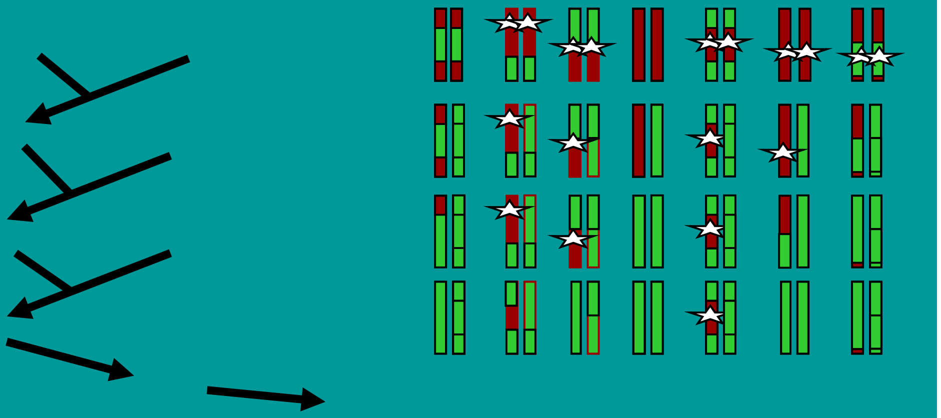
WGIN2 JIC Update

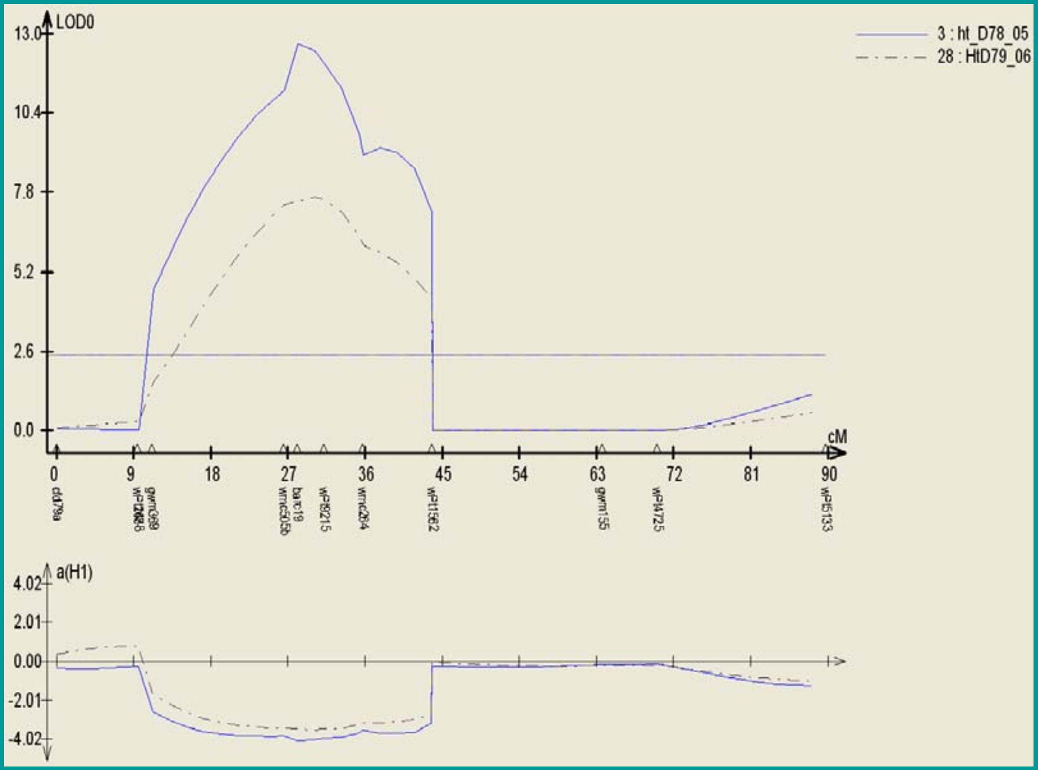
Management meeting Feb ‘10

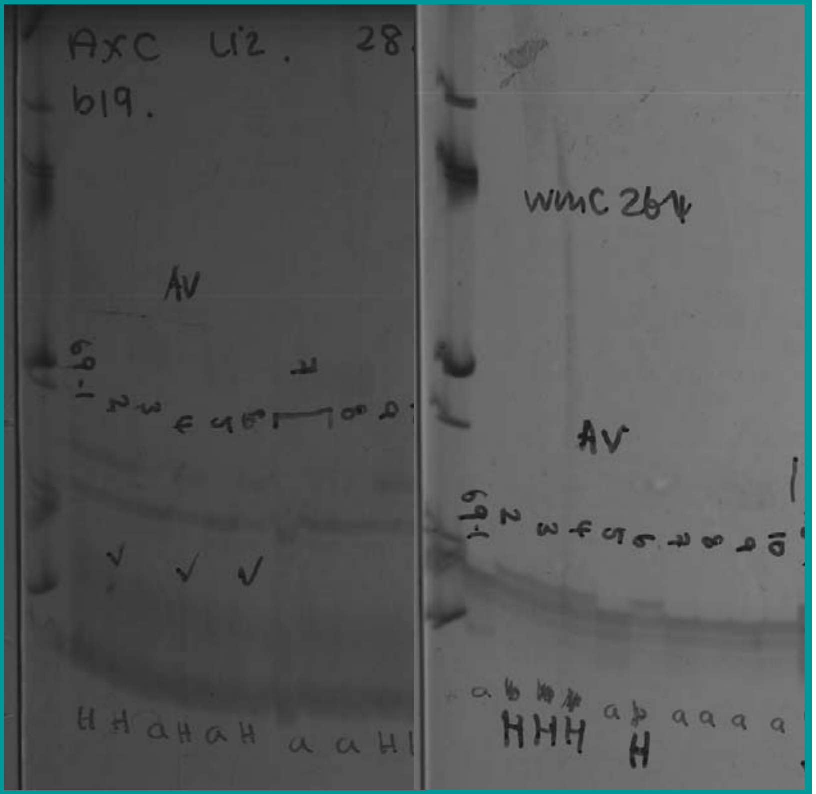
Development of Near Isogenic Lines

Near Isogenic Line development
-Example of strategy for 3A height



WGIN1 → WGIN2

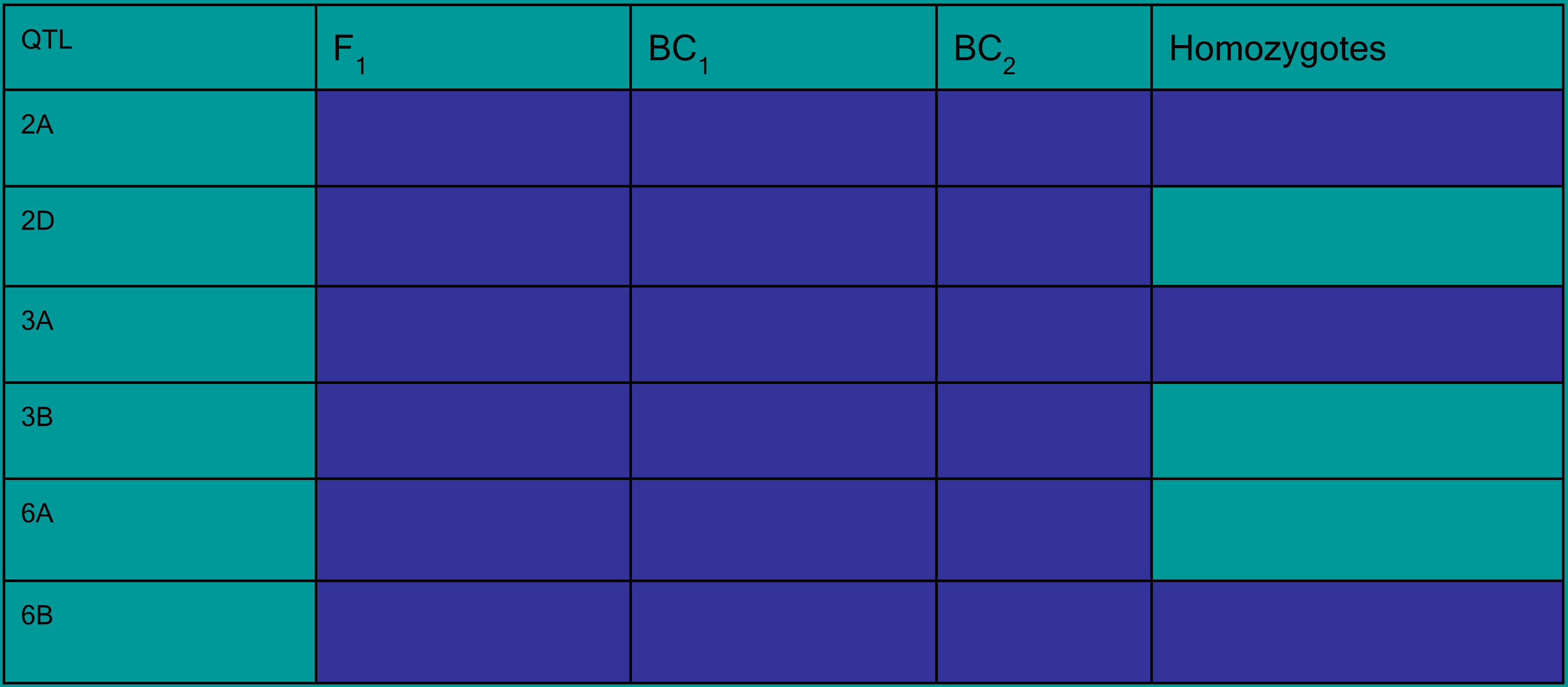




Avalon x Cadenza Near isogenic line development- Yield

QTL	F_1	BC_1	BC_2	Homozygotes
2D	■	■	■	
3B	■	■	■	
5A	■	■	▨	
7B	■	■	■	
7D	■	■	■	■

Avalon x Cadenza Near isogenic line development- Crop height



QTL	F_1	BC_1	BC_2	Homozygotes
2A	■	■	■	■
2D	■	■	■	
3A	■	■	■	■
3B	■	■	■	
6A	■	■	■	
6B	■	■	■	■

Avalon x Cadenza Near isogenic line development- Ear emergence

QTL	F_1	BC_1	BC_2	Homozygotes
1B	🟦	🟦	🟦	🟦
1D	🟦	🟦	🟦	
6B	🟦	🟦	🟦	

Wheat Functionality QTL

- QTL prioritised from Functionality LINK
- Only Malacca x Hereward
- Only Spiral White process
- 5 BCs to Malacca and 3 to Hereward
- QTL for: loaf volume (7B and 2D), cell number (6A and 1B), L* (4D), cell wall thickness (7A), and firmness (2B).
- BC_2 seed (Lesley Fish)

Development of *Lr19/Sr25* NILs

Why – Lr19 on segment of *Agropyron elongarum* translocation to 7DL, as well as disease resistance CIMMYT breeders identified a yield advantage in some environments.

CIMMYT donors:

- Wheatear
- Kambari 1
- Oasis- no pigment

UK recurrent parents:

- Alchemy
- Paragon

Progress:

- BC2 made
- Selection with dominant marker
- WGIN COS are co-dominant so ideal for selection of homozygotes

AE Watkins Collection and population development

Ten AE Watkins x Paragon SSD populations- now at F_4

- Extremes- height and ear emergence. Ten SSD populations up to F_4 plants, therefore F_5 seed in June ’10- 4 fully on track, the other six have winter type stragglers!

- Thousand grain weight, grain length and grain width measured on 1100 Watkins lines. Data available on WGIN website.

- Also 2 SSDs Paragon X CS and JIC synthetic up to F_4. F_6 seed now sown and DNA extracted from F5. Already used for mapping. Availability of pops (including AxC) advertised in Graingenes organised by Justin Farris.

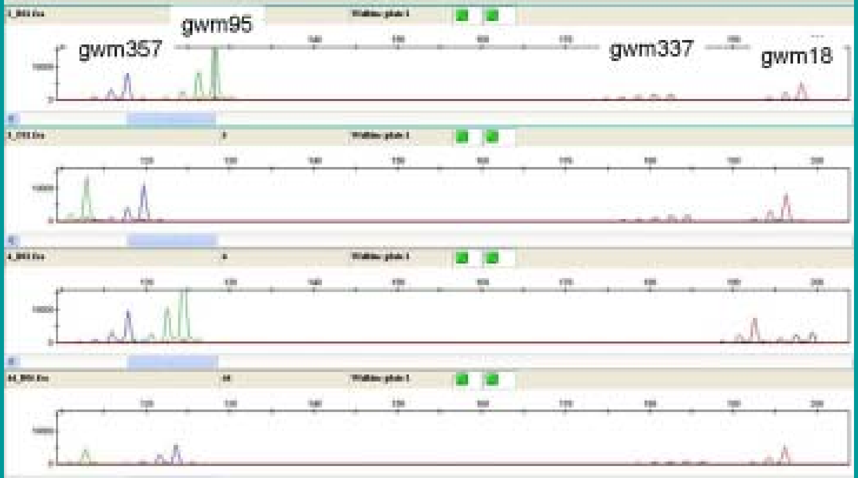
- 1071 Watkins accessions in soil house
- will go into field (Hege 90, 1m2) Autumn ’10.

- DNA extraction of soil house material (bagged) and minimum 800g of Watkins seed for further work.

Development of AE Watkins genotyped core sets

- Originally proposed DArT but more efficient to go for SSRs in anticipation of NGS profiling of relatively small (initially) core sets.

- So far 4 SSRs on full pop



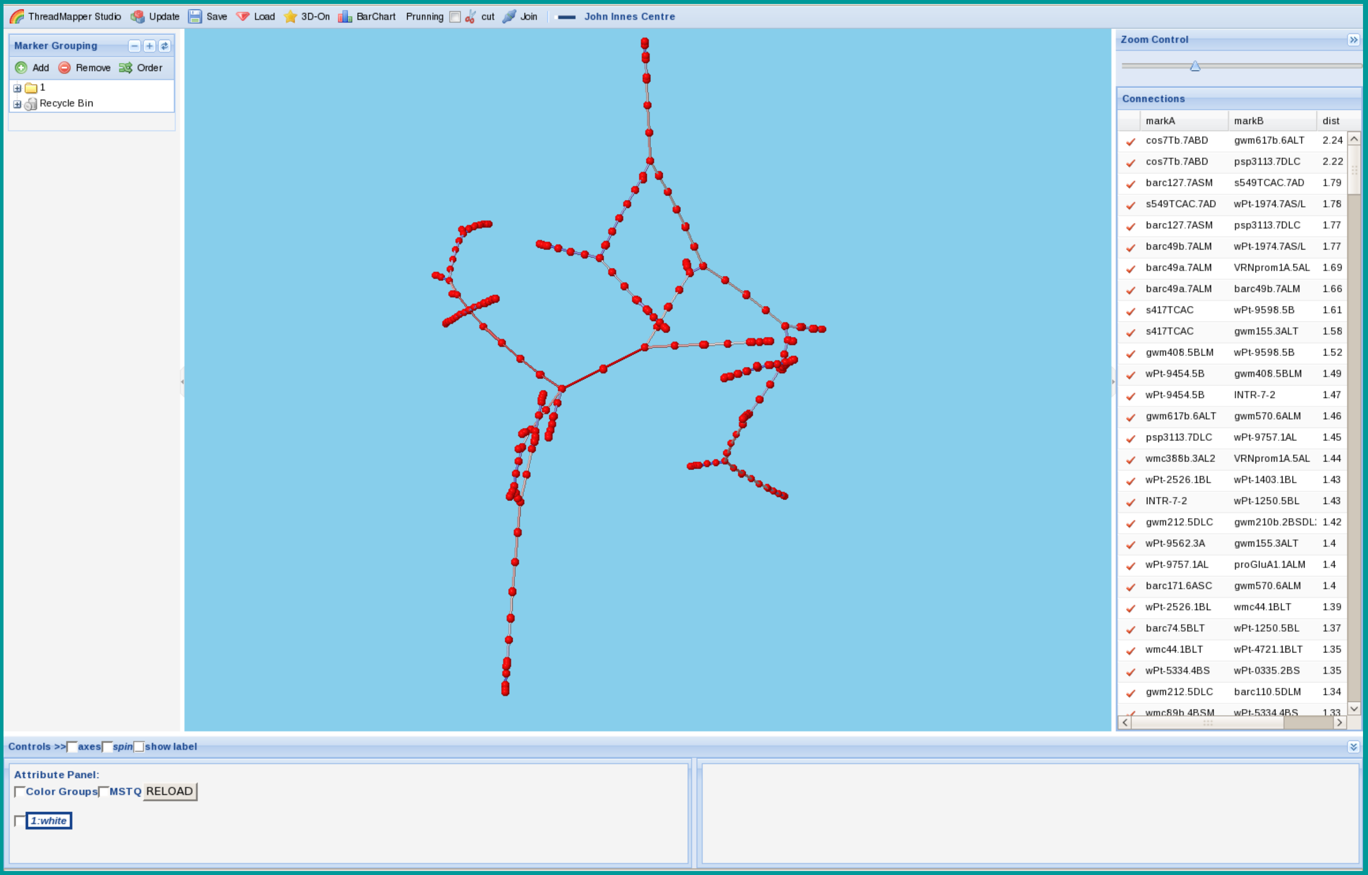
Avalon x Cadenza

- Workshop held on 3rd Nov at JIC- Genome Centre

- JIC continues to maintain stocks

Improved A x C map available on website

- http://cbr.jic.ac.uk/threadmapper



	markA	markB	dist
✓	cos7Tb.7ABD	gwm617b.6ALT	2.24
✓	cos7Tb.7ABD	psp3113.7DLC	2.22
✓	barc127.7ASM	s549TCAC.7AD	1.79
✓	s549TCAC.7AD	wPt-1974.7AS/L	1.78
✓	barc127.7ASM	psp3113.7DLC	1.77
✓	barc49b.7ALM	wPt-1974.7AS/L	1.77
✓	barc49a.7ALM	VRNprom1A.5AL	1.69
✓	barc49a.7ALM	barc49b.7ALM	1.66
✓	s417TCAC	wPt-9598.5B	1.61
✓	s417TCAC	gwm155.3ALT	1.58
✓	gwm408.5BLM	wPt-9598.5B	1.52
✓	wPt-9454.5B	gwm408.5BLM	1.49
✓	wPt-9454.5B	INTR-7-2	1.47
✓	gwm617b.6ALT	gwm570.6ALM	1.46
✓	psp3113.7DLC	wPt-9757.1AL	1.45
✓	wmc388b.3AL2	VRNprom1A.5AL	1.44
✓	wPt-2526.1BL	wPt-1403.1BL	1.43
✓	INTR-7-2	wPt-1250.5BL	1.43
✓	gwm212.5DLC	gwm210b.2BSDL:	1.42
✓	wPt-9562.3A	gwm155.3ALT	1.4
✓	wPt-9757.1AL	proGluA1.1ALM	1.4
✓	barc171.6ASC	gwm570.6ALM	1.4
✓	wPt-2526.1BL	wmc44.1BLT	1.39
✓	barc74.5BLT	wPt-1250.5BL	1.37
✓	wmc44.1BLT	wPt-4721.1BLT	1.35
✓	wPt-5334.4BS	wPt-0335.2BS	1.35
✓	gwm212.5DLC	barc110.5DLM	1.34
✓	wmc89b.4BSM	wPt-5334.4BS	1.33

New WGIN influenced Projects

- Resource Use Efficiency LINK- ADAS, Limagrain, BASF, JIC.
- BBSRC Flowering time and stress in CCPs Reading and JIC.
- BBSRC LOLA prebreeding programme- with BBSRC.

WGIN at JIC


Catherine
Baker


Liz
Sayers


Richard
Goram

Luzie
Wingen

Simon
Orford

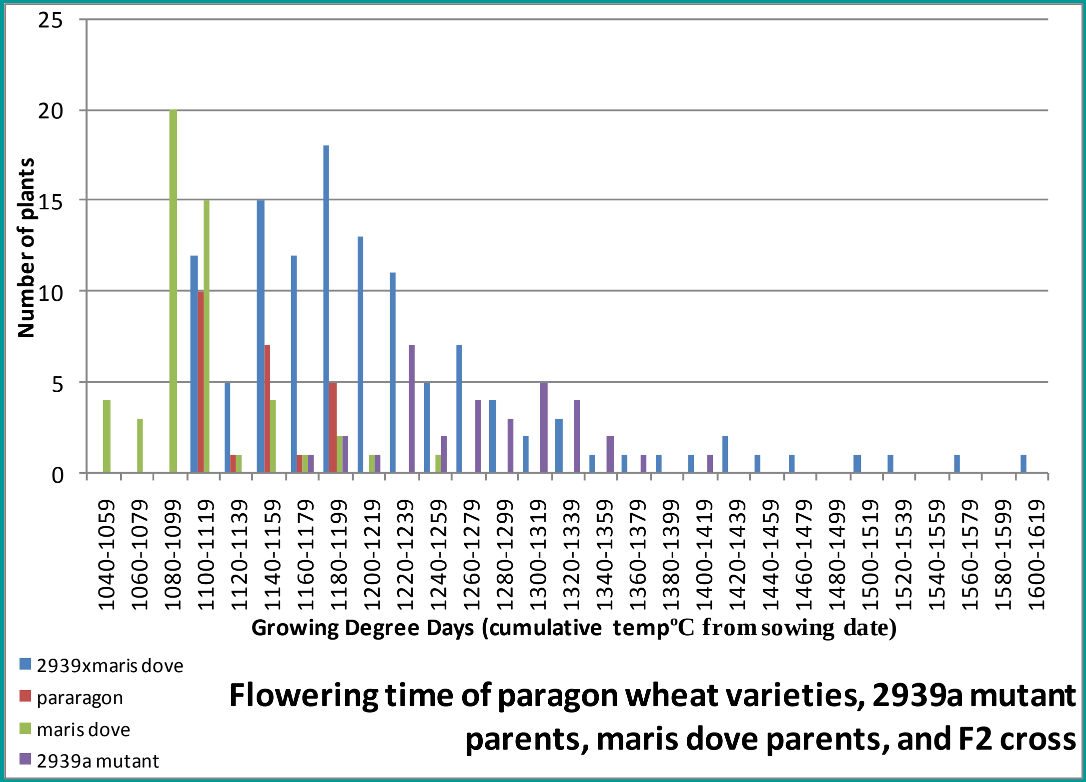
Michelle
Leverington

John
Snape

Lesley
Fish

Debora
Gasperini

Segregation of ear emergence for 2939a



Flowering time of paragon wheat varieties, 2939a mutant parents, maris dove parents, and F2 cross

Heading date DArT data returned for heading date

- 2939a mutant was crossed with Maris Dove
- 2939a mutant was crossed with Wembley
- 423a mutant was crossed with White Fife
- 423a mutant was crossed with Koga

Taking Paragon EMS alleles forward

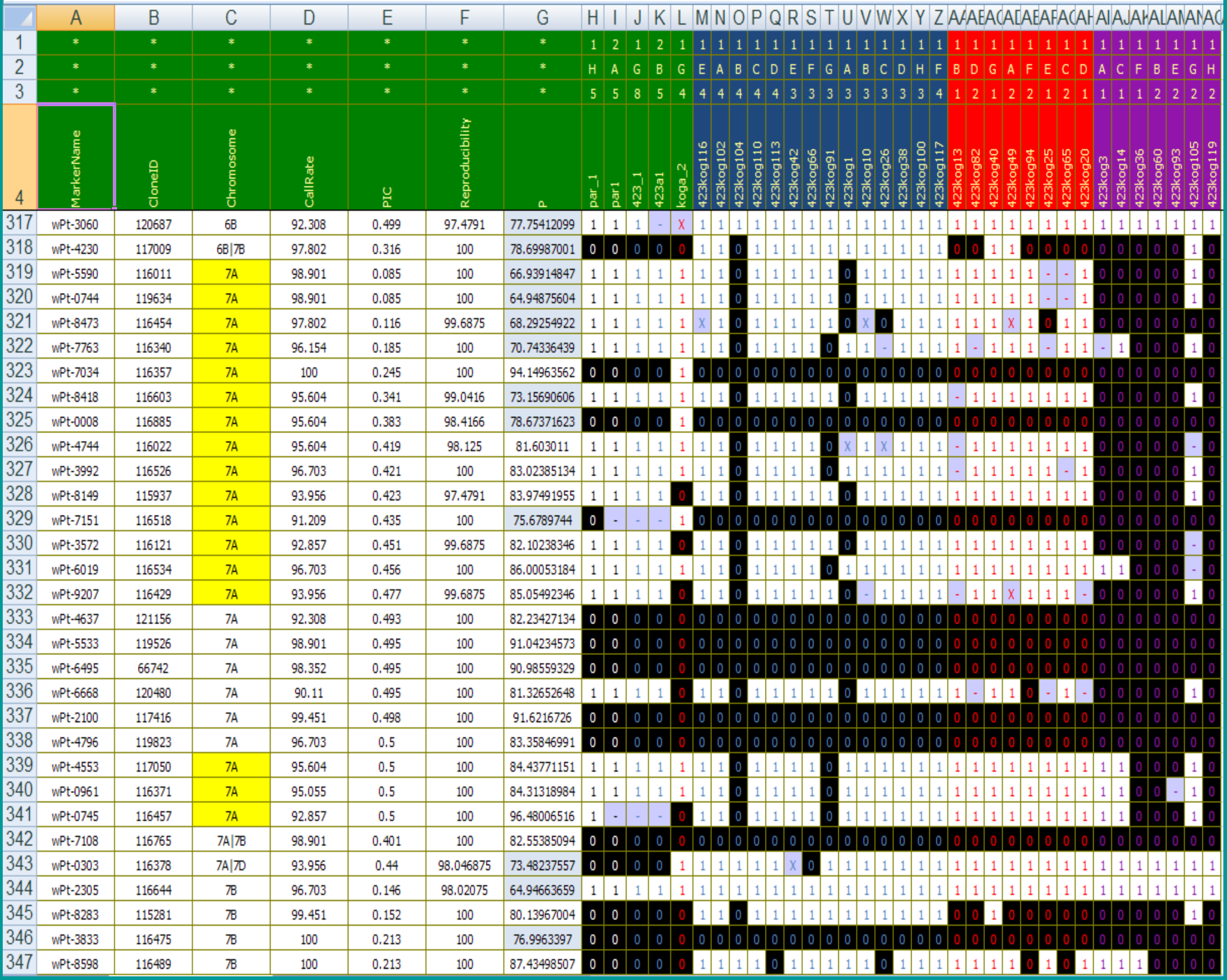
Tagging plants by visiting students for DNA collection and trait scoring

Leaf senescence segregating

Mutant x Spring cultivars: F_2 families
12 families – segregations of mutant types height, flowering, leaf senescence and biomass

Paragon mutants in Hege 80s
Left – flowering time differences (first two plots) Right – leaf senescence

Grp 7 associations for 423a



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO
1	*	*	*	*	*	*	*	1	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	*	*	*	*	*	*	*	H	A	G	B	G	E	A	B	C	D	E	F	G	A	B	C	D	H	F	B	D	G	A	F	E	C	D	A	C	F	B	E	G	H
3	*	*	*	*	*	*	*	5	5	8	5	4	4	4	4	4	4	3	3	3	3	3	3	3	3	4	1	2	1	2	2	1	2	1	1	1	1	2	2	2	2
4	MarkerName	CloneID	Chromosome	CallRate	PIC	Reproducibility	P	par_1	par1	423_1	423a1	koga_2	423kog116	423kog102	423kog104	423kog110	423kog113	423kog42	423kog66	423kog91	423kog1	423kog10	423kog26	423kog38	423kog100	423kog117	423kog13	423kog82	423kog40	423kog49	423kog94	423kog25	423kog65	423kog20	423kog3	423kog14	423kog36	423kog60	423kog93	423kog105	423kog119
317	wPt-3060	120687	6B	92.308	0.499	97.4791	77.75412099	1	1	1	-	X	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
318	wPt-4230	117009	6B|7B	97.802	0.316	100	78.69987001	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0
319	wPt-5590	116011	7A	98.901	0.085	100	66.93914847	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	-	-	1	0	0	0	0	0	1	0
320	wPt-0744	119634	7A	98.901	0.085	100	64.94875604	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	-	-	1	0	0	0	0	0	1	0
321	wPt-8473	116454	7A	97.802	0.116	99.6875	68.29254922	1	1	1	1	1	X	1	0	1	1	1	1	1	0	X	0	1	1	1	1	1	1	X	1	0	1	1	0	0	0	0	0	0	0
322	wPt-7763	116340	7A	96.154	0.185	100	70.74336439	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	-	1	1	1	1	-	1	1	1	-	1	1	-	1	0	0	0	1	0
323	wPt-7034	116357	7A	100	0.245	100	94.14963562	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
324	wPt-8418	116603	7A	95.604	0.341	99.0416	73.15690606	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	-	1	1	1	1	1	1	1	0	0	0	0	0	1	0
325	wPt-0008	116885	7A	95.604	0.383	98.4166	78.67371623	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
326	wPt-4744	116022	7A	95.604	0.419	98.125	81.603011	1	1	1	1	1	1	1	0	1	1	1	1	0	X	1	X	1	1	1	-	1	1	1	1	1	1	1	0	0	0	0	0	-	0
327	wPt-3992	116526	7A	96.703	0.421	100	83.02385134	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	-	1	1	1	1	1	-	1	0	0	0	0	0	1	0
328	wPt-8149	115937	7A	93.956	0.423	97.4791	83.97491955	1	1	1	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0
329	wPt-7151	116518	7A	91.209	0.435	100	75.6789744	0	-	-	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
330	wPt-3572	116121	7A	92.857	0.451	99.6875	82.10238346	1	1	1	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	-	0
331	wPt-6019	116534	7A	96.703	0.456	100	86.00053184	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	-	0
332	wPt-9207	116429	7A	93.956	0.477	99.6875	85.05492346	1	1	1	1	0	1	1	0	1	1	1	1	1	0	-	1	1	1	1	-	1	1	X	1	1	1	-	0	0	0	0	0	1	0
333	wPt-4637	121156	7A	92.308	0.493	100	82.23427134	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
334	wPt-5533	119526	7A	98.901	0.495	100	91.04234573	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
335	wPt-6495	66742	7A	98.352	0.495	100	90.98559329	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
336	wPt-6668	120480	7A	90.11	0.495	100	81.32652648	1	1	1	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	-	1	1	0	-	1	-	0	0	0	0	0	1	0
337	wPt-2100	117416	7A	99.451	0.498	100	91.6216726	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
338	wPt-4796	119823	7A	96.703	0.5	100	83.35846991	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
339	wPt-4553	117050	7A	95.604	0.5	100	84.43771151	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0
340	wPt-0961	116371	7A	95.055	0.5	100	84.31318984	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	-	1	0
341	wPt-0745	116457	7A	92.857	0.5	100	96.48006516	1	-	-	-	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0
342	wPt-7108	116765	7A|7B	98.901	0.401	100	82.55385094	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
343	wPt-0303	116378	7A|7D	93.956	0.44	98.046875	73.48237557	0	0	0	0	1	1	1	1	1	1	X	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
344	wPt-2305	116644	7B	96.703	0.146	98.02075	64.94663659	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
345	wPt-8283	115281	7B	99.451	0.152	100	80.13967004	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
346	wPt-3833	116475	7B	100	0.213	100	76.9963397	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
347	wPt-8598	116489	7B	100	0.213	100	87.43498507	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	0	0	0

‘Late late’ 423a

Gamma M_1 mutants:
CS bagged left (2500) sown). Paragon (2000 sown) currently bagging right
50% fertility rate expected

Now harvested M_2 seed- for sowing in spring 2010
Scope for a further 4000

Koga 7A nullisomics

Gamma mutagenesis

WGIN 2 Objective 10
Take-all Update

Richard Gutteridge
Kim Hammond-Kosack

Wheat
Genetic
Improvement
Network

defra
Department for Environment
Food and Rural Affairs

BBSRC
bioscience for the future

ROTHAMSTED
RESEARCH

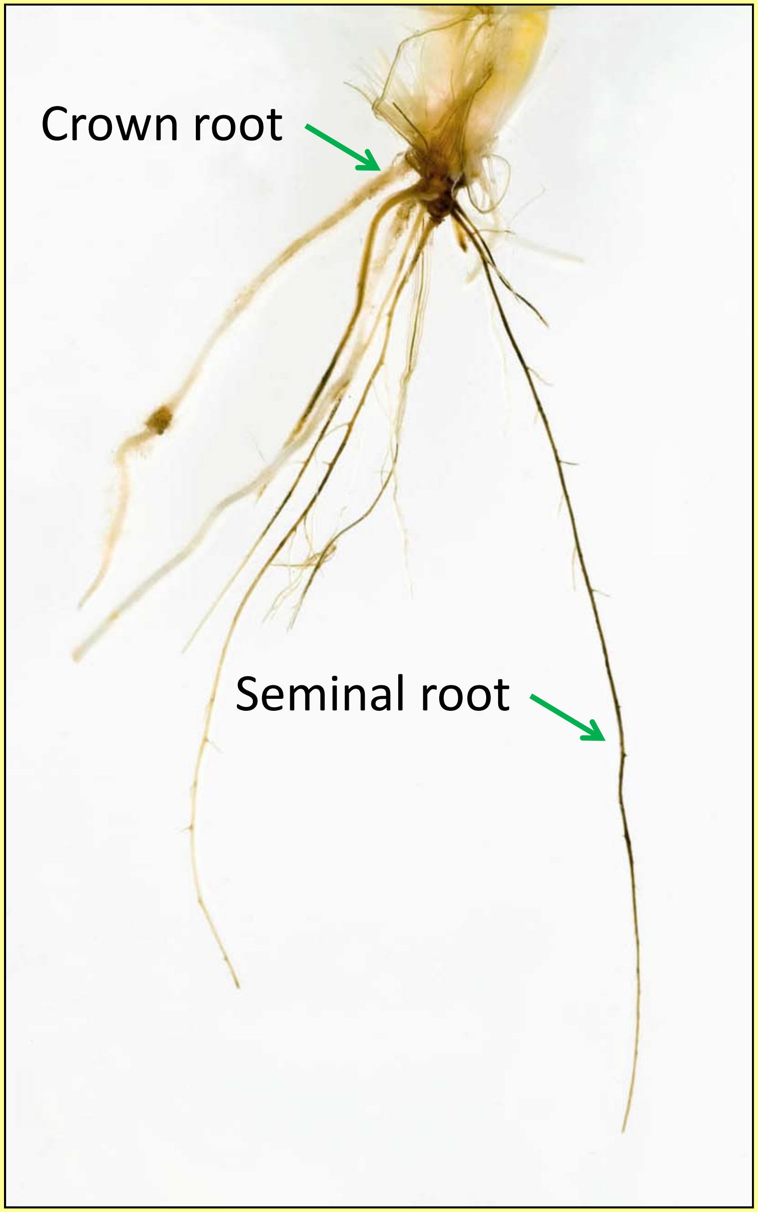
WGIN2
26th Feb 2010

Take-all disease of wheat

- *Gaeumannomyces graminis* var. *tritici* (*Ggt*)

  - ascomycete soil borne fungus

  - related to rice blast fungus *Magnaporthe oryzae* (previously *M. grisea*)



Ggt infected wheat seedling

A *Ggt* infected seminal root

Typical take-all patch showing stunting and premature ripening of the crop

Objective 10 – Take-all

1. Screen Watkins and improved Gediflux collections for take-all resistance under field conditions.
2. Explore the genetic basis for take-all inoculum build-up using the Avalon x Cadenza mapping population

2008 Watkins Experiment

Only growth regulator, herbicides and nitrogen applied

Excellent year foliar diseases:
yellow and brown rust, septoria and mildew
All diseases were scored in July and results can be found on the WGIN website

Also a good year for the root and stem base diseases

Watkins Hexaploid wheat collection
2008 Take-all / eyespot assessments

All 740 lines + controls assessed for stem base diseases

eyespot

sharp eyespot

brown foot rot (fusarium spp.)

74,000 straws assessed

now in stats

Note : the 2008-2009 trial was fungicide treated in the spring 2009 to minimise stem base diseases

Only this one year of data

2008 Eyespot assessment

Hereward control (total - 25 plots)

Percentage of straws infected:	Slight	Moderate	Severe
	33	22	22

Criteria for Resistant lines: slight symptoms and less than 5% moderate infection, no severe infection.

45 Watkins lines were selected

A list of these lines, together with other lines which are fully susceptible, has been sent to PhD student Christopher Burt (JIC) to analyse for the known resistance to eyespot.
Presence of *Pch1* using Wmc14 and Barc97 markers
Presence of *Pch2* using Wmc346, Wmc525 and cfa2040 markers

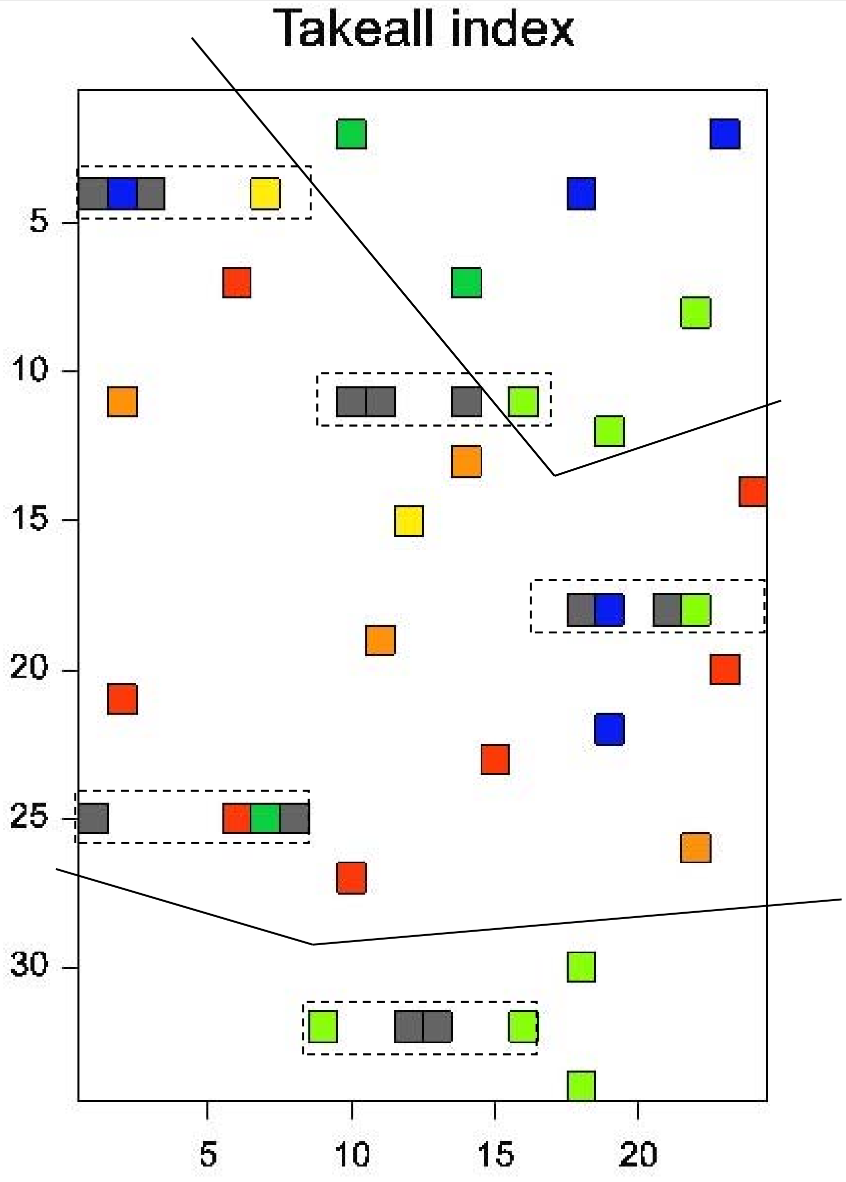
Watkins Experiment 2008 RRes Take-all severity - control plots

5 control blocks

Variety	**TAI**
Hereward	**35.6**
Triticale	**18.4**
Rye	**1.7**
Oats	**0.0**

TAI

⬜	**0-5**
🟦	**6-15**
🟩	**16-25**
🟩	**26-35**
🟨	**36-45**
🟧	**46- 55**
🟥	**> 55**



Hereward - Extra 20 plots - mean TAI = 45.3

Alpha design

N = 800 plots

Mean of all plots
Hereward = 43.4

Watkins Hexaploid wheat collection
2008 Take-all / eyespot assessments

- All assessments completed – 740 lines
  12,000 plants

- Initial findings suggest that 253 lines (34%) can be omitted due to susceptibility to Take-all.

- Further lines may also be discarded once the stats is completed

A.E.Watkins and Gediflux collection
2008 – 2009 field trial

- The same single plot – Alpha design

- **Foliar diseases**

  winter/ spring infection by septoria and mildew but then did not develop further

  late infection by yellow rust and brown rust

  ALL lines affected recorded but not assessed

- overall less FOLIAR disease than in 2007-2008

Watkins / Gediflux collections field experiment 2010

Watkins

500 lines carried forward from the 2008 trial but some may be discarded once statistical analysis complete

78 new lines added not previously tested

Gediflux collection – 60 lines in total

All lines: For 2 years
2008 – 2009 - All assessments completed
2010 experiment was sown October 26th 2009

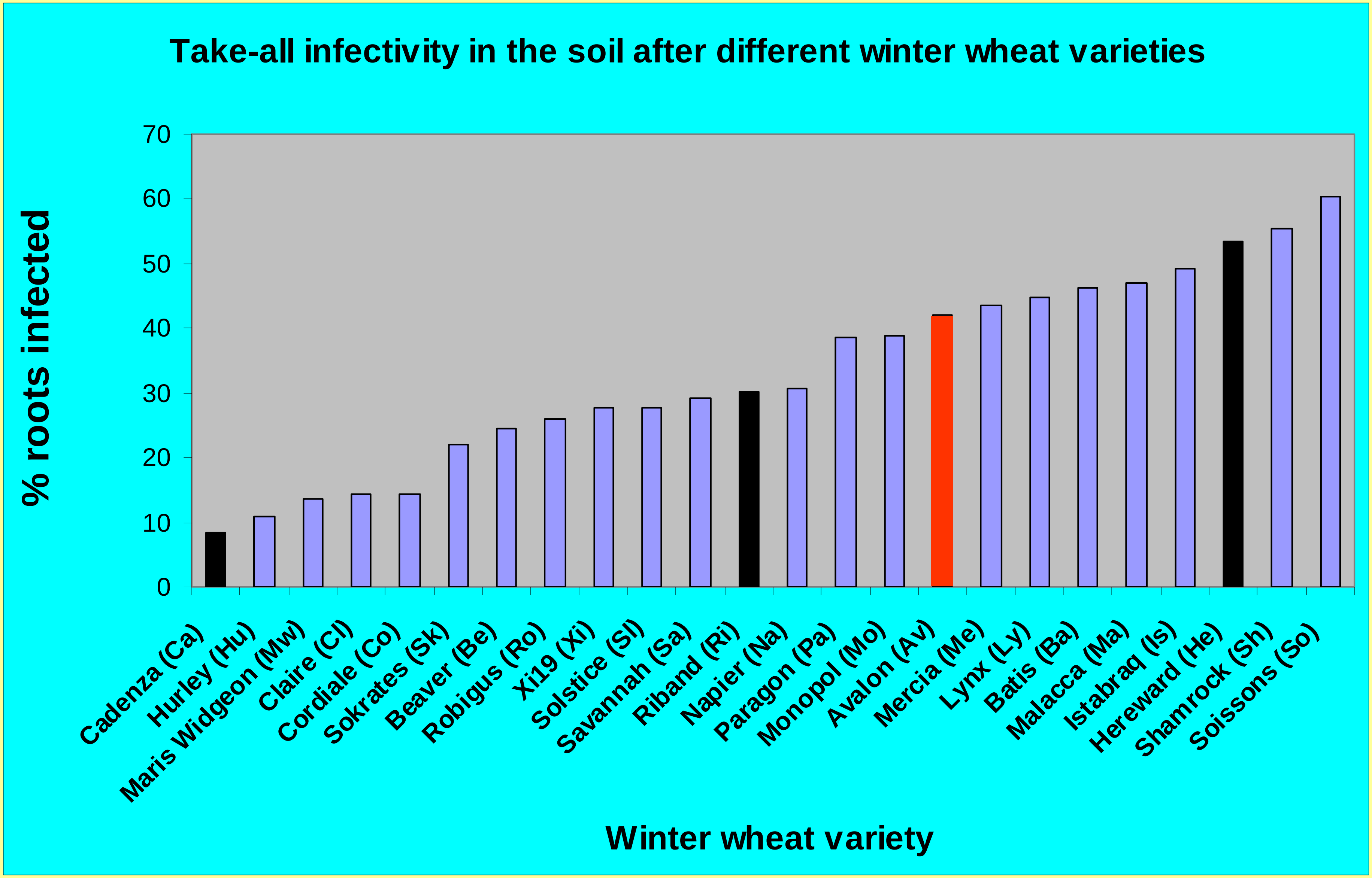
defra

Wheat
Genetic
Improvement
Network

BBSRC
bioscience for the futur

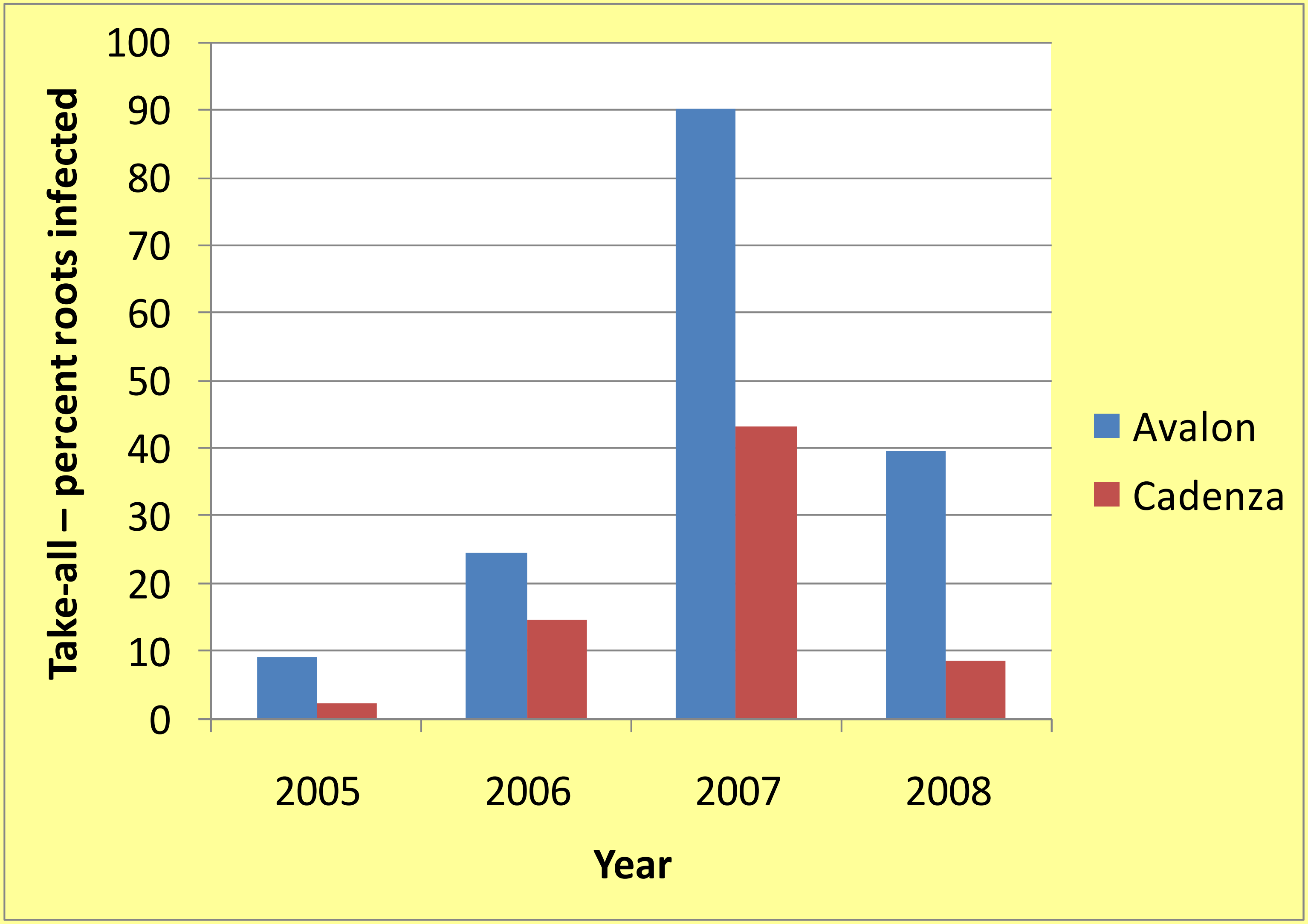
www.WGIN.org.uk

WGIN winter wheat bioassay 2008



Consistent ranking over 4 years

% roots infected with take-all in a soil core bioassay Avalon and Cadenza 2005 - 2008 WGIN Diversity trials



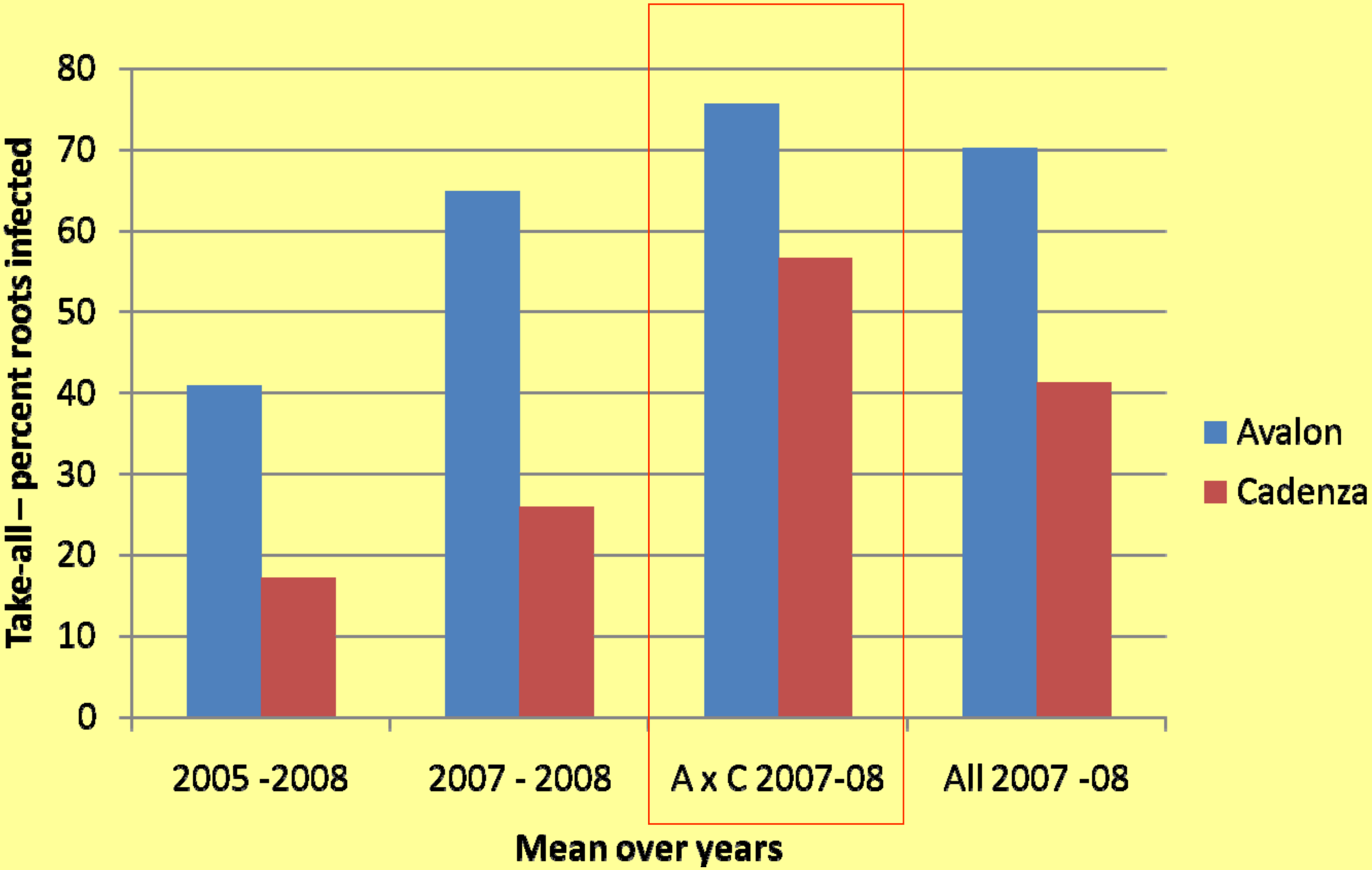
Avalon x Cadenza DH trial

Soil cores, to measure take-all infectivity, were taken from just the parental plots in 2007 and 2008.

The intention was to sample all the A x C DH lines in 2008 but due to the late harvest and the presence of cereal volunteers, this trial was not sampled for the take-all soil infectivity.

In 2009, soil cores were taken from all A x C lines but due to the presence of *Phialophora* take-all inoculum build-up was severely restricted.

% roots infected with take-all in a soil core bioassay Avalon v Cadenza



2008 Seed multiplication trial

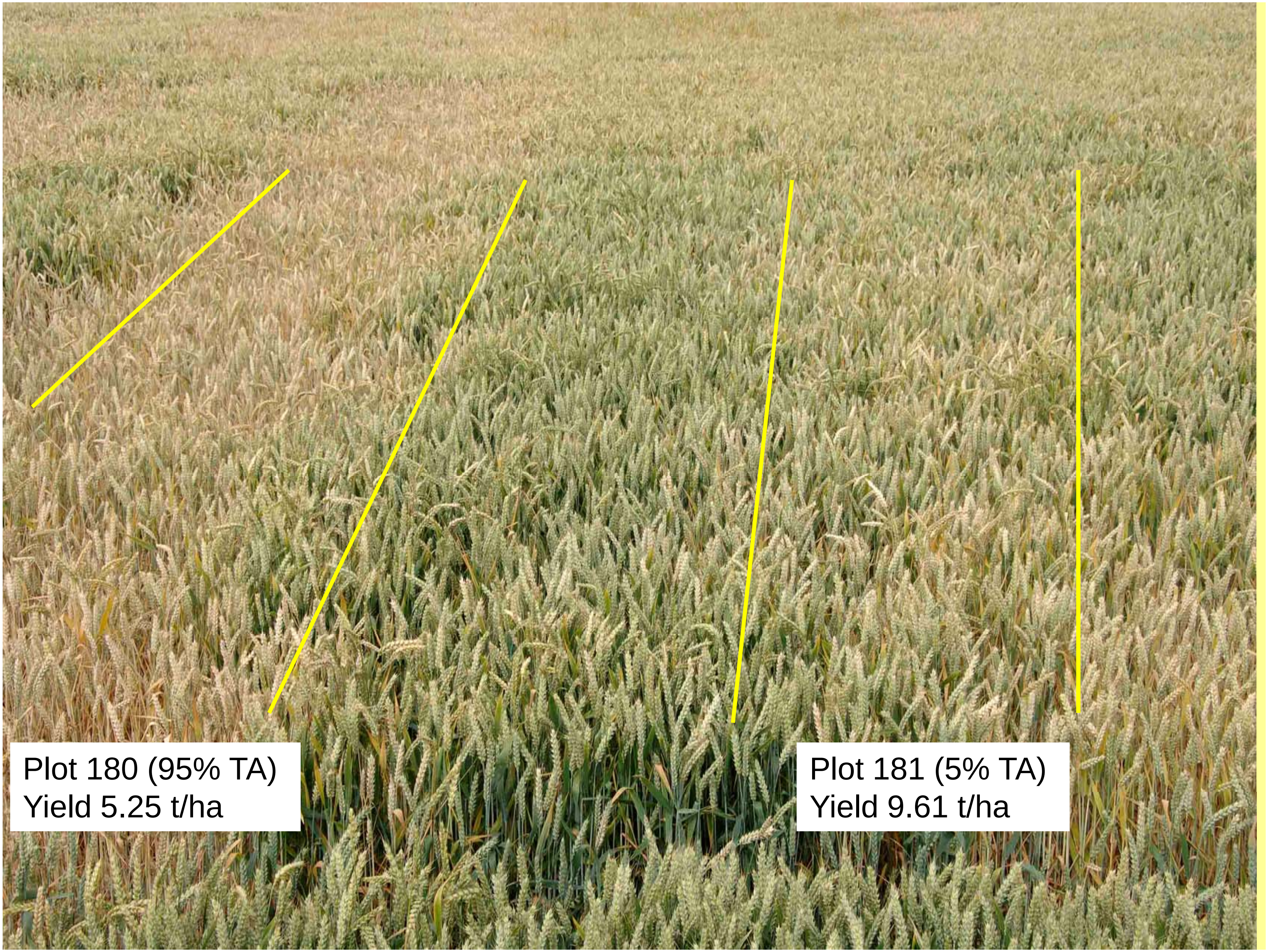
62 A x C lines in a separate field + parental lines (plot size 20m x 2m) for multiplication in 2008 were ploughed and then over sown with Oakley

By July 2009 take-all patches were showing in the areas where the 2008 plots had been sown

These areas were scored for take-all patches as a percentage of area affected

Middle 2 m of each plot was harvested and yield measured


Square Take-all patches



Plot 180 (95% TA)
Yield 5.25 t/ha

Plot 181 (5% TA)
Yield 9.61 t/ha

Can we genetically analyse and map the trait ?

62 DH lines + parentals scored

Can QTL’s help in the analysis?

Can we genetically analyse and map the trait ?

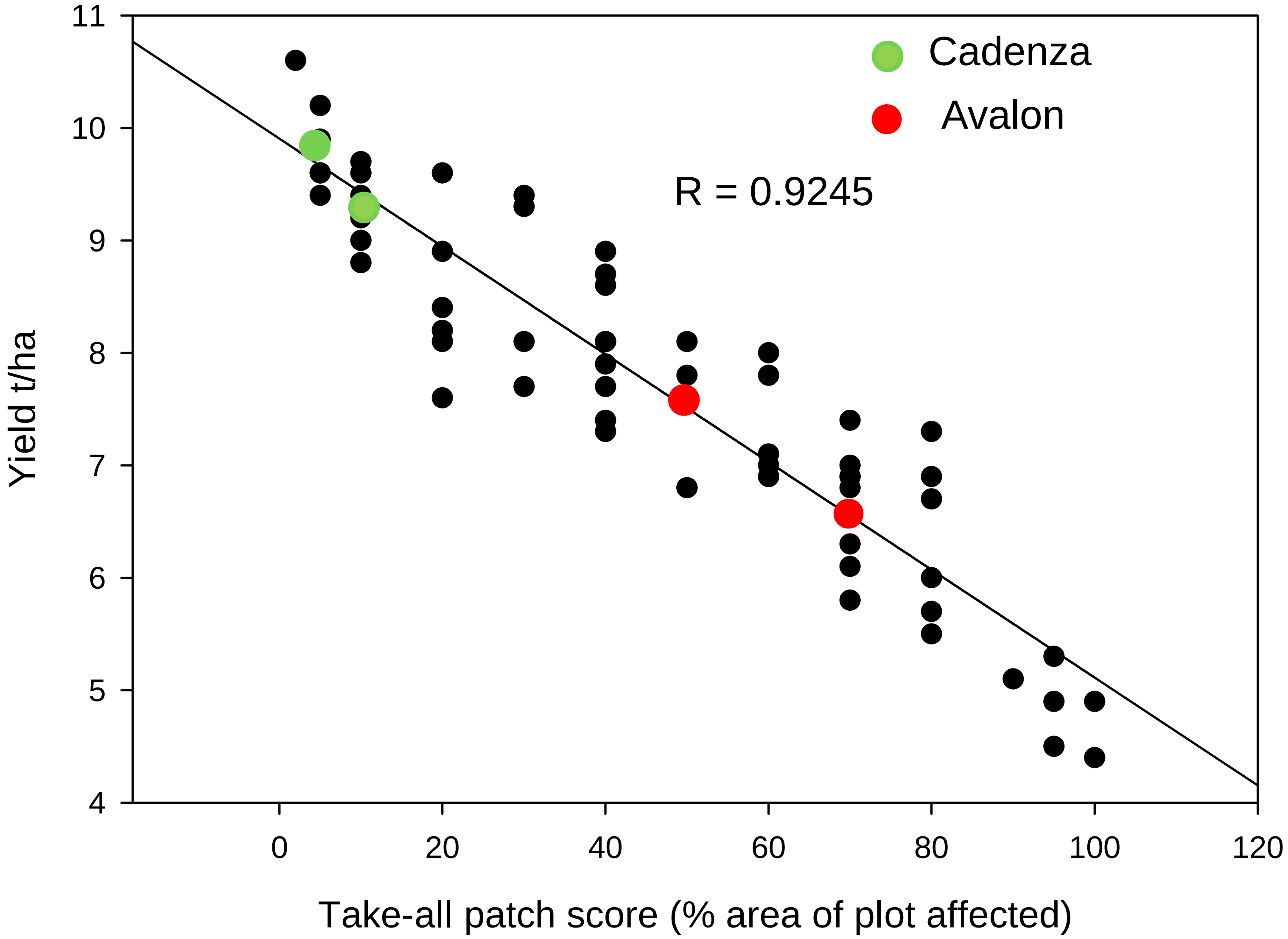
62 DH lines + parentals scored

Can QTL's help in the analysis?

YES!!!

Quality Take-all Lesions

Avalon x Cadenza lines in 2008, oversown with wheat cv. Oakley in 2009. Relationship between take-all patch score and yield



Can we genetically analyse and map the trait ?

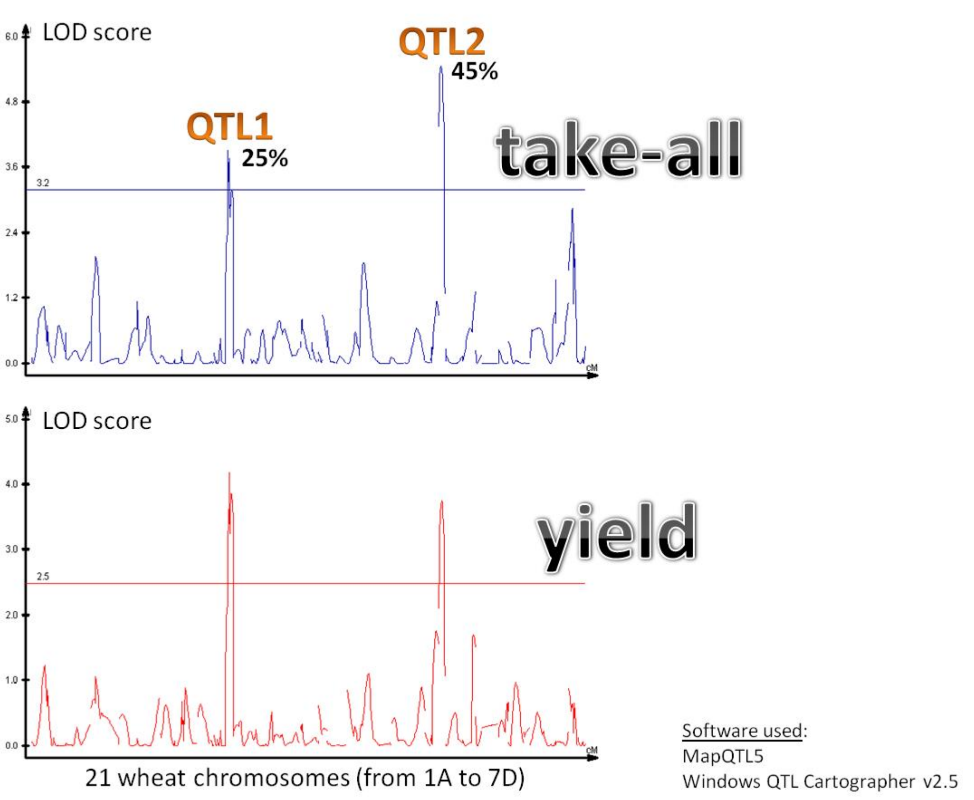
319 markers on the A x C map
62 DH lines + parentals scored

Quantitative trait Loci (QTL) analysis

Can we genetically analyse and map the trait ?

319 markers on the A x C map
62 DH lines + parentals scored

Quantitative trait Loci (QTL) analysis



Software used:
MapQTL5
Windows QTL Cartographer v2.5

Genotyping analysis done by Kostya Kanyuka

Many thanks to…..

Wheat Pathogenomics Team (RRes)

Sanja Treskic
Vanessa McMillan
James Bruce
Adrian Czaban

Kim Hammond-Kosack
Hai-Chun Jing
Kostya Kanyuka
Elke Anzinger

Watkins Exp.

Simon Orford (JIC)
Elke Anzinger
Sarah Usher
Steve Freeman

A x C map (JIC)

Simon Griffiths
Leodie Alberts
Michelle Leverington-Waite

BAB Dept Statistics

Sue Welham
Rodger White

The Rothamsted farm staff

WGIN subcontractor selection

Request for proposals

- Request for proposal was advertised through the WGIN mailing list
- Application form was available on www.wgin.org.uk
- Deadline for application was 1st October 2009
- We received five proposals

Wheat
Genetic
Improvement
Network

Selection Process

- Proposals were reviewed by two independent referees and by Defra
- Management meeting October 2009 – discussion on members for selection panel
- A panel comprising Graham Jellis (chair), David Feuerhelm and Peter Jack was convened in December.
- Applicants were notified of the outcome in January 2010

Selected Projects

Applicant: Richard Weightman

Project :

Non-destructive screening of WGIN Paragon mutants for grain NUE traits

Applicants: John Foulkes, Andy Greenland

Project :

Exploring the use of Δ180 and total mineral ash content in wheat as a new tools for phenotyping wheat with respect to water inputs

5 proposals received

Applicant: Nigel G. Halford

Project : Measuring precursors of acrylamide, reducing sugars and sucrose in diversity trial grain infected with take-all, yellow rust and fusarium ear blight.

Cost: £12,750.--

Applicant: Richard Weightman

Project : Non-destructive screening of WGIN Paragon mutants for grain NUE traits

Cost: £20,680.--

Applicants: Nick Gosman, Eric Ober

Project: Assessing 50 CIMMYT synthetic hexaploid wheat lines for drought tolerance and drought-related traits.

Cost: 42,723.--

Applicants: Peter Isaac, Simon Griffiths, David Laurie, Graham Moore

Project : Gene copy number variation in selected genes (Ppd, Vrn1, Ph1, Rht8, Rht10) in 12 hexaploid wheat varieties

Cost: £48,763,--

Applicants: John Foulkes, Andy Greenland

Project : Exploring the use of Δ180 and total mineral ash content in wheat as a new tools for phenotyping wheat with respect to water inputs

Cost: 49,829.--

Drought tolerance

WGIN-2 SG meeting

Syngenta 26 February 2010

The University of Nottingham

John Innes Centre

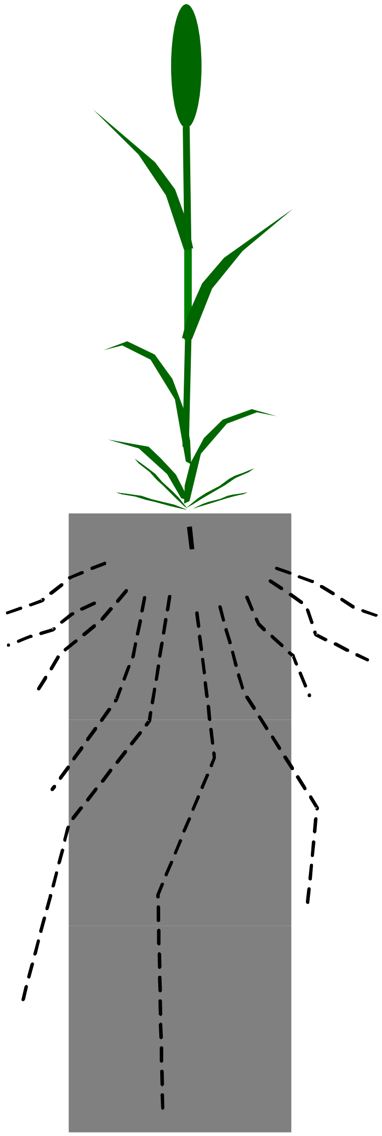
WGIN 2 (Activity 9, Drought tolerance)

1. To identify the physiological traits explaining improved water-use efficiency and drought tolerance in elite winter wheat varieties.
2. To identify robust QTLs for water-use efficiency and drought-tolerance traits using one existing DH population in an elite background.
3. To develop one new DH population in an elite modern background segregating for drought-tolerance traits.
4. To identify novel genes and alleles controlling water-use efficiency and drough tolerance using the AE Watkins and Gediflux collections.
5. To collate a diverse germplasm collection (cultivars, advanced lines) from worldwide drought-tolerance wheat breeding programmes as a resource for future association genetics studies.

Drought Resistance: Target traits

Grain yield	=	Crop water use	X	Water-use efficiency (biomass)	X	Harvest index
Target traits →		• Stay-green • Root density		• Transpiration efficiency (^{13}C Δ) • Awns		• Flowering • Stem CHO reserves

Ideotype for high sustainable yield under drought



OPTIMIZE WUE

- High ^{13}C Δ
- Pn capacity
- Specific leaf N

MAXIMIZE HARVEST INDEX

- Stem CHO reserves
- Stay green

MAXIMIZE WATER CAPTURE

- RLD at depth
- β (distribute roots deeper)
- Specific root length

EARLINESS

- Extend stem elongation phase
- Early onset GS31

WUE trial 2009-10

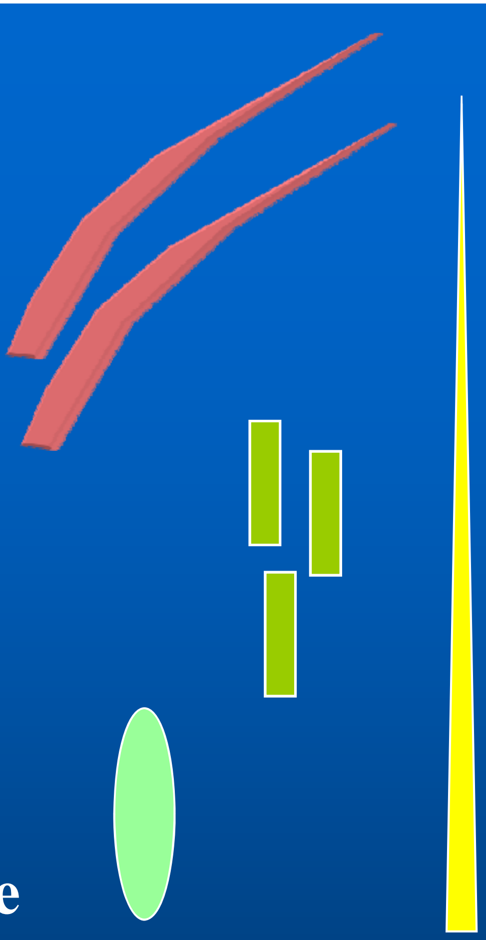
Split plot design (3 reps): plot size 1.6 x 12 m
Main plot: Fully irrigated (trickle irrigation)
Unirrigated

Split plot (variety):

1. Avalon *
2. Beaver
3. Cadenza *
4. Cappelle Deprez
5. Cordiale
6. Gallant *
7. Glasgow
8. Hereward *
9. Hobbit
10. Istabraq *
11. M. Widgeon
12. Oakley *
13. Panorama
14. Paragon
15. Cappelle Deprez
16. Soissons
17. Xi 19 *
18. Zebedee

* Common with NUE trial

Measurements

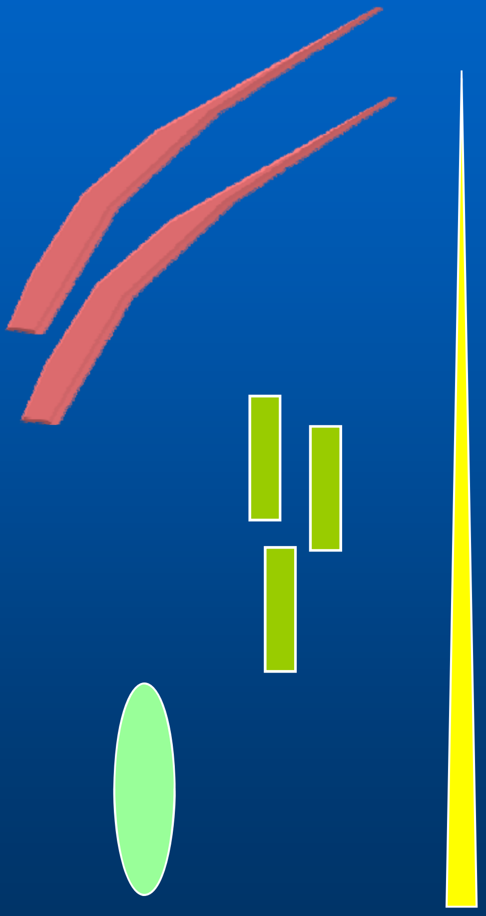


- Combine grain yield, yield components
- DM and partitioning at GS31, GS61, harvest
- % stem WSC at GS61+10d
- Leaf senescence kinetics for flag-leaf, L2 and L3.
- Stomatal conductance/photosynthetic rate using Licor (unirrigated subset , 7 varieties)
- Water use ~ gravimetric analysis of soil cores (unirrigated subset, 7 varieties)

Measurements: isotope analysis of plant samples

Analyses on all varieties in both irrigation trts (108 plots):

- **$\Delta\ ^{13}C$ ~ flag leaf @ flowering and grain @ harvest**
- **$\Delta\ ^{18}O$ ~ flag leaf @ flowering**
- **Total mineral ash content ~ flag leaf @ GS61 and grain @ harvest**



WGIN 2 (Drought tolerance, 9.2)

	Project Month	Milestone
30/11/2012	48	Act 9 Obj2: QTL analysis to identify genome locations associated with WUE and drought tolerance traits completed.

Imminent tasks:

~ select DH population for phenotying for yield physiological triats (94 lines and 2 parents) informed by data analysis from LK0986 project

~ seed supply 2 sites (Nottingham and JIC 2010/11 & 2011/12)

~ extend molecular map as necessary with Dart and SSR

WGIN 2 (Drought tolerance, 9.3)

	Project Month	Milestone
31/03/2012	40	Act 9 Obj3. Complete development of one new DH population in an elite modern background segregating for drought-tolerance traits.

Imminent tasks:

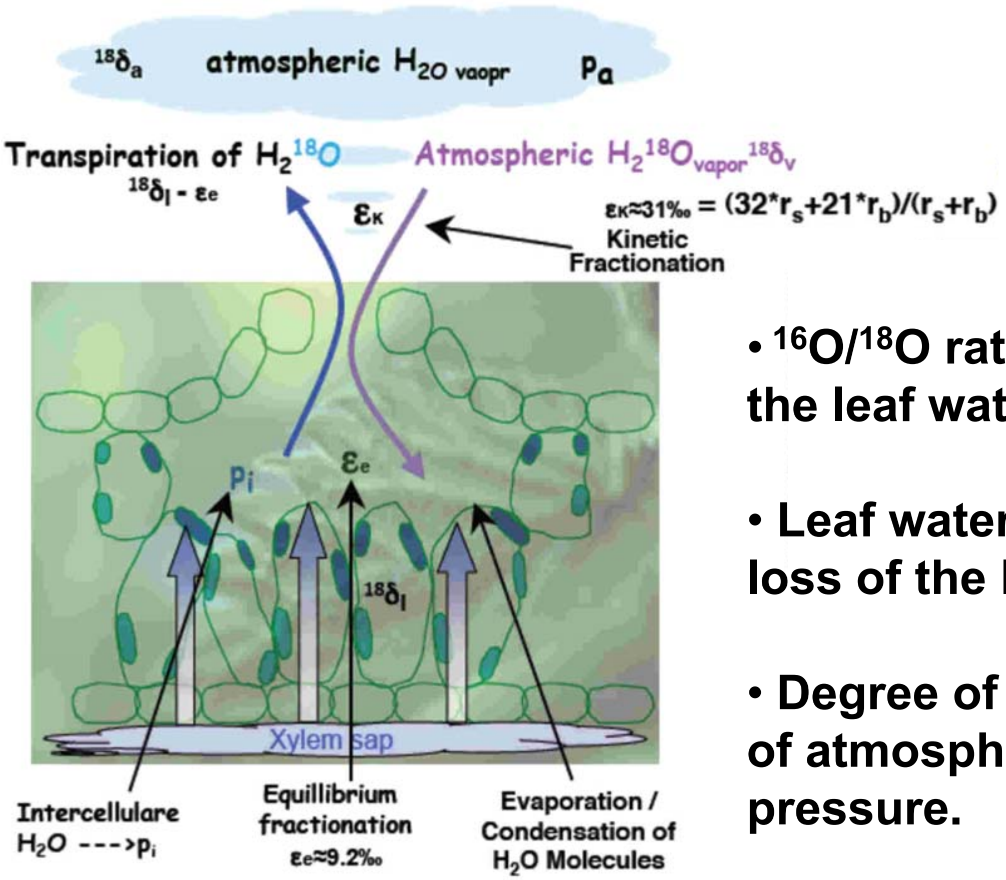
~ select candidate F1(s) to initiate crosses to maize informed by by data analysis from LK0986 project

Evaluation of novel genetic resources

- NIAB backcrossing CIMMYT synthetic wheats (shown in Mexico to have improved drought tolerance) in Xi19 background.

- 29 BC1F3 families grown in small plots (4 x 1 m) and the recurrent parent (Xi19) on the drought-prone light sand at Bunny Park Farm.

Techniques: Oxygen isotope ratio ~ <u>leaf transpiration</u>



- **$^{16}O/^{18}O$ ratio determined by enrichment in the leaf water due to transpiration.**

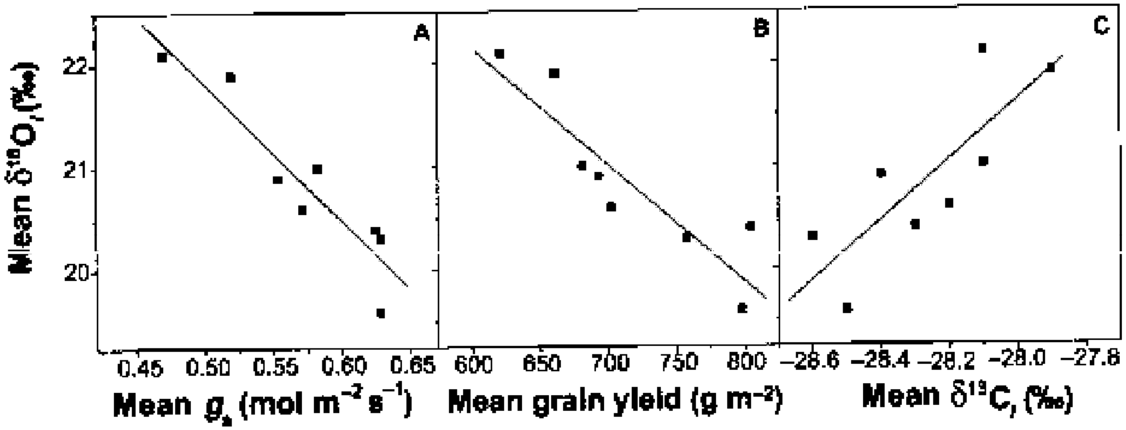
- **Leaf water enriched due to the preferential loss of the lighter $H_2{}^{16}O$ during evaporation.**

- **Degree of enrichment is related to the ratio of atmospheric to intercellular vapour pressure.**

- **An increase in stomatal conductance should decrease leaf temp. hence intercellular vapour pressure, resulting in less enrichment at the evaporating site.**

Barbour et al. (2000) Aust. J. Plant Physiol., **27**, 625–637

Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat



The relationship between the 3 year mean of cultivar $\delta^{18}O_{leaf}$ and corresponding means of stomatal conductance (gs) measured pre-anthesis, grain yield and δ 13C_{leaf} for eight CIMMYT spring wheat cvs

δ^{18}O is not thought to be strongly influenced by photosynthetic rate, so that combined measurement of both δ^{13}C and δ^{18}O should allow stomatal and photosynthetic effects on δ13C to be teased apart

The analysis of mineral ash content analysis is significantly less expensive than $\Delta^{13}C$ or $\Delta^{18}O$, so may be a useful alternative for these screens.

Techniques: Mineral ash content **~ <u>water use</u>**

- Total leaf ash content of plant tissues is suggested as a useful tool to predict yield under drought.
- The mechanism of mineral accumulation in plant tissues appears to be explained through the passive transport of minerals via xylem driven by transpiration.
- Thus, ash content measured in plant tissues provides an indicator of the total water transpired.
- The analysis of mineral ash content analysis is significantly less expensive than Δ13C or Δ18O, so may be a useful alternative for these screens in trials.

Proposed assessments in WGIN drought tolerance trial in 2009/19 and 20010/11

	Chemical analysis			Total
No. samples	**13C**	**18O**	**Ash%**	
Flag leaf @ GS61	**108**	***108***	***108***	**324**
Grain @ harvest	***108***		***108***	**216**

- **Genetic variability in chemical analysis traits will be compared with grain yield (all varieties) and water use (subset of 7 varieties, capacitance probes) in the WGIN drought trial in 2009/10 and 2010/11.**

Monneveux et al. 2005 Eur. J. Agron. 22 231-242

Relationship between leaf δ**13C** (at anthesis) and leaf ash content (at anthesis and maturity) and grain yield and grain δ**13C** for 20 CIMMYT spring wheat cultivars

	Treatment	
	Irrigated	**Residual moisture**
Phenotypic correlation with grain yield		
δ13C leaf anth	**0.069 NS**	**0.527***
Ash% leaf mat	**0.489***	**0.678*****
Ash% grain mat	**0.362**	**0.071 NS**
Phenotypic correlation with δ13C grain		
δ13C leaf anth	***0.001***	**0.498***
Ash% leaf mat	***0.134***	**0.620****
Ash% grain mat	***0.057***	**0.108 NS**

WGIN 2 (Activity 9, Drought tolerance)

	Project Month	Milestone
30/11//2011	36	Act 9 Obj1: Complete phenotyping and data analysis for drought tolerance traits in elite winter wheat varieties in 2009/10 &10/11.
30/11/2012	48	Act 9 Obj2: QTL analysis to identify genome locations associated with WUE and drought tolerance traits completed.
31/03/2012	40	Act 9 Obj3. Complete development of one new DH population in an elite modern background segregating for drought-tolerance traits.
28/02/2013	51	Act 9 Obj4: Association genetics analysis of drought tolerance traits using AE Watkins & Gediflux collections completed.
28/02/2013	51	Act 9 Obj5: Collation of diverse germplasm collection (cultivars, advanced lines) from worldwide drought-tolerance wheat breeding programmes completed.

Non-destructive screening of WGIN Paragon mutants for grain NUE traits

Richard Weightman, ADAS Boxworth

WGIN management meeting 26/02/10

ADAS

Agenda

- Partners
- Background, grain NUE traits
- Development of NIR calibrations
- Performance of existing calibrations
- Work programme

Partners

ADAS	R Weightman, D Kindred
JIC	Simon Griffiths
SCRI	Stuart Swanston
Aunir	Chris Piotrowski

Aunir

- developers & suppliers of NIR calibrations, support contracts and consultancy
- NIR software products used in >35 countries covering diverse range of food & feed applications
- work closely with FOSS (& others)

GREEN grain Project

Genetic Reduction of Energy use and Emissions of Nitrogen through cereal production

LINK collaborative research

HGCA

defra Department for Environment Food and Rural Affairs

The Scottish Government

syngenta

The Scotch Whisky Research Institute

ADAS

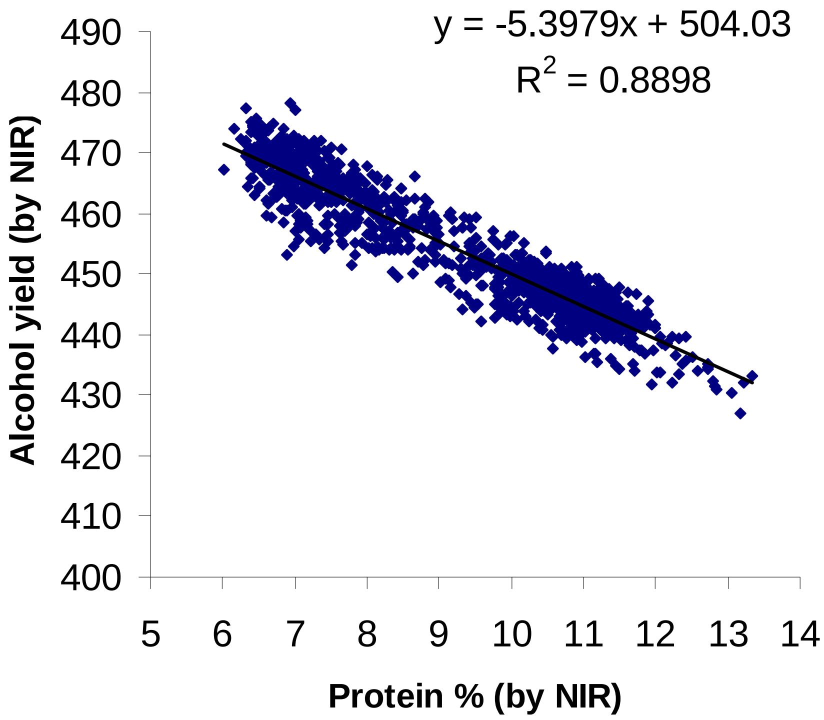
Scottish Crop Research Institute

The University of Nottingham

Grampian Country Food Group Traditional Goodness

Wessex Grain

FOSS

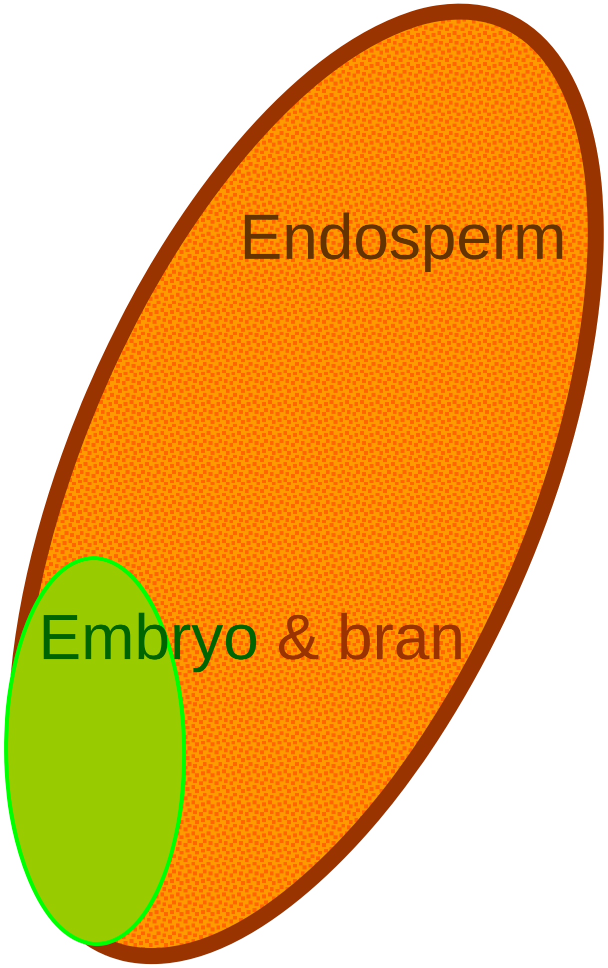


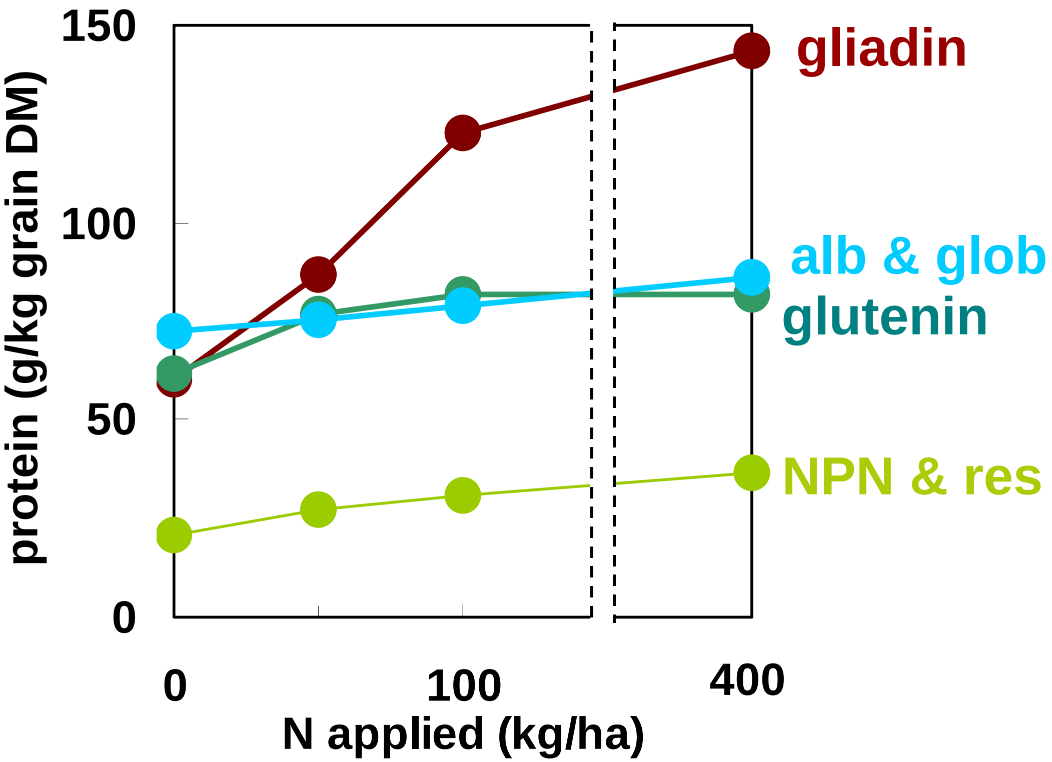
ADAS

Storage protein fractions

- **GG hypothesis relates to reducing gliadins in grain**
    - **Increase in response to applied N**
    - **Major storage protein**
    - **Need rapid screening tool**
    - **Tool could then be used to screen for ‘mutants’ or null gliadin lines**

Wheat grain … response to N supply



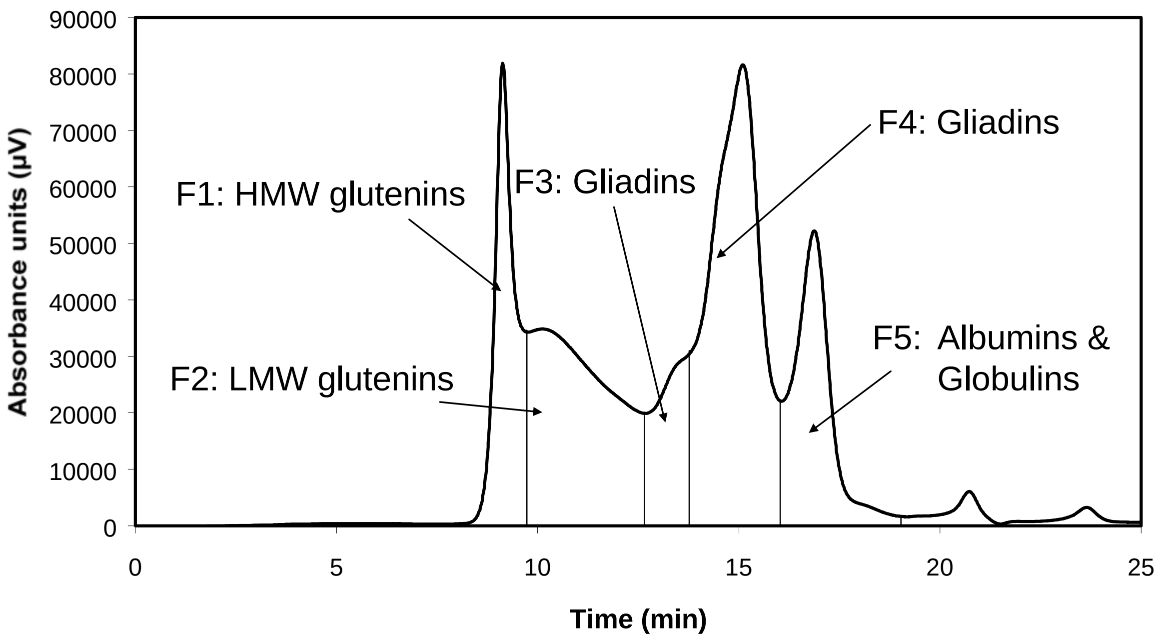


Dubetz, Gardiner, Flynn & de la Roche (1979).

Storage proteins - methods

- **Gel electrophoresis**
  - **not quantitative**
- **Fractionation (salt, alcohol soluble etc)**
  - **Laborious, variable**
- **SE-HPLC**
  - **Relatively expensive, sensitive, quantititifies all fractions following single extraction**

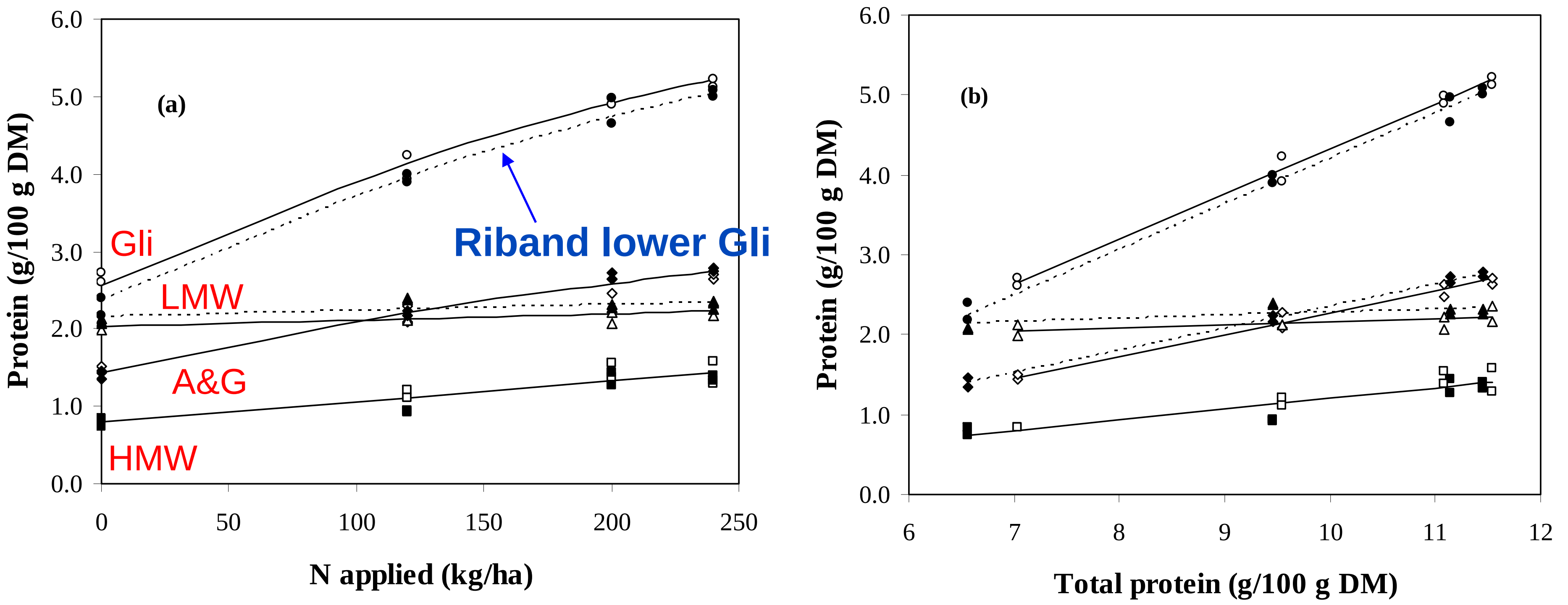
Typical chromatogram



Typical SE-HPLC chromatogram for wheat protein

Response of storage proteins to applied N

SE-HPLC method – Riband and Option data set



Gli

LMW

A&G

HMW

Riband lower Gli

Kindred *et al*., Journal of Cereal Science, 48; 46-57 2008

NIR calibrations

FOSS Infratec

Ref dataset selection rationale (1)

- **Ensured both +/- N represented,**
- **Initial sample selection within sites selected genotypes with contrasting protein, TGW and width:length combinations:**

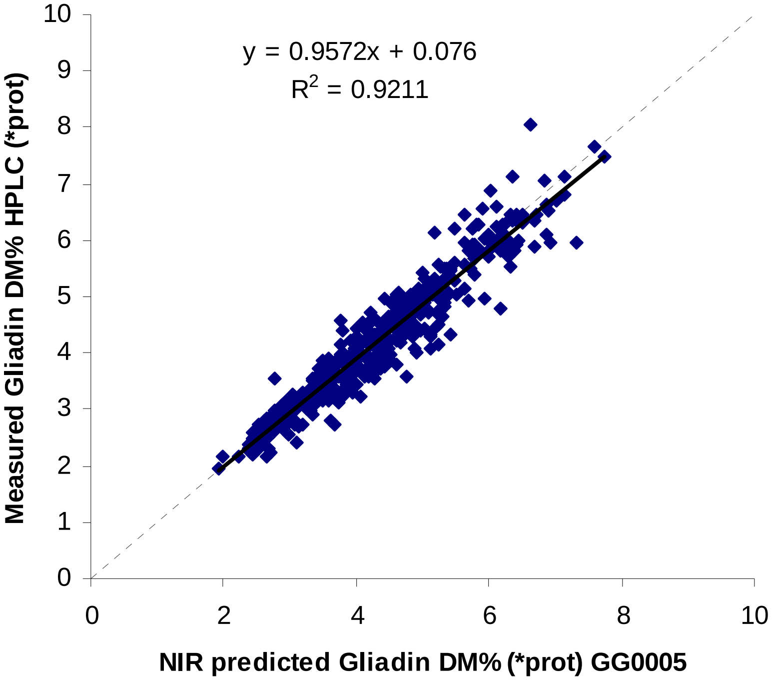
P:TGW:LW code	Protein	TGW	LW
1	H	H	H
2	H	H	L
3	H	L	H
4	L	H	H
5	L	L	H
6	L	H	L
7	H	L	L
8	L	L	L

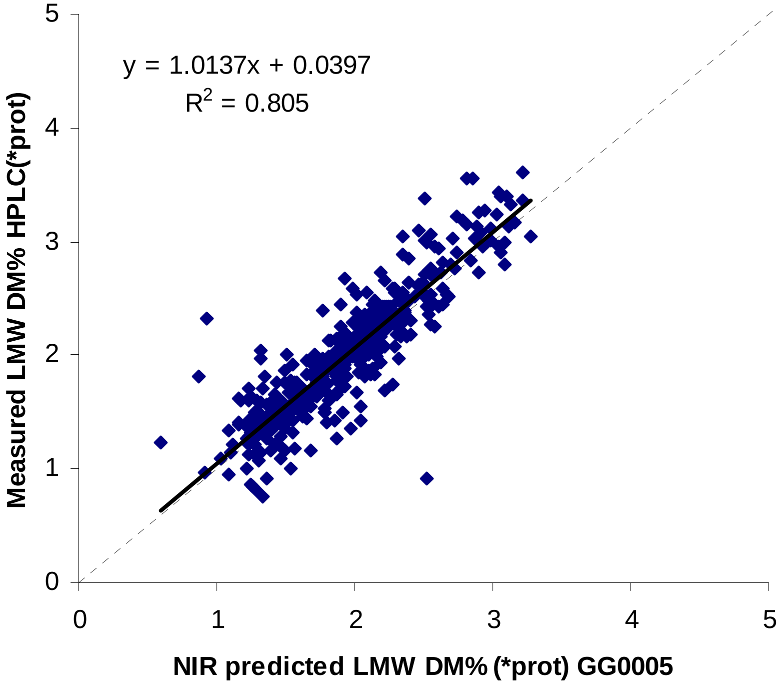
Ref dataset selection rationale (2)

- From season 2
- Included samples based on year 1 NIR predictions for AY and gliadin

P:AY:GLI code	Protein	AY	Gliadin
1	H	H	H
2	H	H	L
3	H	L	H
4	L	H	H
5	L	L	H
6	L	H	L
7	H	L	L
8	L	L	L

NIR calibrations – protein fractions

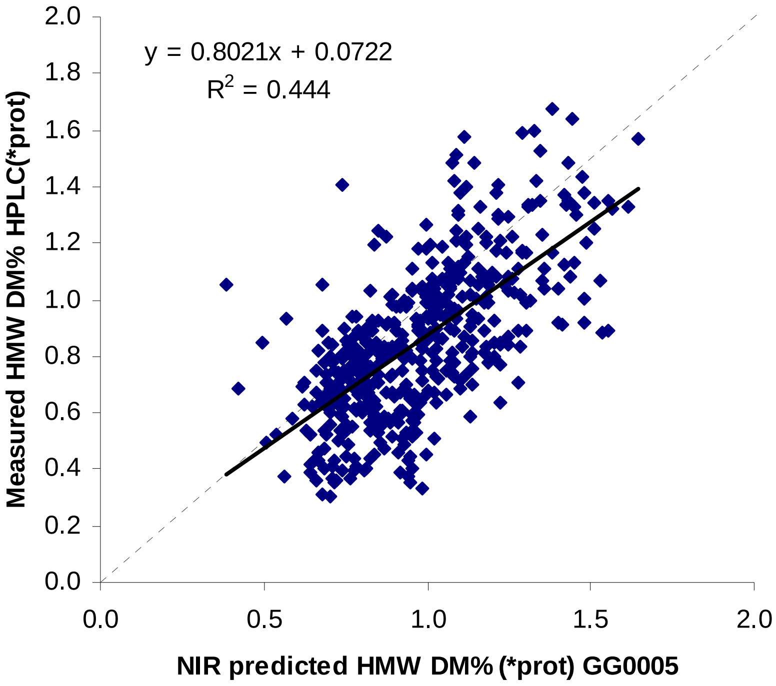


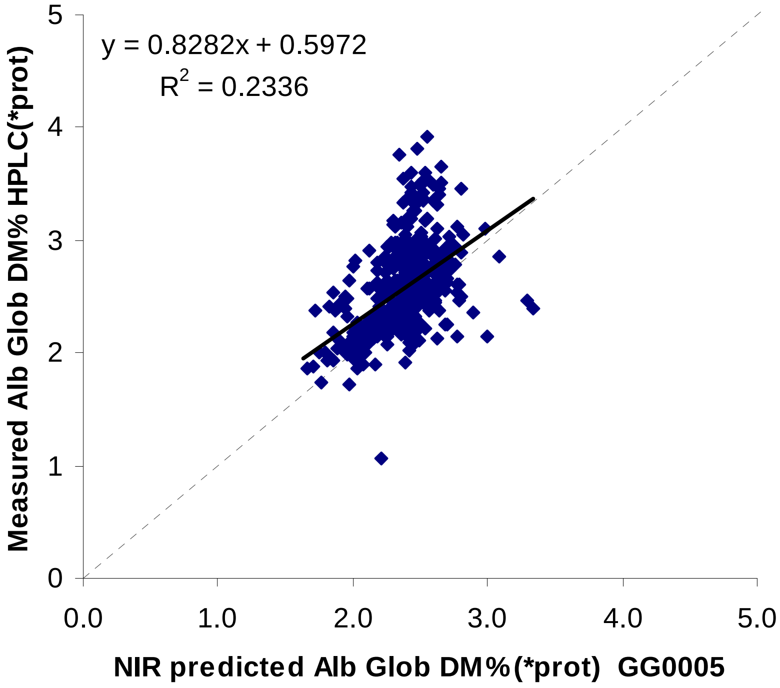


Reasonable calibrations for gliadins and LMW glutenins

… but don't know if the calibration is mainly detecting total protein

NIR calibrations – protein fractions





Poorer calibrations for HMW glutenins and albumins&globulins

NIR calibrations – protein fractions

- Have not been able to fully test the calibrations, in the sense that:
  - Need null gliadin lines to break any relationship between total protein and gliadin in the calibration
  - Attempted to screen Paragon mutants at JIC
    - not possible logistically

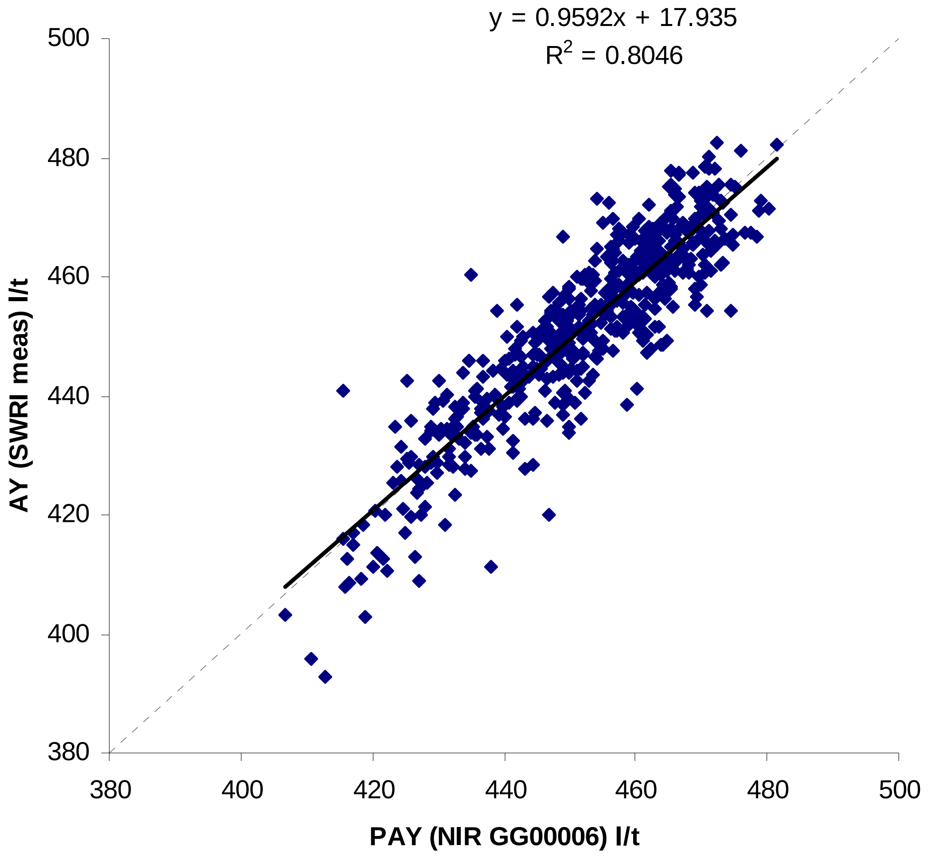
EMPP

- EMS mutated Paragon population
  - If mutants can be identified with reduced gliadin/protein but without damage to starch formation, this would allow testing of whether low grain N storage improves N use
- Large number of lines (~6500)
- Seed samples valuable
  - Quantities for phenotyping small (<50g)
  - Standard Infratec takes 1 kg samples

Calibrations which have been developed

- Alcohol yield (L/t)
- Residue viscosity
- Gliadin content (%DM)
- LMWG, A&G, HMWG (%DM)
- Protein fractions (% of total protein)
- (+ standard protein & starch)
  - Need to set up on FOSS 6500 model (wider spectral range, smaller cell size)

NIR calibrations – Alcohol yield



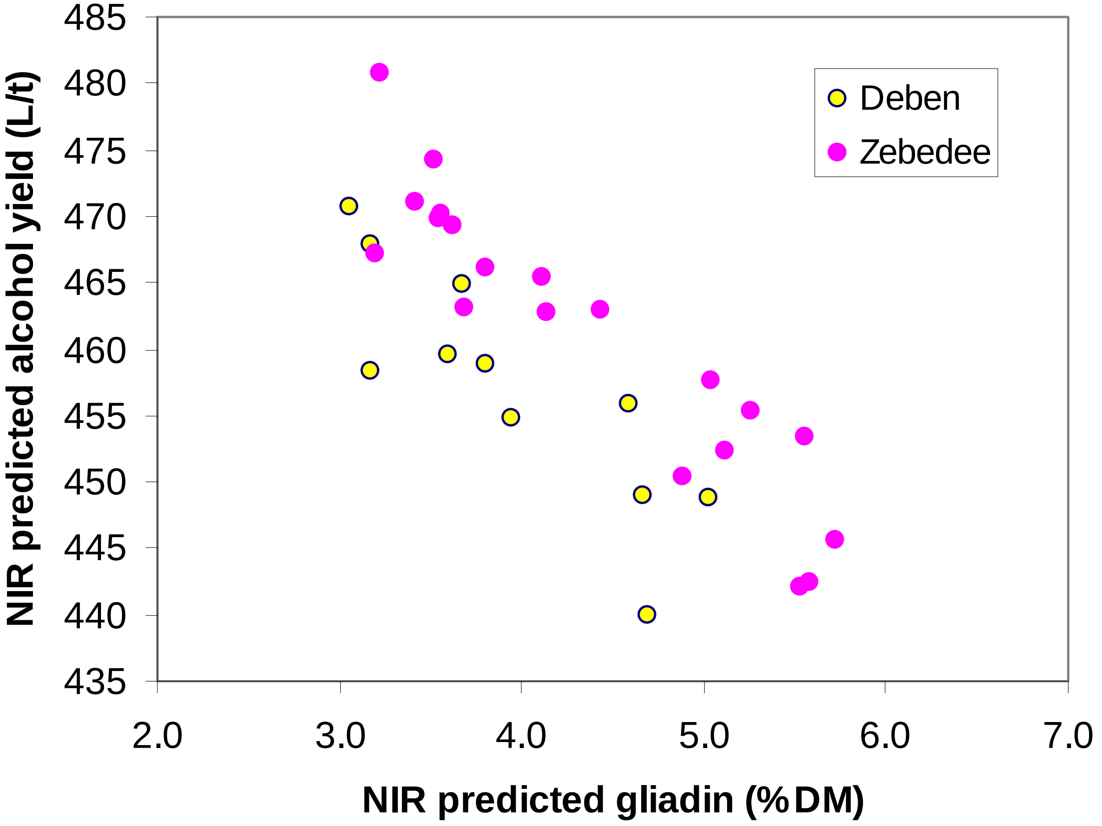
Have a useful NIR tool

Appears difficult to get higher R^2 than ~0.8

However adds a 'variety' factor on top of predicting AY from protein only

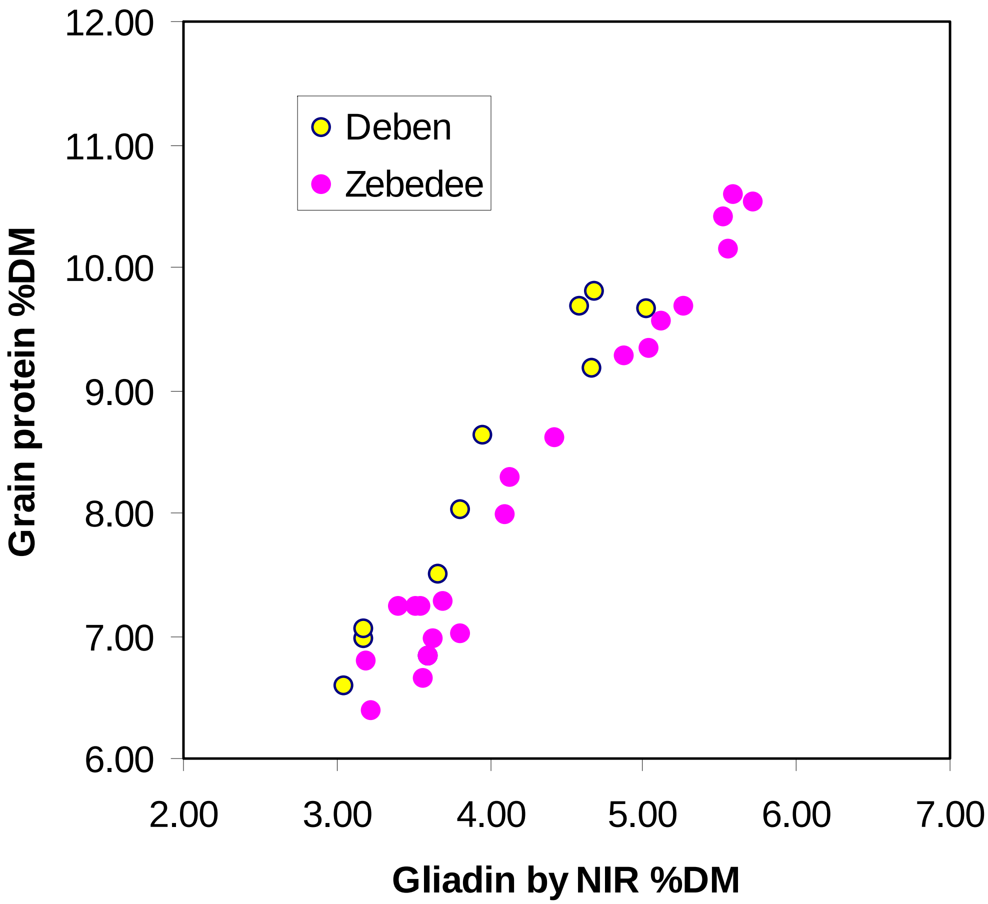
Calibration based on full ref dataset and CEL data

Use of NIR models to detect variety differences



Green Grain data 2005-2008 – site means from HM, TT, SCRI

Use of NIR models to detect variety differences



Work plan (1)

- Transfer ref samples SCRI to Aunir (month 1)
- Development of calibrations at Aunir for advanced platform (FOSS 6500, XDS) able to scan small samples (month 1)
- Transfer machine to JIC, train staff

Work plan (2)

- Scan minimum 3000 samples from EMPP (months 2-4)
- Predictions transferred to ADAS study relationships between AY and protein, gliadins and protein etc (month 5)
- Final report: findings, conclusions, recommendations for future research (month 6)

Thank you

richard.weightman@adas.co.uk