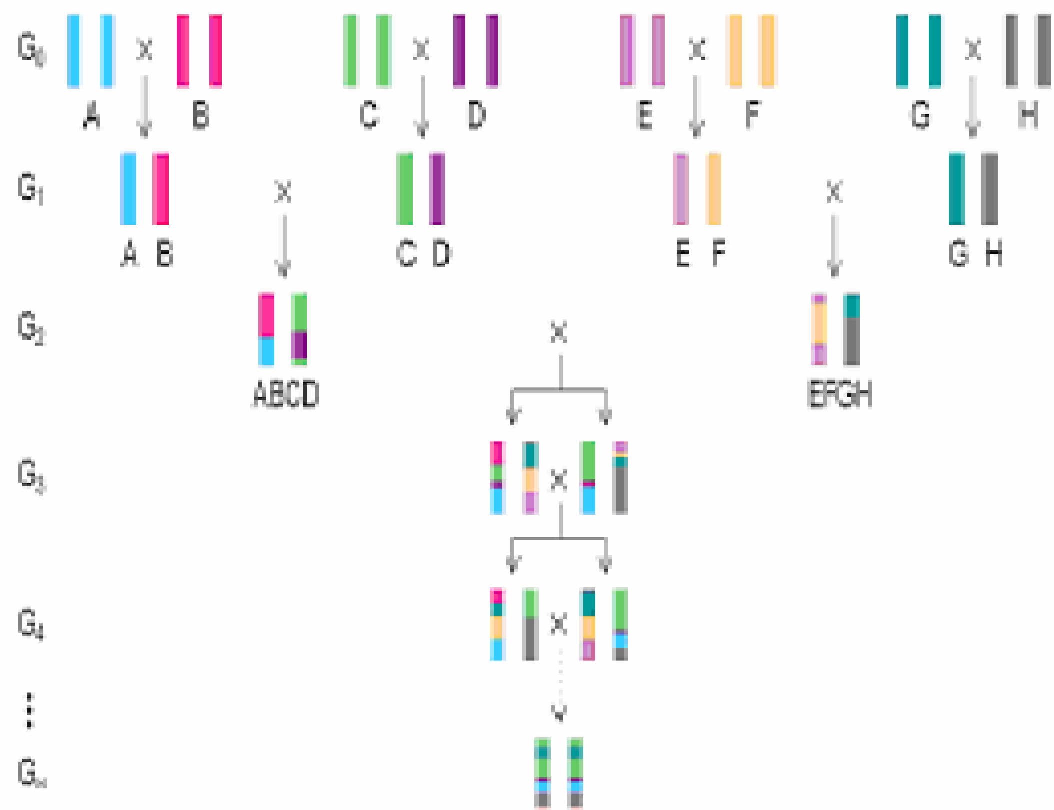
Population development

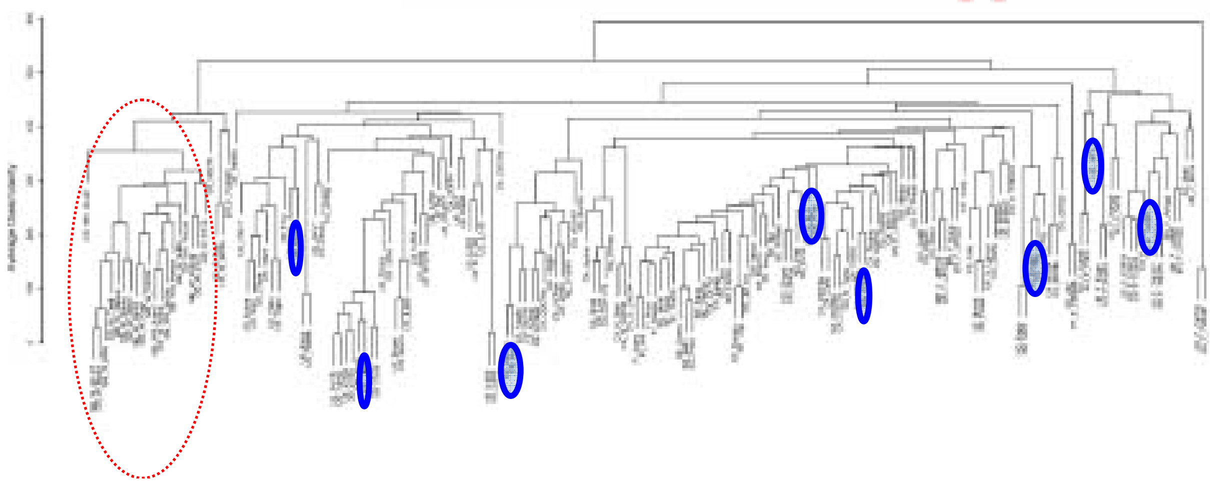
- Bi-parental
- Association mapping
- MAGIC population
- Nested Association mapping
- Wild relatives
- TILLING
- QTL-NILs
- Breeding programme crosses for testing/ validation of MAS

Novel population development; MAGIC

(multiparent advanced generation inter-cross)

8 spring oats chosen to sample world-wide genetic diversity (highlighted in blue in dendrogram from results from DArT analysis)





MAGIC population development

2009: 1st generation of crosses successfully completed (28 x 2 way crosses)

		1	2	3	4	5	6	7	8
		Ogle	TAM O-301	Ac Assiniboia	HiFi	CDC Dancer	Firth	Pol	SolFi
1	Ogle								
2	TAM O-301	12							
3	Ac Assiniboia	13	23						
4	HiFi	14	24	34					
5	CDC Dancer	15	25	35	45				
6	Firth	16	26	36	46	56			
7	Pol	17	27	37	47	57	67		
8	SolFi	18	28	38	48	58	68	78	

2010: 2nd generation successfully completed (28 crosses combining 4 genotypes)

2011: 3rd generation successfully completed (42 crosses combining 8 genotypes)

8 seeds from each of these 42 crosses sown for single seed descent (SSD) summer 2011 and seed harvested October 2011 (population size 336 individuals)

2012: 2nd generation of SSD sown February 2012

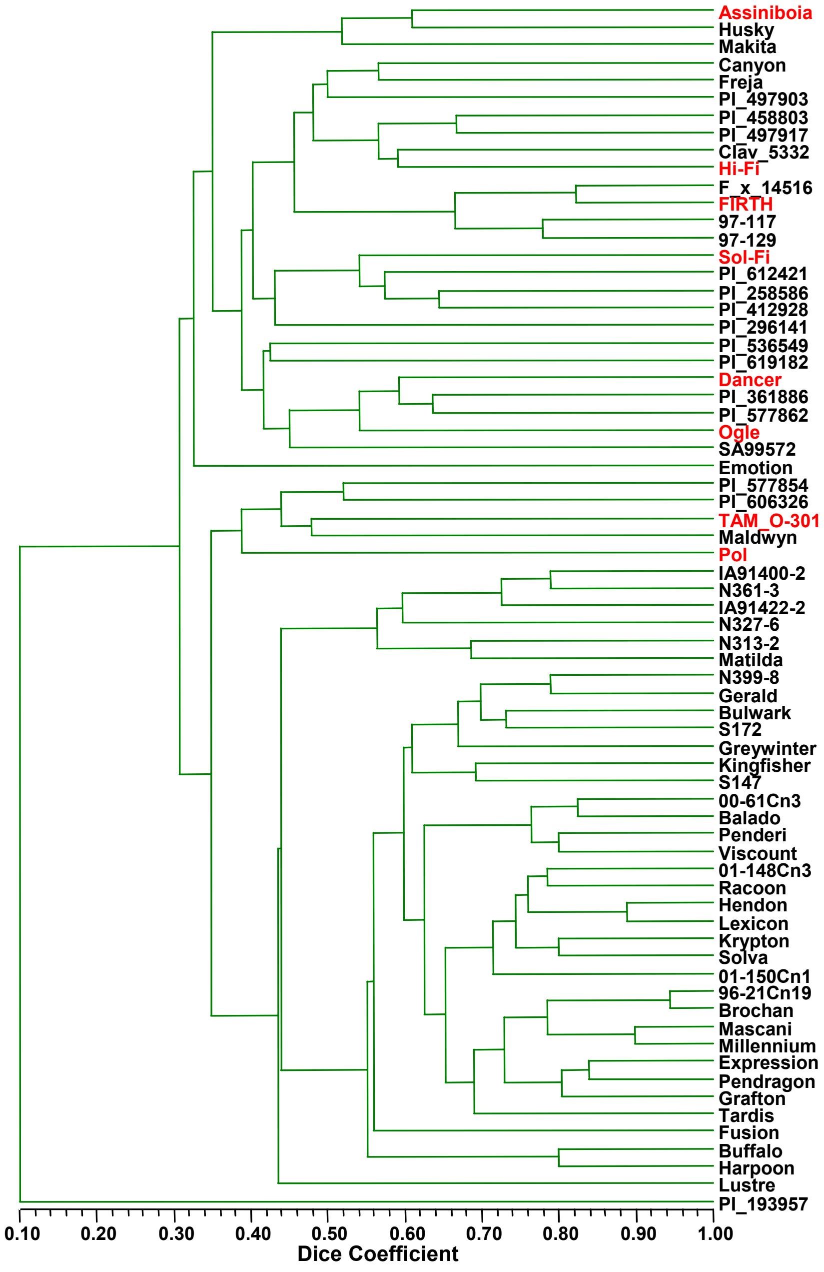
Population on schedule for first field sowing in Spring 2014

NAM hexaploid populations

- Common parent is Firth (spring)
- 15 populations currently at F4 (most have 60 progeny, some less)
- F5 expected to be grown in glasshouse for preliminary phenotyping of at least some populations in summer 2012.

Parents of F4 populations all spring - 7 high β-glucan, 2 low β-glucan, 4 landraces (1 tetraploid), 4 wild species introgressions, 1 naked

Genetic diversity of MAGIC, NAM parents and selected winter oats (63 SSRs)



Population development

- Bi-parental
- Association mapping
- MAGIC population
- Nested Association mapping
- **Wild relatives**
- **TILLING**
- **QTL-NILs**
- **Breeding programme crosses for testing/ validation of MAS**

Avena atlantica

- Collected on Atlantic coast of Morocco by M. Leggett
- Early flowering (primary stem)
- Few productive tillers at harvest
- Seed shed
- Covered seed
- High β-glucan

Avena strigosa

- Cultivated; Ceirch Llwyd (Welsh), Corc beag (Gaelic), sand, grey, black or small oat
- Late flowering (primary stem)
- Many productive tillers at harvest
- Seed held
- Naked seed

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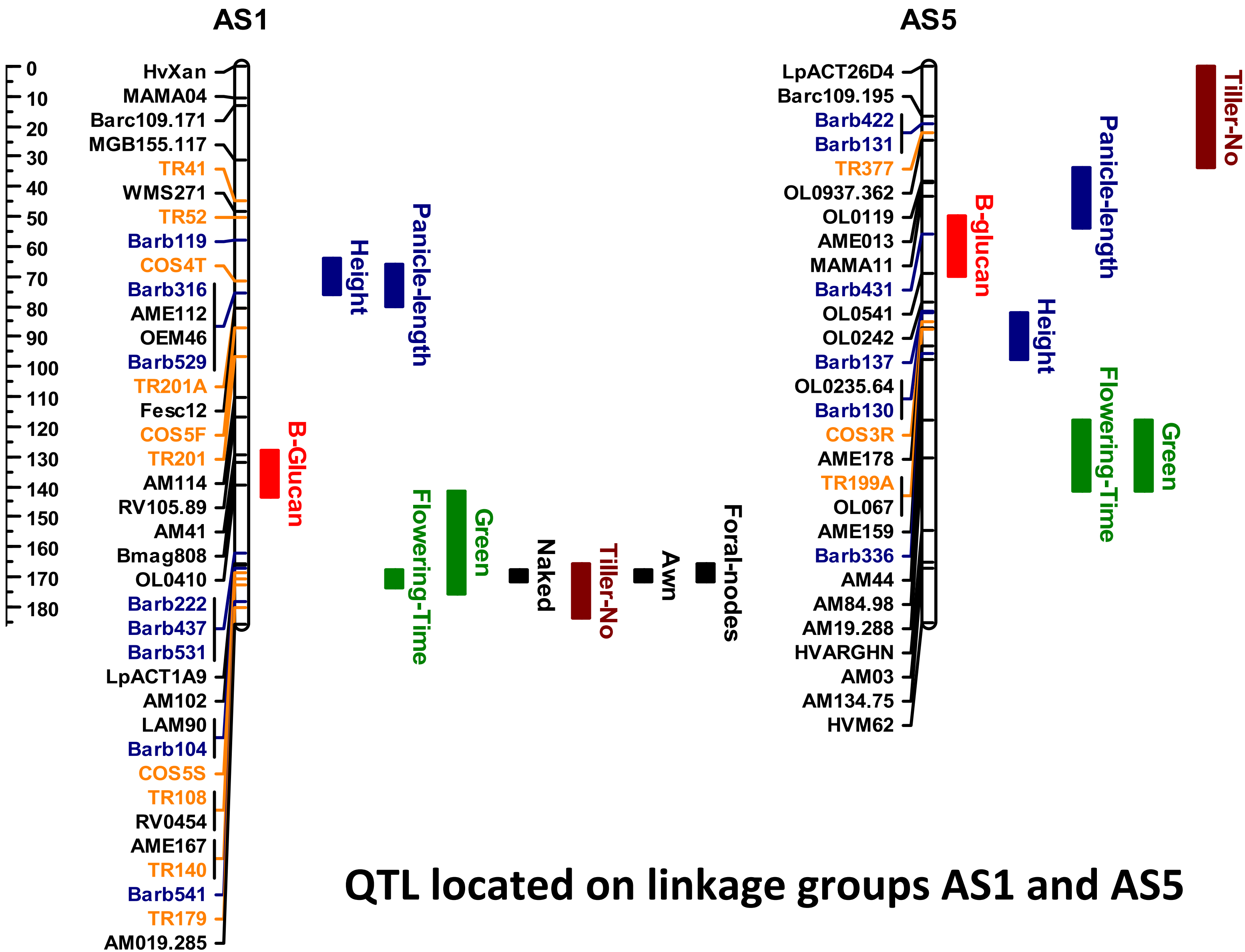
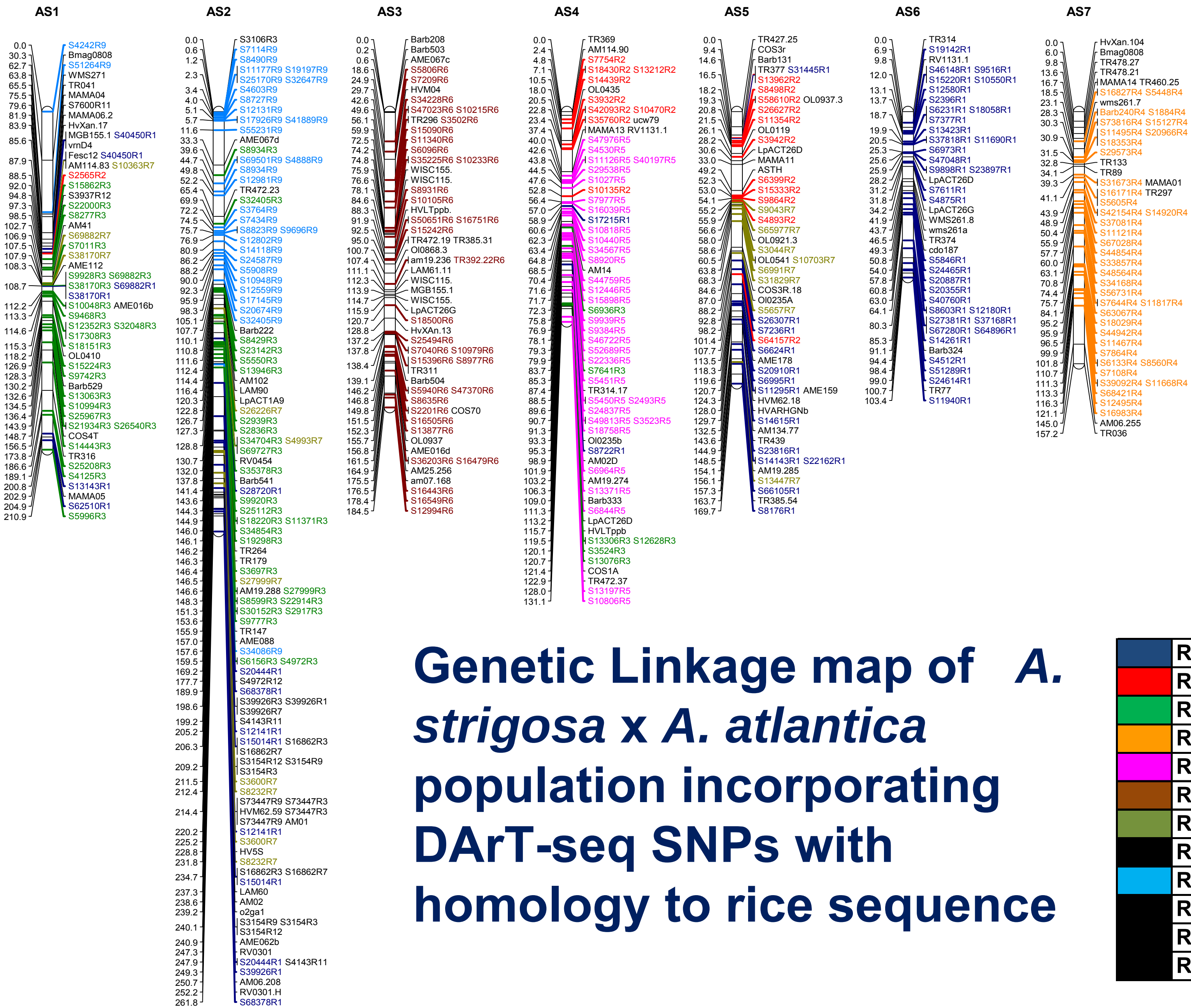


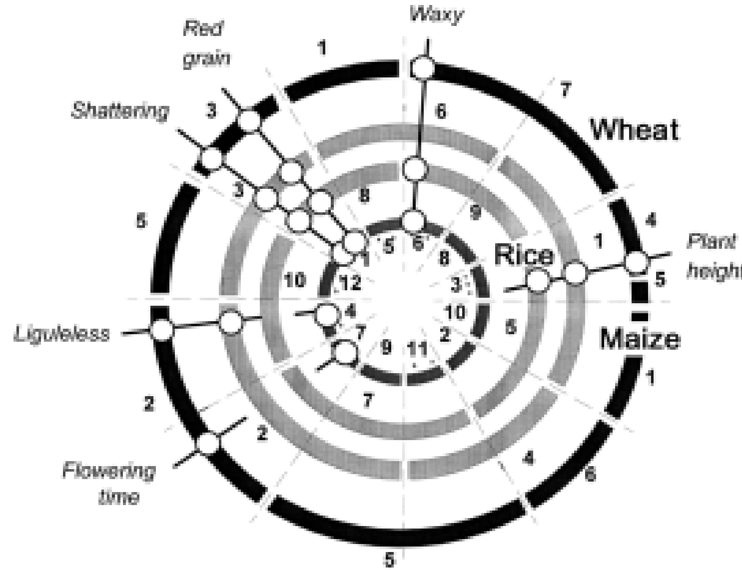
QTL located on linkage groups AS1 and AS5

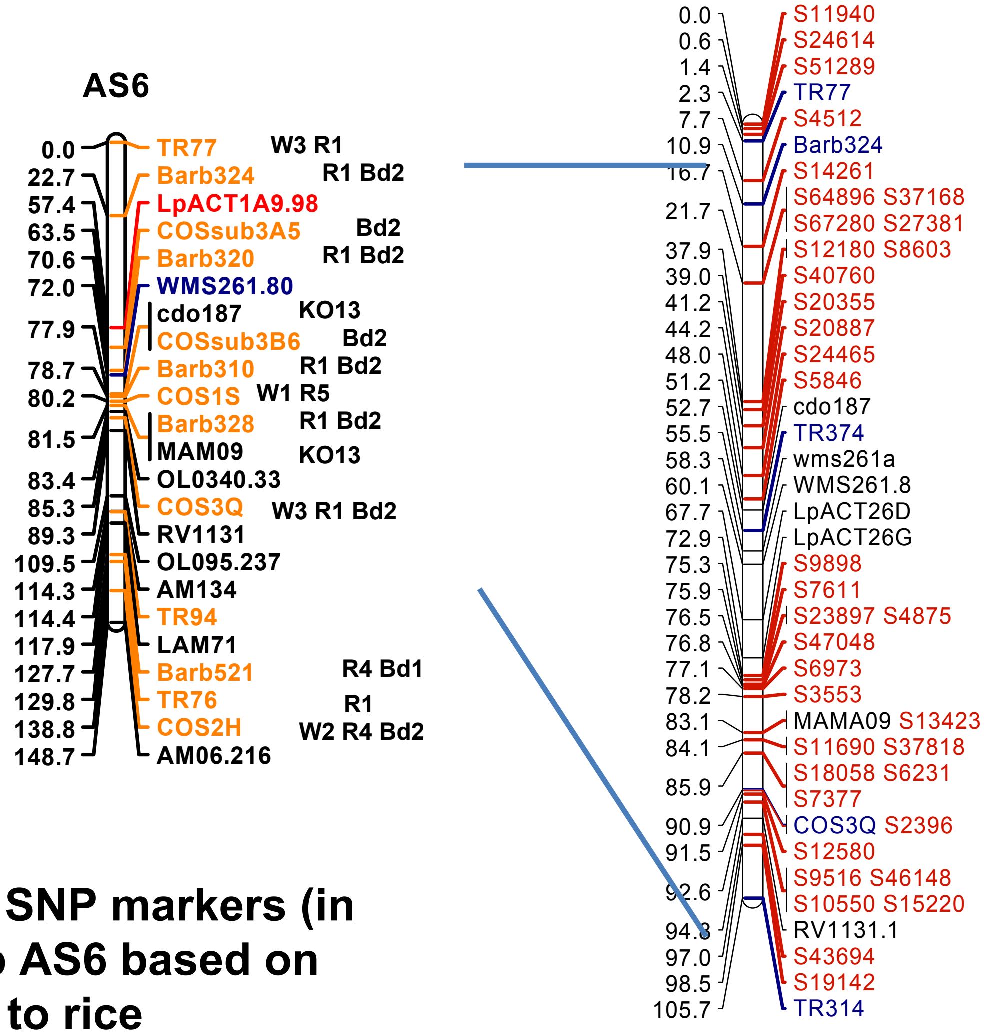


Genetic Linkage map of *A. strigosa* x *A. atlantica* population incorporating DArT-seq SNPs with homology to rice sequence

	Rice 1
	Rice 2
	Rice 3
	Rice 4
	Rice 5
	Rice 6
	Rice 7
	Rice 8
	Rice 9
	Rice 10
	Rice 11
	Rice 12

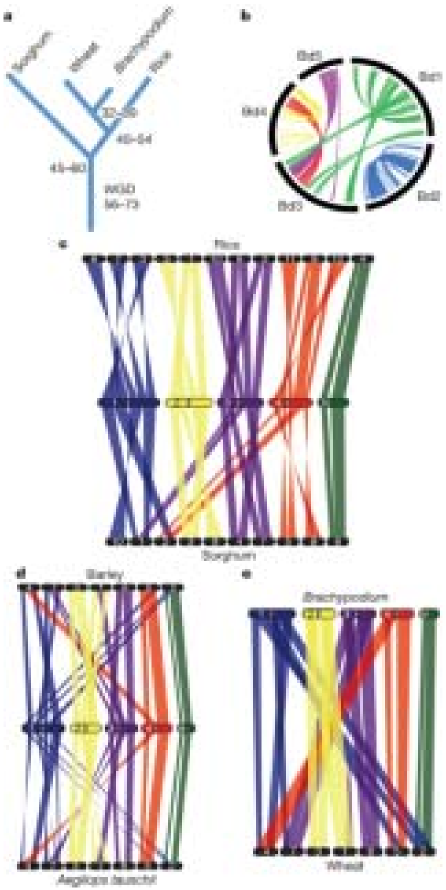
- **Leveraging information from model genomes**
- **Predicting gene location**
- **Validation**





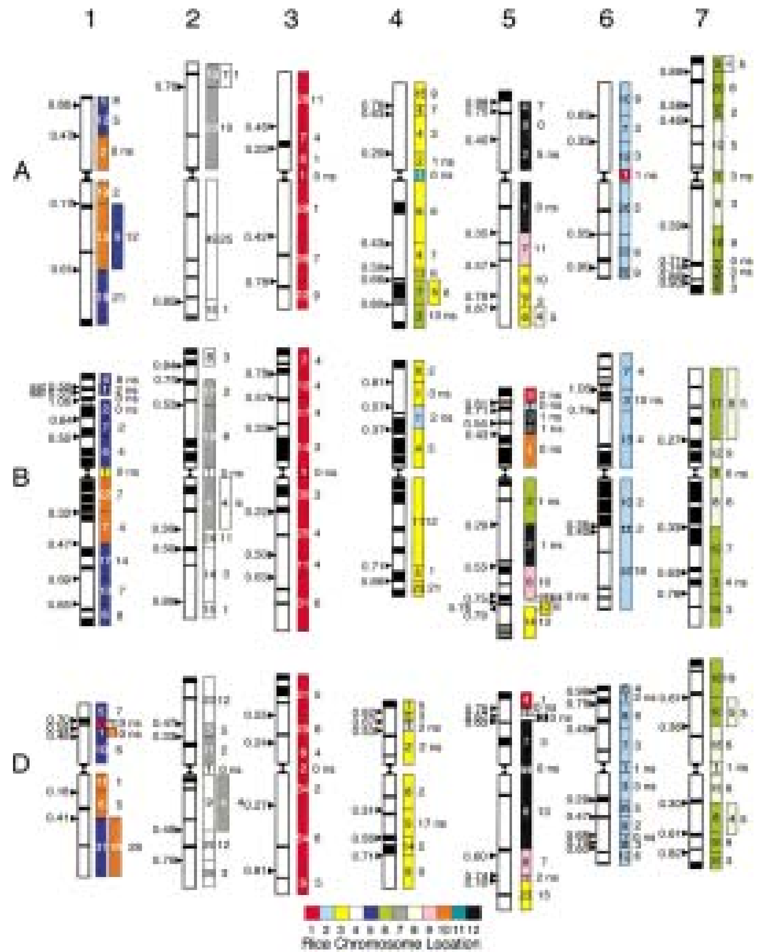
Targeted addition of SNP markers (in red) to linkage group AS6 based on sequence homology to rice chromosome 1, and *Brachypodium* chromosome 2

Brachypodium genome evolution and synteny between grass subfamilies.



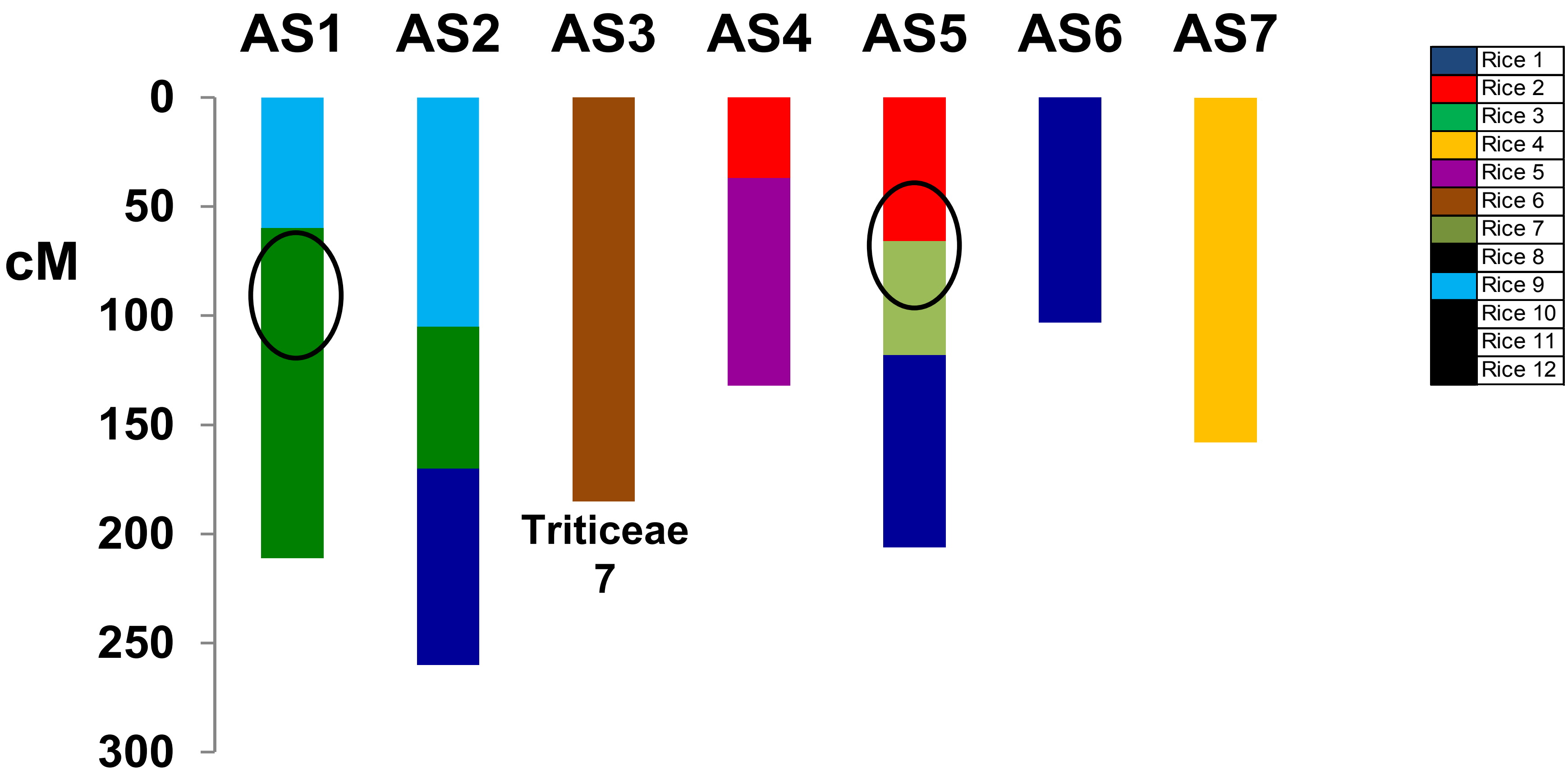
JP Vogel *et al.* *Nature* 463, 763-768 (2010) doi:10.1038/nature08747

Wheat–rice genome relationships.



Sorrells M E et al. Genome Res. 2003;13:1818-1827

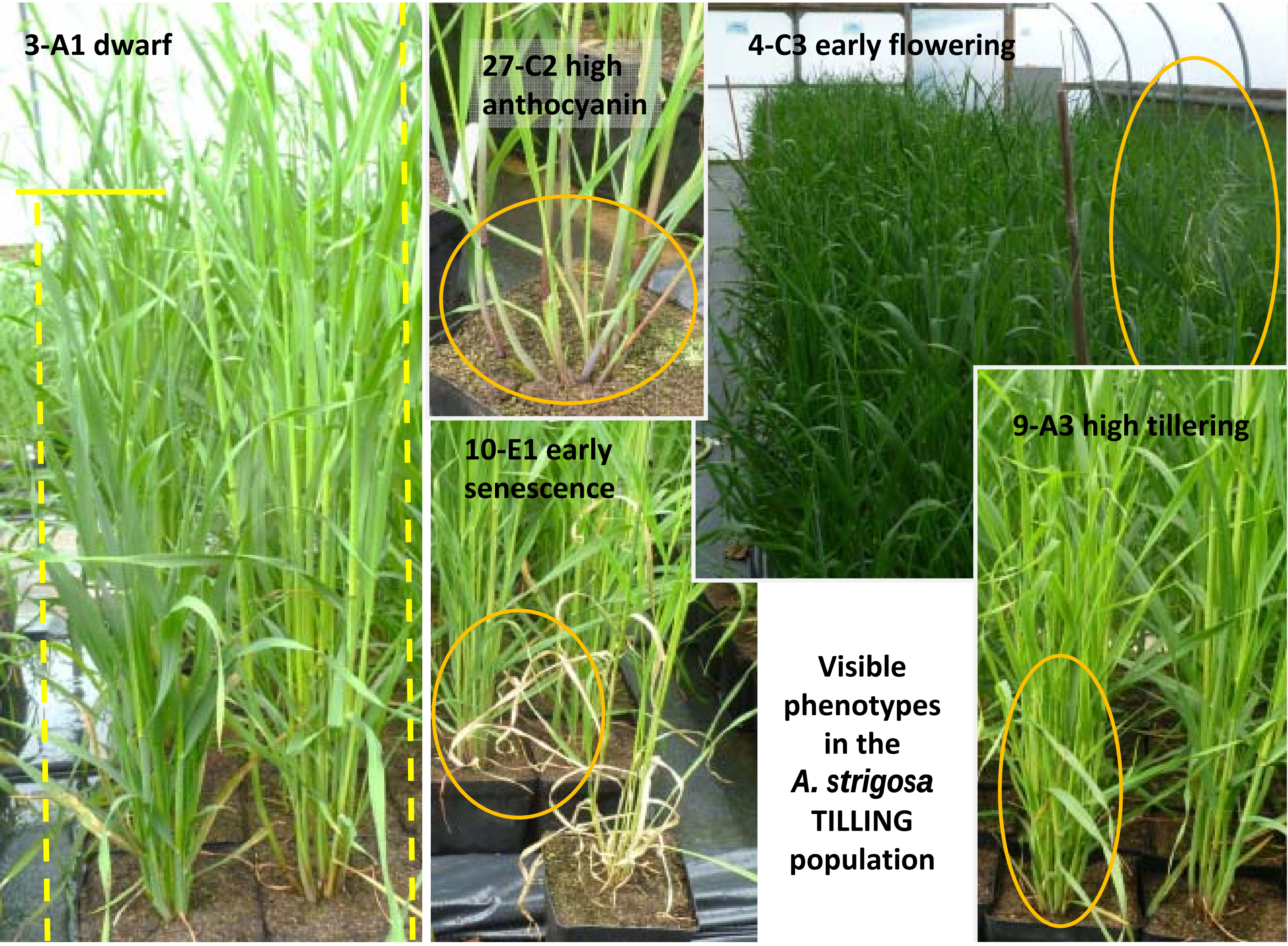
Diploid oat- rice genome relationships



TILLING (Targeting Induced Local Lesions IN Genomes)

Azide treated *A. strigosa* (Anne Osbourn, JIC)
Validated by recovery of root metabolite mutants
>800 plants grown 2011, DNA and seed taken
>1600 plants growing 2012, DNA taken
DNA stocks and screening at JIC (Trevor Wang)

Allows identification of plants with mutations in given gene (NB plus 1000 other mutations!)
Can also screen for phenotypes (eg disease mimic, high tillering, low lignin, panicle architecture)


3-A1 dwarf
27-C2 high anthocyanin
4-C3 early flowering
10-E1 early senescence
9-A3 high tillering
Visible phenotypes in the *A. strigosa* TILLING population

Use of markers in breeding programme

- **Follow beneficial alleles associated with traits of interest in breeding programme**
- **Identify suitable parents for crossing**
- **Confirm success of crossing**
- **Cultivar identification**

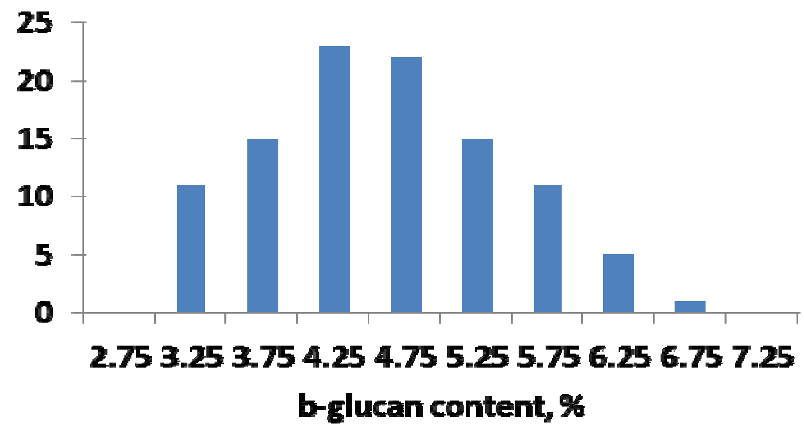
		011-SO2011		038-SO2011		047-SO2011	
		1488Cn2/1/1	Canyon	Ac Assiniboia	Gandalf	1478Cn3	Circle
OL0702	103						
	105	1	1		1	1	1
	107			1			
	112						
	113						
	114		1	1		1	
	117						
	119						
MAMA1	164						
	174						
	184						
	186						
	188						
	192				1		
	196	1	1				1
	198						
	200						
	204					1	
	208			1			
	212						
	216						
MAMA3	334						
	368						
	366						
	368						
	374						
	376			1	1	1	
	380						
	384						
	388						
	392	1	1				1
	395						
	399						
	404						
	464						
	467						
	471	1		1	1	1	1
	[illegible]		1				
	479						
	483						
	487						
	504						

Populations for validating marker-trait associations

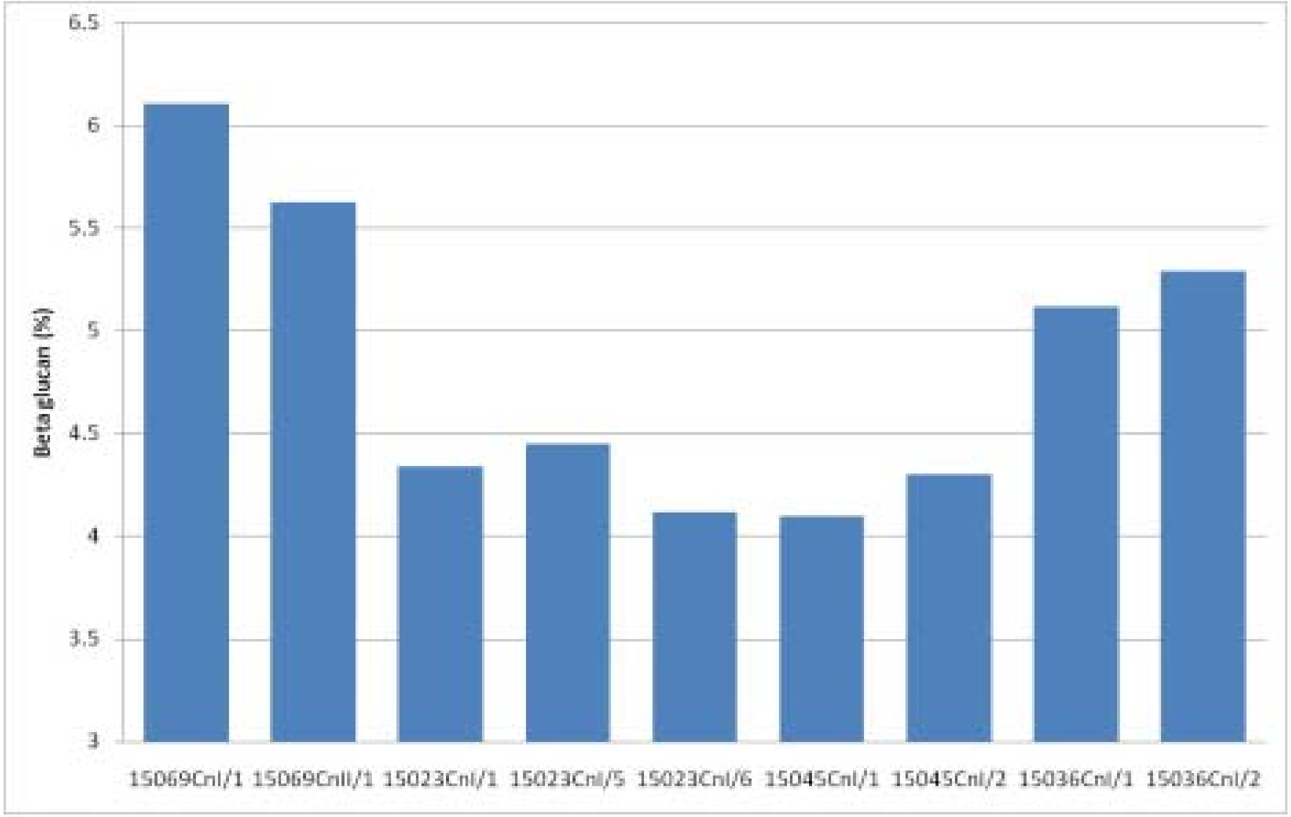
2010: 3 F2 populations selected for β-glucan:

- 08-187Cn1 (HiFi x 01-150Cn1)
- 08-60Cn2 (SolFi x 01-150Cn1)
- 08-56Cn1 (ND9508252-9 x 01-15Cn1)

2102: 12 F2 populations selected for MAS for range of traits including β-glucan, oil and disease resistance



Advanced spring oat trial – β- glucan



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