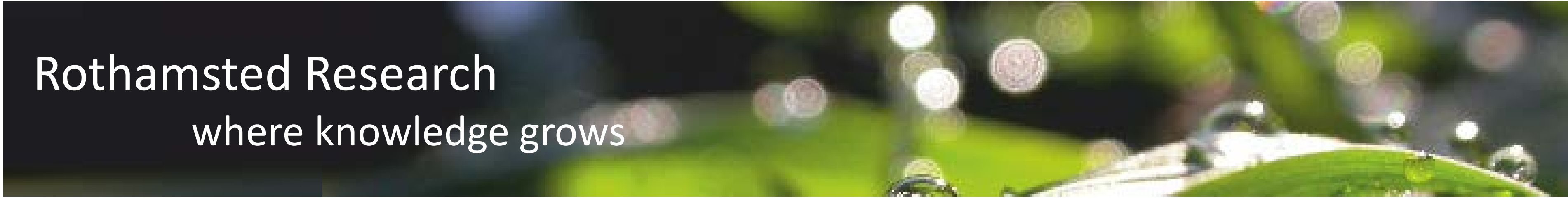


The use of NIRS to identify nitrogen content

WGIN Diversity Trial data update

Robert Jackson

Whittlesford, 1st May 2014

Extension project aims

Original Objective 8 The new **NUE** objectives for the 4 month extension will be:

- NIRS (near infra red spectroscopy) analysis of archived grain and create calibration curves against the standard Dumas combustion.
- Evaluate year to year stability of the calibration curves generated, and usefulness of using the same curve in multiple years.
- Assess usefulness of NIRS for future wheat NUE studies.
- Evaluate effectiveness of NIRS for monitoring straw N content

WGIN: The Nitrogen-Diversity trial

- 2004-13
- 51 varieties
- 14 varieties in at least 9 years
- 4 N levels in all except 2 years
- Grain and straw, yield and %N
- Archived fresh grain
- Archived dry milled grain and straw

Year	Varieties (core of 9)	N-levels	kg N/ha
2007	24	4	0,100,200,350
2008	24	4	0,100,200,350
2009	24 (include 6 x A x Cs)	4	0,100,200,350
2010	25 (include 6 x A x Cs)	4	0,100,200,350
2011	25 (include 4 x A x Cs)	4	0,100,200,350
2012	25 (include WUE/take-all lines)	4	0,100,200,350
2013	25 (include WUE/take-all lines)	4	0,100,200,350




Nitrogen determining methods

- Kjeldahl method
  - a three step process
  - Requires:
    - Acid digestion with a heavy metal catalyst
    - Caustic dilution
    - Titration
- Dumas combustion method
  - requires the combustion of the sample and detection of the nitrogen produced
- Both require destructive sampling of samples and present health risk to operators health

Near-infrared spectroscopy (NIRS)

Agrispec NIRS equipment

Muglite

High-intensity contact probe

analytik.co.uk

- Low cost, low input, high-throughput
- NIR spectra generation is comparison of the radiation reflectance of a sample, with the reflectance radiation of a reference.
- Comparison is performed by software packages (e.g. GRAMS IQ)
- Numerous characteristics can be analysed
- Here NIR spectra generated from milled grain sample will be used to identify %N
- %N reference data obtained by Dumas combustion

Single year predictions

ROTHAMSTED RESEARCH

Evaluate year to year stability of the calibration curves generated, and usefulness of using the same curve in multiple years.

- Calibration produced from NIR spectra and %N of ≈200 samples from one year
- %N of samples from remaining 6 years of WGIN trial predicted from their NIR spectra using calibrations
- Predicted %N values were correlated against reference %N.

2007

Predicting	r^2	SEP	P
2008	0.96	0.9	0
2009	0.97	0.42	0
2010	0.93	0.87	0
2011	0.98	1.07	0
2012	0.89	1.13	0
2013	0.97	1.17	0

2008

Predicting	r^2	SEP	P
2007	0.96	0.94	0
2009	0.97	0.42	0
2010	0.93	1.39	0
2011	0.99	0.18	0
2012	0.85	1.34	0
2013	0.97	0.93	0

2009

Predicting	r^2	SEP	P
2007	0.89	1.05	0
2008	0.93	0.41	0
2010	0.86	1.69	0
2011	0.99	0.35	0
2012	0.74	1.72	0
2013	0.94	1.04	0

2010

Predicting	r^2	SEP	P
2007	0.94	0.42	0
2008	0.93	0.51	0
2009	0.97	0.73	0
2011	0.99	0.03	0
2012	0.97	0.51	0
2013	0.97	0.32	0

2011

Predicting	r^2	SEP	P
2007	0.91	1.01	0
2008	0.94	0.28	0
2009	0.97	0.38	0
2010	0.89	1.56	0
2012	0.75	1.68	0
2013	0.97	1.4	0

2012

Predicting	r^2	SEP	P
2007	0.93	0.59	0
2008	0.88	0.44	0
2009	0.97	0.78	0
2010	0.96	0.91	0
2011	0.99	0.82	0
2013	0.97	0.48	0

2013

Predicting	r^2	SEP	P
2007	0.95	0.65	0
2008	0.96	0.6	0
2009	0.97	0.02	0
2010	0.98	0.83	0
2011	0.99	0.43	0
2012	0.97	0.67	0

BBSRC bioscience for the future

GRAMS IQ calibrations

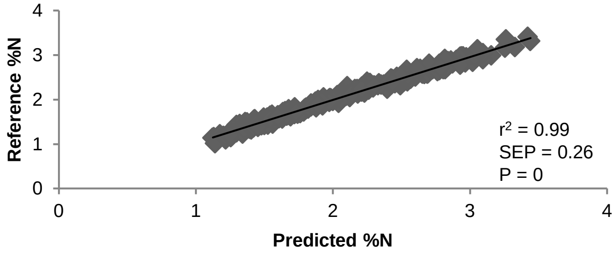
Assess usefulness of NIRS for future wheat NUE studies.

- Calibration produced between reference %N and NIR spectra of samples from 5 years of WGIN project
- Calibrations used to predict %N from NIR spectra of samples not used in calibration
- Effectiveness of calibration/model is assessed by correlating predicted with reference %N

Multi-year GRAMS IQ calibration

Model	Year predicted	r^2	SEP	P
-2010, -2012	2010	0.96	0.24	0
-2010, -2012	2012	0.96	0.19	0
-2011, -2013	2011	0.99	0.26	0
-2011, -2013	2013	0.97	0.54	0
-2008, -2009	2008	0.96	0.48	0
-2008, -2009	2009	0.97	0.2	0
-2007, -2011	2007	0.96	0.65	0
-2007, -2011	2011	0.99	0.34	0

Correlation of 2011 reference %N and predicted %N from Multi-year GRAMS IQ -2011, -2013 calibration



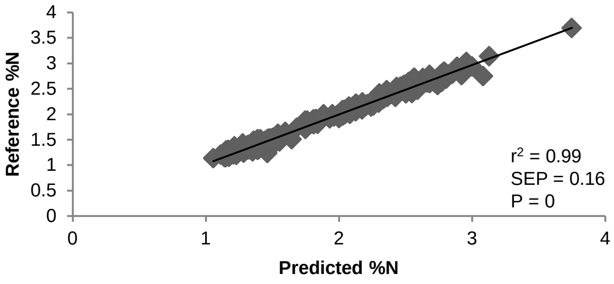
GenStat model

$$\%N_{ij} = x + b \times NIR\ spectra + year_i + variety_j + (NIR\ spectra.variety)_j + (year.variety)_{ij}$$

Assess usefulness of NIRS for future wheat NUE studies.

- Taking into account effect of each variety and year data gives a more precise model

Correlation of 2013 reference %N and predicted %N from -2011, -2013 Complex GenStat model



Multi-year GRAMS IQ calibration					Complex GenStat model				
Model	Year predicted	r^2	SEP	P	Model	Year predicted	r^2	SEP	P
-2010, -2012	2010	0.96	0.24	0	-2010, -2012	2010	0.98	0.08	0
-2010, -2012	2012	0.96	0.19	0	-2010, -2012	2012	0.97	0.09	0
-2011, -2013	2011	0.99	0.26	0	-2011, -2013	2011	0.99	0.19	0
-2011, -2013	2013	0.97	0.54	0	-2011, -2013	2013	0.99	0.16	0
-2008, -2009	2008	0.96	0.48	0	-2008, -2009	2008	0.97	0.26	0
-2008, -2009	2009	0.97	0.2	0	-2008, -2009	2009	0.96	0.22	0
-2007, -2011	2007	0.96	0.65	0	-2007, -2011	2007	0.97	0.34	0
-2007, -2011	2011	0.99	0.34	0	-2007, -2011	2011	0.99	0.3	0

Conclusion

- %N predictions based on NIRS software calibrations produce large errors and do not take into account other variables
- GenStat model taking into account effect of each variety and year presents a high-throughput technique to predict %N of milled grain from NIRS
- NIRS presents a cheap, non-destructive, safe alternative for %N determination
- NIRS is also effective at predicting %N of milled straw samples
- Other factors (e.g. yield, NutE, ears etc.) can also be predicted by NIRS

Original Objective 8, extension funding aim:

Evaluate existing NUE/yield datasets for indications of year to year stability of traits

Compiling the WGIN Nitrogen-Diversity trial data

ROTHAMSTED RESEARCH

- Diversity trial funding is coming to end
- Currently yield and %N data available for 2004-2013 trials
- 2004-2007 data has been published (Barraclough et al., 2010)
- Statistical analysis will focus on yield, uptake and N use efficiency data from 2007-2013 trials

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ELSEVIER

Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement

Peter B. Barraclough[a,*], Jonathan R. Howarth[a], Janina Jones[a], Rafael Lopez-Bellido[b], Saroj Parmar[a], Caroline E. Shepherd[a], Malcolm J. Hawkesford[a]

[a] Plant Science Department, Rothamsted Research, West Common, Harpenden, Hertfordshire, AL5 2JQ, UK
[b] Departamento de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, Edificio C4 "Celestino Mutis", Ctra. 14071 Córdoba, Spain

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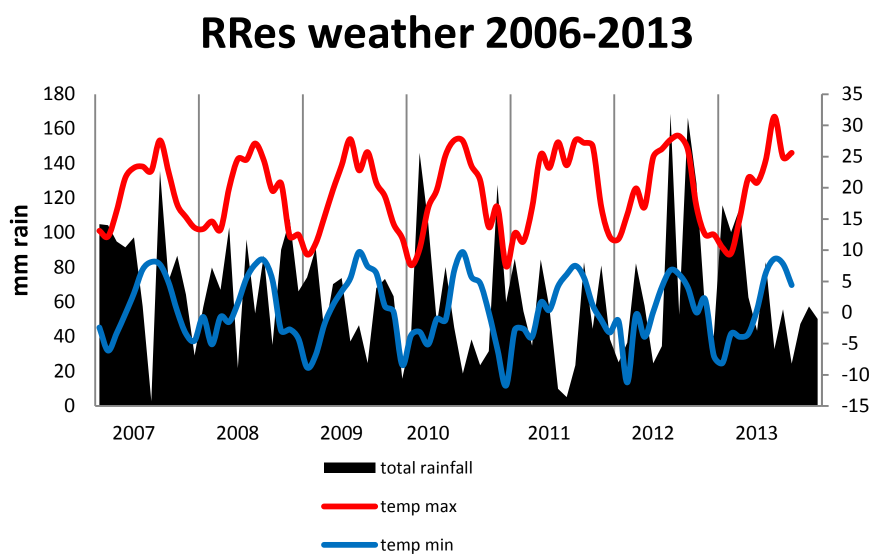
Stats

ROTHAMSTED RESEARCH

- Rothamsted stats department producing a model for yield, grain/straw %N content, HI, N uptake, NHI, NutE
- Model will take into account year variation and plot/block variation
- Model should allow us to rank variety performance.



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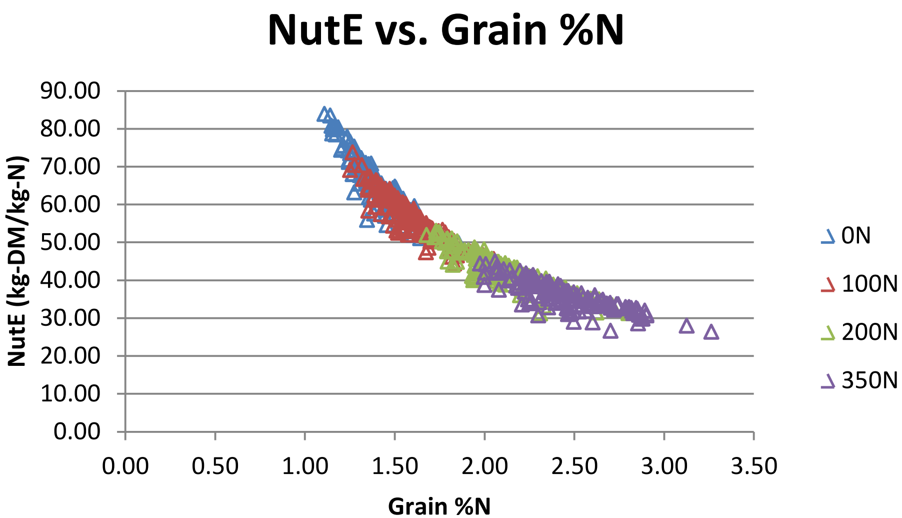
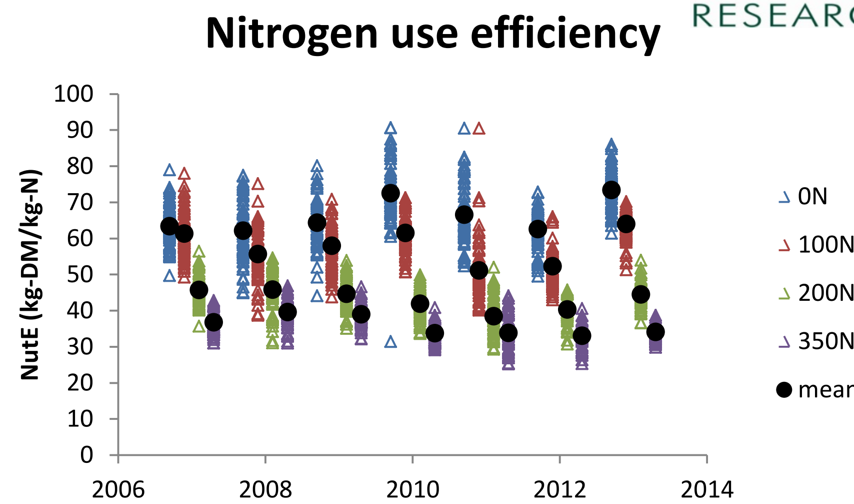
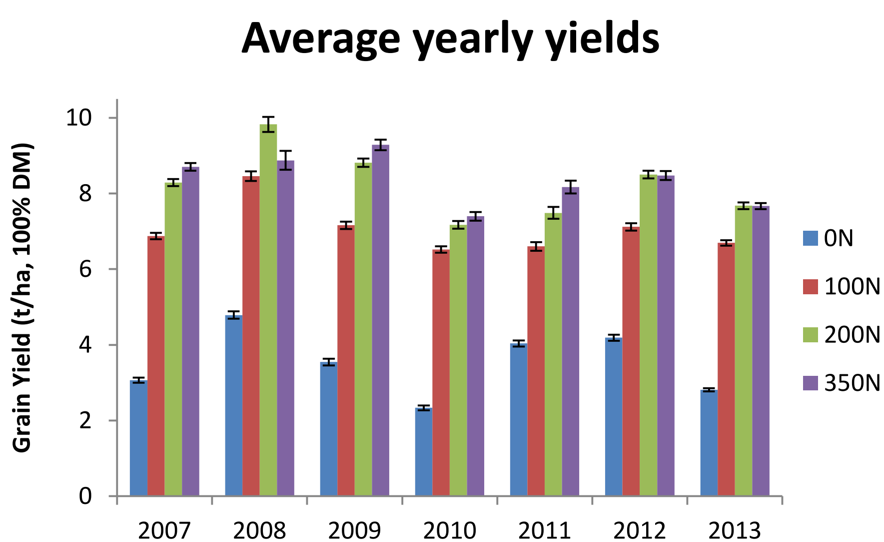
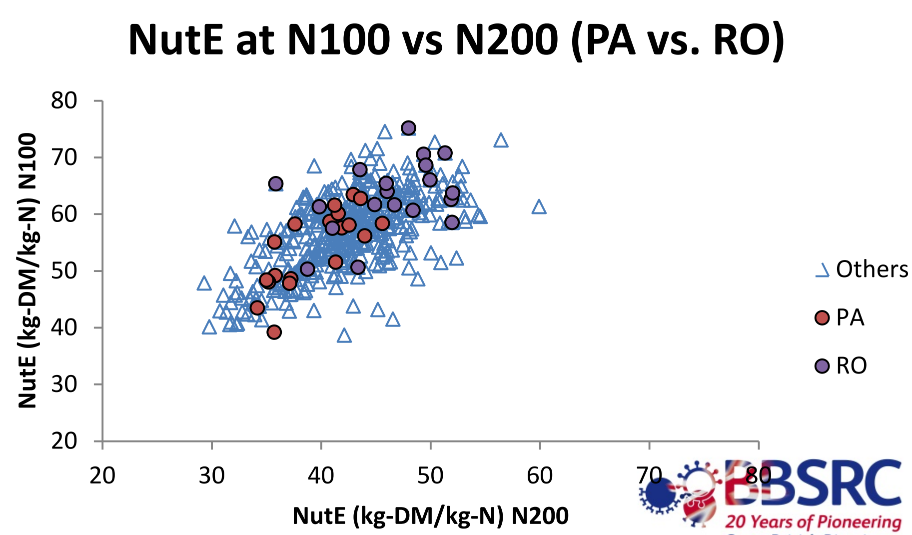
2013 data


NutE vs. Grain %N
NutE (kg-DM/kg-N)
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
Grain %N
0N
100N
200N
350N


Nitrogen use efficiency
NutE (kg-DM/kg-N)
100
90
80
70
60
50
40
30
20
10
0
2006
2008
2010
2012
2014
0N
100N
200N
350N
mean


Average yearly yields
Grain Yield (t/ha, 100% DM)
10
8
6
4
2
0
2007
2008
2009
2010
2011
2012
2013
0N
100N
200N
350N


NutE at N100 vs N200 (PA vs. RO)
NutE (kg-DM/kg-N) N100
80
70
60
50
40
30
20
20
30
40
50
60
70
80
NutE (kg-DM/kg-N) N200
Others
PA
RO

Thanks

ROTHAMSTED RESEARCH

- Malcolm Hawkesford
- Peter Buchner, Saroj Parmar, Andrew Riche, Yongfang Wan, Peter Barraclough
- Stats: Rodger White, Stephen Powers
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- RRes Farm staff
- Summer students and casuals
- Peter Shewry
- WISP, WGIN and 20:20 teams

20:20 Wheat®

defra
Department for Environment Food and Rural Affairs

Wheat Genetic Improvement Network

HGCA

WISP

BBSRC
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Objective 10
Take-all Disease

Vanessa McMillan
Richard Gutteridge
Kim Hammond-Kosack

Wheat Genetic Improvement Network

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ROTHAMSTED RESEARCH

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

Take-all disease of wheat

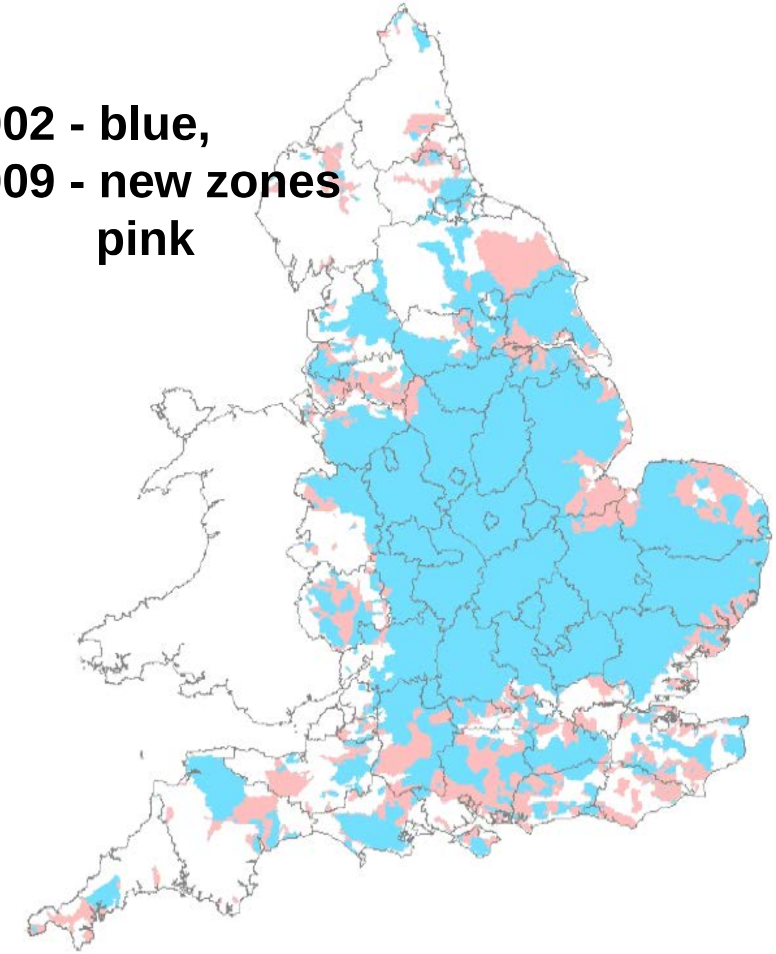
- *Gaeumannomyces graminis* var. *tritici* (*Ggt*)
- ascomycete soil borne fungus
- *Ggt* infects the roots
- No genetic solution to the control of take-all disease available

Severely take-all infected plant

The ‘ HIDDEN IMPACT ’ of take-all root infections

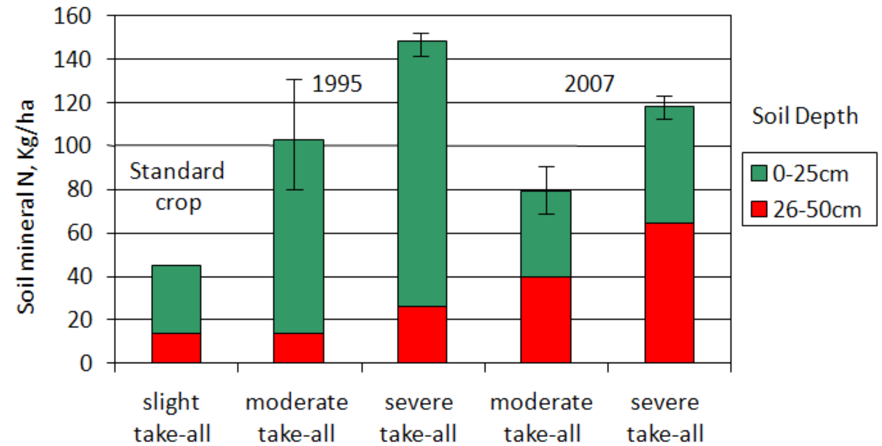
NITRATE VULNERABLE ZONES

2002 - blue,
2009 - new zones pink



Source
http://www.defra.gov.uk/environment

Effects of Take-all on soil mineral N at harvest



Rainfall (mm)		1995	2007
	April	11.3	2.8
	May	**28.4**	**135.8**
	June	**28.2**	**72.4**
	July	18.6	86.8

Eutrophication

- **Increased biomass – algal blooms**
- **Decreased oxygen concentrations**
- **Ecosystem collapse**
- **GHG emissions**

Disease development

- **1st wheat crop-** very little disease provided break crop is free from take-all carriers

- **2nd – 4th wheat crop-** severe disease can occur during this period

- **5th wheat crop onwards-** take-all severity decreases compared to a crop at its peak. This is known as Take-all Decline (TAD)

THE SOIL CORE BIOASSAY

1. Soil core taken angled underneath row

2. Core inverted into plastic cup

3. Ten bait wheat (cv Hereward) seeds sown

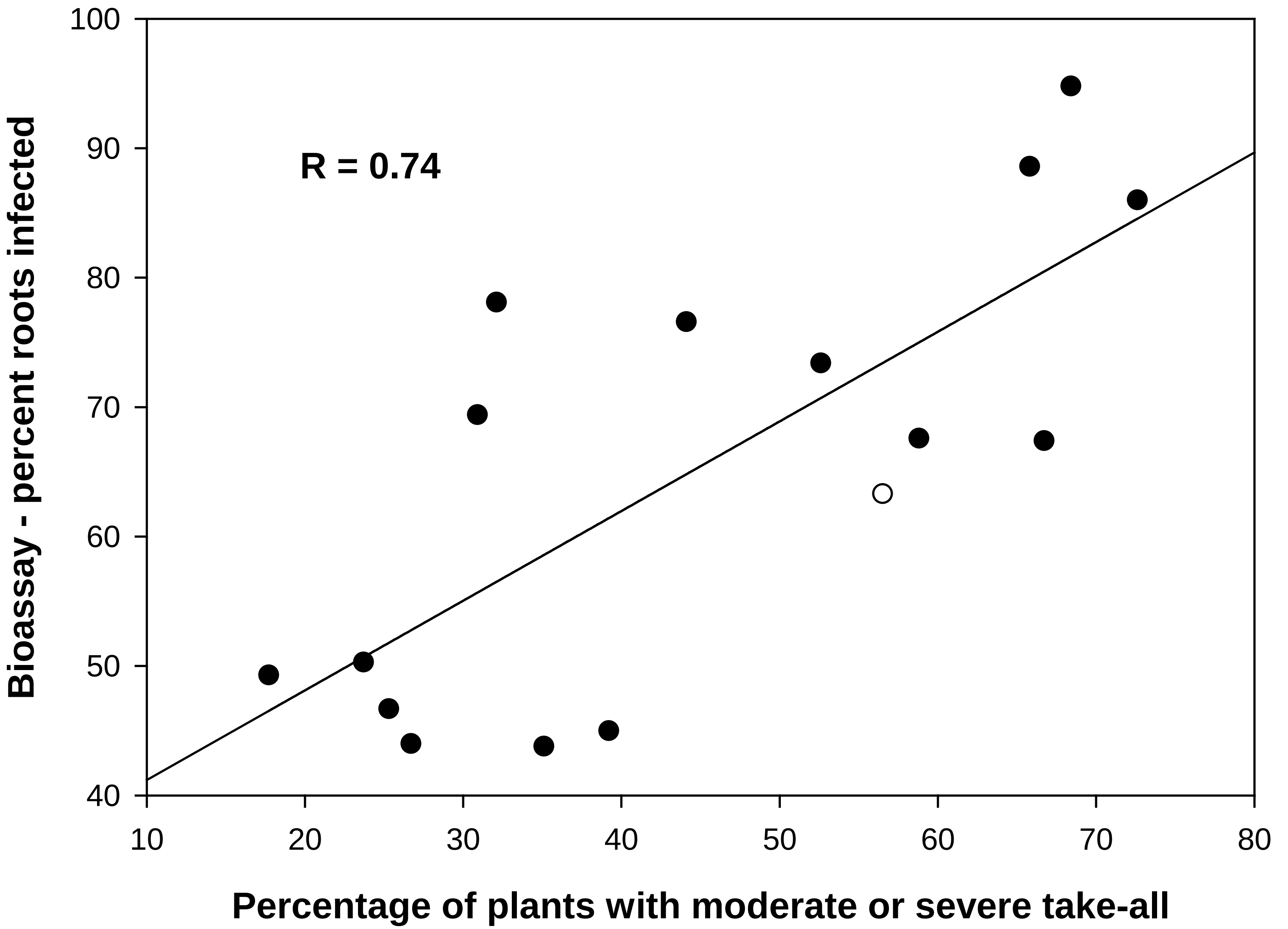
4. Growth room for 5 weeks

Soil core bioassay plants

Severe take-all infection

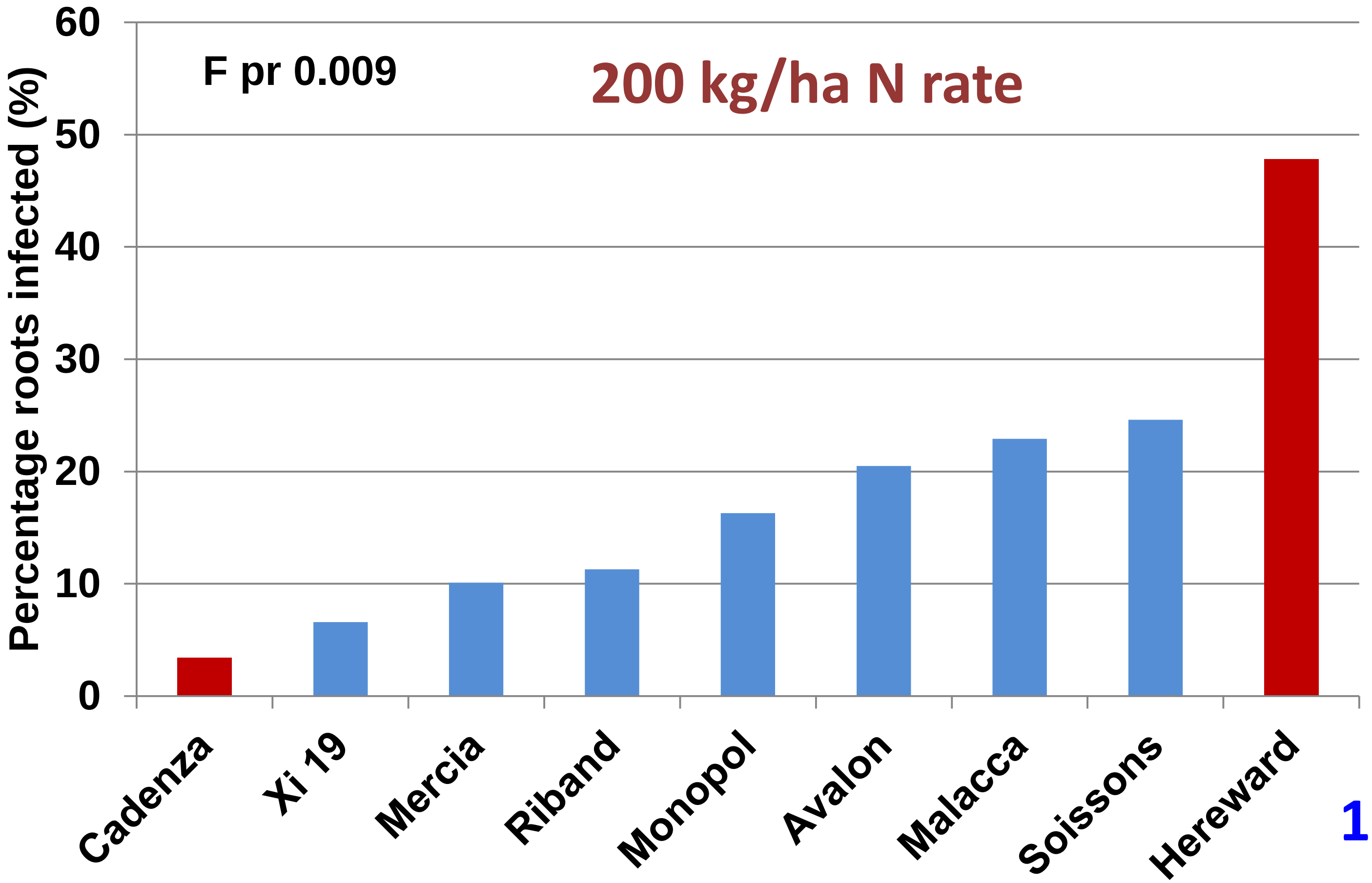
Slight take-all infection

The relationship between percentage of roots infected in the autumn bioassay and the disease in the following crop



WGIN winter wheat soil core bioassay (4 year means)

New trait is called **TAB** (**T**ake-**A**ll inoculum **B**uild-up)

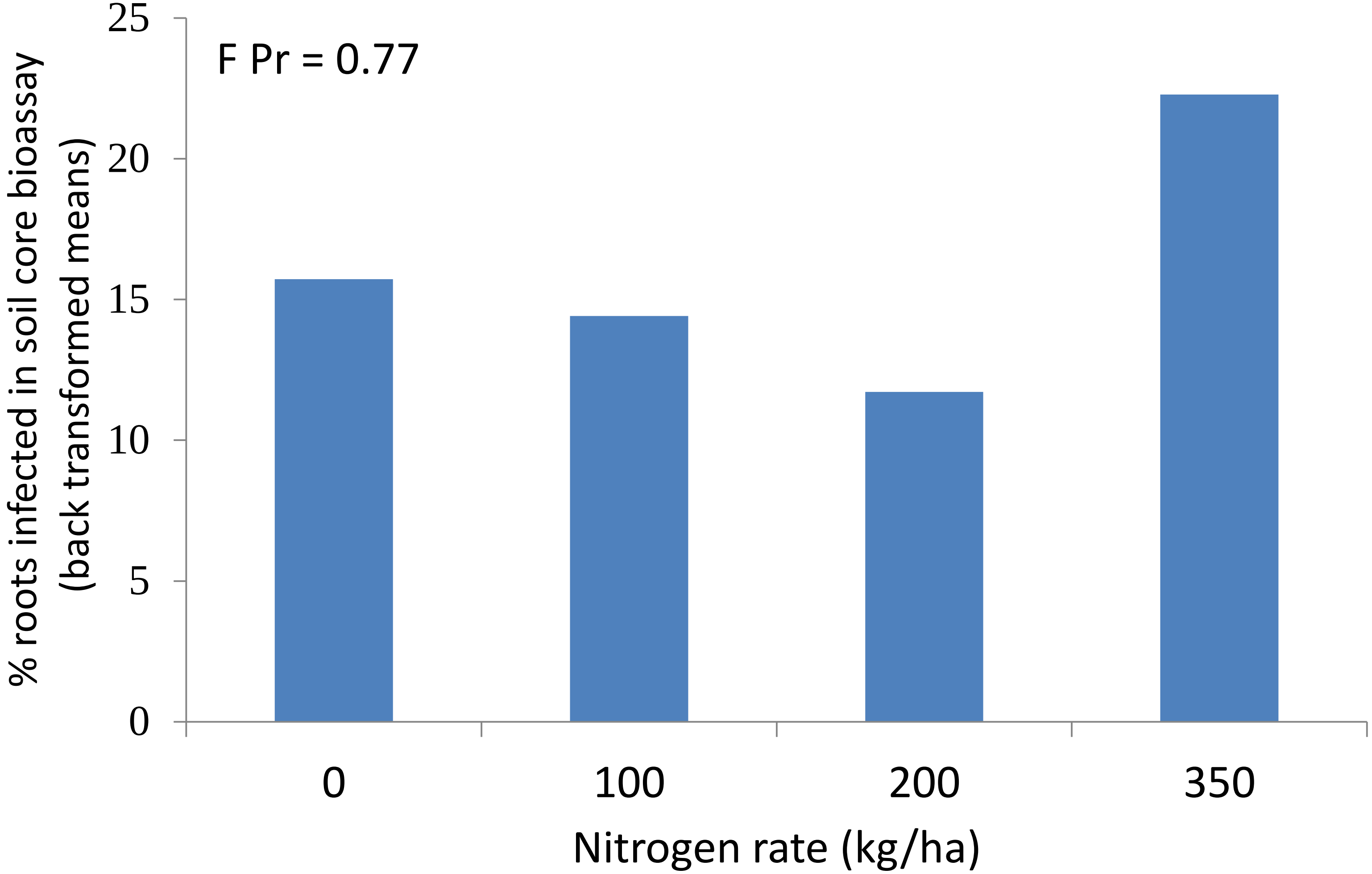


1st wheats

McMillan *et al*. 2011, Plant Pathology, **60**, 200-206

Effect of N rate on TAB trait

WGIN Diversity trial 2011/R/WW/1102 Field: Meadow



WGIN Diversity trial 2013

2013/R/WW/1316 Field: Blackhorse

June and July

Cadenza (Low TAB) and Hereward (High TAB) plots sampled at all 4 N rates

Post harvest sampling

All 25 cultivars sampled at 200 kg/ha N

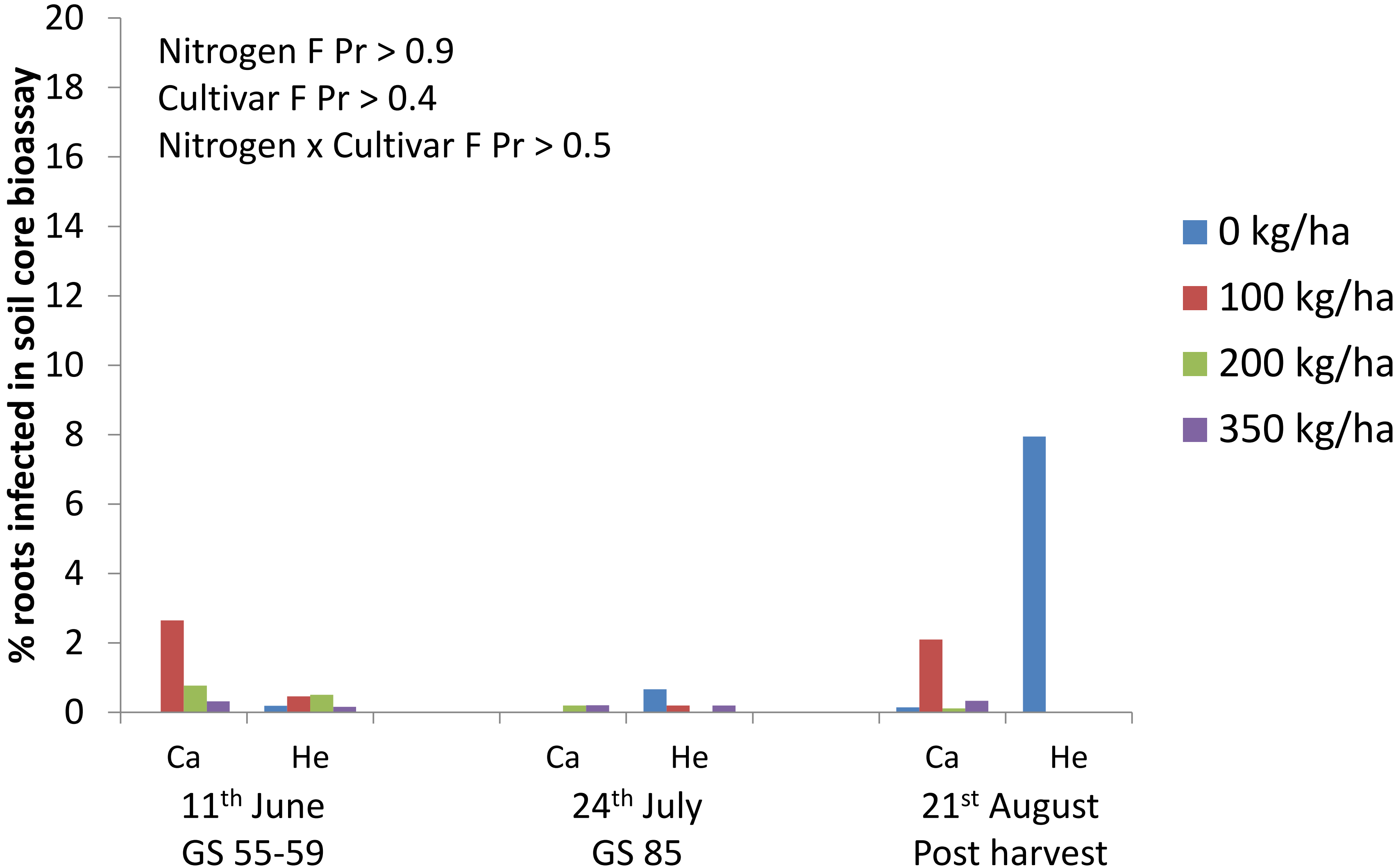
6 cultivars sampled at all 4 N rates – 0, 100, 200 and 350 kg/ha

Aerial photograph June 2013

WGIN Diversity trial 2013

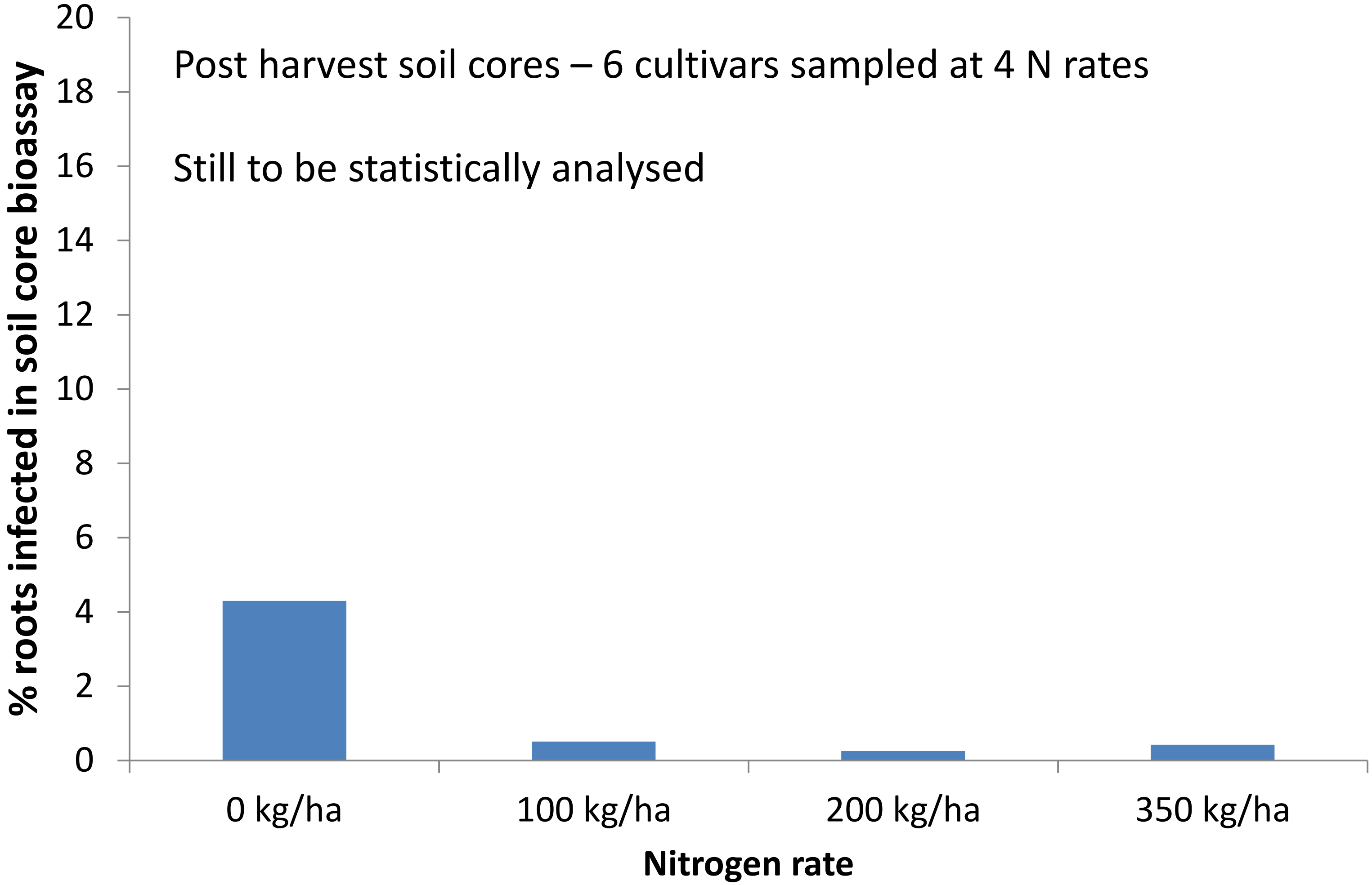
2013/R/WW/1316 Field: Blackhorse

Cadenza and Hereward monthly samples



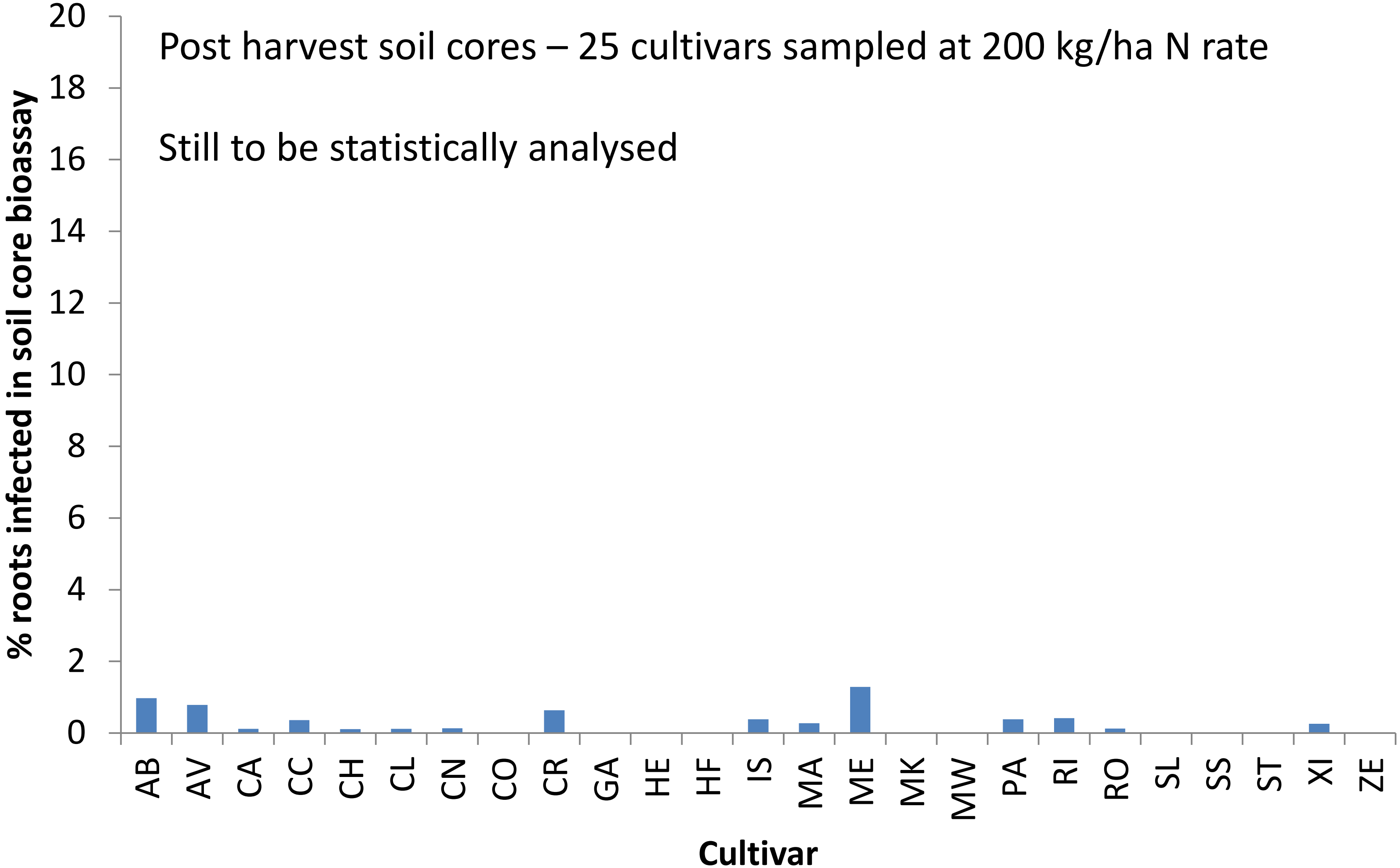
Effect of N rate on TAB trait

WGIN Diversity trial 2013/R/WW/1316 Field: Blackhorse

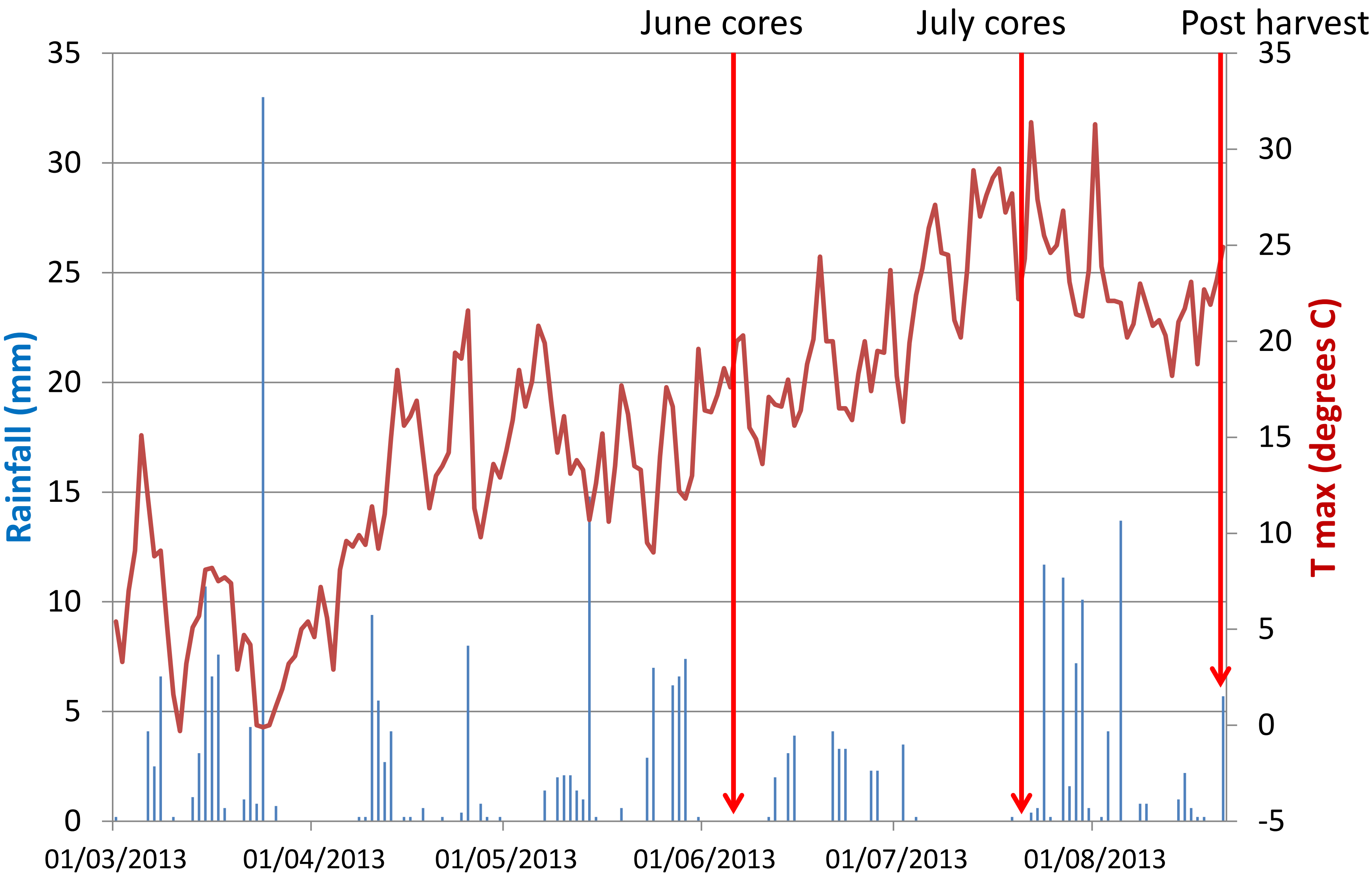


Effect of cultivar on TAB trait

WGIN Diversity trial 2013/R/WW/1316 Field: Blackhorse



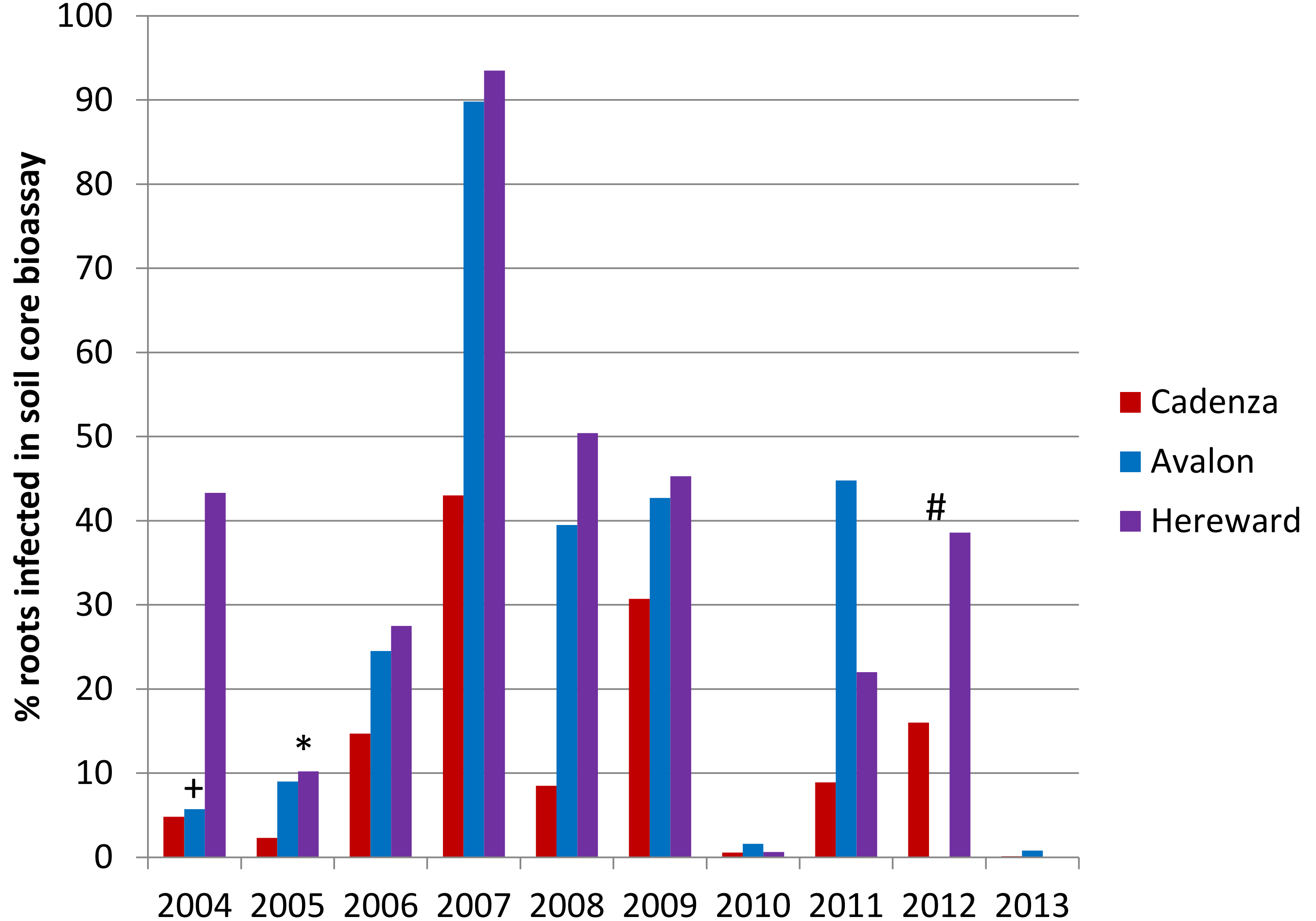
Rainfall and temperature data March – August 2013



Rainfall in the last month of the season is not sufficient to build-up take-all inoculum in the soil

A decade of Take-all build up data from the WGIN 1st wheat diversity trial

% roots infected with take-all in a soil core bioassay Avalon, Cadenza and Hereward 2004 - 2013 WGIN Diversity trials at 200 kg N/ha



+ In 2004 cv. Avalon was sown late and the emerging seedlings were dislodged by rooks, *Corvus frugilegus*

* High incidence of *Phialophora* in 2005 trial site reduced TAB

2012 data from WGIN cultivar rotation trial, Ca and He only

Objective 10 – Take-all disease
Project extension objectives

1. Explore the most resistant *T. monococcum* (*Tm*) lines and a selection of the susceptible *Tm* for seedling root architecture using the root roll bioassay

2. Explore the seedling root architecture of selected A x C lines with and without the QTLs controlling the Low TAB trait using the root roll assay

3. Take the two newly developed *Tm* populations to the F5 generation – F3 seed currently in the glasshouse to produce the F4

MDR031 (R) x MDR043 (S)
MDR043 (S) x MDR046 (R)

4. Continue the *Tm* take all resistance introgression from MDR 31, MDR 46 and MDR 229 into Paragon wheat to the next generation.

Many thanks to

RRes Farm staff

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Gail Canning

Rodger White (Stats)

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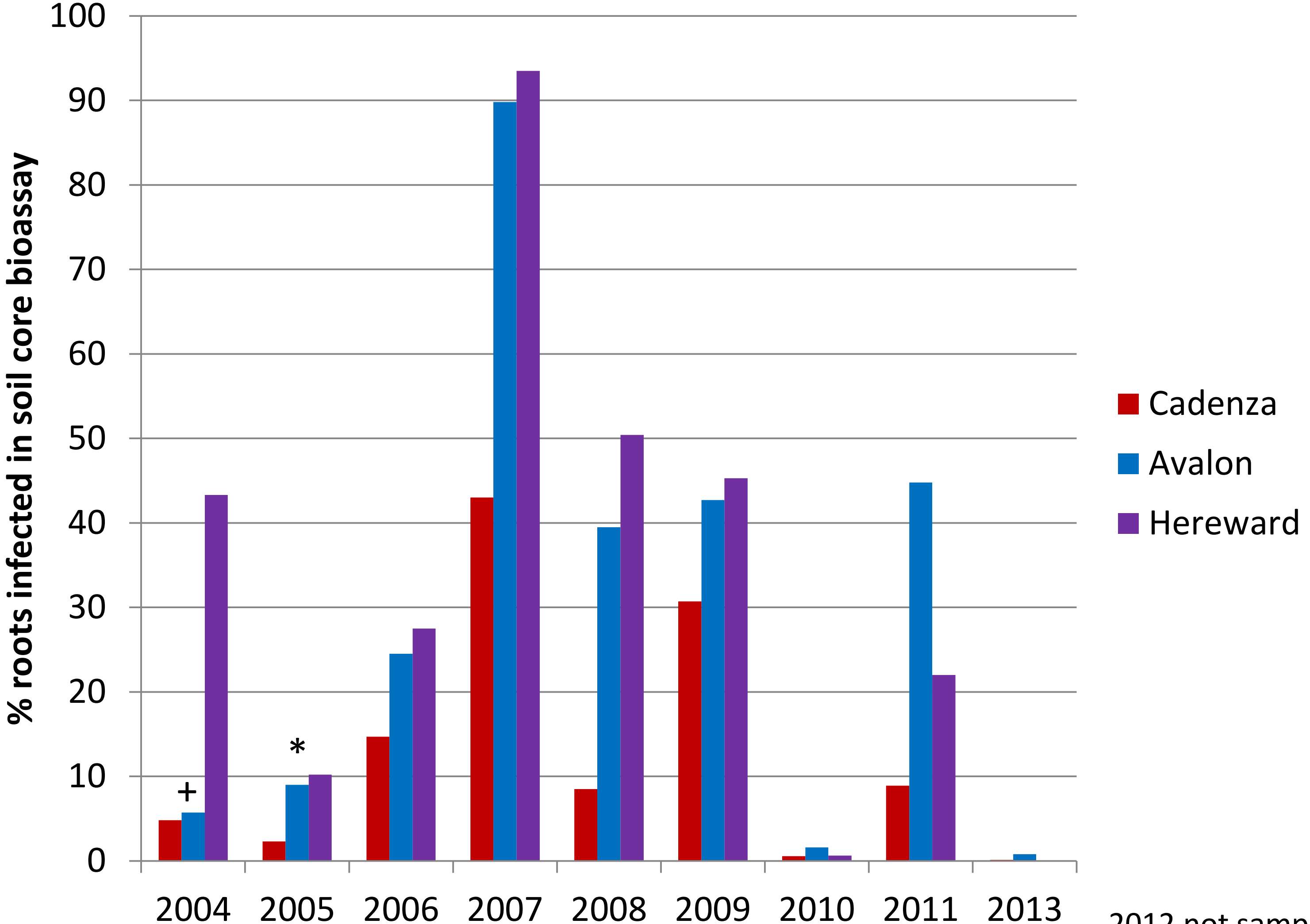
Martha Jones (WGIN)
Nicola Phillips (HGCA)
Joseph Whittaker (BBSRC)
Adrian Czaban (WGIN)
Marcin Czaban (WGIN)
James Bruce (HGCA-BBSRC)
Steve Freeman (WGIN)
Carl Halford (WGIN)
Daniela Izera (WGIN)
Mike Hammond-Kosack (TSB)
Mike Hall (TSB-WGIN)
Aisling Clifford (WGIN)

% roots infected with take-all in a soil core bioassay Avalon, Cadenza and Hereward 2004 - 2013 WGIN Diversity trials at 200 kg N/ha



2012 not sampled. Reason Summerdell known to have a high incidence of Phialophora

+ In 2004 cv. Avalon was sown late and the emerging seedlings were dislodged by rooks, *Corvus frugilegus*

* High incidence of *Phialophora* in 2005 trial site reduced TAB

Drought tolerance

WGIN-2 SG meeting

Syngenta Whittlesford 1 May 2014

The University of Nottingham

JOHN INNES CENTRE

defra
Department for Environment Food and Rural Affairs

Wheat Genetic Improvement Network

Activity 9, Drought tolerance (2009-14)

Obj 1. Identify traits for WUE and drought tolerance (DT) in elite winter wheat varieties. (*Yrs 1-2*)

Obj 2. Identify QTLs for WUE and DT traits using one DH pop in an elite background. (*Yrs 2-3*)

Obj 3. Develop one new DH pop for drought research. (*Yrs 2-4*)

Obj 4. Identify novel genes and alleles for WUE and DT using the AE Watkins and Gediflux collections. (*Yrs 2-4*)

Obj 5. Collate germplasm (cvs, advanced lines) for future genetics studies. (*Yrs 4 -5*)

WGIN Objective 9.2 QTL Detection

2010-11, 2011-12 and 2012-13 expts

- Rialto x Savannah DH population for phenotying for yield physiological traits (94 lines and 2 parents)
- 2 sites: Nottingham - irrigated & unirrigated; JIC - unirrigated
- Target traits
    - $^{13}C\ \Delta$ grain
    - senescence kinetic
    - stem WSC

Measurements on DH pop

- **Combine grain yield, yield components**
- **% stem WSC at GS61+10d (unirrigated)**
- **Leaf senescence kinetics for flag-leaf, L2 and L3.**
- **Canopy temperature**
- **grain Δ 13C (unirrigated)**
- **NDVI**

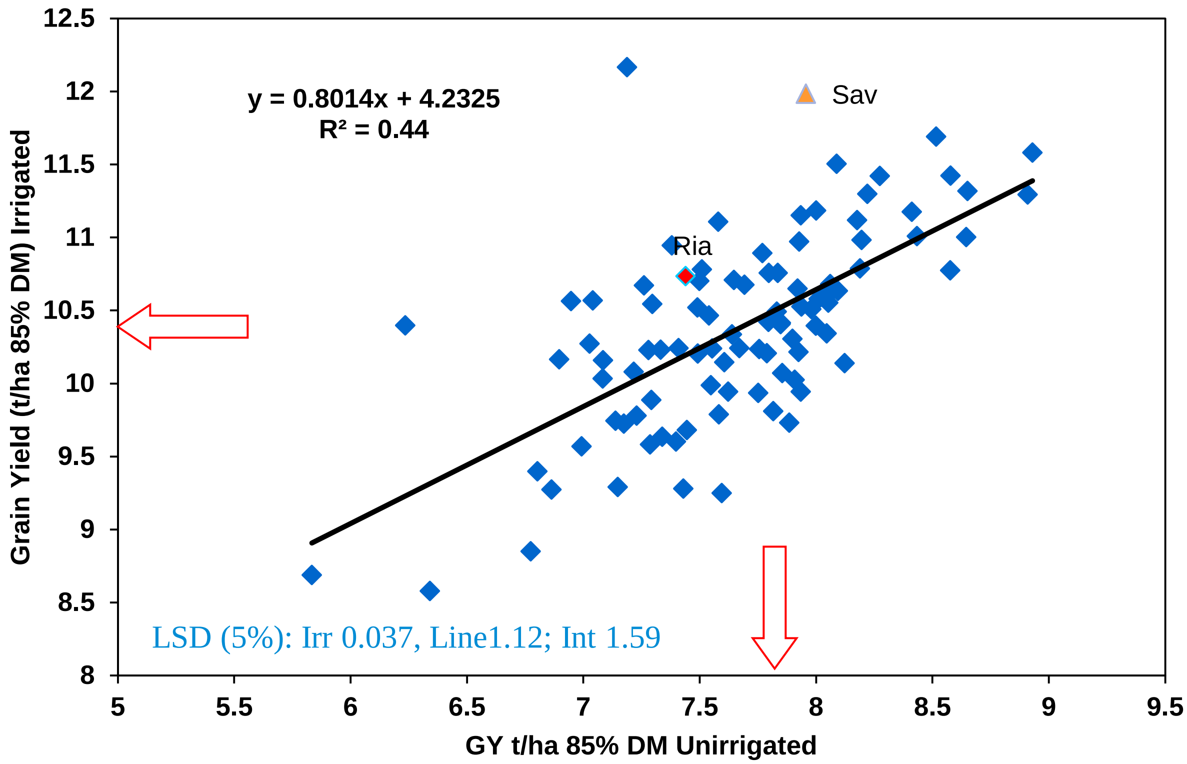
Drought effects 11 July 2011

L2

L39

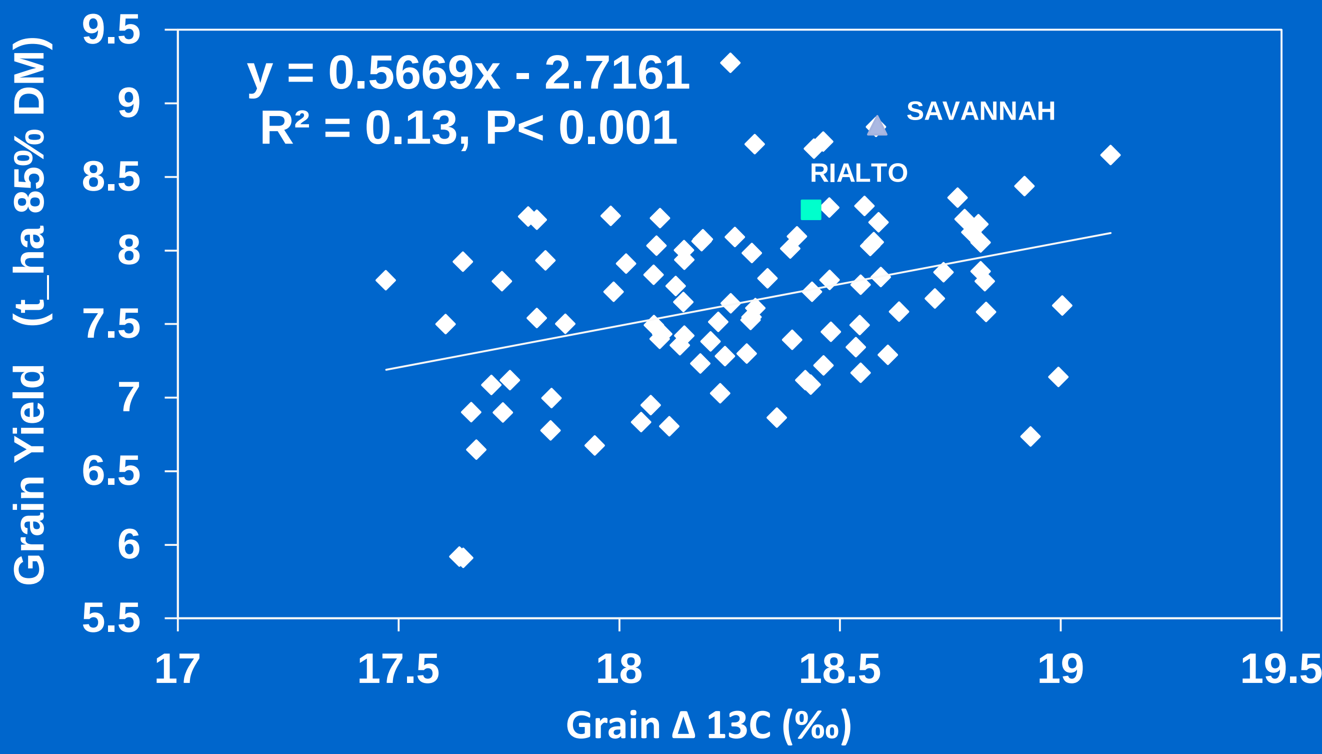
L47

Grain yield responses to irrigation



Sutton Bonington 2010-11

Δ ^{13}C vs grain yield: Savannah x Rialto DH 2010-11



High WUE ← Low WUE

L2

L39

L47

SavXRia Sutton Bonnington QTLs 2010-11

chr	pos	LOD	var	mean	add eff	on	marker	trait	env
1D	28	3.1	12.9	0.5	-0.008	S	RAC875_c78062	HI	IRRI11
4D	17.8	2.7	11.1	0.5	0.008	R	GENE-2812	HI	IRRI11
4A	191.6	2.5	7.7	50.8	-0.8	S	BS00023164	TGRWT	IRRI11
5A	70.8	5.8	20.1	50.8	-1.35	S	Kukri_c61108	TGRWT	IRRI11
6A	129	4	13.1	50.8	-1.1	S	EXcalibur_c52196	TGRWT	IRRI11
2D	3	3	12	343	12.9	R	Kukri_c2912	EARNBpsqm	UNIRRI11
7A	0	3.9	16	343	15.1	R	EXcalibur_c48636	EARNBpsqm	UNIRRI11
2D	3	4.4	17.3	16267	703	R	Kukri_c2912	GRpsqm	UNIRRI11
3B	261	3	11.3	16266	-585	S	Ex_c14162_22093694	GRpsqm	UNIRRI11
3B	260	2.4	11.3	7.6	-0.19	S	Ex_c14162_22093694	GRYLD	UNIRRI11
3A	144.9	2.6	7.2	47	1.2	R	BS00110564	TGRWT	UNIRRI11
5A	337.3	2.8	7.7	47	-1.3	S	IAAV7514	TGRWT	UNIRRI11
7D	45	2.9	8.1	47.2	-1.1	S	Kukri_c48125	TGRWT	UNIRRI11
2D	34	8.2	30.3	4.4	0.28	R	BS00049370	LFCURL	UNIRRI11
2B		3.0	17.5	4.4	0.18	R	BS00098024	LFCURL	UNIRRI11
5A	119.8	1.4	4.2	4.4	0.18	R	RAC875_c61493	LFCURL	UNIRRI11
5A	249.8	3.2	15.1	0.32	-0.011	S	EXcalibur_rep_c68005	NDVI	UNIRRI11
2A	161	3.4	16.0	18.2	0.135	R	Tdurum_contig66015	DeltaC	UNIRRI11

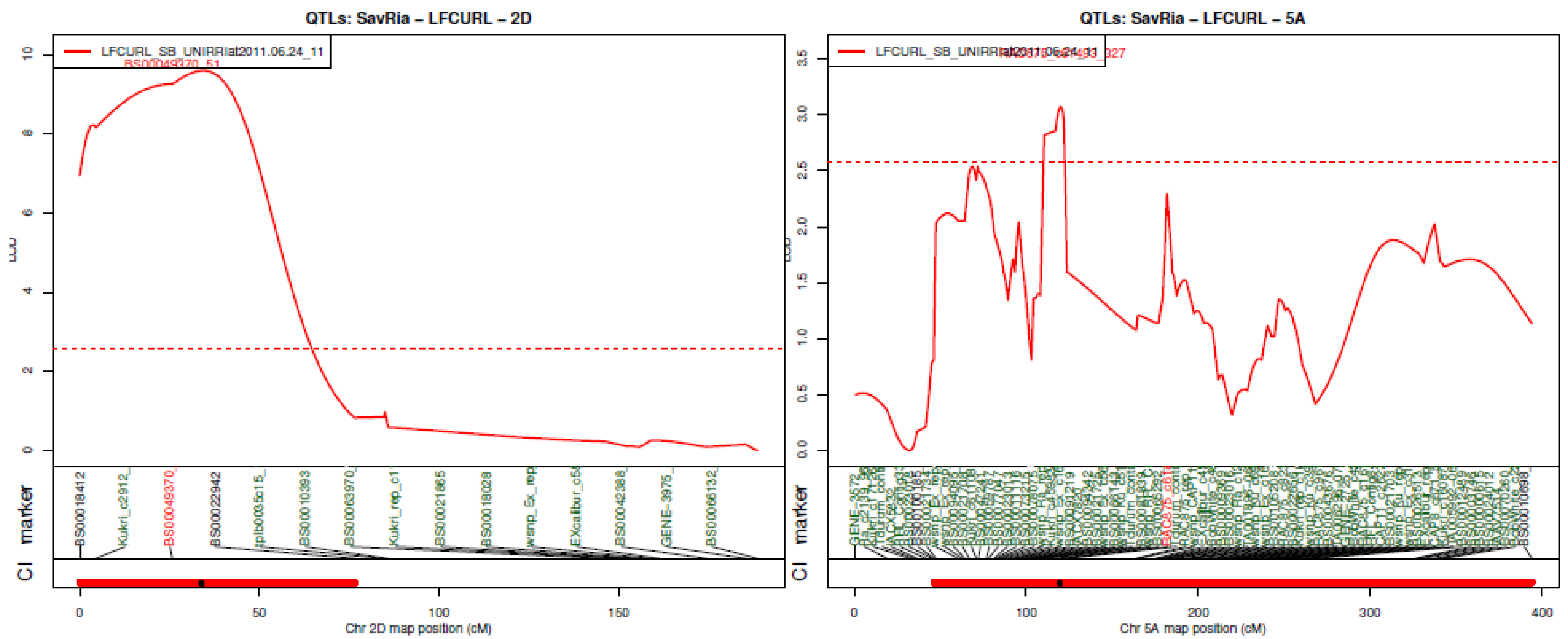
- both environments: TGRWT (5A)
- irrigated: HI (1D, 4D), TGRWT (4A,6A)
- unirrigated: EARpsqm (2D, 7A), GRpsqm (2D,3B), GRYLD (3B), LFCURL (2B, 2D, 5A), NDVI (5A), Cdelta (2A)
- no qtls: AGDM, CTEMP, StemWSC

SavXRia Sutton Bonnington QTLs 2010-11

chr	pos	LOD	var	mean	add eff	on	marker	trait	env
1D	28	3.1	12.9	0.5	-0.008	S	RAC875_c78062	HI	IRRI11
4D	17.8	2.7	11.1	0.5	0.008	R	GENE-2812	HI	IRRI11
4A	191.6	2.5	7.7	50.8	-0.8	S	BS00023164	TGRWT	IRRI11
5A	70.8	5.8	20.1	50.8	-1.35	S	Kukri_c61108	TGRWT	IRRI11
6A	129	4	13.1	50.8	-1.1	S	EXcalibur_c52196	TGRWT	IRRI11
2D	3	3	12	343	12.9	R	Kukri_c2912	EARNBpsqm	UNIRRI11
7A	0	3.9	16	343	15.1	R	EXcalibur_c48636	EARNBpsqm	UNIRRI11
2D	3	4.4	17.3	16267	703	R	Kukri_c2912	GRpsqm	UNIRRI11
3B	261	3	11.3	16266	-585	S	Ex_c14162_22093694	GRpsqm	UNIRRI11
3B	260	2.4	11.3	7.6	-0.19	S	Ex_c14162_22093694	GRYLD	UNIRRI11
3A	144.9	2.6	7.2	47	1.2	R	BS00110564	TGRWT	UNIRRI11
5A	337.3	2.8	7.7	47	-1.3	S	IAAV7514	TGRWT	UNIRRI11
7D	45	2.9	8.1	47.2	-1.1	S	Kukri_c48125	TGRWT	UNIRRI11
2D	34	8.2	30.3	4.4	0.28	R	BS00049370	LFCURL	UNIRRI11
2B		3.0	17.5	4.4	0.18	R	BS00098024	LFCURL	UNIRRI11
5A	119.8	1.4	4.2	4.4	0.18	R	RAC875_c61493	LFCURL	UNIRRI11
5A	249.8	3.2	15.1	0.32	-0.011	S	EXcalibur_rep_c68005	NDVI	UNIRRI11
2A	161	3.4	16.0	18.2	0.135	R	Tdurum_contig66015	DeltaC	UNIRRI11

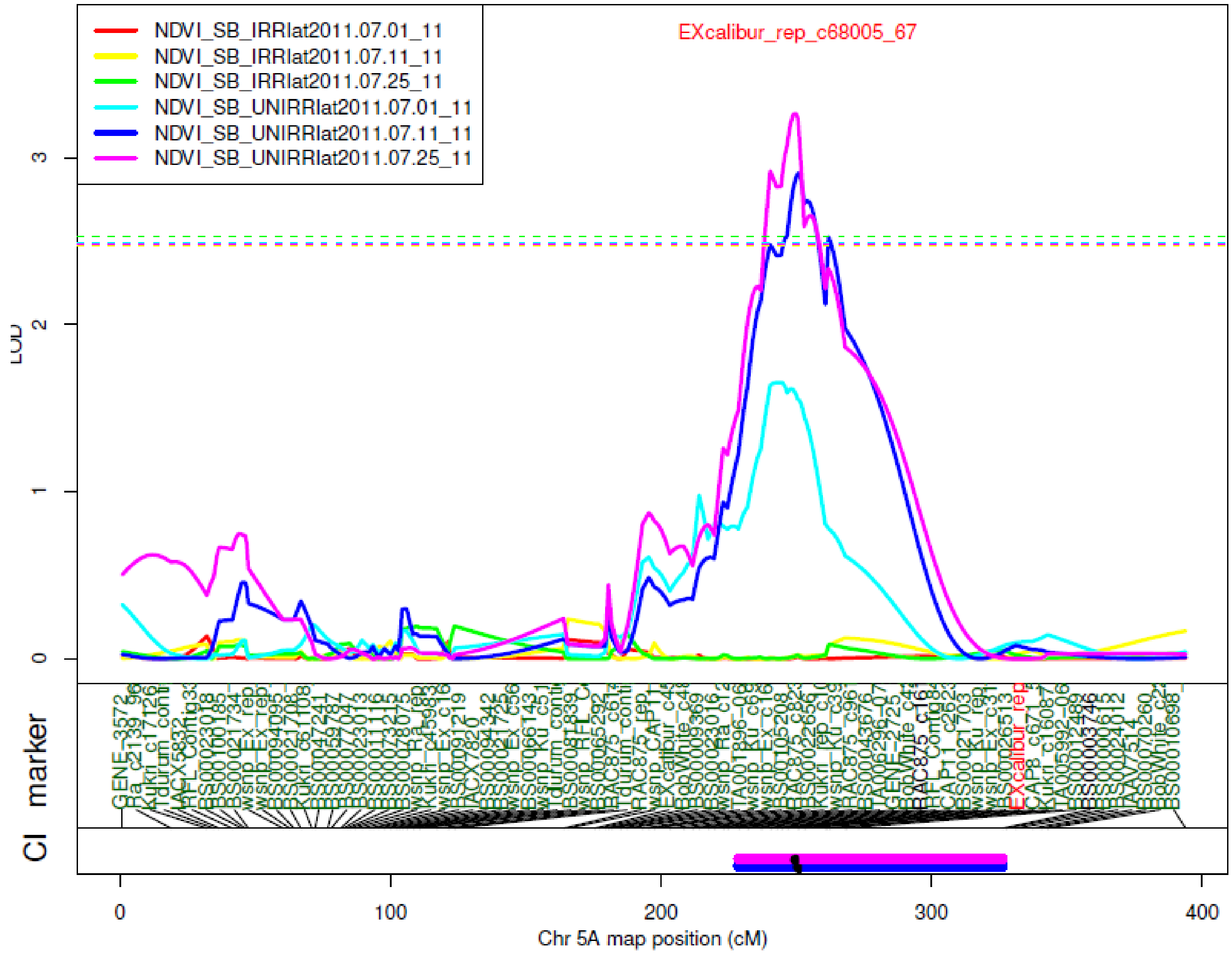
- both environments: TGRWT (5A)
- irrigated: HI (1D, 4D), TGRWT (4A,6A)
- unirrigated: EARpsqm (2D, 7A), GRpsqm (2D,3B), GRYLD (3B), LFCURL (2B, 2D, 5A), NDVI (5A), Cdelta (2A)
- no qtls: AGDM, CTEMP, StemWSC

QTLs for LFCURL



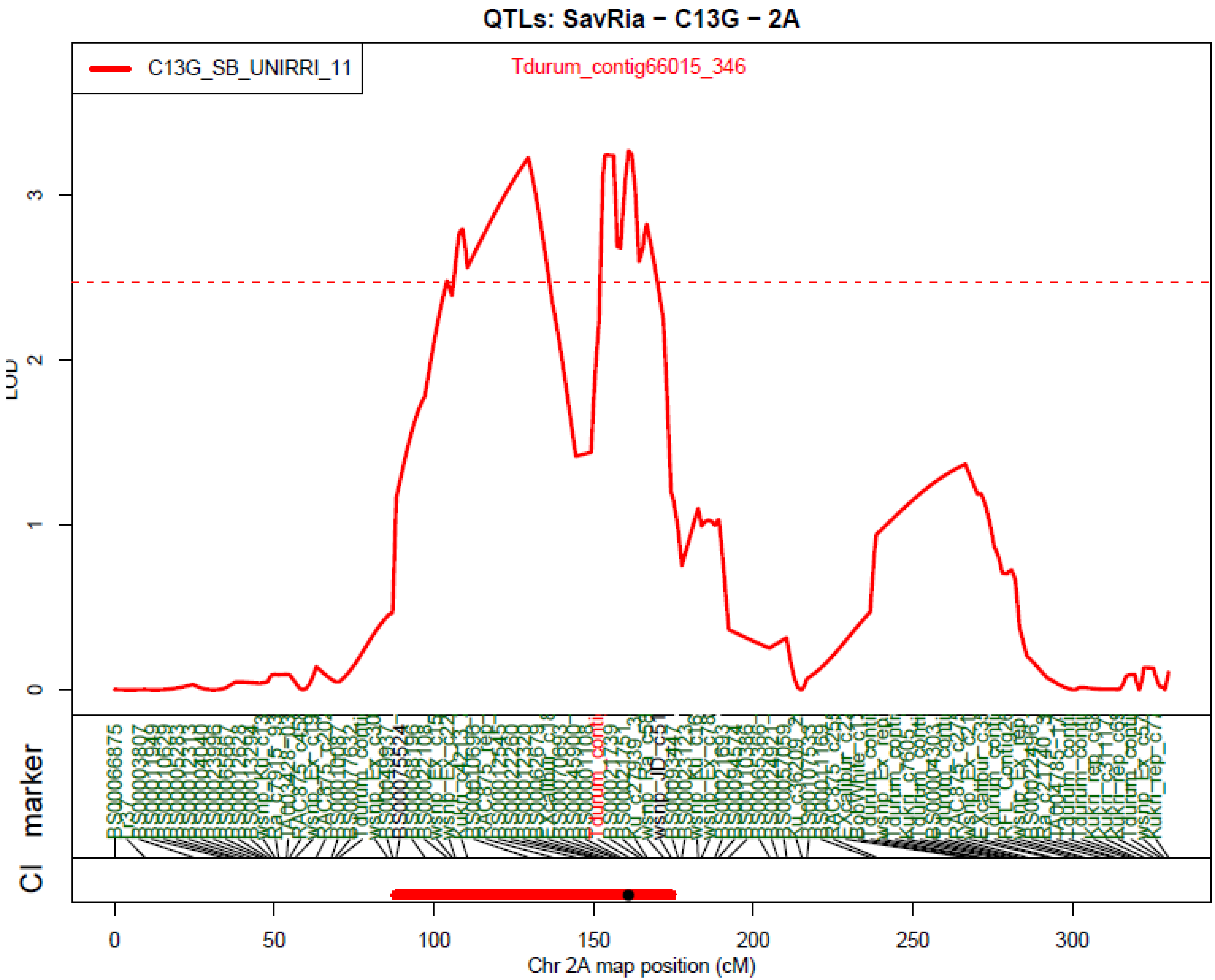
LEAFCURL QTL 2D coincides with GRpsqm, 5A NOT with NDVI.

QTL 5A for NDVI

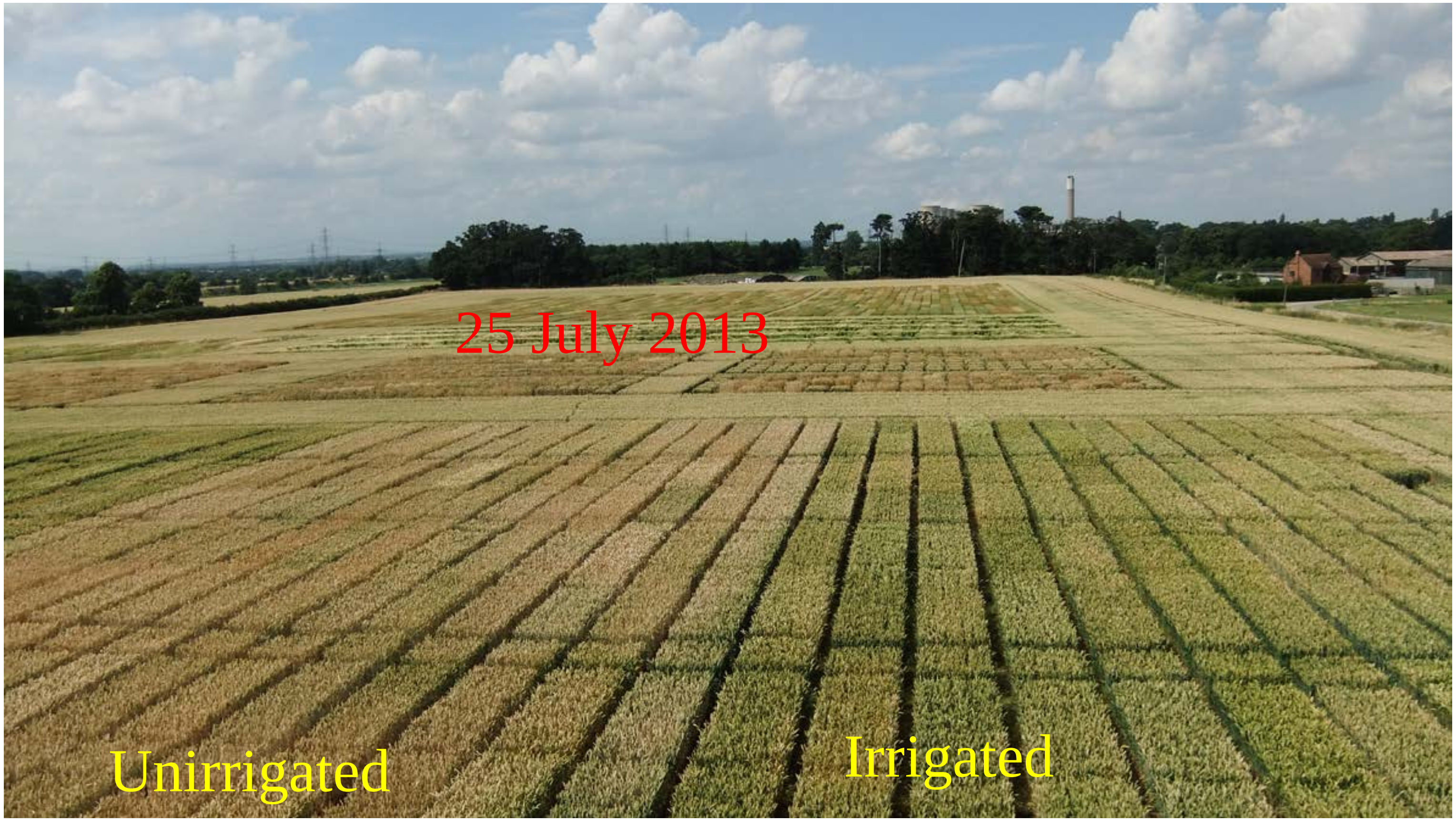


NDVI QTL 5A only under 'drought'.

QTLs for Cdelta



Rialto x Savannah DH pop 2012-13



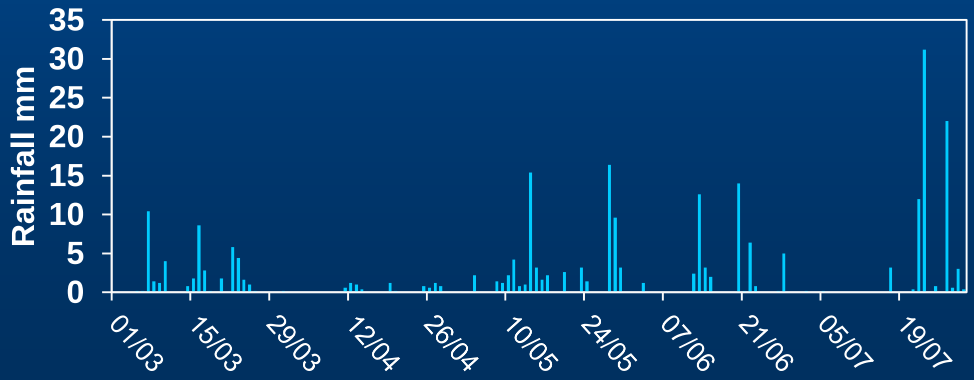
9.2 QTL Detection

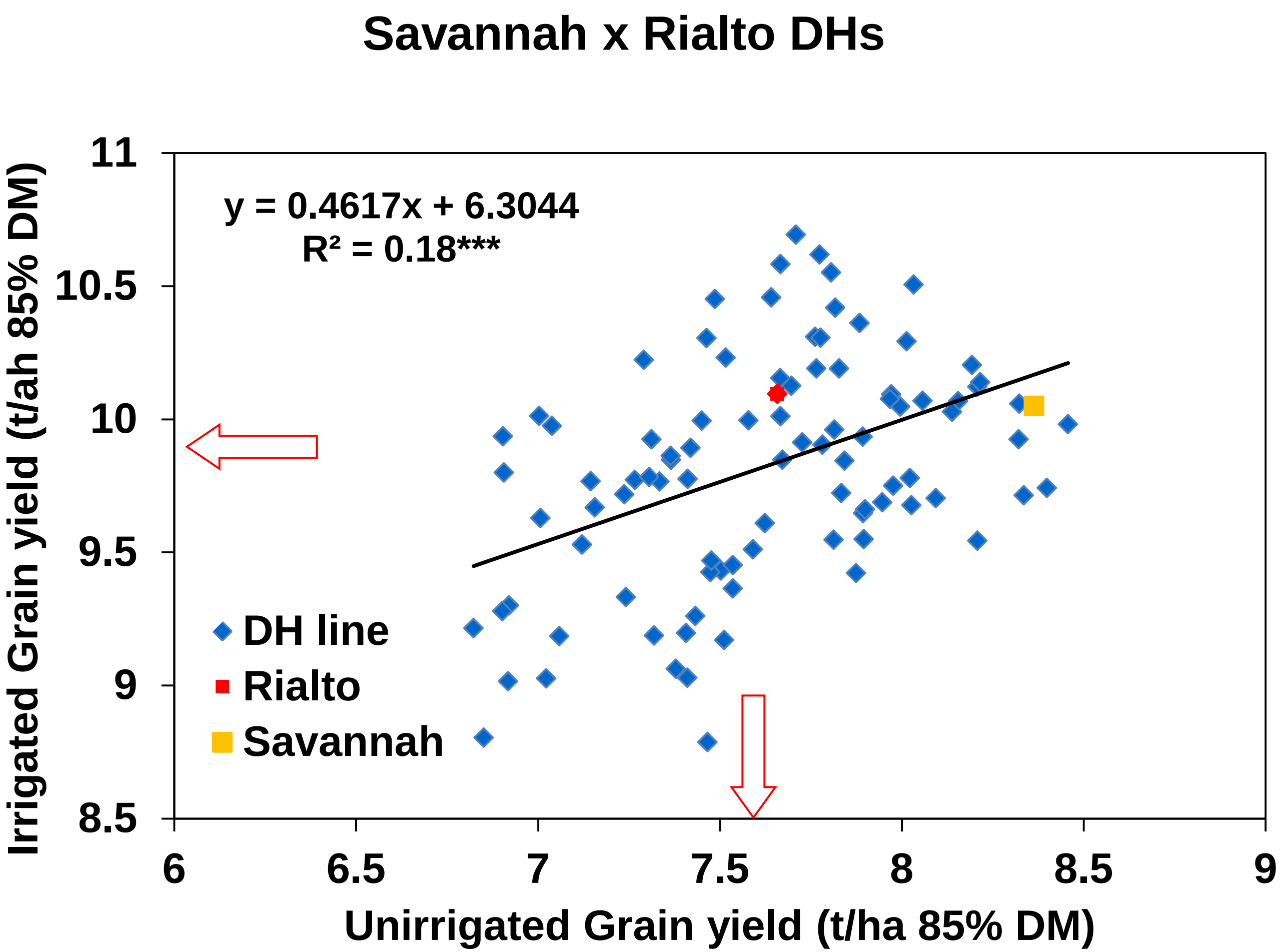
2012-13 expt (Sown 16 October 2012)

• Rialto x Savannah DH population (94 lines and 2 parents) - irrigated & unirrigated

• Traits

- $^{13}C\ \Delta$ grain (unirrigated)
- senescence kinetics (flag leaf visual score,NDVI)
- stem WSC at GS61+7d (unirrigated)
- flag leaf Licor 6400 : Rialto, Savannah , 6 DH lines contrasting for $^{13}C\ \Delta$ grain (Licor 6400) (unirrigated)

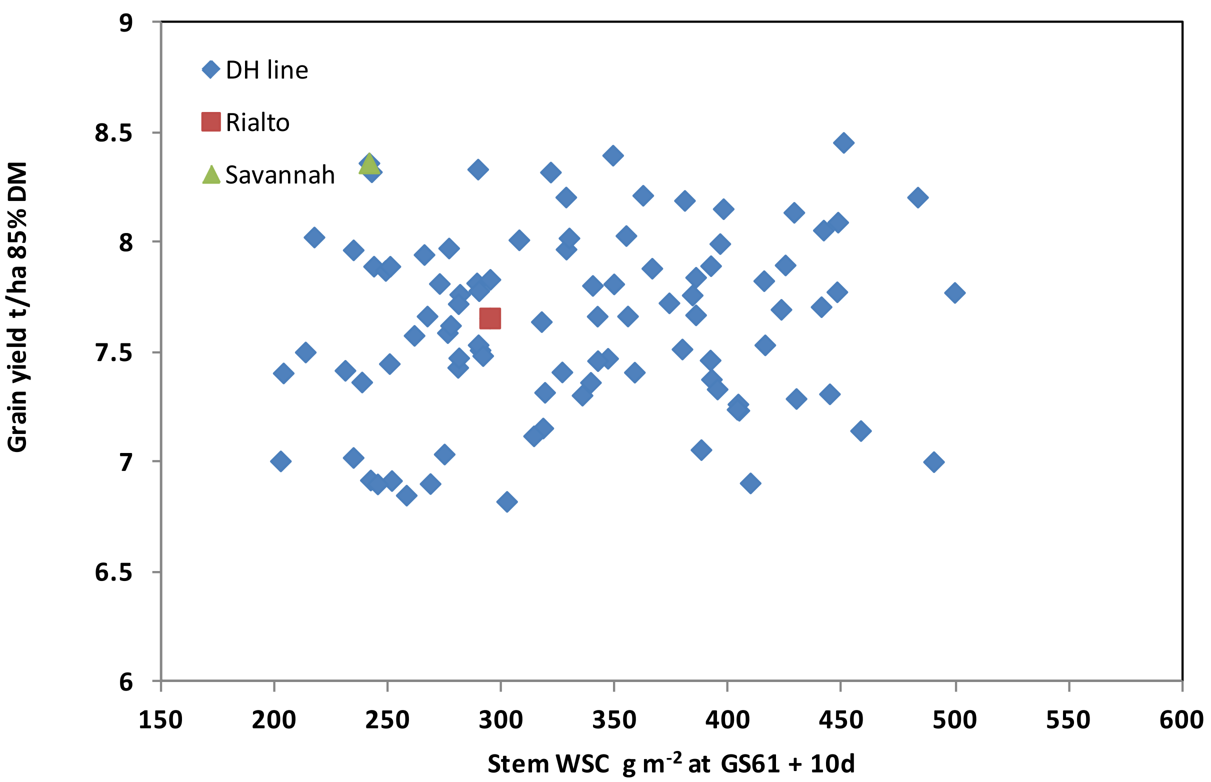




LSD (5%): Irr 2.97 (P = 0.06) , Line 0.51 (P< 0.001) ; Int 0.95 (P< 0.01)

Sutton Bonington 2012-13

Grain Yield versus Stem water sol.CHO (Savannah x Rialto DHs)

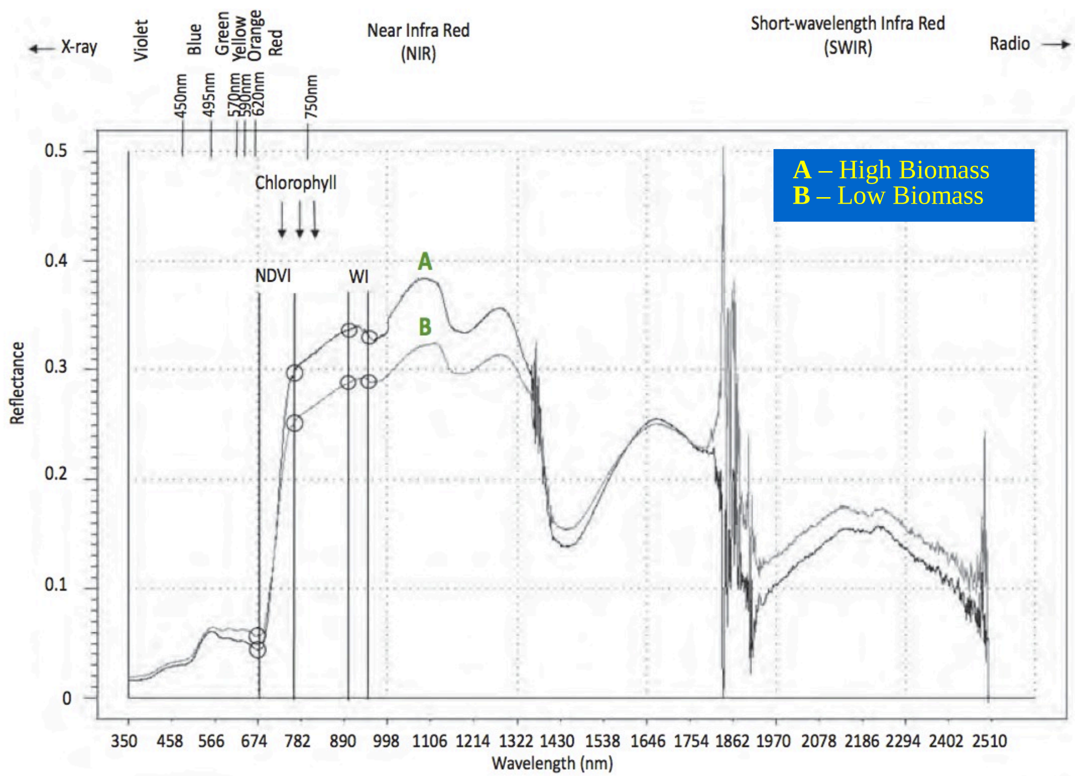


Sutton Bonington 2012-13 Unirrigated

Normalized Difference Vegetation Index (NDVI)

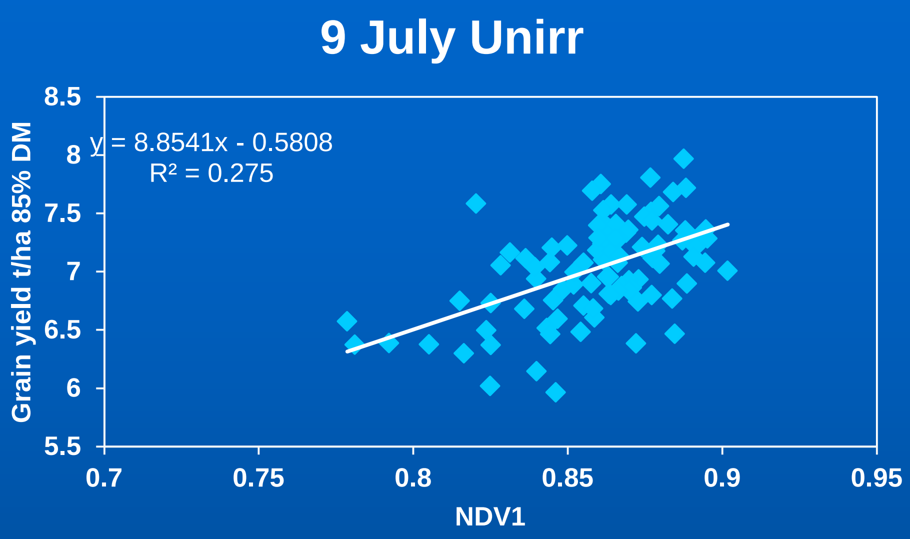
$$\mathbf{NDVI} = \frac{R_{NIR} - R_{RED}}{R_{NIR} + R_{RED}} = \frac{R_{900} - R_{680}}{R_{900} + R_{680}}$$

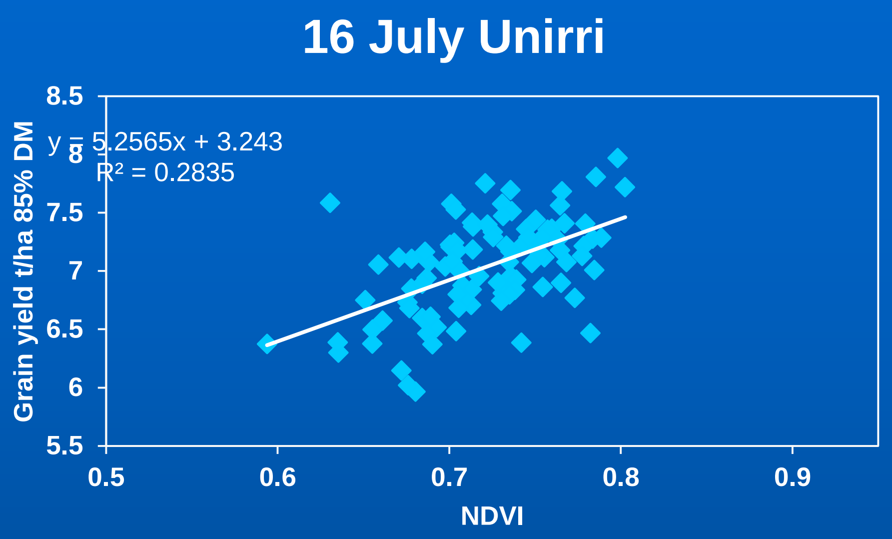
- Green area
- Photosynthetic capacity
- N status

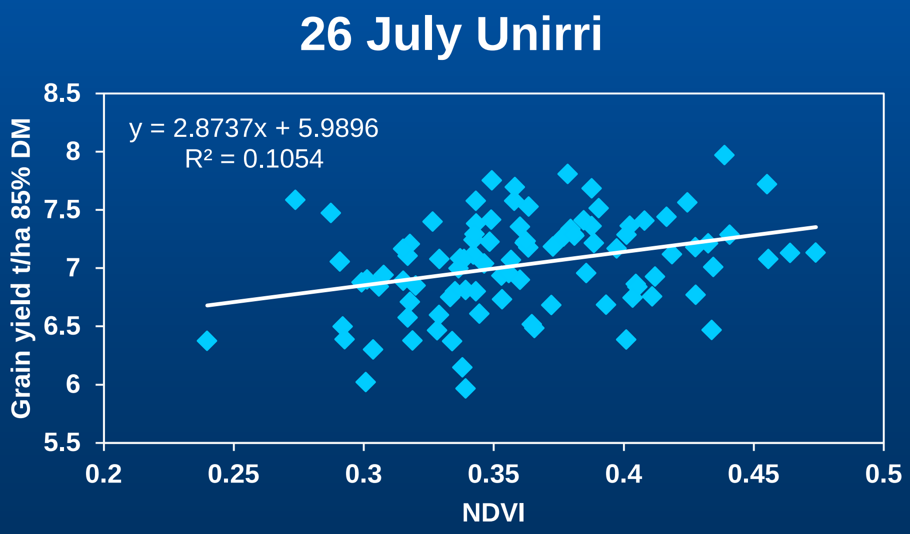


Adapted from: Reynolds M, Pask A, Mullan D (2012) Physiological breeding I: Interdisciplinary approaches to improve crop adaptation.

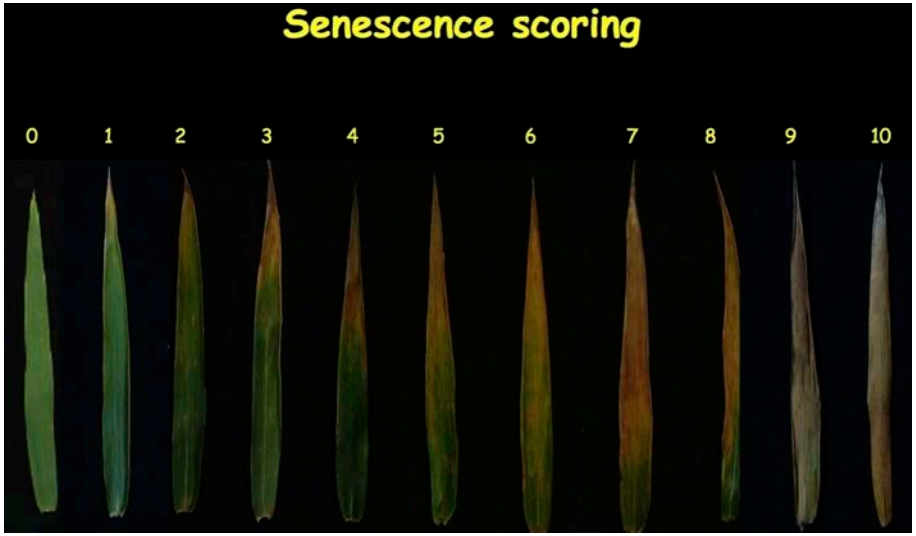
Grain Yield versus NDVI (Savannah x Rialto DHs)

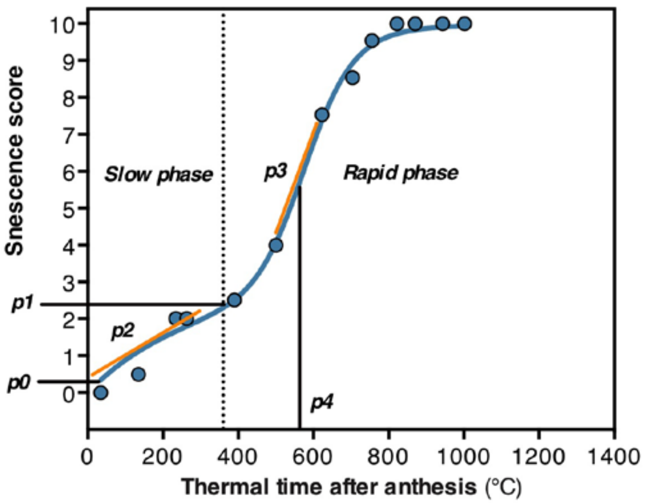






Flag Leaf Senescence Kinetics





Fitting the senescence data

score = p0 + p1 x (1 – exp((-p2 x STA/ p1)) + (10 – p1 – p0/ (1 + exp(-4 x p3 (STA – p4)/ (10 – p1 – p0)))

Score: visual senescence score

STA: thermal time after anthesis (˚ C days)

p0: score at anthesis

p1: score at the end of the slow phase

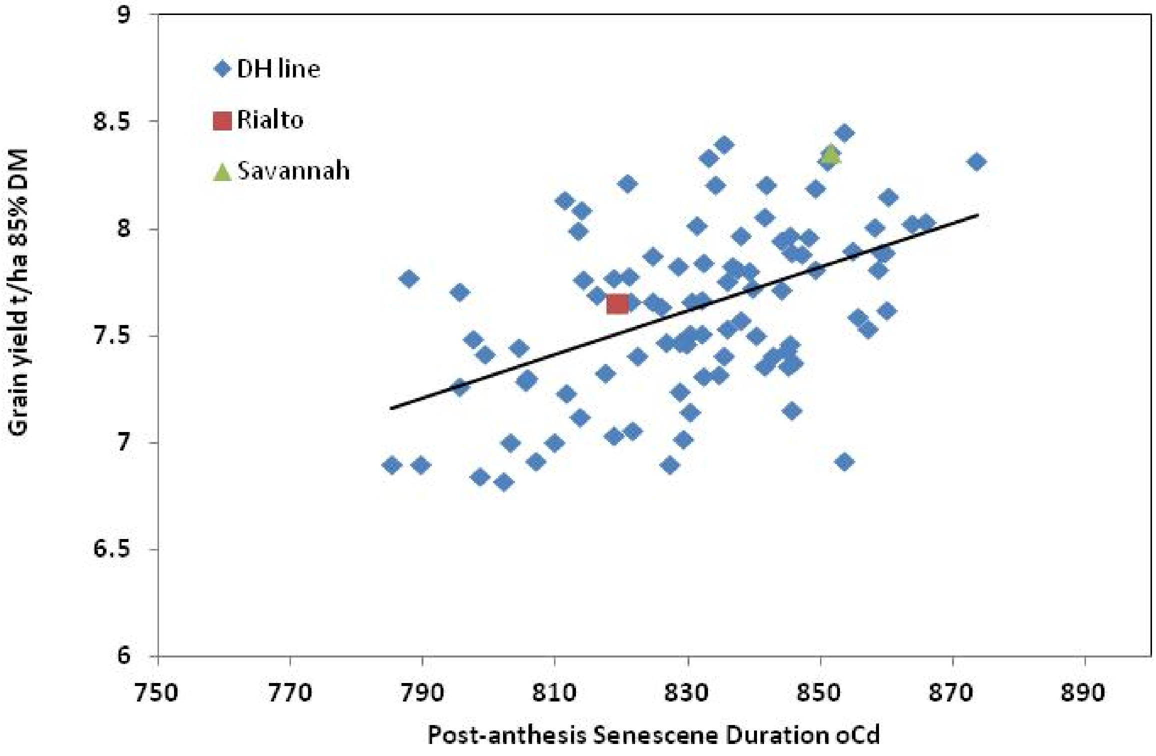
p2: maximum rate of the slow phase

p3: maximum rate at rapid phase

p4: date at which ***p3*** is reached

Stay-green associated with ***p4***.

Grain yield versus Flag leaf senescence duration (Savannah x Rialto DHs)



Sutton Bonington 2012-13 Unirrigated

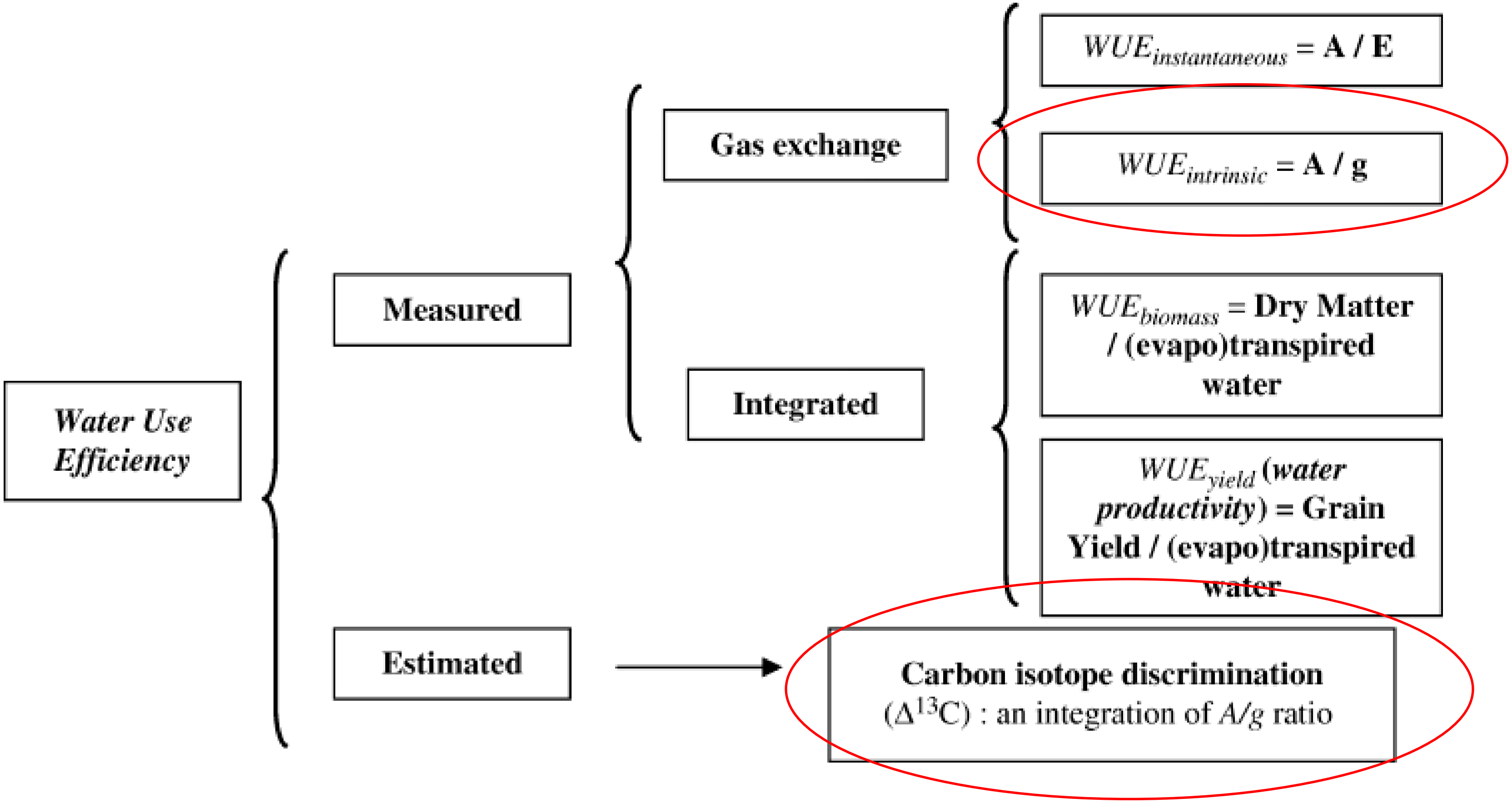
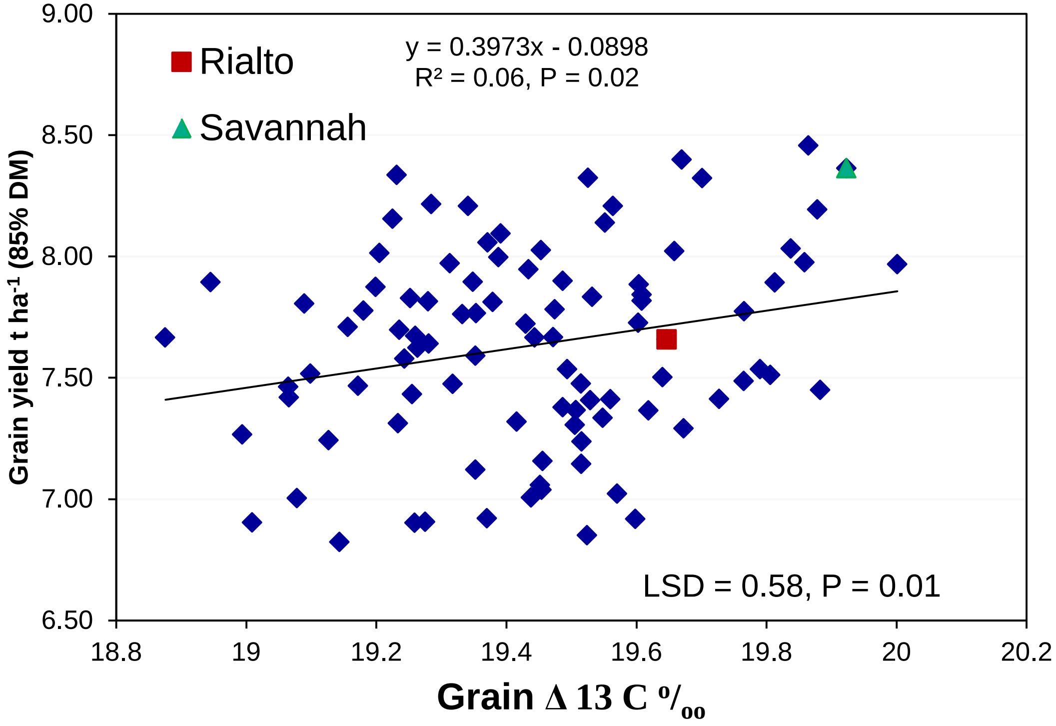


Figure 1. Definitions of 'water use efficiency'. The scheme represents the several definitions of water use efficiency (WUE) used in the text. *A*, net photosynthetic rate expressed as μmol CO_2 m^{-1} s^{-1}; E, transpiration rate expressed as mmol H_2O m^{-2} s^{-1}; $\Delta^{13}C$, carbon isotope discrimination.

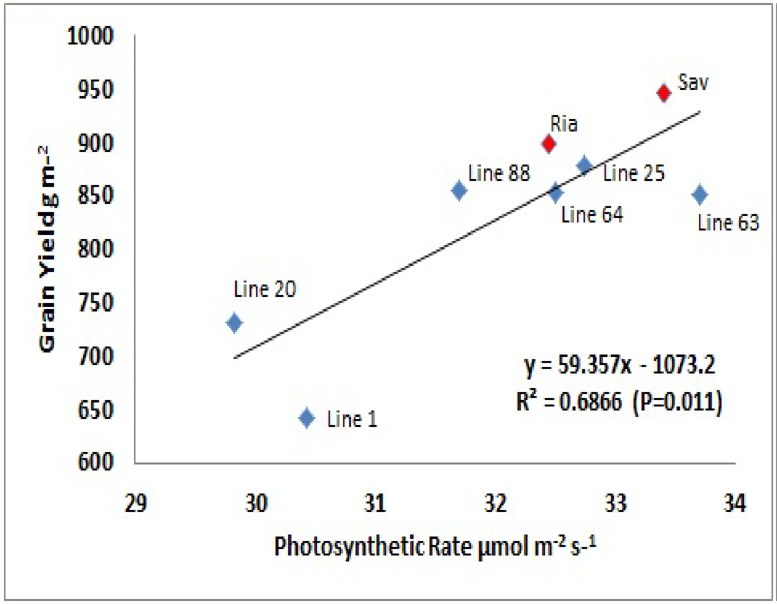
Tambussi et al. 2007 Field Crops Res

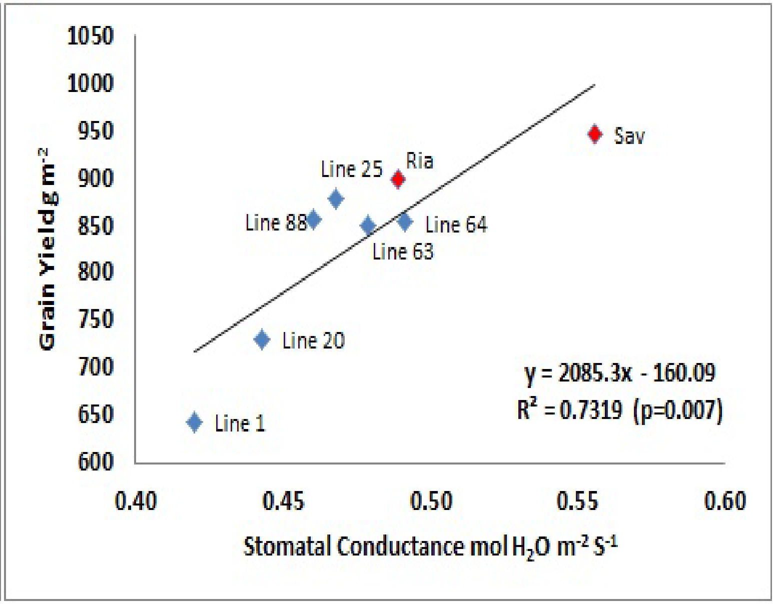
Δ ^{13}C vs grain yield: Savannah x Rialto DH 2012-13



High WUE ← Low WUE

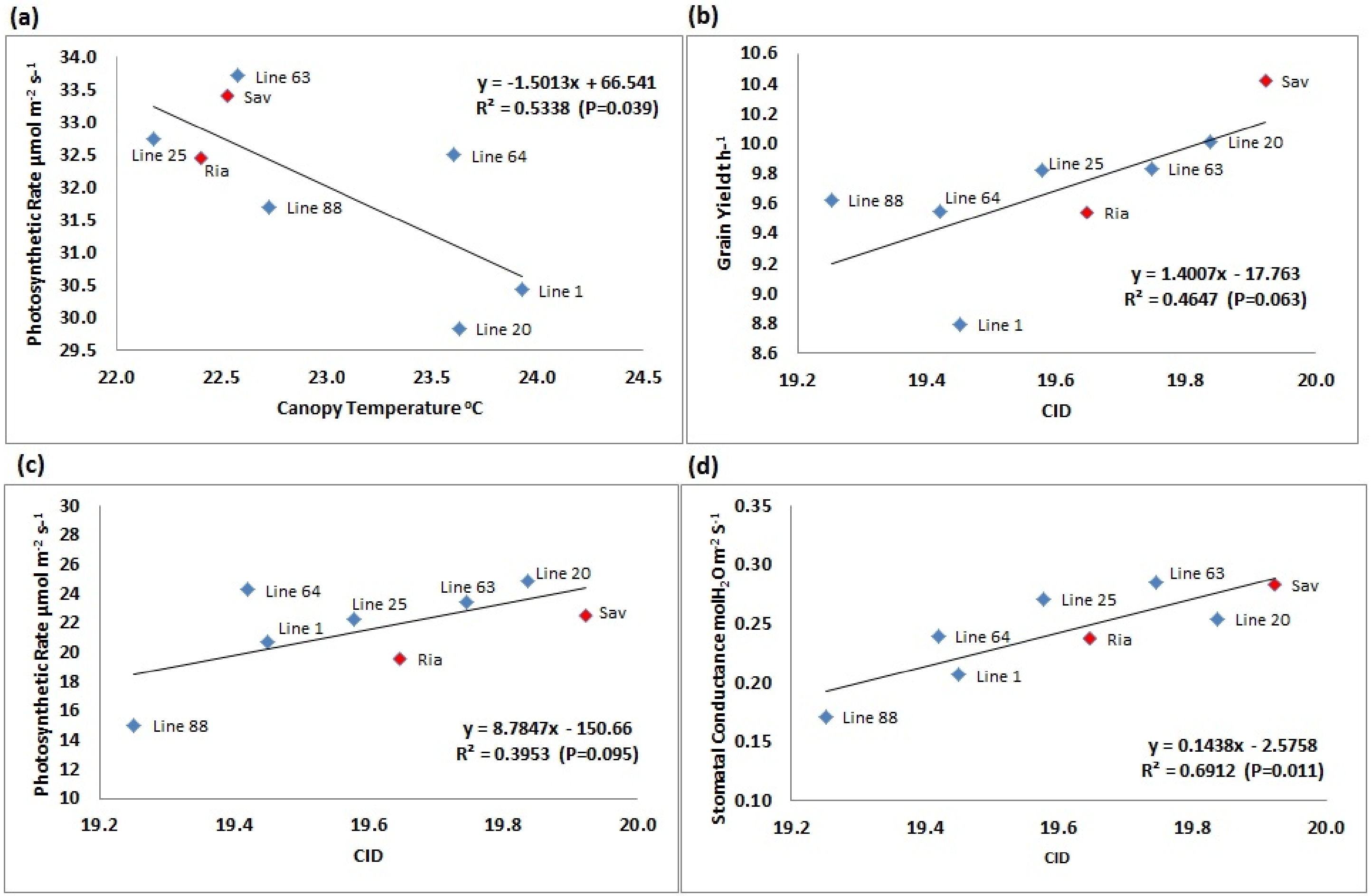
Stomatal aperture traits assessed in subset of DH lines Unirrigated trt





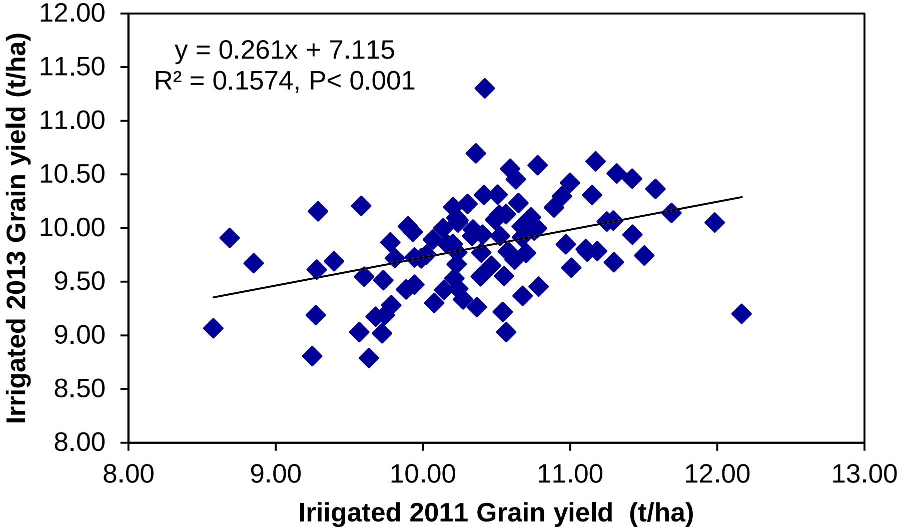
SB 2012-13 Unirrigated

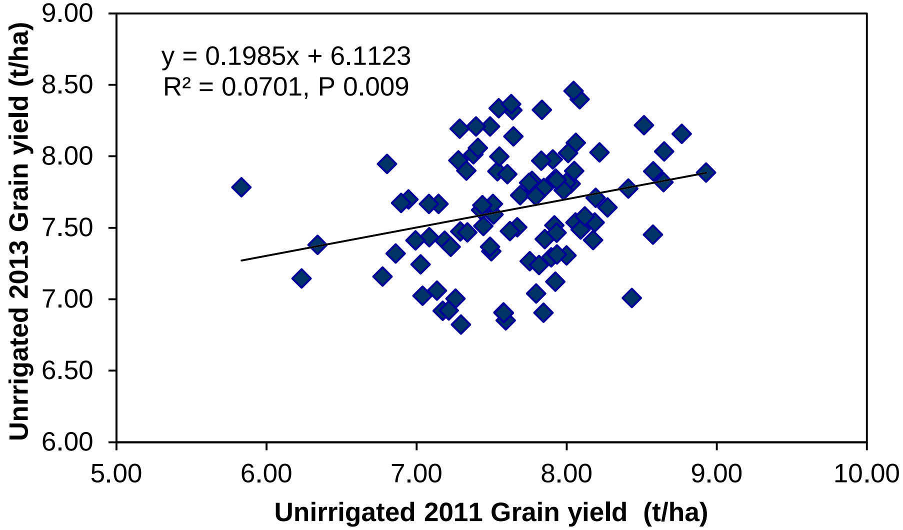
SATs assessed in subset of DH lines Unirrigated trt



SB 2012-13 Unirrigated

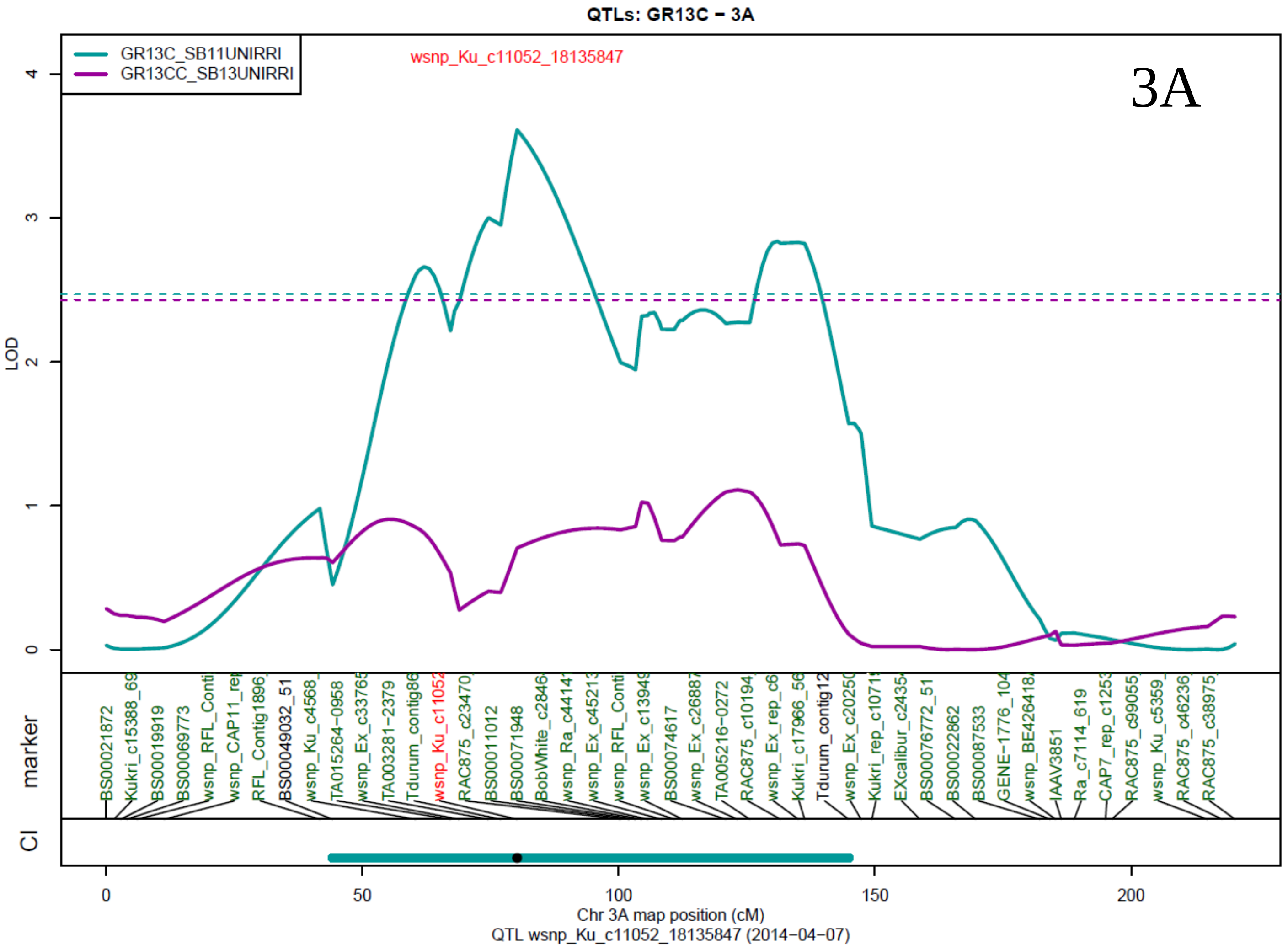
Comparison of grain yields in 2011 and 2013





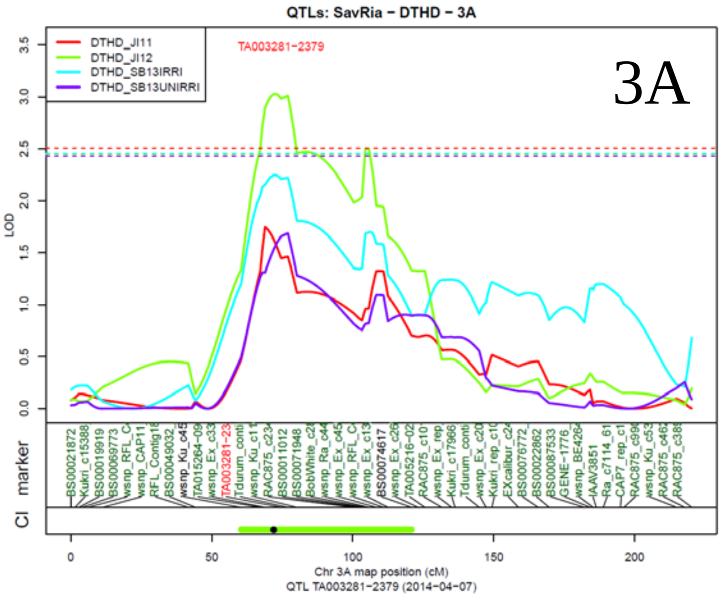
QTL analysis 2010-11 and 2012-13

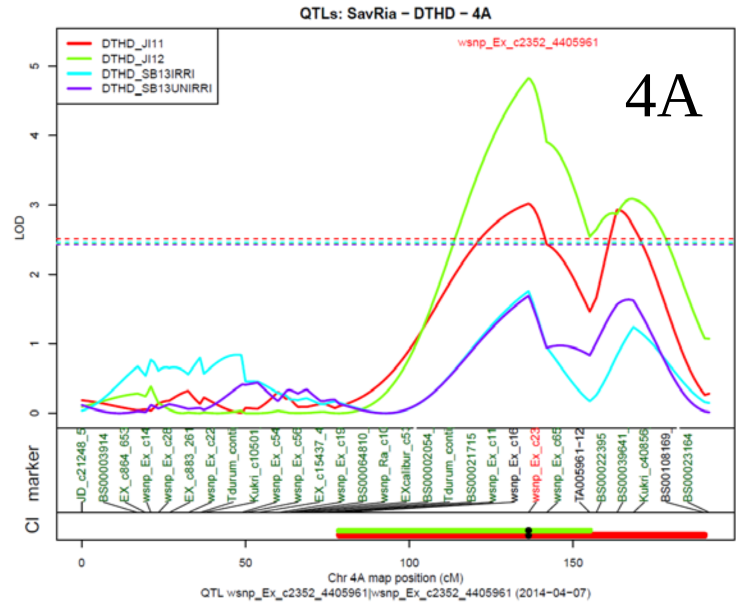
Grain Δ 13 C

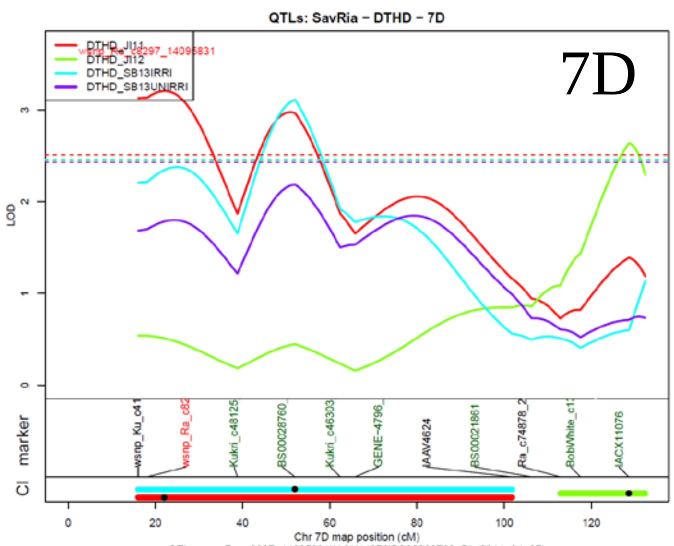


QTLs: GR13C – 3A
GR13C_SB11UNIRRI
GR13CC_SB13UNIRRI
wsnp_Ku_c11052_18135847
3A
LOD
marker
CI
Chr 3A map position (cM)
QTL wsnp_Ku_c11052_18135847 (2014–04–07)

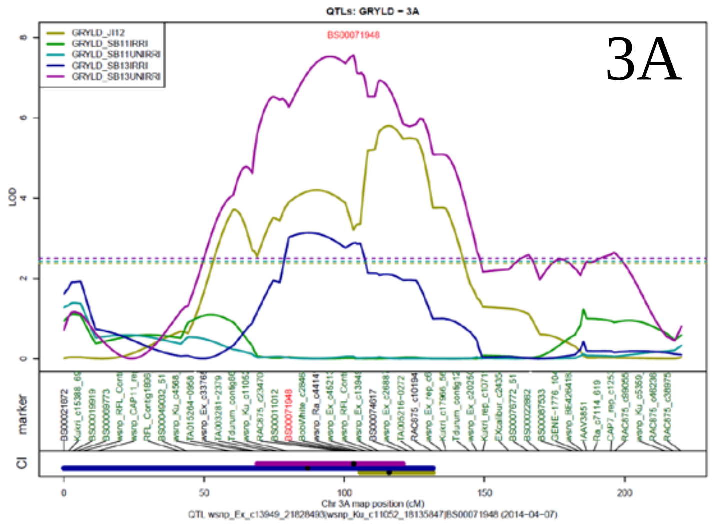
Heading date



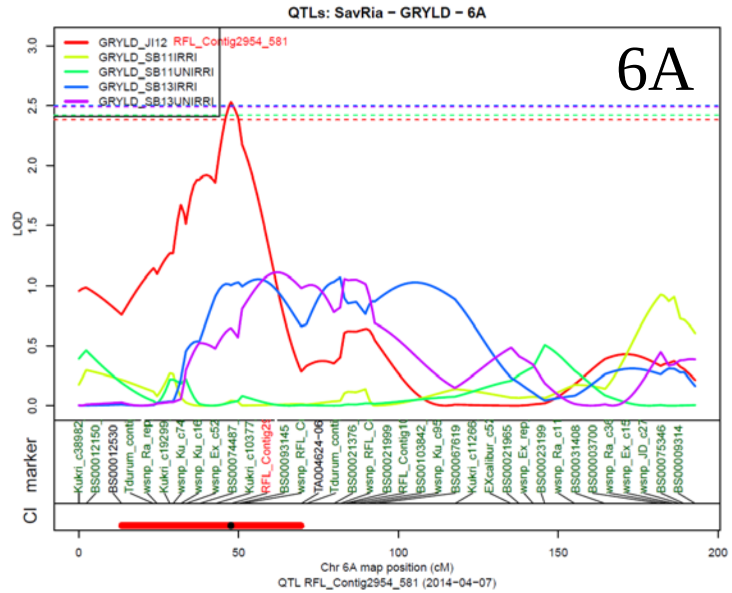




Grain yield

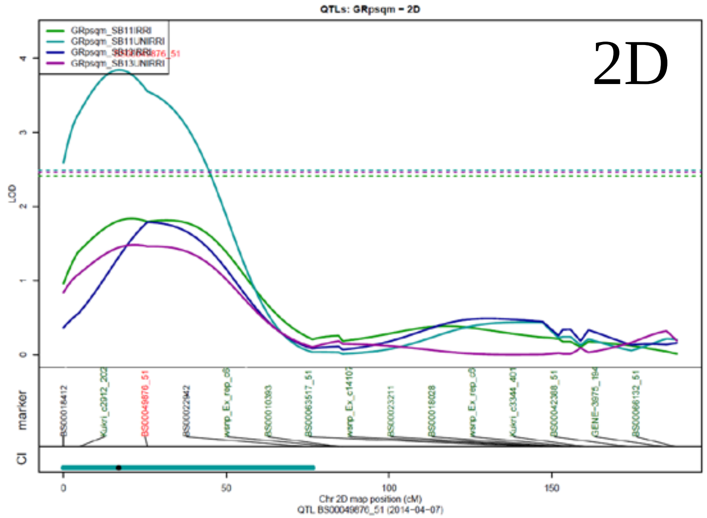


QTLs: GRYLD – 3A
3A
BS00071948
GRYLD_JI12
GRYLD_SB11IRRI
GRYLD_SB11UNIRRI
GRYLD_SB13IRRI
GRYLD_SB13UNIRRI
LOD
marker
CI
Chr 3A map position (cM)

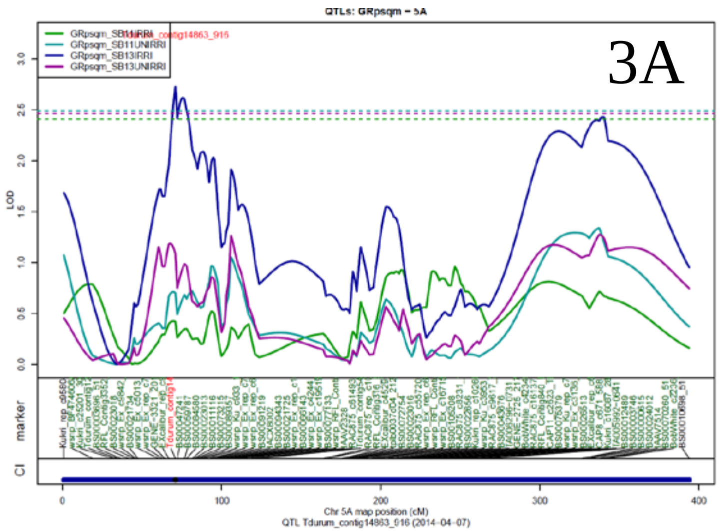


QTLs: SavRia – GRYLD – 6A
6A
RFL_Contig2954_581
GRYLD_JI12
GRYLD_SB11IRRI
GRYLD_SB11UNIRRI
GRYLD_SB13IRRI
GRYLD_SB13UNIRRI
LOD
marker
CI
0
50
100
150
200
Chr 6A map position (cM)
QTL RFL_Contig2954_581 (2014-04-07)

Grains m^{-2}



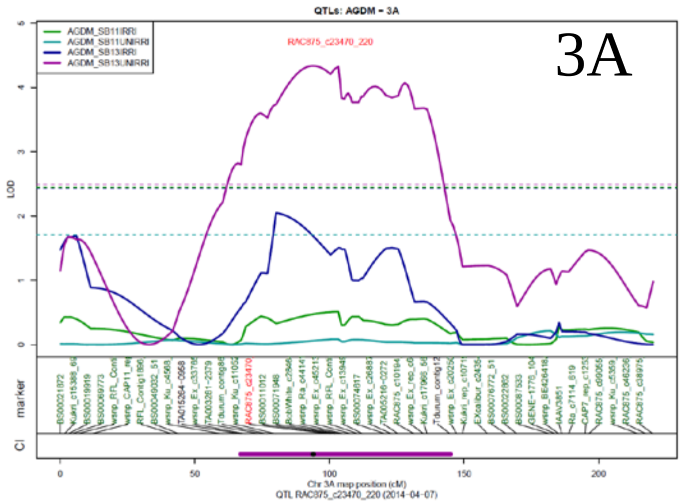
QTLs: GRpsqm – 2D
GRpsqm_SB11IRRI
GRpsqm_SB11UNIRRI
GRpsqm_SB13IRRI
GRpsqm_SB13UNIRRI
2D
LOD
marker
CI
0
50
100
150
Chr 2D map position (cM)
QTL BS00049876_51 (2014-04-07)

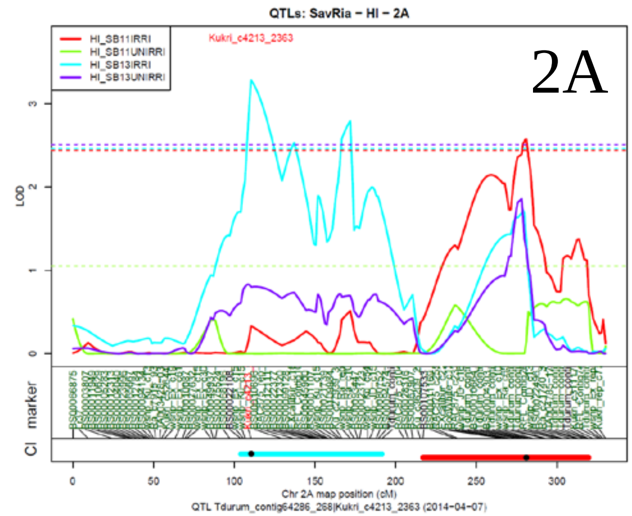


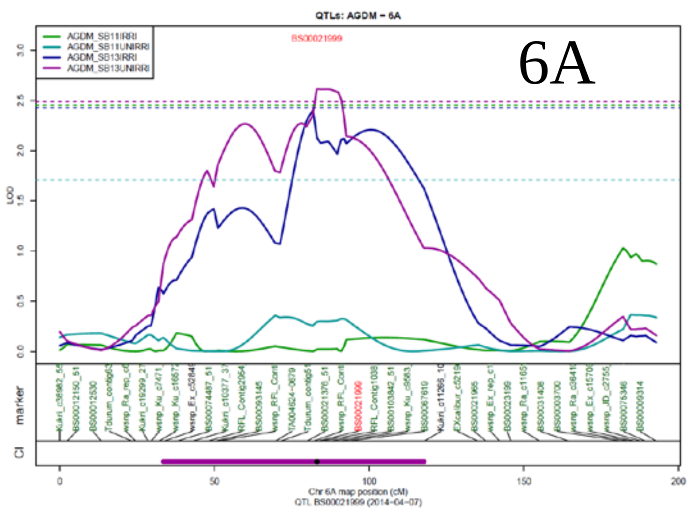
QTLs: GRpsqm – 5A
GRpsqm_SB11IRRI
GRpsqm_SB11UNIRRI
GRpsqm_SB13IRRI
GRpsqm_SB13UNIRRI
3A
LOD
marker
CI
0
100
200
300
400
Chr 5A map position (cM)
QTL Tdurum_contig14863_916 (2014-04-07)

Above-ground Biomass

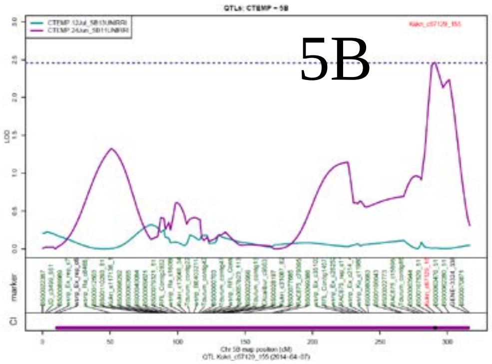
Harvest index

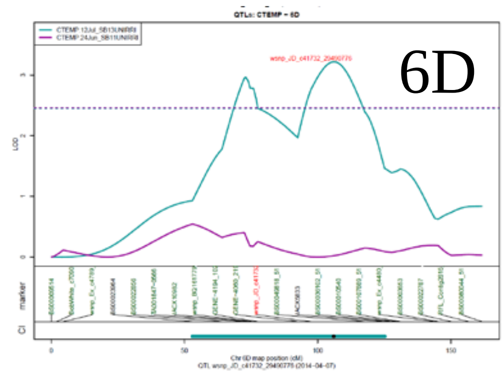




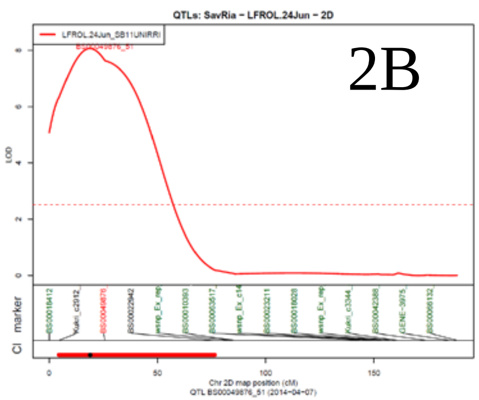


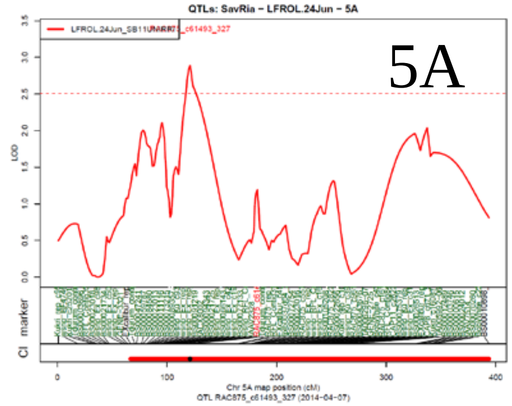
Canopy ToC





Leaf rolling





Conclusions

- **Ability to access water appears to be a key driver for productivity under UK drought.**
- **High 13C Δ correlated with grain yield under drought. Physiological basis ~ increased stomatal conductance, deeper roots?**
- **Physiological framework linking stomatal aperture traits to grain Δ13 C and grain yield under drought confirmed on subset of DH lines.**
- **QTL for grain Δ13 C, grain yield, biomass and anthesis date on chr 3A under drought – pleiotropoic effects?**

Original Objective 9 The new **Drought Tolerance** objectives for the 4 month extension will be:

- Develop physiological ideotype framework predicting beneficial combinations of traits influencing drought tolerance and water-use efficiency
- Report on physiological mechanisms to break the negative relationship between water use and water-use efficiency.
- Report on high-throughput phenotyping methodologies developed for practical screening for drought tolerance and water-use efficiency target traits for breeding.

Jayalath DeSilva
Pedro Carvalho

PhD student: Y.adgar Mahmood

Simon Griffiths
Simon Orford
Luzie Wingen

9.3 Develop SSD population

- Paragon x Garcia (contrasting for drought tolerance traits)
- Population segregating for *Ppd1a*:
  - use WGIN resource to select against *PpdD1a* - ie make the pop photoperiod sensitive.
  - keep the pop large, so we can have flowering time strata and perform analysis within them
- F3 sown in October 2012, population is in excess of 350 lines
  -

John Innes Centre

Obj 9.5. Collate germplasm (cvs, advanced lines) for genetics studies. (*Yrs 4 -5*)

CIMMYT Semi-arid Trials/Nurseries

Trial Name	Target environment	No Entries	No. Reps		
Semi-Arid Wheat Yield Trial (SAWYT)	Low rainfall environments	50	2	SAWYT*1 (Yield Trial with one 85g envelope/entry/rep, for machine sowing)	
Semi-Arid Wheat Screening Nursery (SAWSN)	Low rainfall environments	150	1	SAWSN (Screening Nursery with one 10g envelope/entry)	

- Standard Material Transfer Agreement (SMTA) signature

- Any additional declaration to be stated in the phytosanitary certificate issued by the Mexican phytosanitary authorities for the requested crop?

Jayalath DeSilva

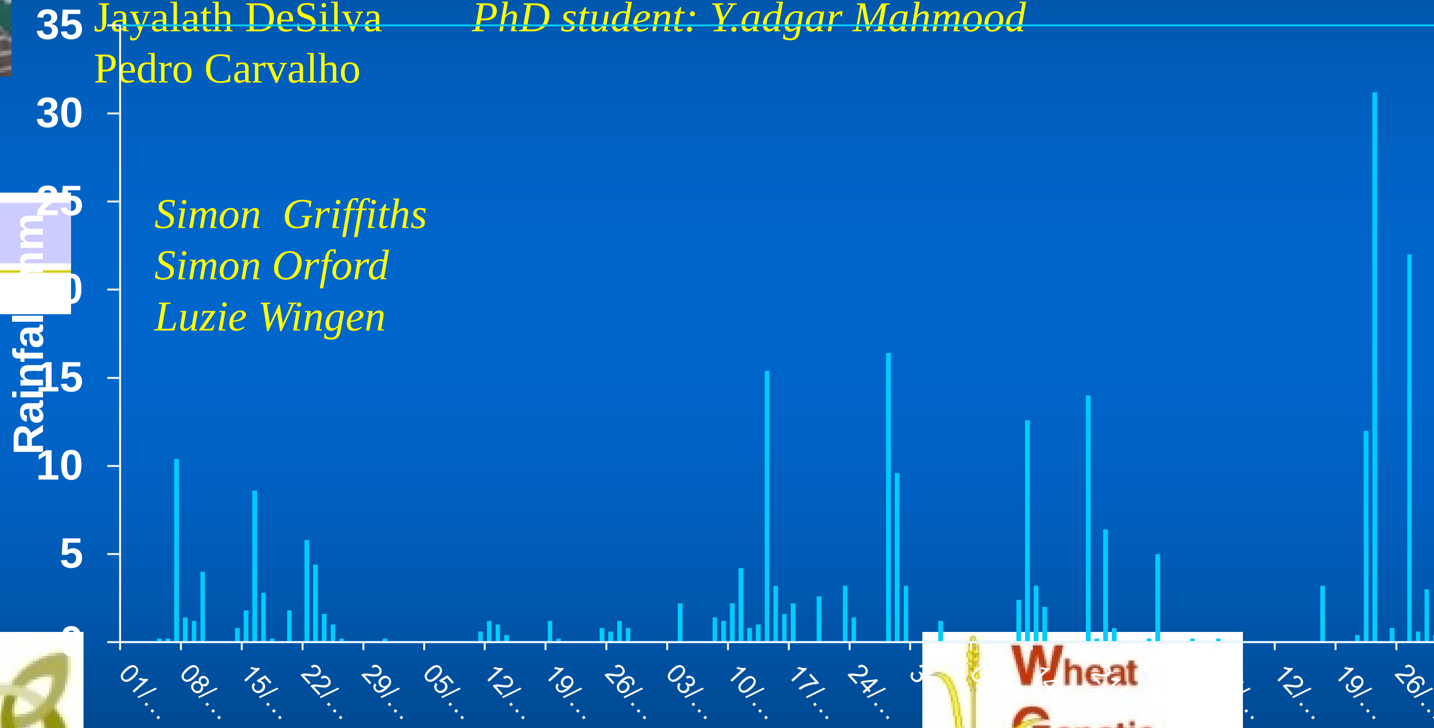
Pedro Carvalho

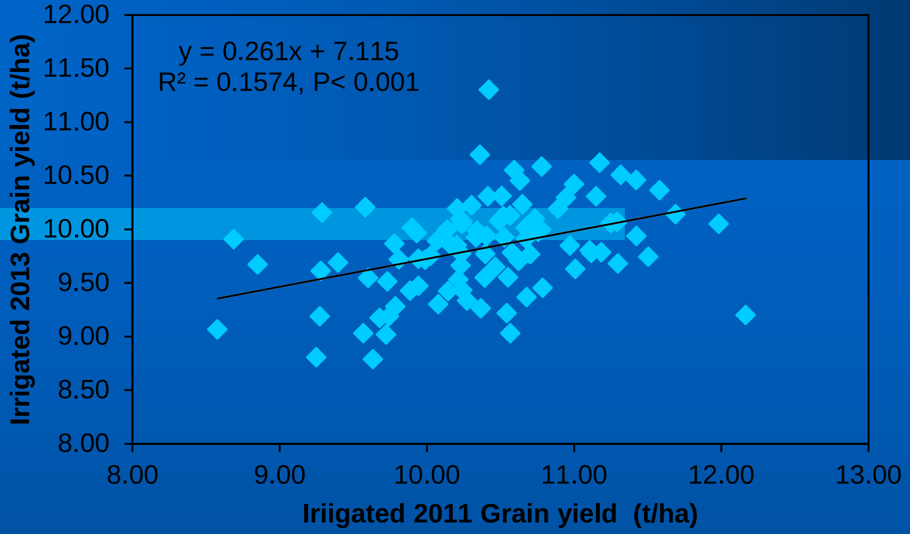
PhD student: Y.adgar Mahmood

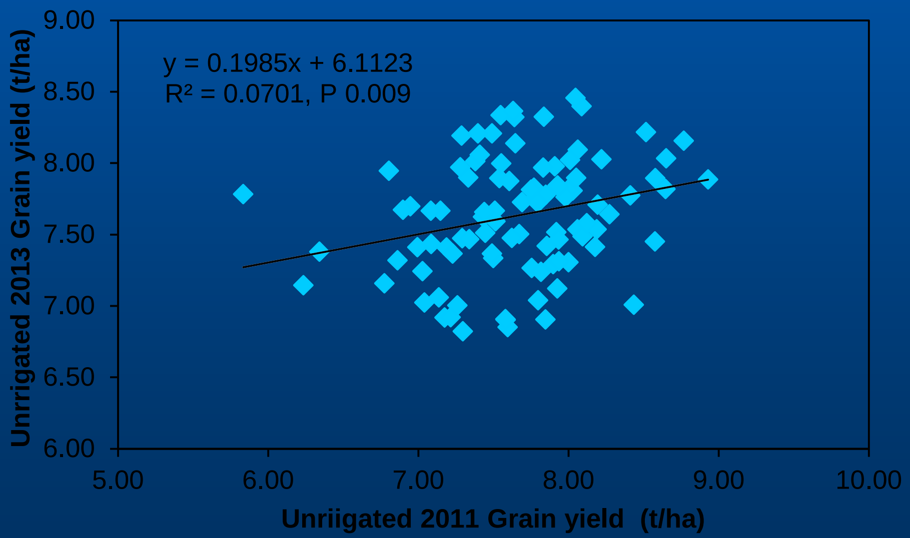
Simon Griffiths

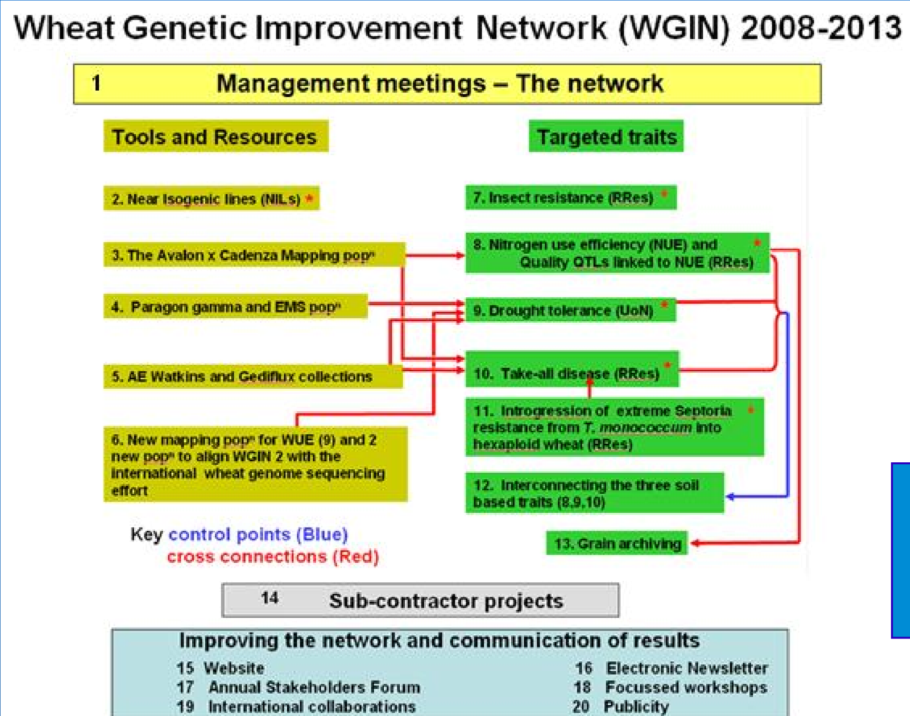
Simon Orford

Luzie Wingen




y = 0.261x + 7.115
R² = 0.1574, P< 0.001
Irrigated 2013 Grain yield (t/ha)
Iriigated 2011 Grain yield (t/ha)
12.00
11.50
11.00
10.50
10.00
9.50
9.00
8.50
8.00
8.00
9.00
10.00
11.00
12.00
13.00


y = 0.1985x + 6.1123
R² = 0.0701, P 0.009
Unrrigated 2013 Grain yield (t/ha)
Unriigated 2011 Grain yield (t/ha)
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.00
6.00
7.00
8.00
9.00
10.00



ncy: definitio
urement

Genetic Improvement of Drought Tolerance

Target physiological traits:

- Grain Δ 13 C (WUE)
- Flag Δ 18 O (Water Use)
- Stem WSC Reserves (HI)
- Canopy water status (NIR - WI)

GY = Water Use x WUE x HI

Passioura 1977

Traits associated with main drivers of yield under drought

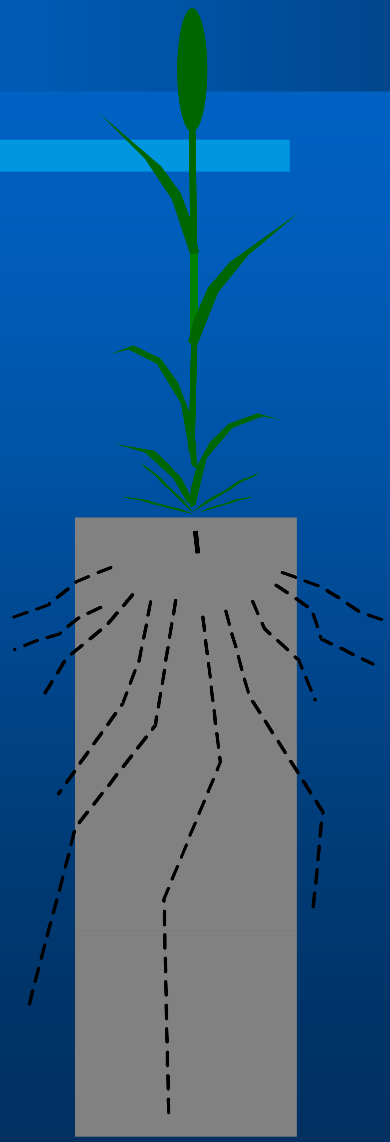
Yield = WU x WUE x HI

OPTIMIZE WUE

- WUE of leaf photosynthesis
- Low $^{13/12}C$ discrimination

MAXIMIZE HARVEST INDEX

- Pre-antheis partitioning to stem CHO reserves
- Functional stay green



MAXIMIZE WATER CAPTURE

- Increase root density at depth
- Distribute roots deeper
- Access to water by roots indicated by cooler canopy

Optimised ABA root signalling

EARLINESS

- Extend stem elongation phase
- Early onset GS31

18 Cultivar wheat panel selection informed by LINK 0986 Wheat WUE project, Eric Ober

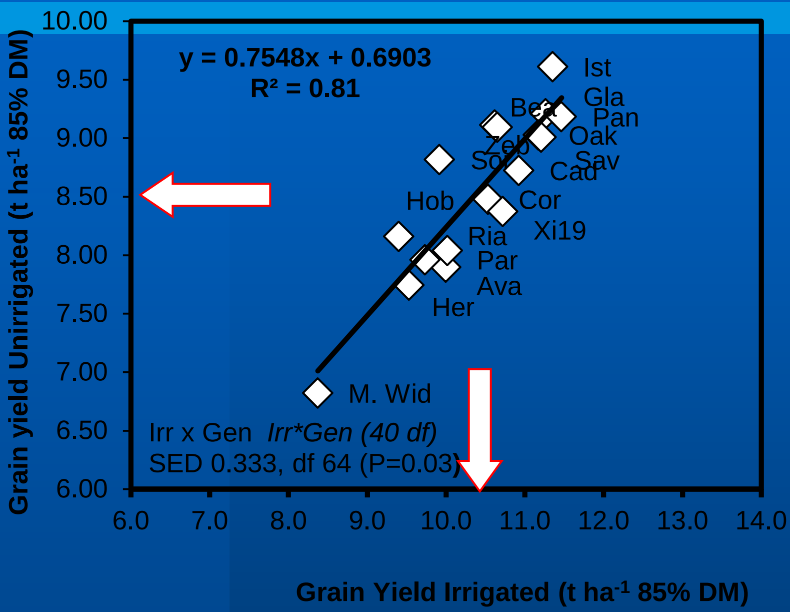
Wheat Genetic Improvement Network

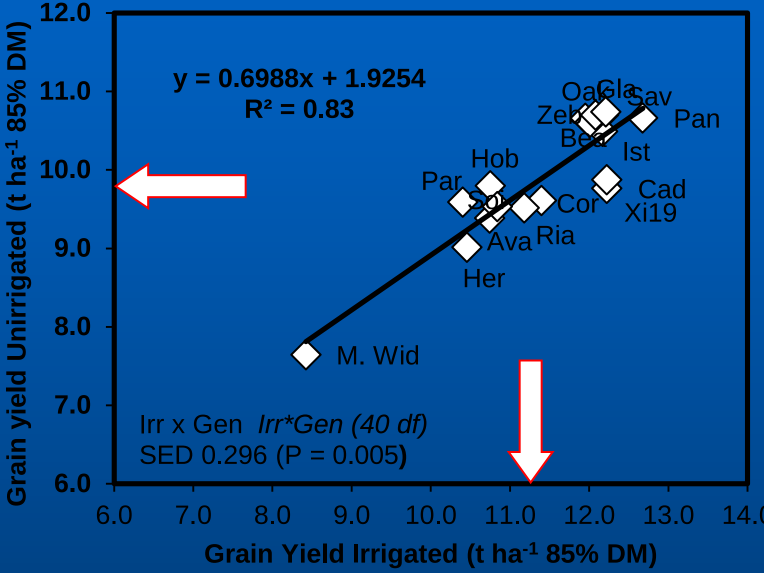
Years: 2009-10 & 2010-11
Split plot design (3 reps): plot size 1.6 x 12 m
Main plot: Fully irrigated (trickle irrigation)
Unirrigated

Split plot (variety):

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

Grain yield responses to Drought

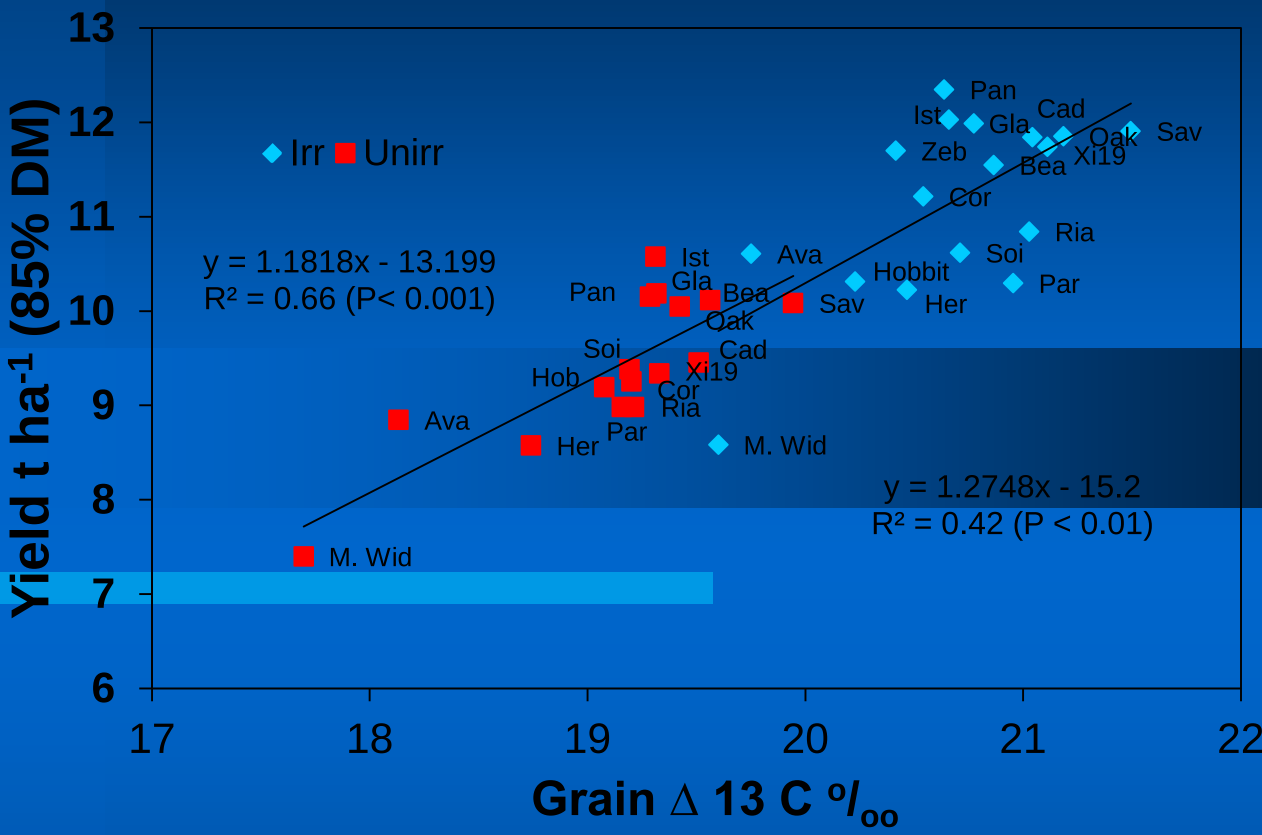




	Rainfall mm (% LTM)	
	2010	2011
January	33.0 (62)	33.2 (62)
February	41.6 (95)	44.6 (101)
March	36 (67)	1.2 (2)
April	24 (55)	23 (53)
May	16.2 (35)	27.8 (61)
June	69.2 (152)	45.4 (100)
July	42.6 (86)	17.8 (36)

Irrigated vs Unirrigated 19 July

Δ ^{13}C vs grain yield in 18 wheat cultivars



High WUE ← Low WUE

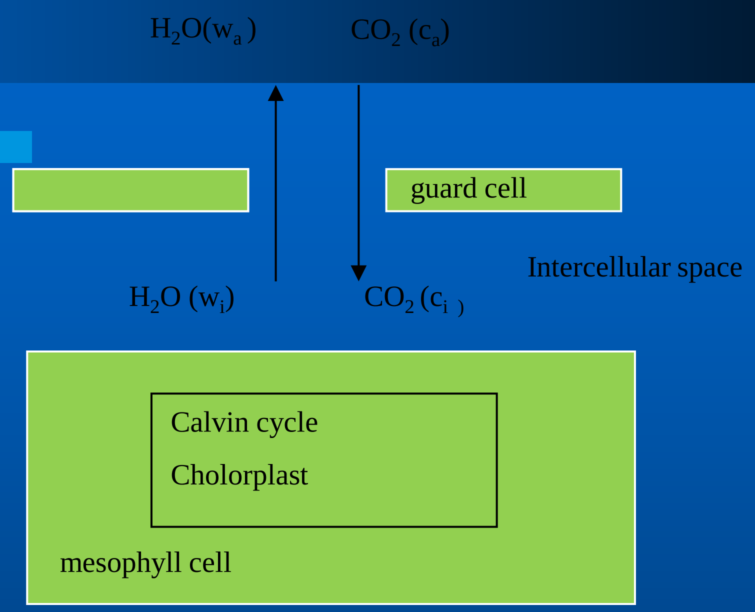
- ❖ ***Grain $^{13C}\Delta$ positively associated with yield under drought – indicator of ability to access water***
- ❖ ***Trade-off between WUE and season-long water use***

Sutton Bonington Mean 2009-10 & 2010-11

- ***Stomatal conductance:***. Lower conductance ↓ ci hence ↑ TE.

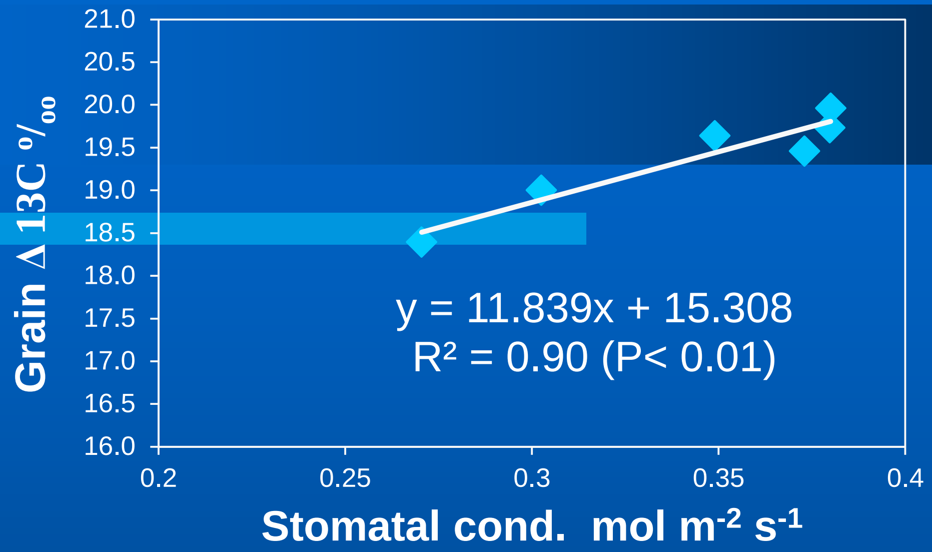
- ***N or Rubisco content per unit leaf area:*** Greater photosynthetic activity ↓ c_i , hence ↑ TE.

Condon et al. (2002). Crop Science

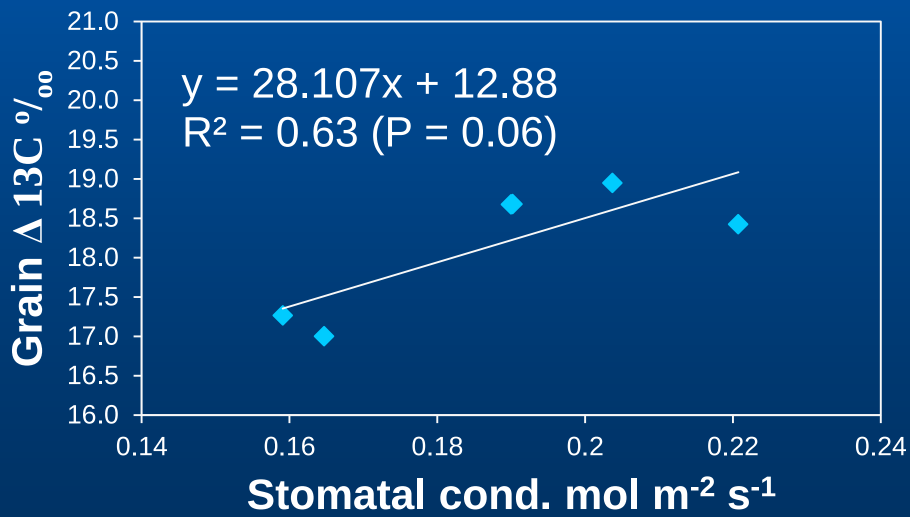


Grain Δ13C versus stomatal conductance (Unirrigated)

2009-10



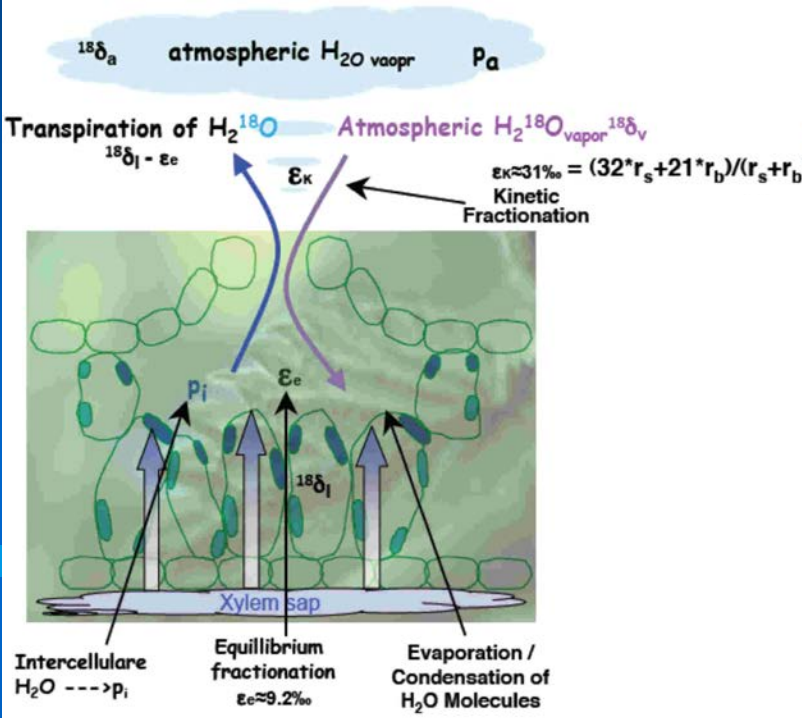
2010-11



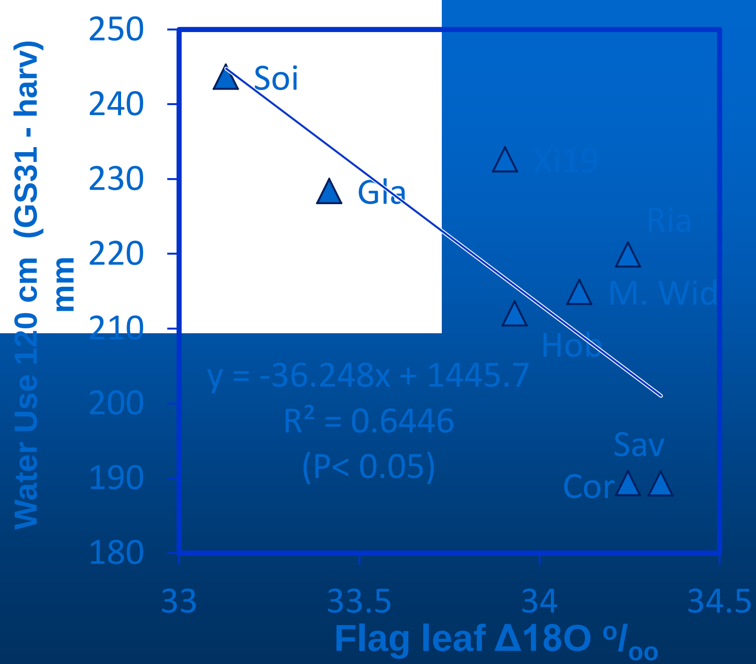
Trade off between water-use efficiency and water use

Use Δ18O as an indicator of transpiration to allow stomatal and Ps effects on Δ13C to be teased apart

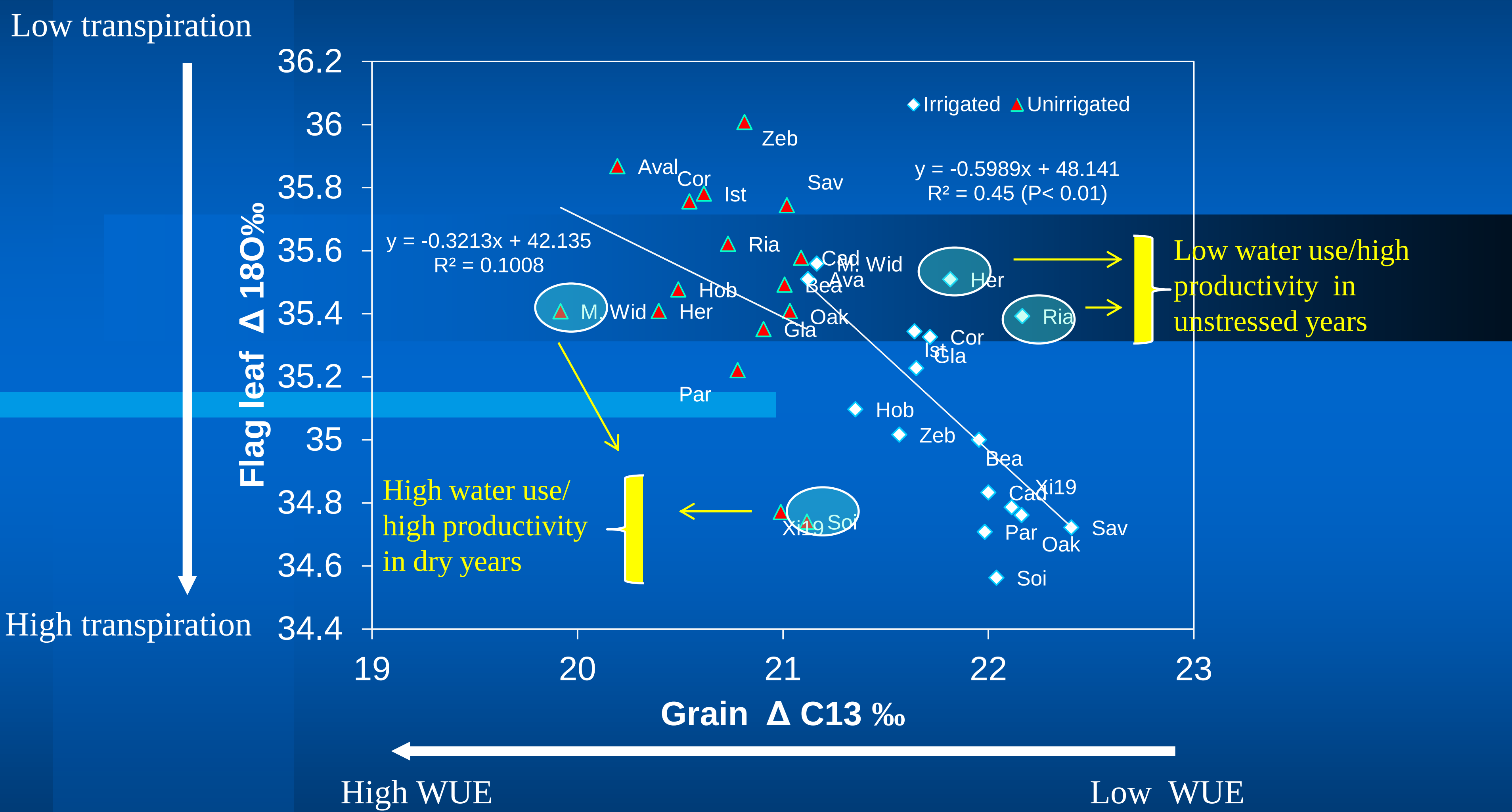
Oxygen isotope ratio technique ~ leaf transpiration



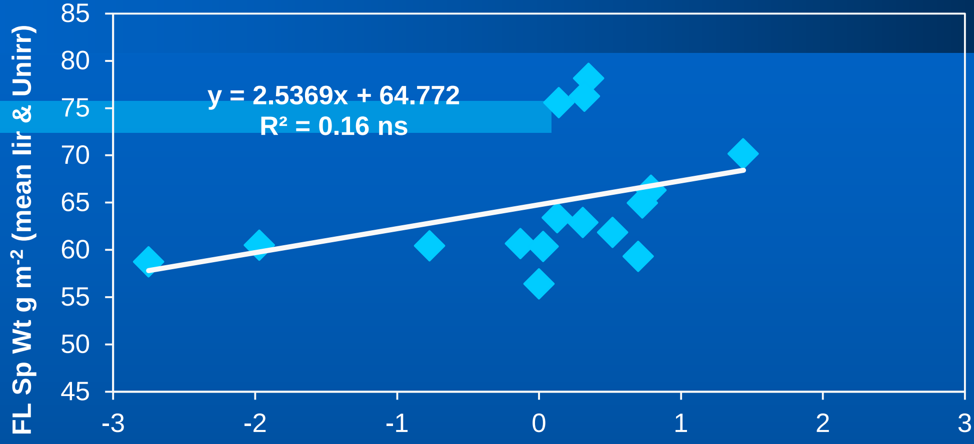
- $^{18}O/^{16}O$ ratio determined by enrichment in the leaf water due to transpiration.
- Leaf water enriched due to preferential loss of the lighter $H_2{}^{16}O$ during evaporation.
- An increase in leaf transpiration decreases leaf T°C (hence intercellular vapour pressure) resulting in less $H_2{}^{18}O$ enrichment at the evaporating site.



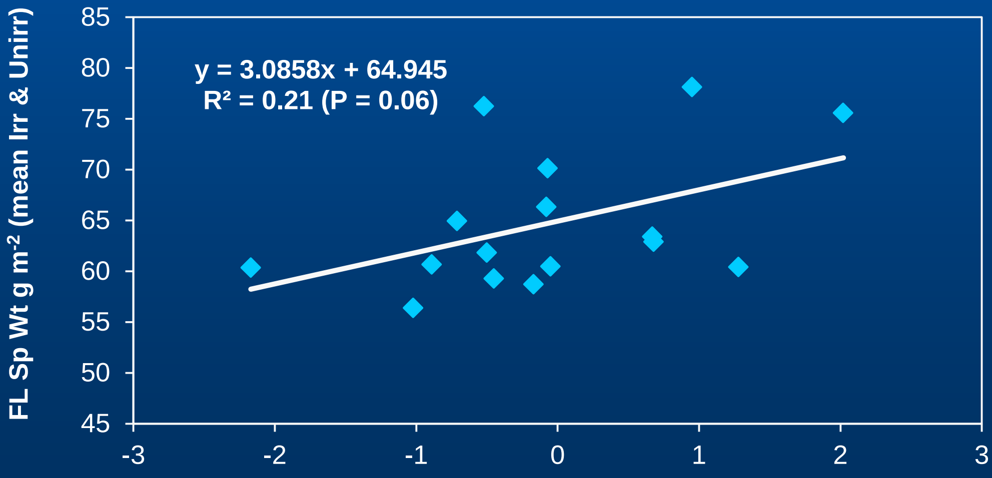
Relationship between $\Delta^{13}C$ and $\Delta^{18}O$ in 18 wheat cultivars (mean 2010 and 2011)



Residual flag leaf $\Delta^{18}O$ vs grain $\Delta^{13}C$ relationship versus flag leaf specific weight at GS61



Residual FL Δ 18O versus grain Δ 13 C Unirrigated



Residual FL 18O versus grain 13C Irrigated

WGIN 2 (9.2 QTL Detection)

2010-11 and 2011-12 expts

• Rialto x Savannah DH population for phenotying for yield physiological traits (94 lines and 2 parents)

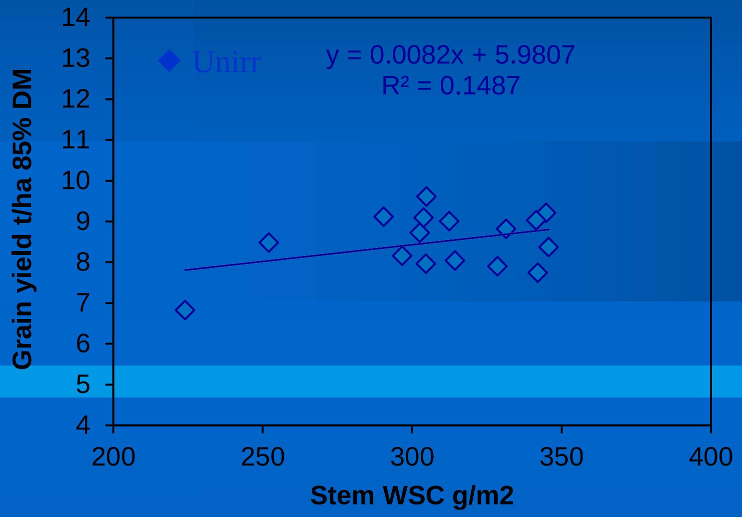
• 2 sites: Nottingham - irrigated & unirrigated; JIC - unirrigated

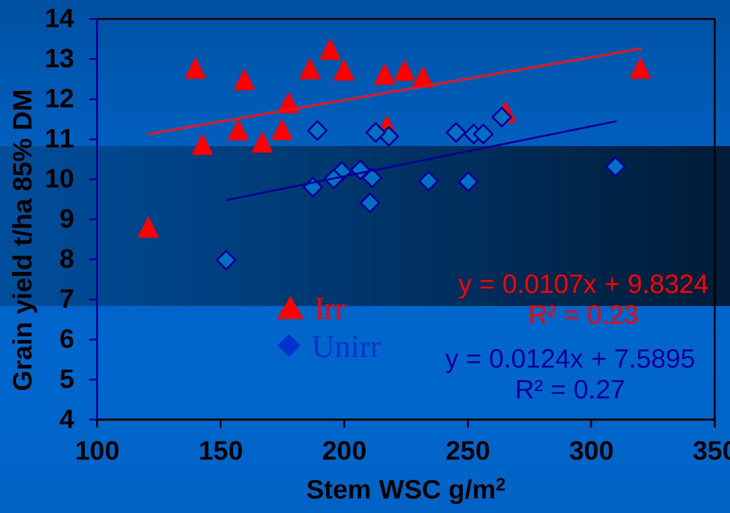
• Target traits

- $^{13}C\ \Delta$ grain
- senescence kinetics
- canopy temperature
- stem WSC

Other traits correlations: Stem WSC @ GS61+9d

Grain yield versus stem WSC reserves





Sutton Bonington 2009-10 *Sutton Bonington 2010-11*

Flag leaf senescence score

Fitting the senescence data

$$score = p0 + p1 * (1 - \exp((-p2 * STA / p1)) + (10 - p1 - p0 / (1 + \exp(-4 * p4(STA - p5) / (10 - p1 - p0)))$$

score : visual senescence score

STA : thermal time after anthesis (°C.days)

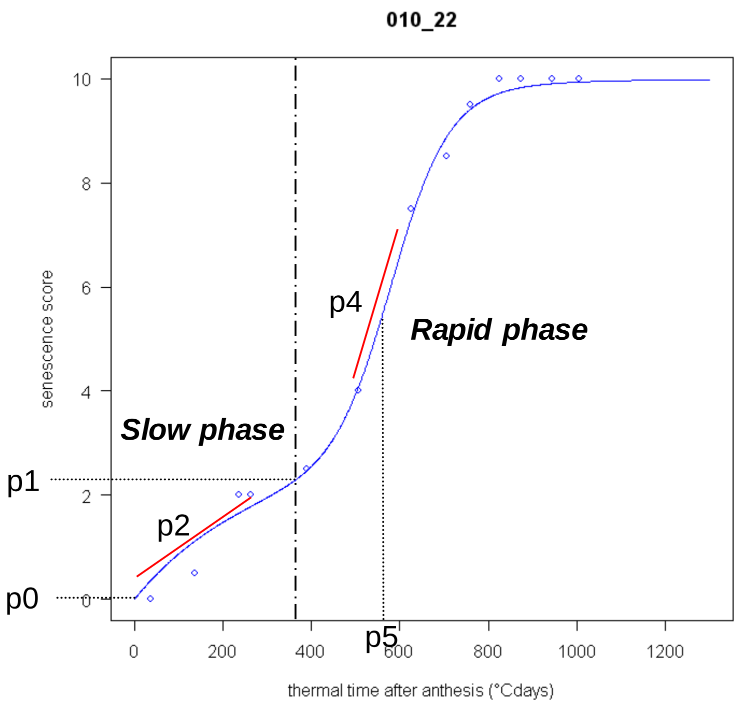
p0 : score at anthesis

p1 : score at the end of the slow phase

p2 : max rate of the slow phase

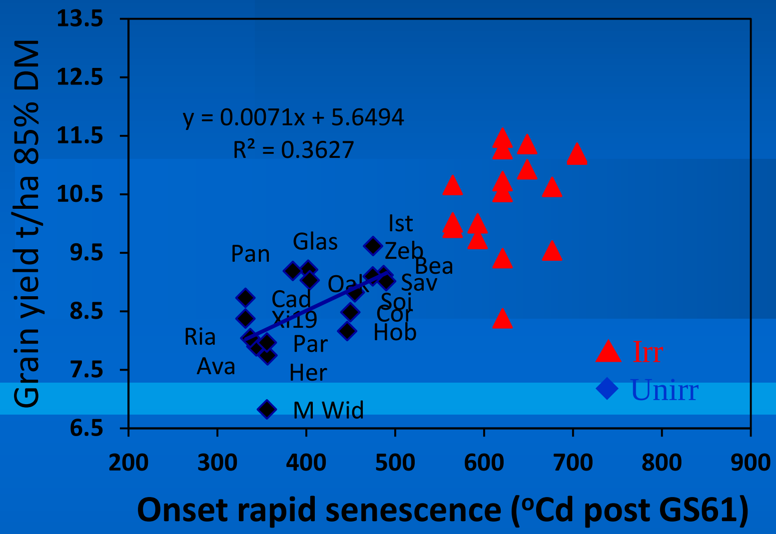
p4 : max rate of the rapid phase

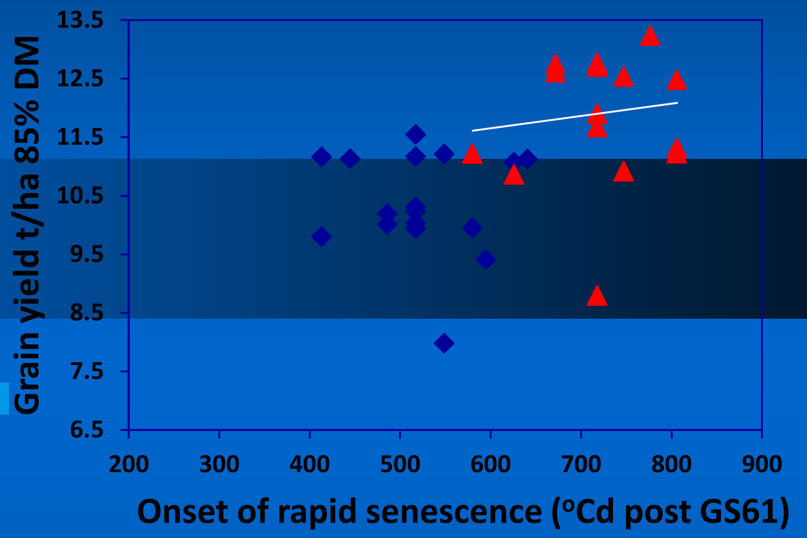
p5 : date at which p4 is reached



Other traits correlations: Flag leaf senescence

Grain yield versus Onset of Senescence

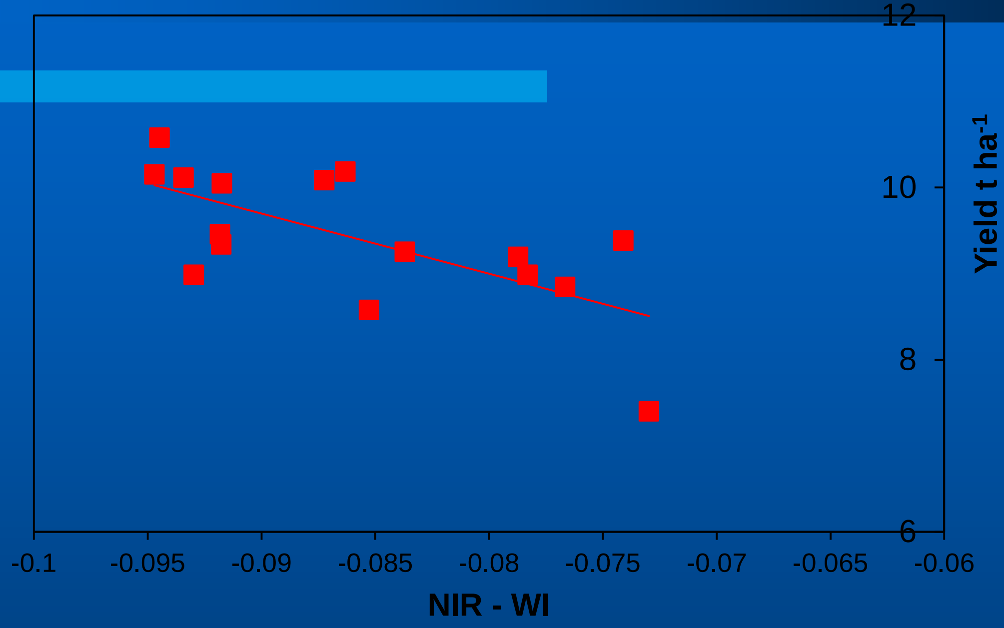




Sutton Bonington 2009-10

Sutton Bonington 2010-11

Spectral reflectance: Water index at GS61+14 d



WI = (R970-R900/)/(R970+R900)

Sutton Bonington Mean 2009-10 & 2010-11

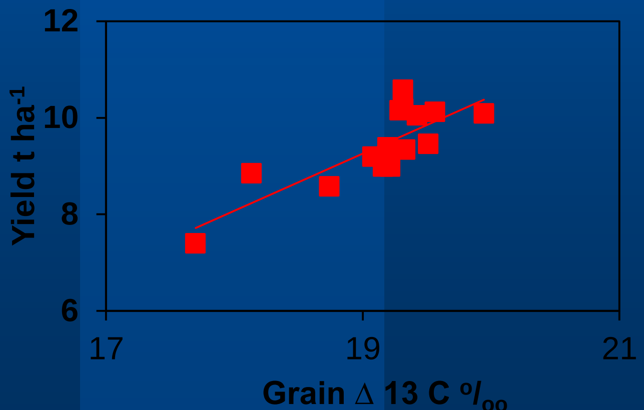
Drought tolerant plant ideotype

- **Multiple linear regression (P<0.001) accounted for 71% of the yield under drought and showed that:**

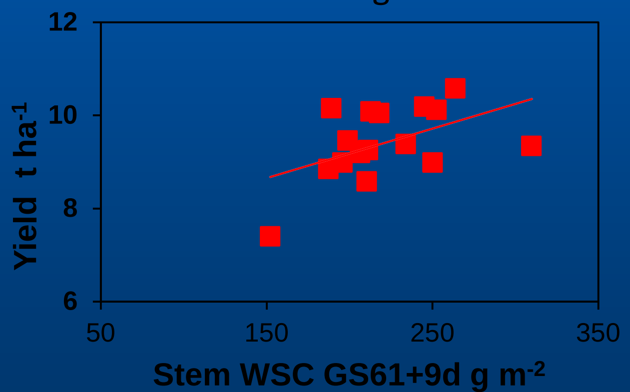
$$GY = -41.9\ x_1 + 0.08\ x_2 + 12.3\ x_3$$

Unirr

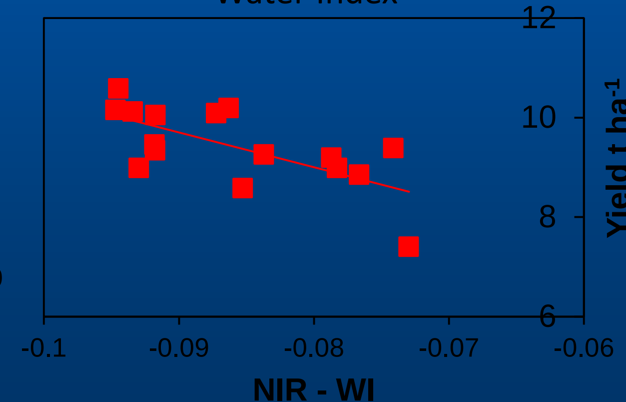
GY increases with increasing Δ 13 C (↓WUE) :



Grain yield increases with increasing stem WSC



GY increases with decreasing Water Index



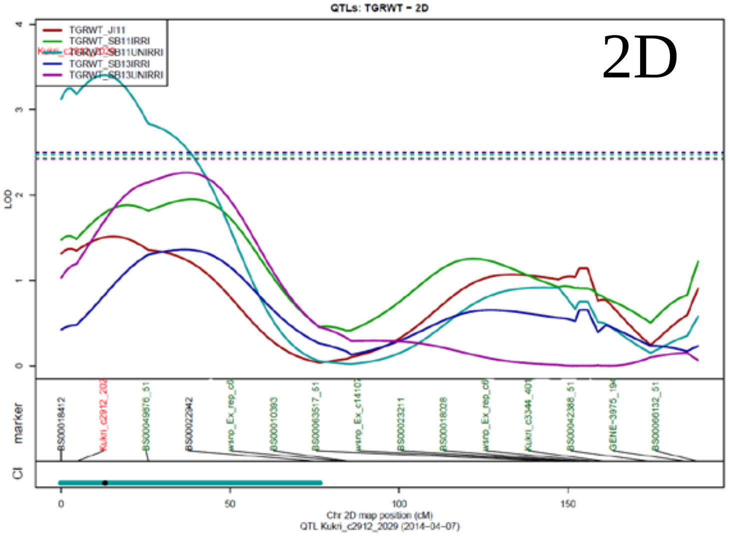
WI = (R970-R900/)/(R970+R900)

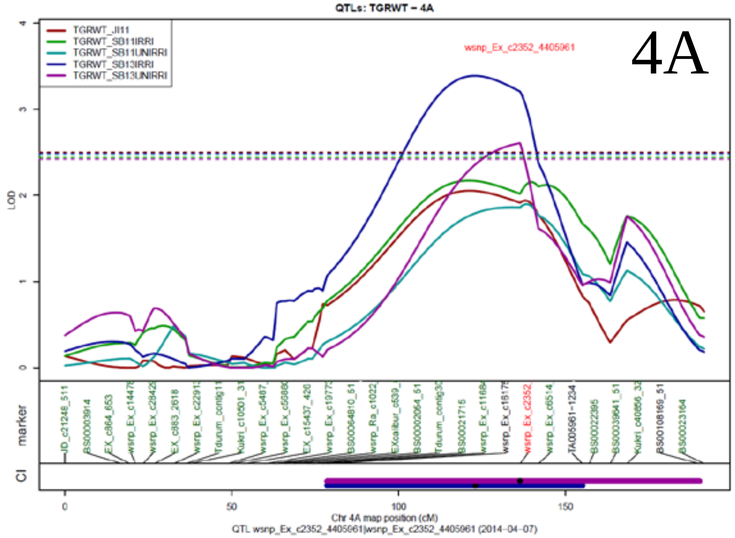
Traits summary

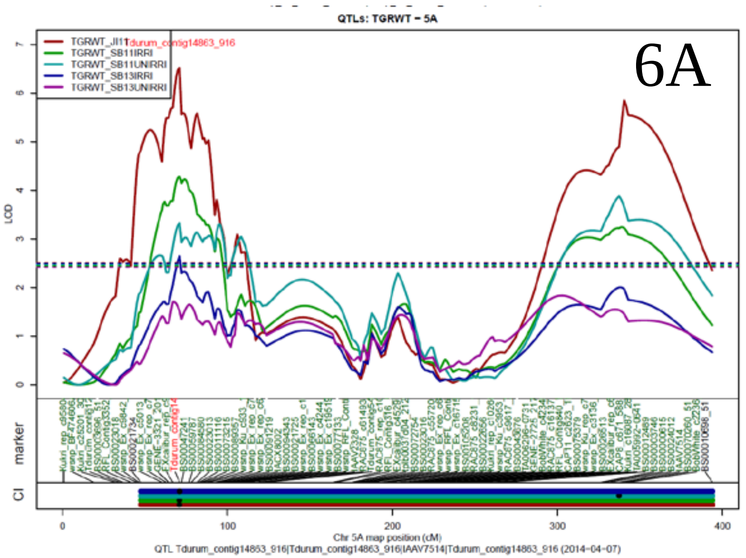
Estimated value of traits to avoid or minimise effects of drought in UK

Variety character	How it might work	Value
High ^{13}C Δ grain	Captures extra water	High
Flag leaf 'stay-green'	Extends grain filling during late drought	High
Low canopy T°C/ deep roots	Captures extra water	High
High stem sugars	Buffers effects of post-flowering drought on grain filling.	Moderate
Early flowering	Advances grain filling before the drought risk period.	Neutral
Awns	Use less water per unit growth.	Slight

Thousand grain weight







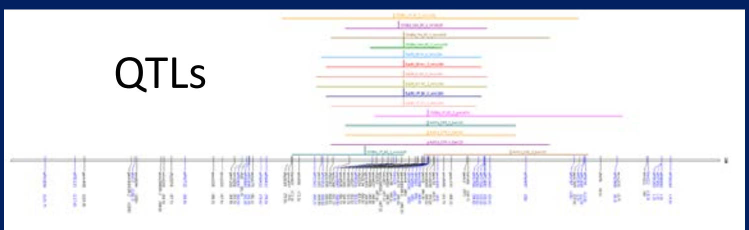

WGIN resource development update

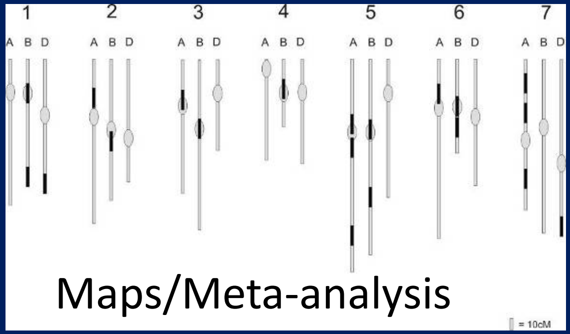
WGIN Dissection of genetic gain in UK winter wheat

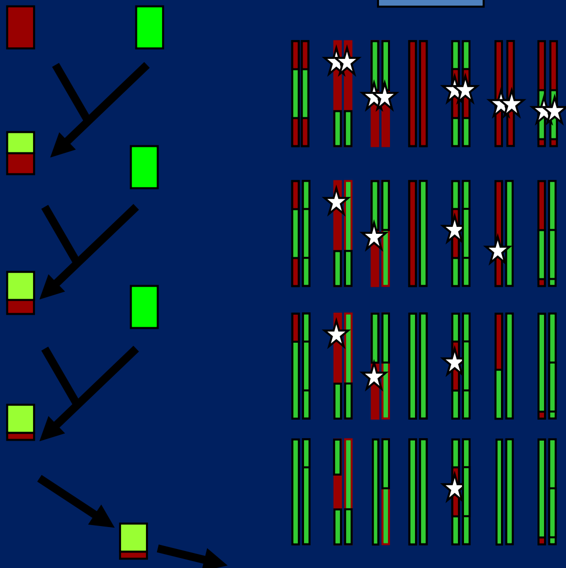
crosses

- Spark x Rialto
- Avalon x Cadenza
- Buster x Charger
- Charger x Badger
- Savannah x Rialto
- Shango x Shamrock
- Malacca x Charger
- Savannah x Renesansa
- Lynx x Cadenza
- Beaver x Soissons
- Weebil x Bacanora
- Milan x Catbird

What genes control these traits?
How do alleles
work in combination for genetic gain and
trait stability?







Isogenics

WGIN resources

- **Germplasm Collections**
  - AE Watkins
  - Gediflux
- **Segregating Populations**
  - Avalon x Cadenza
  - Paragon x Chinese Spring
  - Paragon x synthetic
  - Paragon x Garcia
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  - A x C (height, heading, yield)
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  - *Aegilops uniaristata* 3N
  - Seed dimensions (SxS, AxC, BxS)
  - Bread making functionality (MxH)

Theoretical Applied Genetics manuscript No.
(will be inserted by the editor)

Establishing the A. E. Watkins landrace cultivar collection as a resource for systematic gene discovery in bread wheat.

Luzie U. Wingen · Simon Orford · Richard Goram · Michelle Leverington-Waite, · Lorelei Bilham · Theofania S. Patsiou · Mike Ambrose · Jo Dicks · Simon Griffiths

Received: date / Accepted: date

Author contribution

LUW wrote the paper; SO, MA, and TSP provided phenotypic scores; SO developed mapping populations; RG, LB, and MLW conducted the genotyping; LUW conducted the genetic diversity analysis; LUW, MLW, JD, and SG designed the research.

Key message

A high level of genetic diversity was found in the A. E. Watkins bread wheat landrace collection. Genotypic information was used to determine the population structure and to develop germplasm resources.

Abstract In the 1930s A. E. Watkins acquired landrace cultivars of bread wheat (*Triticum aestivum* L.) from official channels of the board of Trade in London, many of which originated from local markets in 32 countries. The geographic distribution of the 826 landrace cultivars of the current collection, here called the Watkins collection, covers many Asian and European countries and some from Africa. The cultivars were genotyped with 41 microsatellite markers in order to investigate the genetic diversity and population structure of the collection. A high level of genetic diversity was found, higher than in a collection of modern European winter bread wheat varieties from 1945-2000. Furthermore, although weak, the population structure of the Watkins collection reveals nine ancestral geographical groupings. An exchange of genetic material between ancestral groups before commercial wheat-breeding started would be a possible explanation for this. The increased knowledge regarding the diversity of the Watkins collection was used to develop resources for wheat

L.U. Wingen, S. Orford, R. Goram, M. Leverington-Waite, L. Bilham, M. Ambrose, T. S. Patsiou, J. Dicks, S. Griffiths
John Innes Centre
Norwich Research Park
Norwich, UK
Tel.: +1603-450-508
Fax: +1603-450-045
E-mail: luzie.wingen@jic.ac.uk

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Mutant Alleles of *Photoperiod-1* in Wheat (*Triticum aestivum* L.) That Confer a Late Flowering Phenotype in Long Days

Lindsay M. Shaw[*¤a], **Adrian S. Turner**, **Laurence Herry**[¤b], **Simon Griffiths**, **David A. Laurie**

Department of Crop Genetics, John Innes Centre, Norwich, United Kingdom

Abstract

Flowering time in wheat and barley is known to be modified by mutations in the *Photoperiod-1* (*Ppd-1*) gene. Semi-dominant *Ppd-1a* mutations conferring an early flowering phenotype are well documented in wheat but gene sequencing has also identified candidate loss of function mutations for *Ppd-A1* and *Ppd-D1*. By analogy to the recessive *ppd-H1* mutation in barley, loss of function mutations in wheat are predicted to delay flowering under long day conditions. To test this experimentally, introgression lines were developed in the spring wheat variety 'Paragon'. Plants lacking a *Ppd-B1* gene were identified from a gamma irradiated 'Paragon' population. These were crossed with the other introgression lines to generate plants with candidate loss of function mutations on one, two or three genomes. Lines lacking *Ppd-B1* flowered 10 to 15 days later than controls under long days. Candidate loss of function *Ppd-A1* alleles delayed flowering by 1 to 5 days while candidate loss of function *Ppd-D1* alleles did not affect flowering time. Loss of *Ppd-A1* gave an enhanced effect, and loss of *Ppd-D1* became detectable in lines where *Ppd-B1* was absent, indicating effects may be buffered by functional *Ppd-1* alleles on other genomes. Expression analysis revealed that delayed flowering was associated with reduced expression of the *TaFT1* gene and increased expression of *TaCO1*. A survey of the GEDIFLUX wheat collection grown in the UK and North Western Europe between the 1940s and 1980s and the A.E. Watkins global collection of landraces from the 1920s and 1930s showed that the identified candidate loss of function mutations for *Ppd-D1* were common and widespread, while the identified candidate *Ppd-A1* loss of function mutation was rare in countries around the Mediterranean and in the Far East but was common in North Western Europe. This may reflect a possible benefit of the latter in northern locations.

Citation: Shaw LM, Turner AS, Herry L, Griffiths S, Laurie DA (2013) Mutant Alleles of *Photoperiod-1* in Wheat (*Triticum aestivum* L.) That Confer a Late Flowering Phenotype in Long Days. PLoS ONE 8(11): e79459. doi:10.1371/journal.pone.0079459

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: lindsayshaw@csiro.au

¤a Current address: CSIRO Plant Industry, Canberra, Australian Capital Territory, Australia
¤b Current address: Université Paris Descartes, Paris, France

Introduction

Wheat (*Triticum* species) and barley (*Hordeum vulgare*) in their ancestral forms are quantitative long day plants. That is, they are stimulated to flower by increasing day length [1]. In barley, a recessive mutation at the *Photoperiod-H1* (*Ppd-H1*) locus allele attenuates the long day photoperiod response to confer a later flowering phenotype [2,3]. This has a yield benefit in environments with long growing seasons such as North Western Europe [2].

Colinear with the *Ppd-H1* gene on barley chromosome 2H are a homoeologous series of *Ppd-1* genes on the group 2 chromosomes of durum and bread wheat (*T. turgidum ssp. durum*, *T. aestivum*). In contrast to barley, the previously characterized mutations in wheat are partially dominant and confer an early flowering phenotype in short day (SD) or long day (LD) conditions. This day neutral or "photoperiod insensitive" (PI) phenotype is widely used in environments where the optimal growing conditions occur under SD conditions or where early flowering is desirable to avoid summer temperature and drought stresses [4–6]. PI is associated with promoter deletions [7–9], a transposon insertion in the promoter of *Ppd-1* [9] or increased gene copy number of *Ppd-1* [10]. PI alleles are designated by an *a* suffix (*Ppd-1a*) while alleles conferring a photoperiod sensitive phenotype have a *b* suffix (*Ppd-1b* [11]).

The expression of *Ppd-1b* alleles follows a diurnal cycle with very low expression at dawn followed by a morning peak in expression which falls to very low levels at night. Early flowering PI (*Ppd-1a*) alleles lose this diurnal pattern and have constantly elevated expression which is closely associated with the increased expression of *TaFT1* (also called *VRN-3*), a wheat orthologue of the Arabidopsis *FT* gene [7,8,12,13]. In contrast, the late flowering *ppd-H1* mutation in barley is associated with reduced expression of *HvFT1* [3,14]. In Arabidopsis, FT protein is a mobile signal produced in leaves which moves to the shoot apex where it interacts with the FD protein to induce flowering by promoting the expression of *APETALA1* [15–19]. The cereal orthologues are predicted to act in a similar manner [20].

Ppd-1 is a member of the pseudo-response regulator (*PRR*) gene family. PRR proteins have two conserved domains. The first is the

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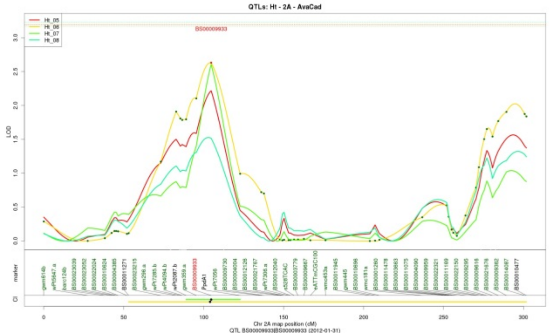
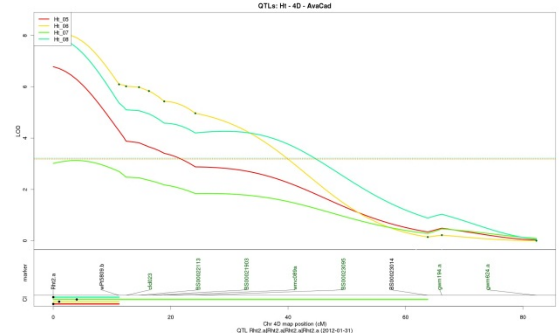
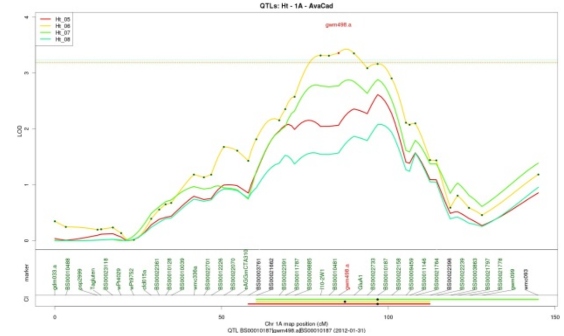
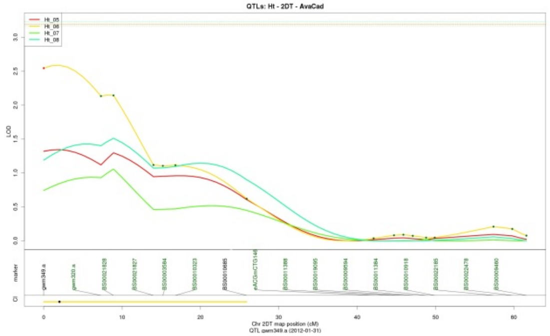
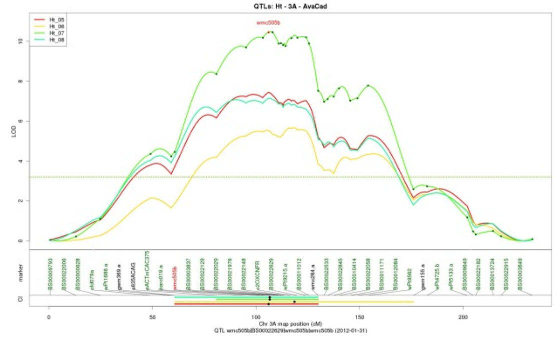
Avalon x Cadenza QTL summary- height

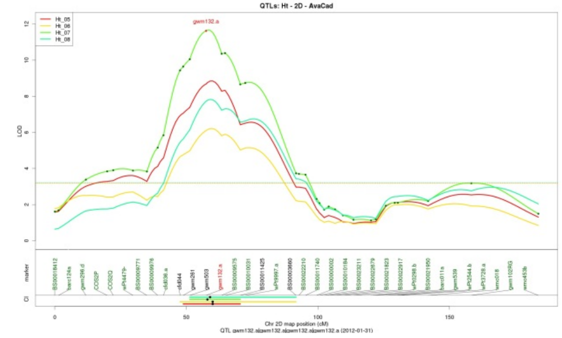


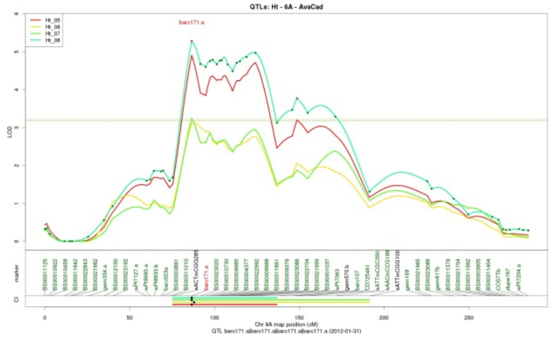




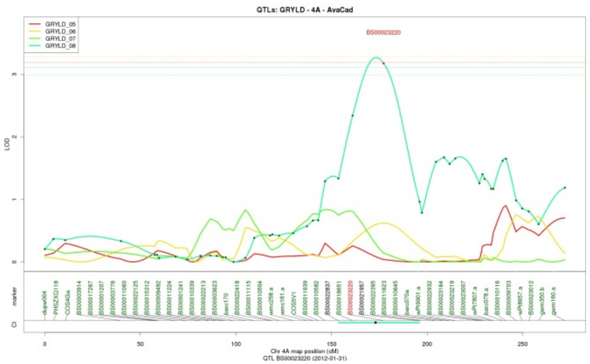


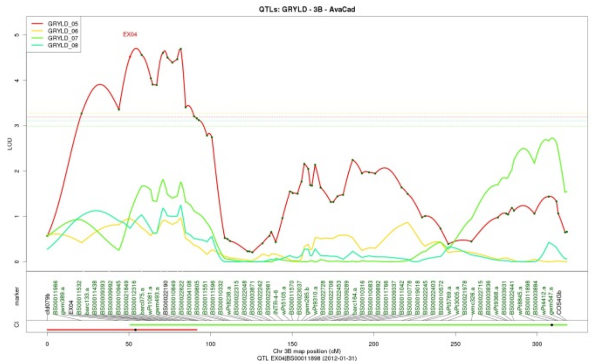


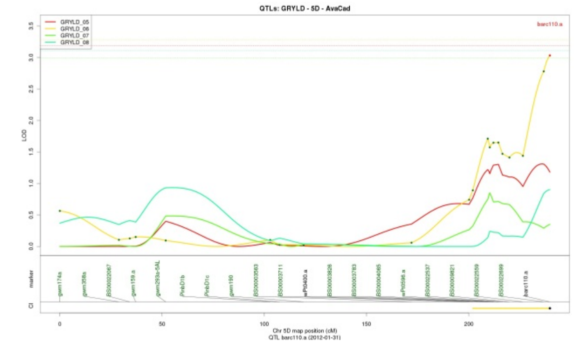


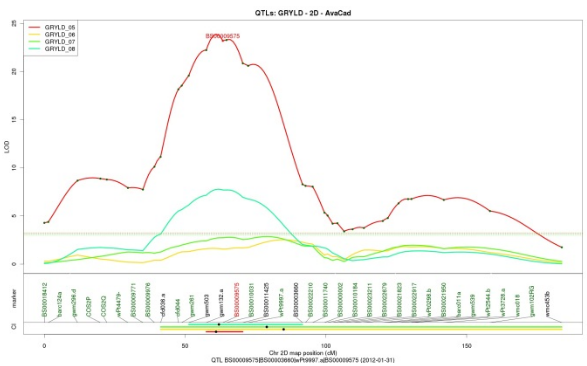


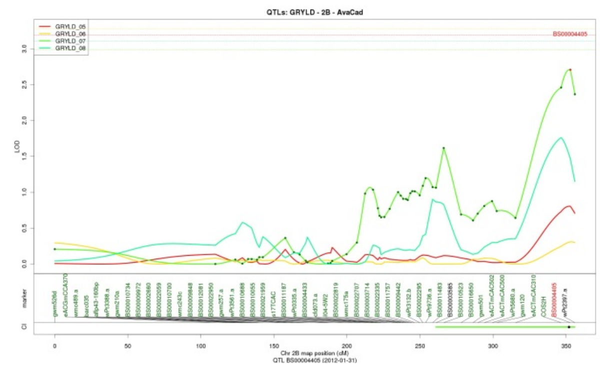
Avalon x Cadenza QTL summary- grain yield

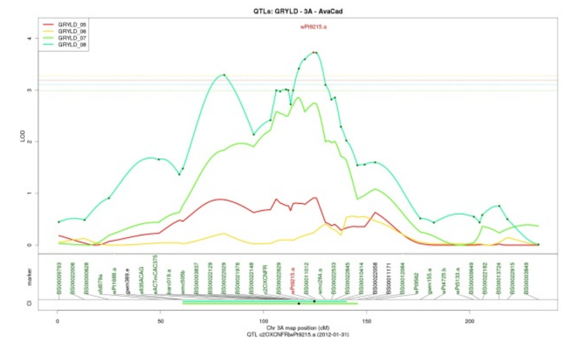


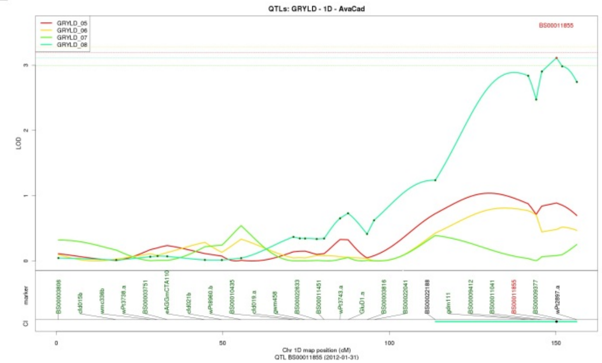




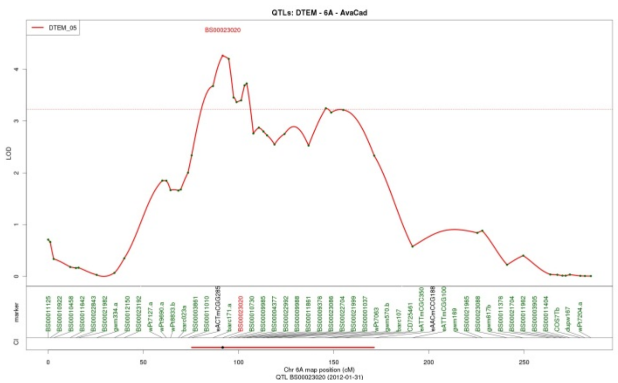


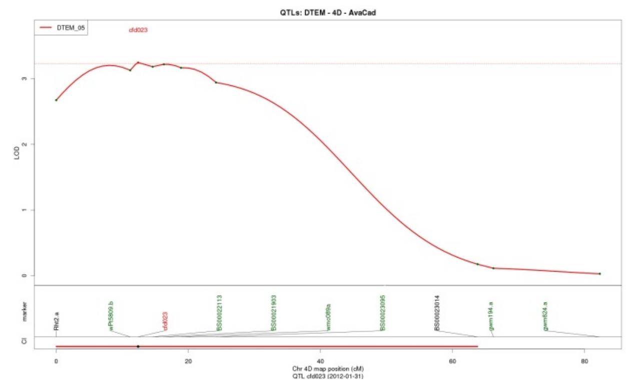


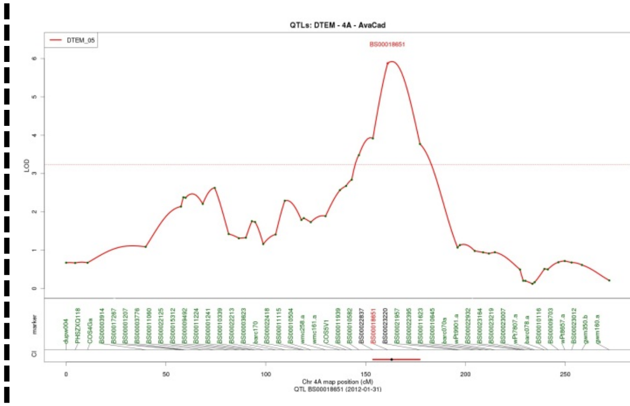


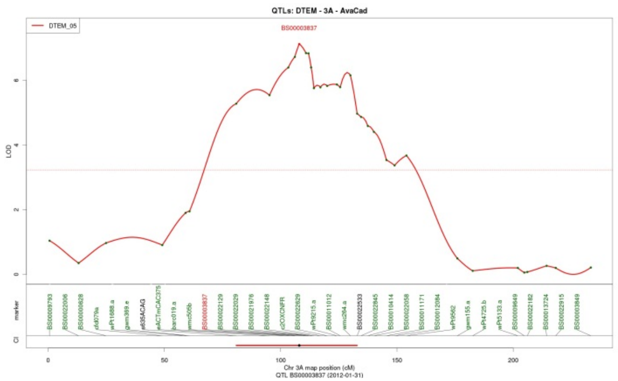


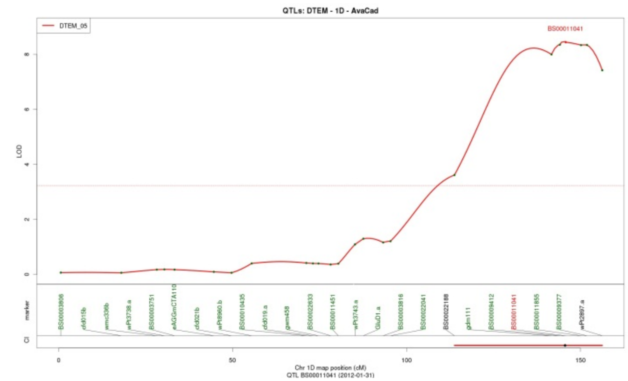
Avalon x Cadenza QTL summary- ear emergence

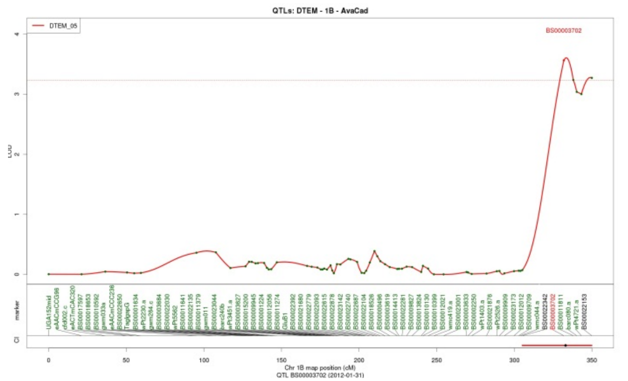












2012-13 WGIN Near Isogenic Line results

Heading date

QTL	trait	stream	BCparent	no A	no B	t-stat	df	Ava mean	Cad mean	A>B	p.val	signif
1B	FT	1B	Ava	9	20	-1.16	11.5	46.1	47.2	FALSE	0.2681	
1B	FT	1B	Cad	24	27	-3.2	48.7	43.1	44.8	FALSE	0.0024	***
1D	FT	1D	Ava	26	14	3.97	27.7	47.3	44.5	TRUE	5.00E-004	***
1D	FT	1D	Ava	26	6	2.88	6.7	47.3	44	TRUE	0.0247	**
1D	FT	1D	Ava	14	6	0.42	7.9	44.5	44	TRUE	0.6889	
1D	FT	1D	Cad	56	62	5.61	115.9	46.1	44	TRUE	0	***
3A	FT	3A	Ava	44	37	-4.74	71	45.3	47.8	FALSE	0	***
3A	FT	3A	Cad	84	73	-4.61	154.5	43.7	45.4	FALSE	0	***
3B	FT	3B	Cad	42	50	-0.47	90	44.9	45.1	FALSE	0.6412	
6A	FT	6A	Ava	67	24	-0.54	31.8	46.6	47	FALSE	0.5899	
6A	FT	6A	Cad	76	63	-2.95	125.6	43.3	44.4	FALSE	0.0037	***
6B	FT	6B	Ava	73	54	-2.74	117.1	46.1	47.3	FALSE	0.0072	***
6B	FT	6B	Cad	50	77	-1.03	89.7	43.7	44.1	FALSE	0.3039	
6B	FT	6B	Cad	50	3	2.46	3.7	43.7	42	TRUE	0.0747	*

Crop height

QTL	trait	stream	BCparent	no A	no B	t-stat	df	Ava mean	Cad mean	A>B	p.val	signif
2A	Ht	2A	Ava	27	6	-0.09	13.3	68.4	68.5	FALSE	0.9274	
2D	Ht	2D	Ava	107	108	-4.72	212	70.4	73.3	FALSE	0	***
3A	Ht	3A	Ava	44	37	-6.96	73.2	69.3	76.1	FALSE	0	***
5A	Ht	5A	Ava	24	23	-2.86	41.5	67.2	70.4	FALSE	0.0065	***
6A	Ht	6A	Ava	67	24	2.5	31.8	70.4	67.4	TRUE	0.0178	**
6B	Ht	6B	Ava	73	58	-0.97	109.4	70.3	71	FALSE	0.3349	
2A	Ht	2A	Cad	24	15	0.5	27.7	74.7	74.1	TRUE	0.624	
2D	Ht	2D	Cad	80	96	-6	170	70.3	74.6	FALSE	0	***
3A	Ht	3A	Cad	85	74	-6.57	157	67.4	72.2	FALSE	0	***
3B	Ht	3B	Cad	43	51	2.15	91.8	75.5	73.5	TRUE	0.0341	**
6A	Ht	6A	Cad	76	62	5.8	131.4	75.2	70.8	TRUE	0	***
6B	Ht	6B	Cad	51	78	-4.53	101.1	67.8	71.9	FALSE	0	***
6B	Ht	6B	Cad	51	3	2.15	5.5	67.8	65.3	TRUE	0.0794	*
6B	Ht	6B	Cad	78	3	6.31	3.8	71.9	65.3	TRUE	0.004	***

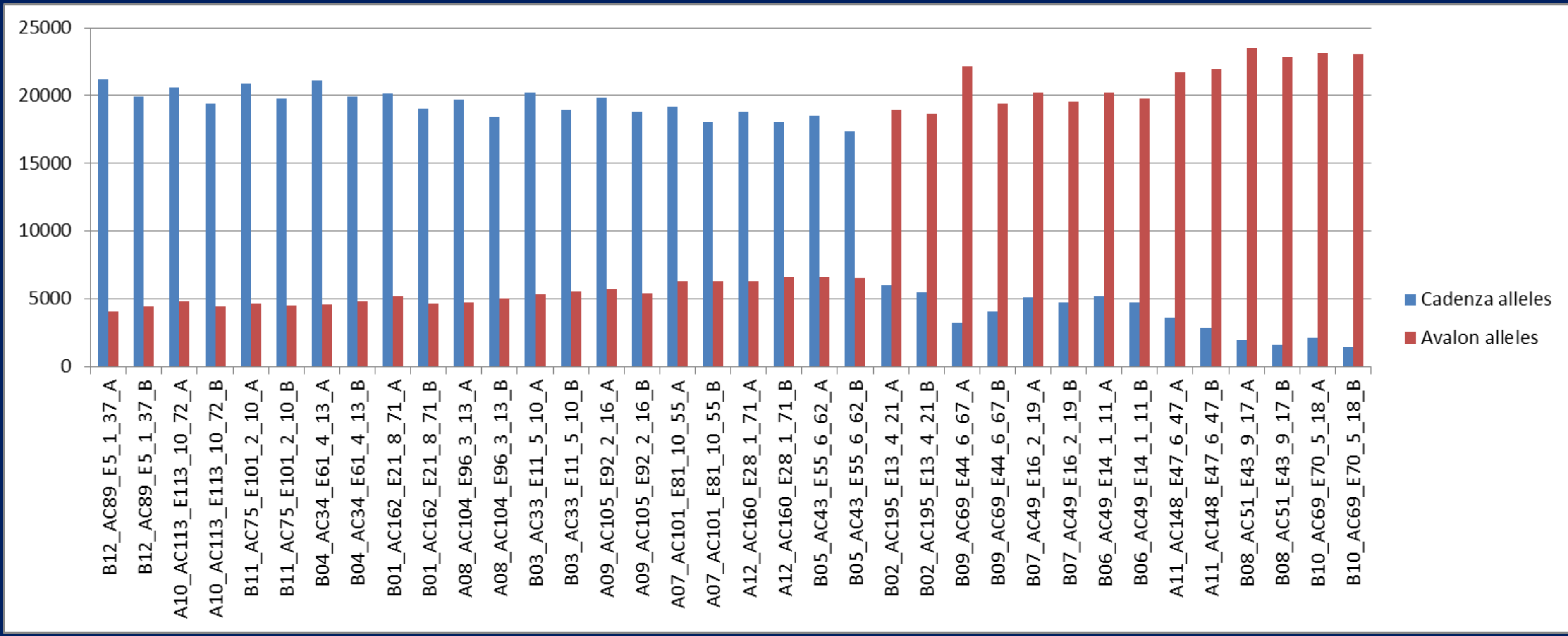
570 NILs in the field trial in 3 replicates trial repeated in 2013-14.

Pleiotropy and linkage

Other traits scored:

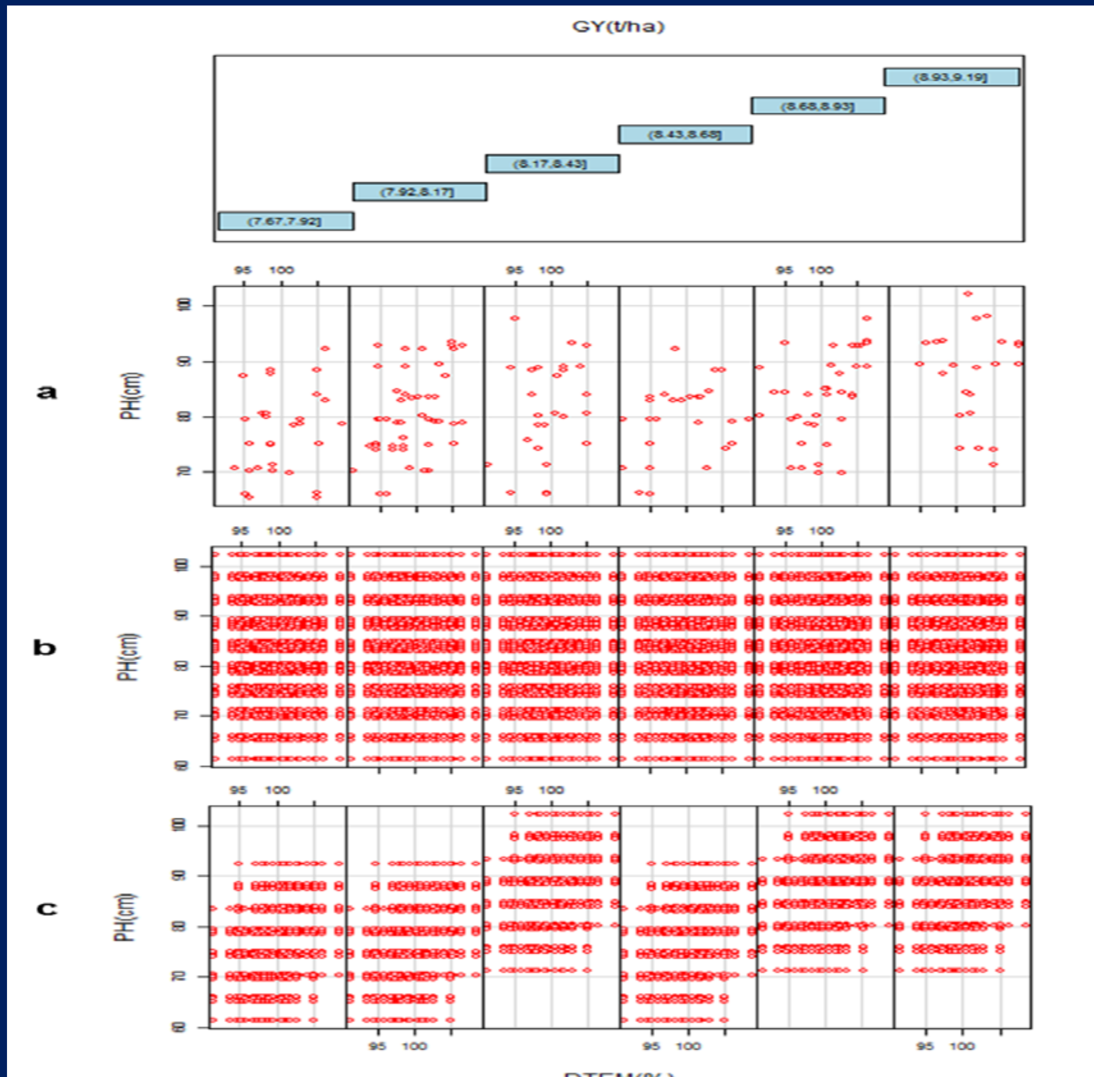
- Yield
- GS31: length of internodes
- Ear length
- Spikelet number/Infertile spikelet number
- Peduncle and internode length of mature plant
- Harvest index for selected 2D and 3A NILs
- Leaf senescence of single reps

Genotypic composition of back/foreground
Axiom 817 K analysis of Avalon x Cadenza Near Isogenic Line sub set



Breeding simulations using WGIN data

The UK reference population Avalon×Cadenza as a platform for simulated breeding strategies for grain yield in elite Western European bread wheat



QTL and genes used in the simulation study and genotypes of DH27, DH61, DH109, DH160, DH182 and the target genotype

QTL Locus	DH27	DH61	DH182	DH109	DH160	Target genotype
qGY-psr-2D.1[a]	qq	QQ	qq	qq	QQ	qq
qGY-psr-3A.2	QQ	qq	QQ	qq	QQ	qq
qGY-psr-3B.2	qq	QQ	QQ	QQ	QQ	QQ
qTGRWT-psr-5A.1	qq	QQ	qq	QQ	qq	qq
qGRLG-psr-5A.1	qq	QQ	qq	qq	qq	qq
qGRWD-psr-5A.2	QQ	QQ	QQ	QQ	qq	QQ
qPH-psr-2A.1[b]	qq	QQ	QQ	qq	QQ	QQ
qPH-psr-3A[c]	QQ	QQ	qq	QQ	QQ	QQ
qPH-psr-3B.1[d]	qq	qq	QQ	qq	QQ	qq
qPH-psr-4D[e]	QQ	QQ	QQ	QQ	QQ	QQ
qPH-psr-5A.1	qq	QQ	qq	QQ	qq	QQ
qDTEM-psr-1B.2	qq	QQ	qq	qq	qq	qq
qDTEM-psr-1D.1	QQ	QQ	QQ	QQ	QQ	QQ
qDTEM-psr-5A[f]	QQ	qq	QQ	QQ	qq	qq
qDTEM-psr-6A.1[g]	qq	QQ	QQ	qq	QQ	QQ
Vrn-A1b[h]	qq	QQ	QQ	qq	QQ	QQ
qTRSA-2A[i]	qq	QQ	qq	QQ	qq	qq
qSDW-5A[i]	qq	QQ	qq	qq	qq	qq
qTRL-5B[i]	qq	qq	qq	qq	QQ	qq
qTRL-6A[i]	qq	QQ	QQ	qq	QQ	QQ
Sbm1-5D[j]	qq	qq	qq	qq	QQ	qq
Yr6	qq	QQ	qq	QQ	qq	qq
Yr7	qq	qq	QQ	qq	QQ	qq

Efficiency of breeding methods and population size of generations

Crosses	Breeding methods	Generation	Population size	Number of target individuals
DH61×DH182	F_2-DH	F_2 Enrichment	2 000	9.64±0.13
		F_2-DH	2 000	
	RIL	F_2 Enrichment	2 000	6.22±0.39
		F_6	2 000	
	Modified SSD	F_2 Enrichment	10 000	2.27±0.06
		F_6	715	
DH27×DH61	F_2-DH	F_2 Enrichment	3 000	6.45±0.11
		F_2-DH	3 000	
	RIL	F_2 Enrichment	3 000	3.82±0.35
		F_6	3 000	
DH109×DH160	F_2-DH	F_2 Enrichment	5 000	7.09±0.12
		F_2-DH	5 000	
	RIL	F_2 Enrichment	5 000	4.57±0.57
		F_6	5 000	

Acknowledgements

HM TREASURY

Dear Chief Secretary,

I'm afraid there is no money.
Kind regards - and good luck!

Liam