

WGIN Update Nov 2011

John Innes Centre – Crop Genetics

Simon Griffiths Group

Luzie Wingen

Michelle Leverington

Sue Freeman

Richard Goram

Simon Orford

Cathy Mumford

Lesley Fish

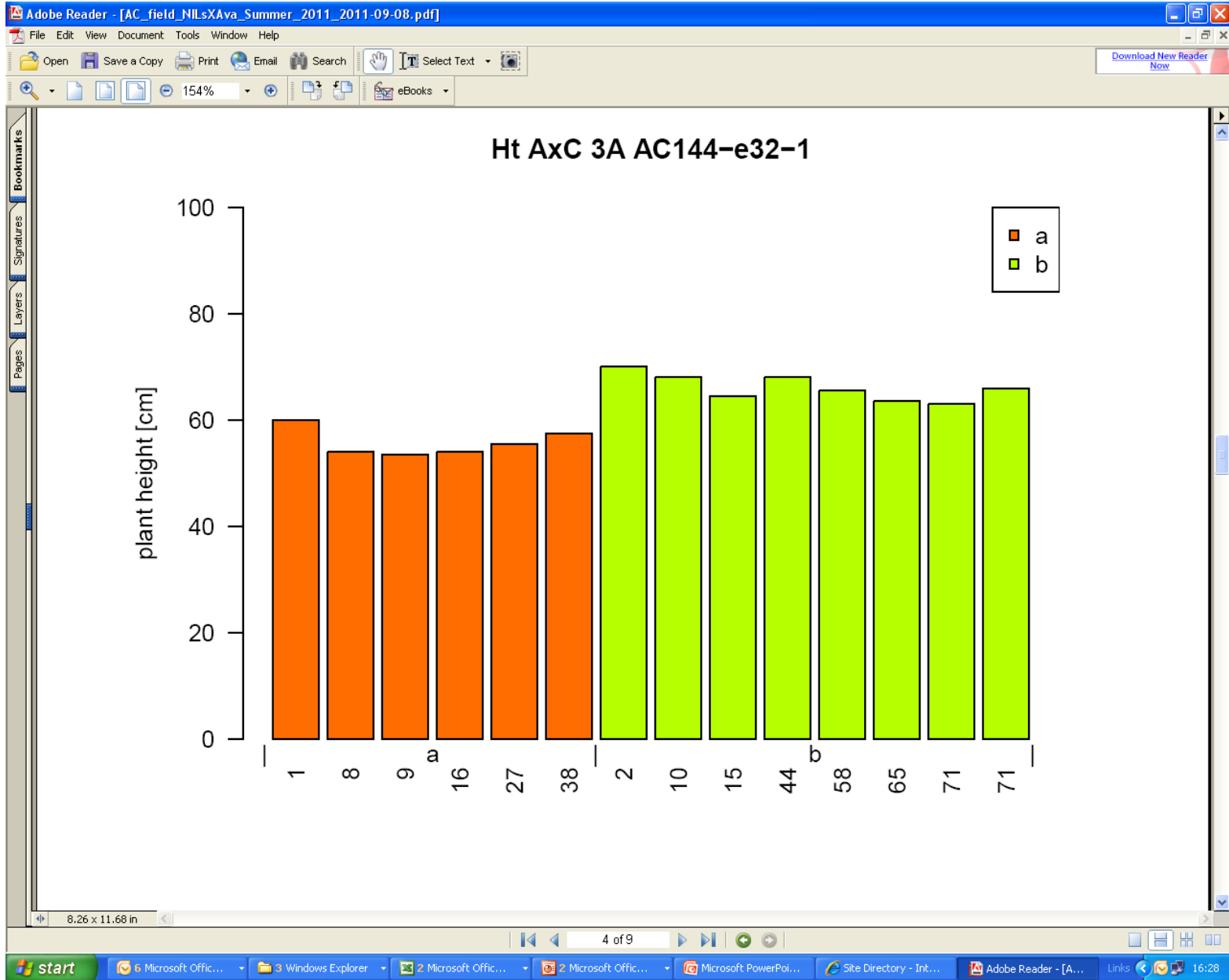
NIL Development

- Avalon x Cadenza 3A NILs for flowering and height. 1x1m plot trials in 2011 successfully completed. Some significance reported
- Other NILs also being developed but less advanced 1A, 1D, 2A, 2D, 3B, 6A, 6B
- Perils of 2011 spring sowing....

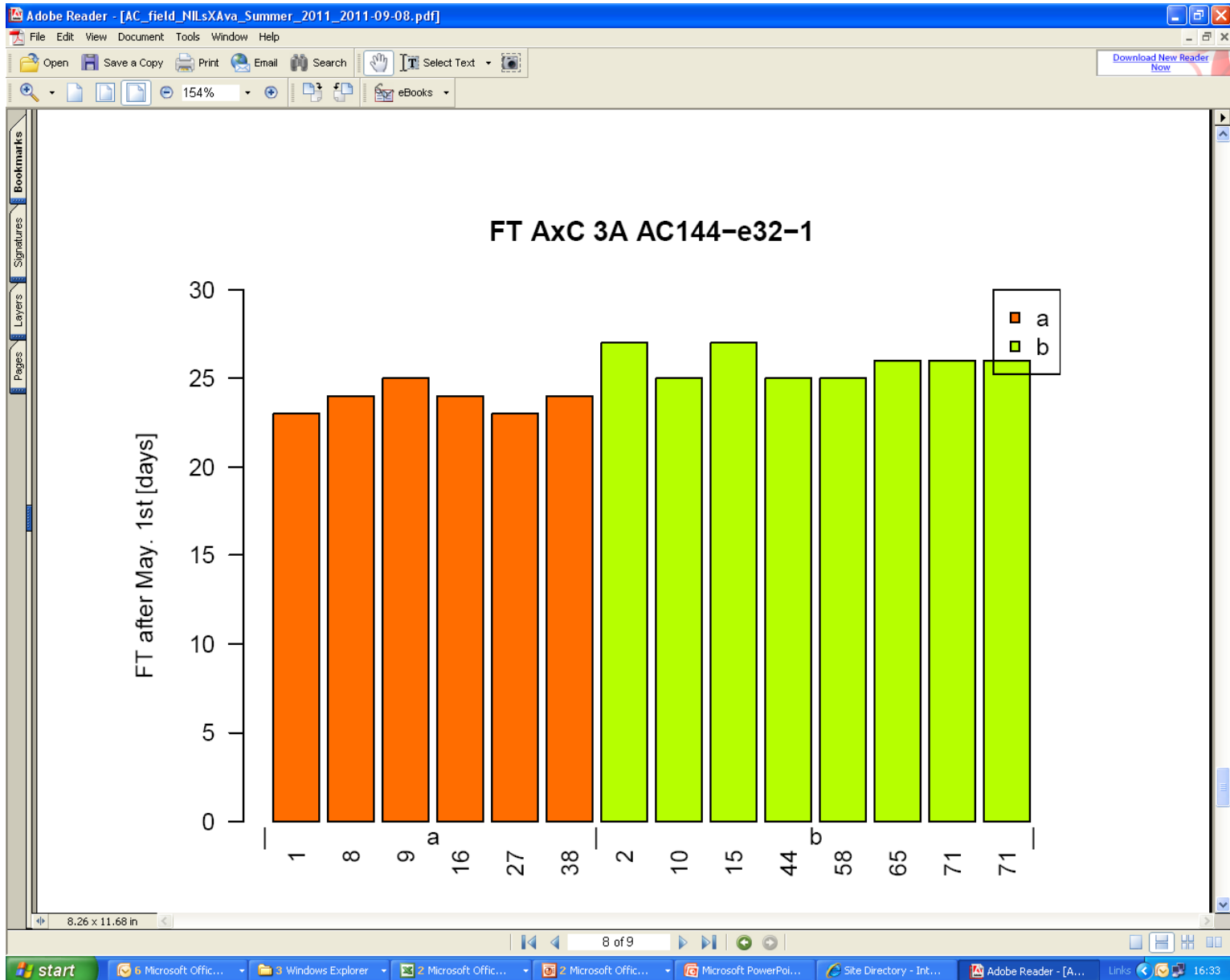
Significant NIL Results from Field Trials 2011

QTL	trait	stream	BCparent	no A	no B	t-stat	df	Ava	Cad	A>B	p.val	signif
								mean	mean			
AxC 3A	Ht	AC113-e113-8	Ava	13	18	-1.77	17.1	56.8	59.7	FALSE	0.094	*
AxC 2D	Ht	AC162-e21-8	Cad	3	3	-2.81	3.4	62	73.5	FALSE	0.0589	*
AxC 2A	FT	AC174-e8-2	Cad	3	3	2.46	3.9	17.3	13	TRUE	0.0712	*
AxC 6A	FT	AC89-e5-1	Cad	3	2	-3.12	2.9	14.3	17.5	FALSE	0.0549	*
AxC 6A	FT	AC104-e6-8	Cad	3	3	-3.13	2.9	10.3	12.7	FALSE	0.0535	*
AxC 6A	Ht	AC104-e6-9	Cad	3	3	3.46	3.7	71	62.3	TRUE	0.0297	**
AxC 3A	Ht	AC113-e113-10	Ava	19	12	-4.69	25.7	59.6	63.5	FALSE	1.00E-004	***
AxC 3A	Ht	AC179-e27-2	Ava	14	5	-7.37	15.8	55.4	62.6	FALSE	0	***
AxC 3A	Ht	AC179-e27-8	Ava	3	8	-6.07	6.1	53.2	61.8	FALSE	8.00E-004	***
AxC 3A	Ht	AC144-e32-1	Ava	6	8	-7.64	10.7	55.8	66.1	FALSE	0	***
AxC 3A	FT	AC113-e113-10	Ava	20	12	-3.31	28.6	23.6	24.3	FALSE	0.0026	***
AxC 3A	FT	AC179-e27-2	Ava	14	5	-5.06	8.1	22.6	23.8	FALSE	9.00E-004	***
AxC 3A	FT	AC179-e27-8	Ava	3	8	-10.58	7	22	24	FALSE	0	***
AxC 3A	FT	AC144-e32-1	Ava	6	8	-4.79	11.5	23.8	25.9	FALSE	5.00E-004	***

Avalon x Cadenza Height NIL Example



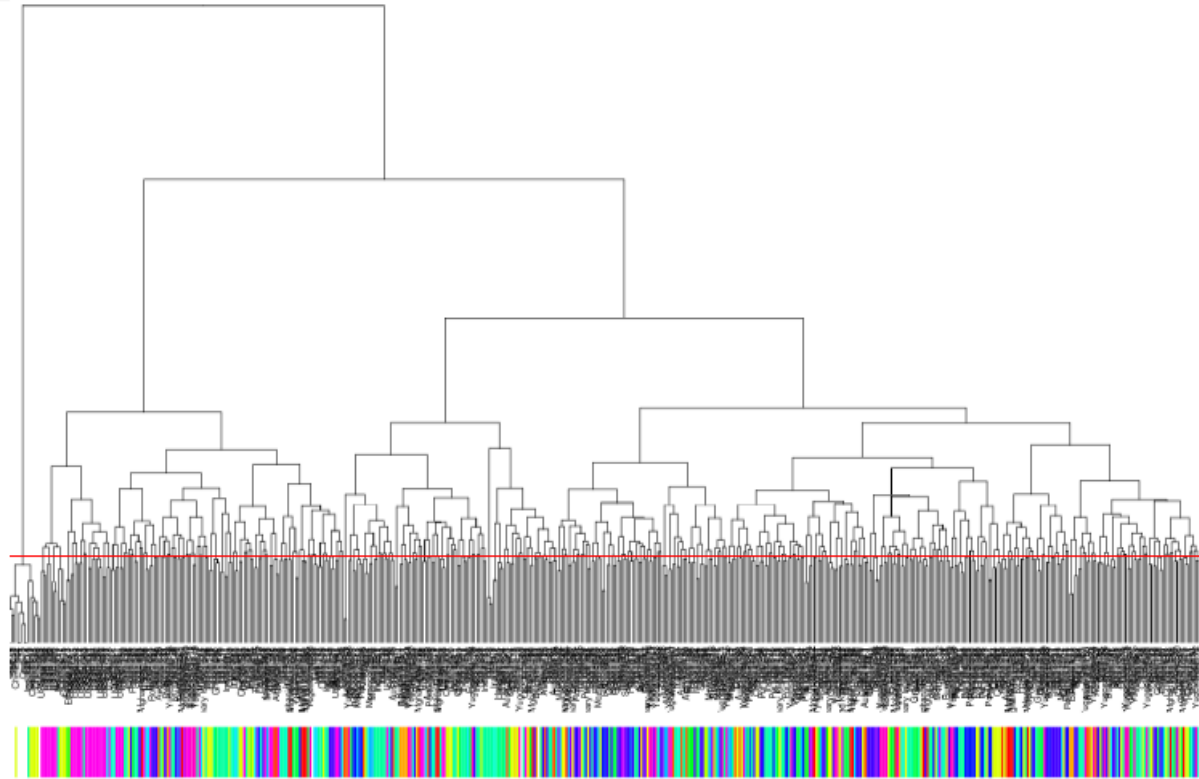
Avalon x Cadenza Flowering NIL Example



NIL Development 2012

- Spring problems being overcome with a three rep trial of 1m plot on all material not covered in 3A 2011 trial
- Will provide enough material for extensive NIL trial using 6m plots in 2012 / 13
- Other NILs being fixed (BC2F2) at this season include LR19 and bread making quality. Grain size and shape following behind

Watkins Core Collection 2010



100 clusters
(height 60)

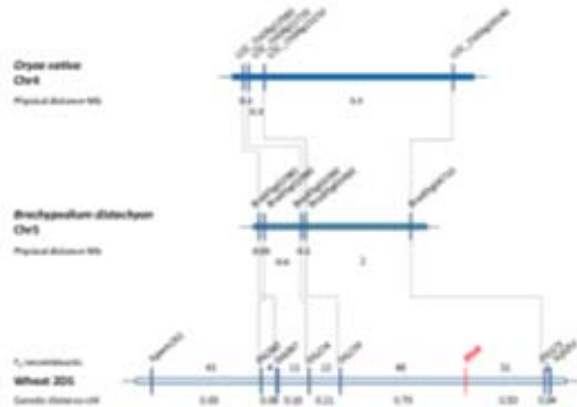
Dendrogram of 512 accessions genotyped by 33 markers.
All countries represented

Watkins Core Collections

- Two independently derived diversity core collections formed
- 120 and 100 lines using differing systems to develop dendrograms
- All being grown at the JIC in 6m beds
2011/12

Direction for Allele mining in Watkins Material

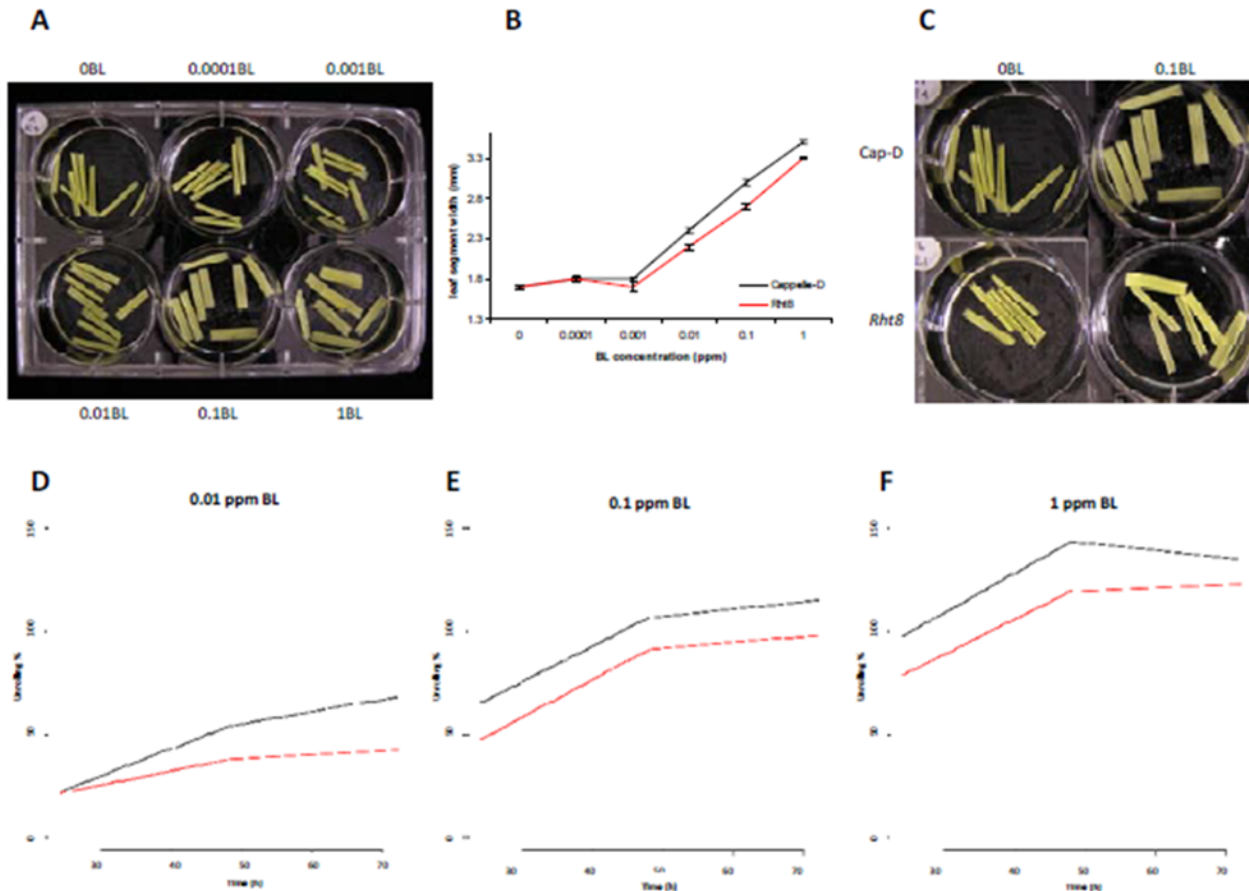
RHt 8 region in the short arm of 2D discovered using predictive comparison with Rice (Gasperini PhD)



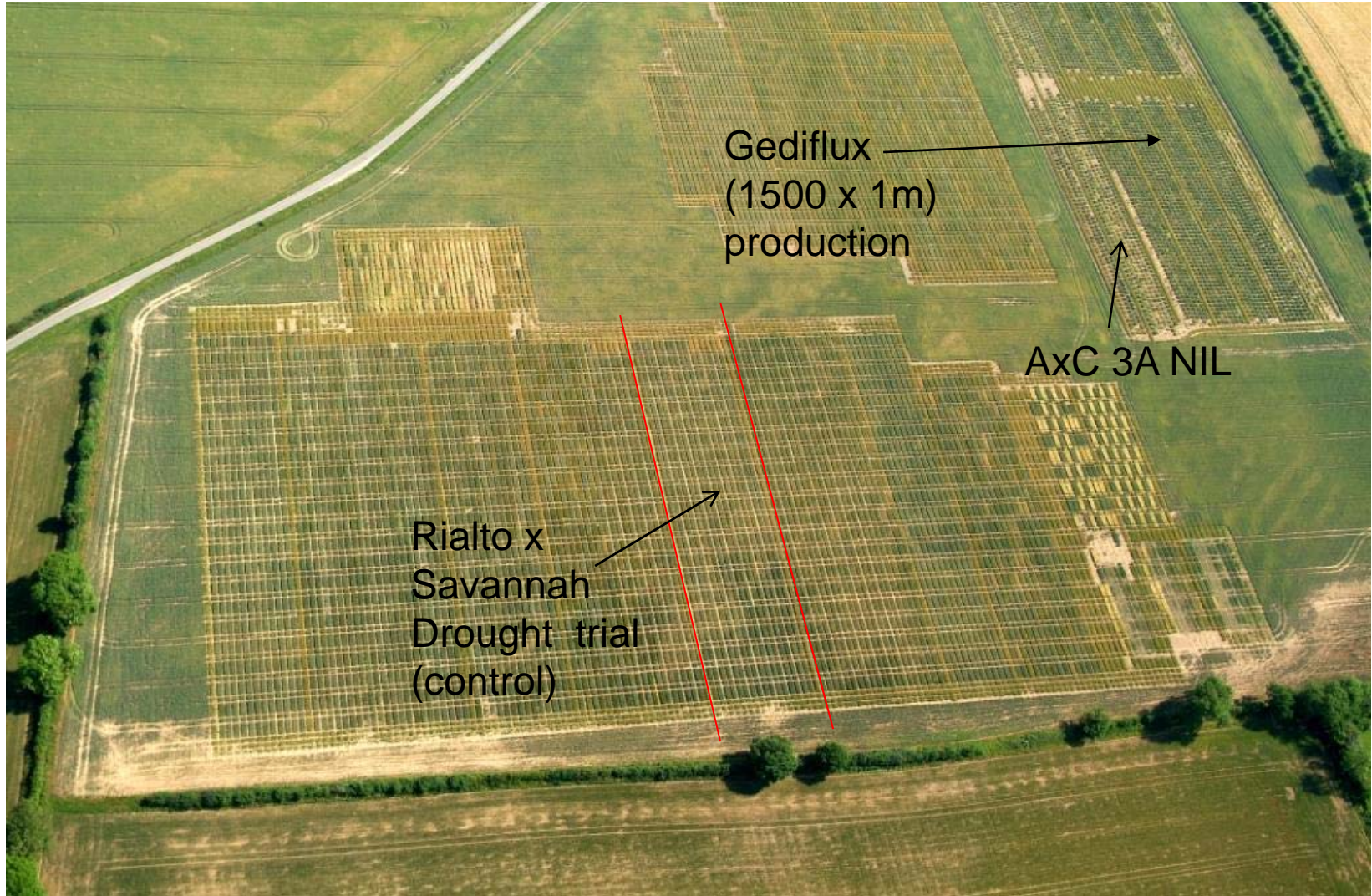
Brassinosteroid screen

- Growth hormone connected specifically with Rht8
- Use of the leaf curling action associated with this hormone to screen Watkins material

Brassinosteroid Screening



WGIN - JIC Field Trials 2011



Gediflux Data Collection

- Good coverage of data from flowering and height.
- Combinable plots for the first time resulting in stocks of around 3kg for all
- Sample tillers taken and currently being assessed for internode length and grain / ear characteristics

Gediflux Genotyping

- Originally planned to use DArT technology
- Direction now planned for Kaspar platform
- Griffiths group has purchased platform technology and installation is planned for later this month

Drought Tolerance

- Paragon x Garcia F2 sown
- Population size in excess of 350
- Unusual germination delay

Objective 8 – Nitrogen update

M J Hawkesford

WGIN Management Meeting

11th November 2011

Diversity trial history

Trial	Year	Varieties (core of 9)	N-levels	kg N/ha
1	2004	32	4	0,50,200,350
2	2005	20	2	0,200
3	2006	24	3	0,100,200
4	2007	24	4	0,100,200,350
5	2008	24	4	0,100,200,350
6	2009	24 (include 6 x A x Cs)	4	0,100,200,350
7	2010	25 (include 6 x A x Cs)	4	0,100,200,350
8	2011	25 (include 4 x A x Cs)	4	0,100,200,350
9	2012	25 (include WUE/take-all lines)	4	0,100,200,350
10	2013	25 (include WUE/take-all lines)	4	0,100,200,350

2012 varieties list (part 1)

Wheat varieties for WGIN-NUE 2010/11

W=WGIN data, D=desk study

Variety	Source	Code	Nabim	Rationale	inclusion in trial requested by	Previous years of trials (harvest year)
1. Avalon	Av	AV	1	WGIN DH parent; Low NupE & NutE (D), High TAB	PB, RG, MJH	05-10
2. Cadenza	Ca	CA	2	WGIN DH parent; Best NupE (W) , Low TAB	PB, RG, MJH	04-10
3. Chablis NEW 09/10	KWS		2	SPRING variety (previous grown in 2004 trial) as very N-MH responsive variety		only in 04 and 10
4. Claire NEW 2005	Nick	CL	3	Biggest area on RL; WGIN DH parent; Good second wheat	PB,PS	05-10
5. Conqueror	KWS	CN	4	New Grp 4, very high yielding	MH	new
6. Cordiale NEW 2006	KWS	CO	2	Good second wheat. BBSRC Quality project	RG	06-10
7. Crusoe NEW 10/11	Nick	CR	2	Carries dicoccoides. Shows the 'stay green' character		
8. Gallant NEW 09/10	Syn	GA	1	new claimed high yield and high protein type	MH	
9. Hereford	Syn /SU	HF	4	Feed (not on RL), high yield, brown rust susceptible, KHK/RG possible low take-all build-up (lowTAB)		new
10. Hereward	RAGT	HE	1	Best protein on RL; benchmark bread variety. BBSRC Quality project	PB,PS	04-10
11. Istabraq NEW 2005	Nick	IS	4	Best yield on RL; Distilling cultivar; In LINK 'GREENgrain'; Good second wheat. BBSRC Quality project. WUE trial		05-10
12. Malacca	KWS	MA	1	Biggest Group 1 area; DH choice; Low NupE, high NutE (W). BBSRC Quality project		04-10
13. Marksman	RAGT	MK	2	new for 2009, PRS request for BBSRC Quality project		only 09 and 10

2012 varieties list (part 2)

W=WGIN data, D=desk study

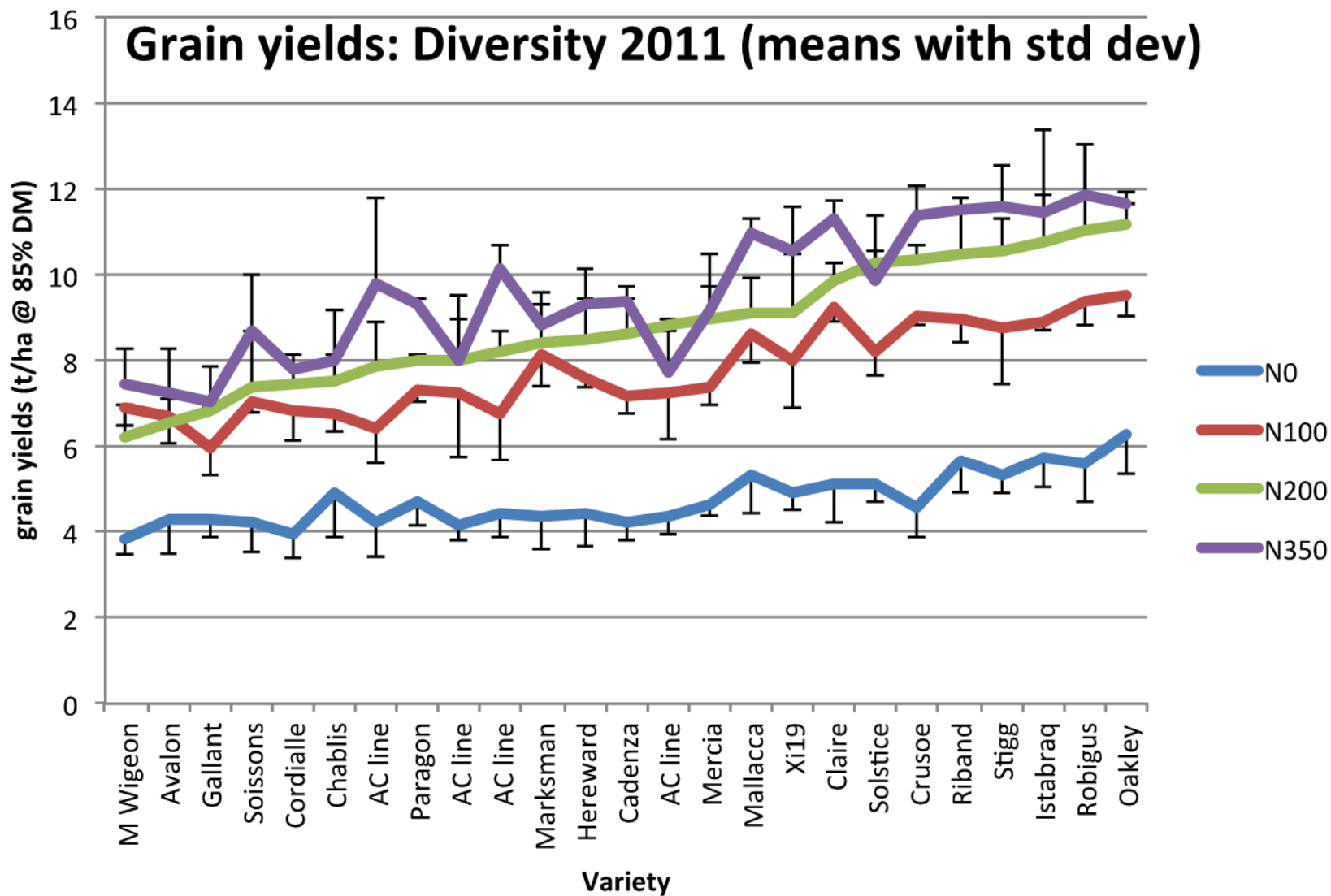
Variety	Source	Code	Nabim	Rationale	inclusion in trial requested by	Previous years of trials (harvest year)
14. Maris Widgeon		MW	1	Tall (rht), old cultivar	PB, AM	04-10
15. Mercia		ME	1	Low NupE & NutE (desk); Low Canopy N requirement; In IGF micro-array. WUE trial . RHT series	RG	04 and 06-10
16. Paragon	RAGT	PA	1	Spring variety; WGIN mutagenesis population; High NupE (W)	PB	04-10
17. Riband	RAGT	RI	3	WGIN DH parent; Distilling cultivar; In LINK 'GREENgrain'; High NutERG (W)		04-10
18. Robigus NEW 2005	KWS	RO	3	Best Group 3 yield; Best NUE, high NupE & NutE (D); Good second wheat. WUE trial	PB, AM	05-10
19. Stigg NEW 10/11	Nick	ST	?4	Carries dicoccoides. High disease resistance. Shows the 'stay green' character		
20 Soissons	Elsoms	SS	2	WGIN DH parent; Early maturing; High NupE, low NutE (W)	PB, RG, AM	04-10
21. Solstice	Nick	SL	2	Biggest Group 2 area; DH choice; Worst NupE (W)	RG	04-10
22. Xi19	Nick	XI	1	Best Group 1 yield; High NUE, NupE, NutE (D); Low NupE (W). BBSRC Quality project. WUE trial	PB, PS	04-10
23. Zebedee	LIM	ZE	3	High WUE, grp 3	JFoulkes	new
24. AxC line 127		D5		new in 2009 - good early export from leaves	MJH	09/10/
25. AxC line 82		D6		new in 2009 - slow early export from leaves	MJH	09/10/

Removed from trial: 2 A x C lines and Oakley

Diversity Trial 2011

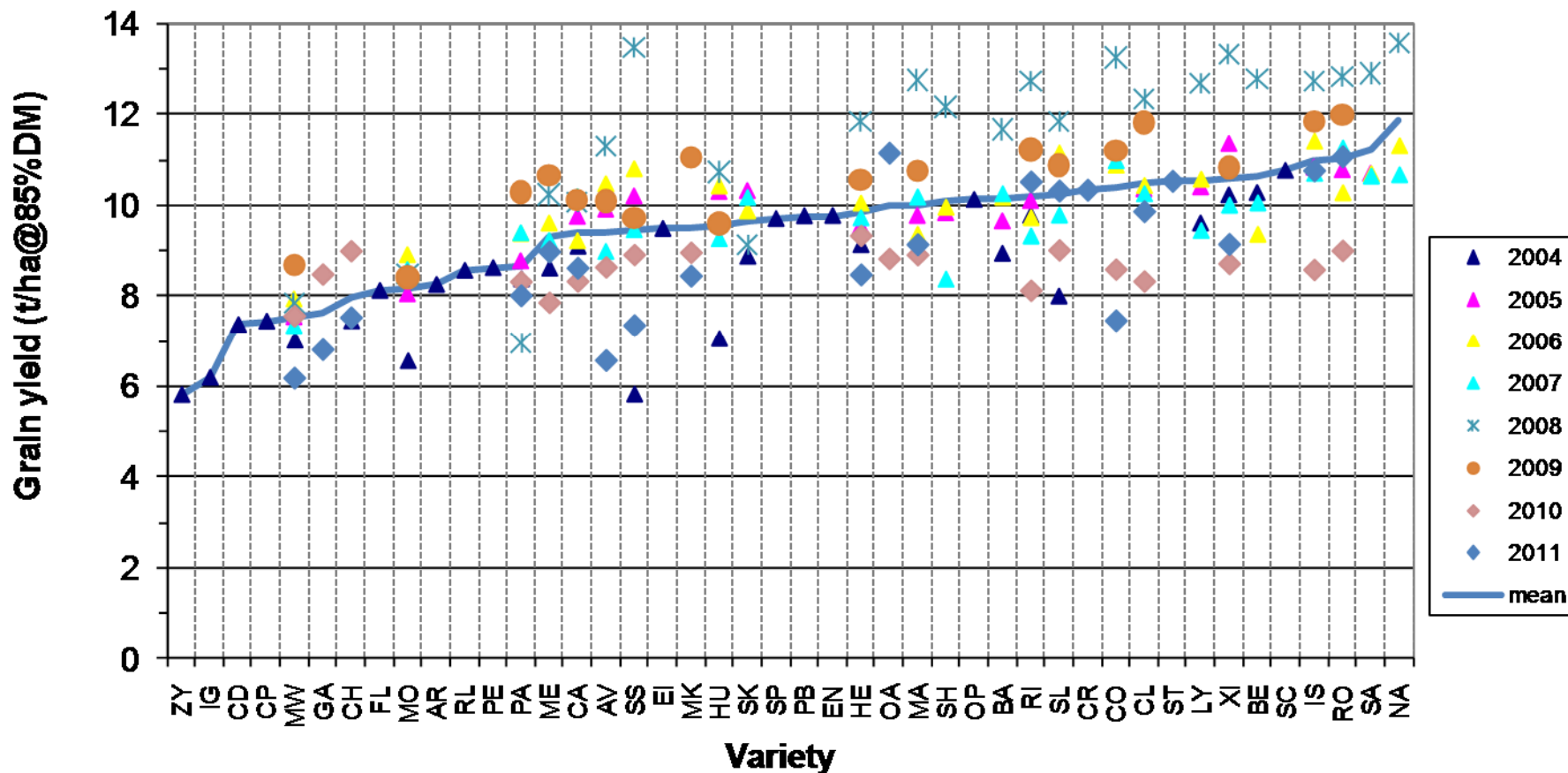


Grain yields: Diversity 2011 (means with std dev)



Rothamsted WGIN-N200

Combine Grain Yield (2004-11)



Avalon x Cadenza Trials

Trial	Harvest year	Sites	Notes	kg N/ha
1	2007	Blackhorse		200
2, 3	2008	Bones and Woburn	Woburn trial split	Both 100
4	2009	Fosters/Summardells	Split over 2 fields (wet)	100
5	2010	Blackhorse	(very dry)	200
6	2011	Great Harpenden	(very dry)	200
7*	2012	Bones		Tbc: 100?
8*	2013?	?		200?

*= not WGIN funded

Avalon x Cadenza 2011



Forward plans....

- 2 years Diversity –varieties now fixed
- NUE/RUE/Take-all interactions to be evaluated
- Addition field trial(s) for Avalon x Cadenza
- Greenhouse N uptake trials for Avalon x Cadenza (one completed)





Thanks



- RRes Farm staff
- Group and field team: Peter Barraclough, Peter Buchner, Andrew Riche, Yongfan Wan, Jonathan Howarth, Mark Durenkamp, Saroj Parmar, Janina Jones, Dan Godfrey, Emmanuelle Cabannes, Adinda Derkx, Fumie Shinmachi, Caihong Bai + many summer students



Drought tolerance

WGIN-2 SG meeting
KWS UK Ltd 11 November 2011



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 a diverse germplasm (cvs, advanced lines) for future association genetics studies. (Yrs 4 -5)**

WGIN 2 (9.1 Trait Identification)

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

* Common with NUE trial

Chemical analysis In WGIN drought tolerance trial in 2009/10 & 2010/11

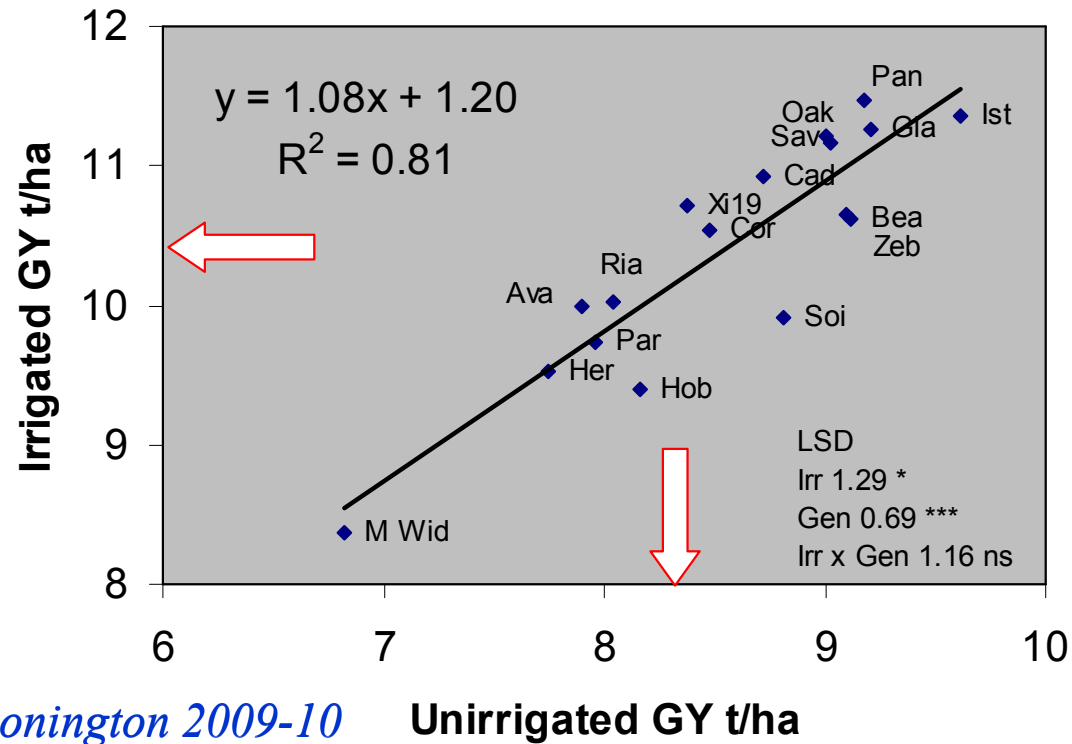
Samples (per year) on 18 cultivars x 2 Irr trts x 3 reps
(=108 plots)

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

Core funding

Sub-contract funding

Grain yield responses to irrigation



Sutton Bonington 2009-10

Unirrigated GY t/ha

	Rainfall (mm)	
	2010 LTM 75-09	
Jan	33	54.1
Feb	41.6	43.4
Mar	36	45.7
Apr	24	44.4
May	18.2	45.6
Jun	69.2	58.7
Jul	42.6	49.8

Glasgow Irrigated vs Unirrigated 19 July



WGIN 2 (Drought tolerance, 9.1)

2010-11 expt

Split plot design (3 reps): plot size 1.6 x 12 m

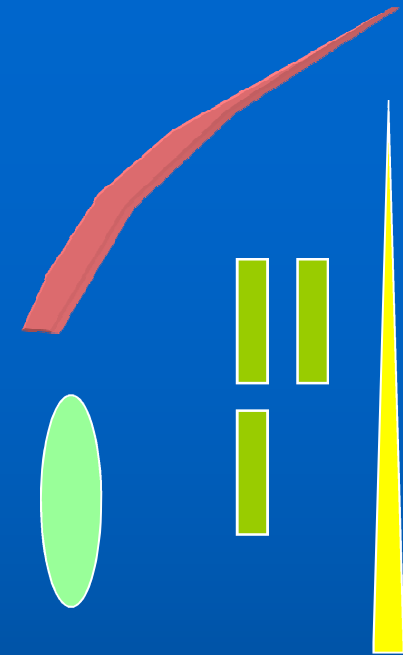
Main plot: Fully irrigated (trickle irrigation)
Unirrigated

Split plot (variety):

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

Measurements

- Combine grain yield, yield components
- DM & partitioning at GS31, GS61, harvest
- % stem WSC at GS61+10d
- Leaf senescence kinetics for L1, L2 and L3.
- Stomatal conductance/photosynthetic rate using Licor 6400 (unirrigated)
- Canopy temperature
- Water use ~ gravimetric analysis of soil cores (unirrigated, 18 varieties)
- ^{13}C Δ grain ~ leaf WUE
- ^{18}O Δ flag leaf ~ leaf transpiration



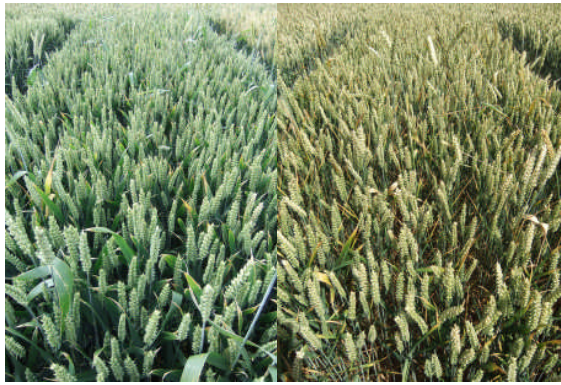
Leaf gas exchange



Canopy temp.



Drought effects 11 July 2011



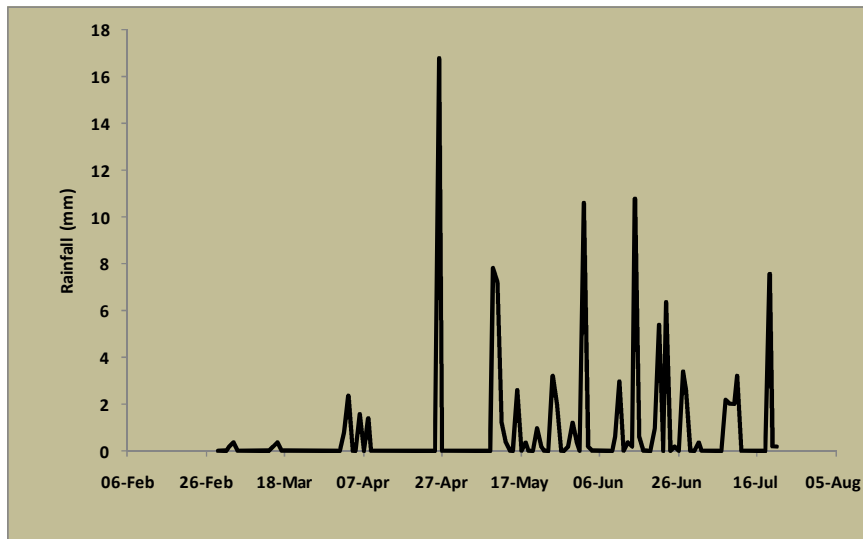
Panorama



Cadenza

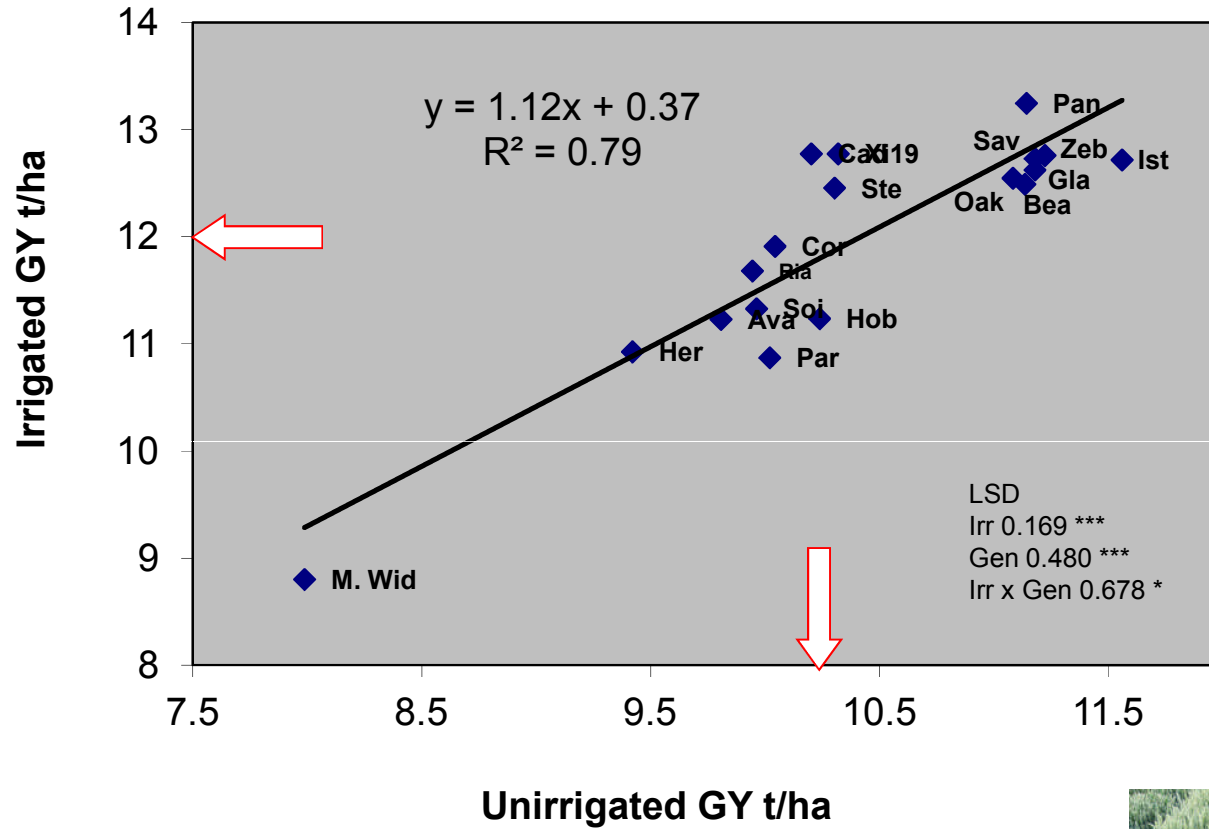


Istabraq



	Rainfal (mm)	LTM
	2011	75-09
March	1.2	54.1
April	23	43.4
May	27.8	45.7
June	45.4	45.6
July	17.8	49.8

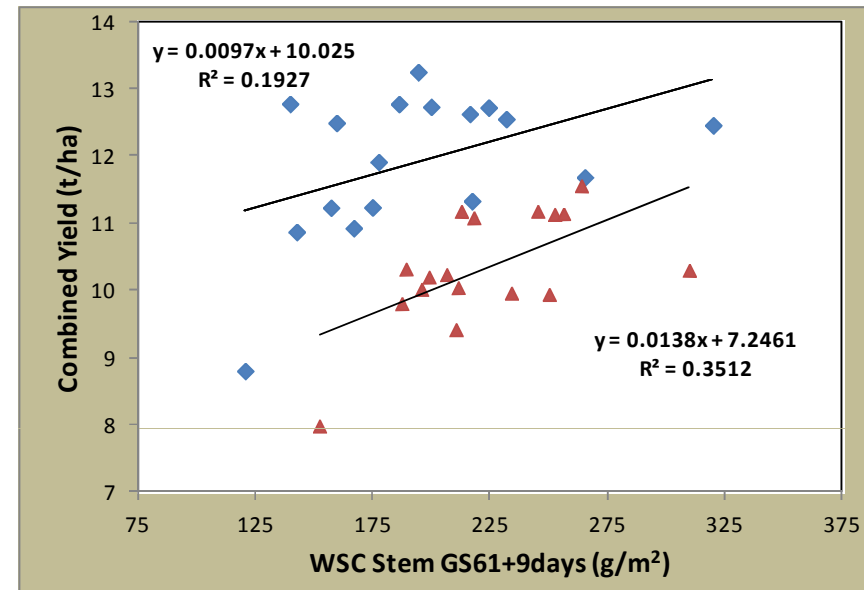
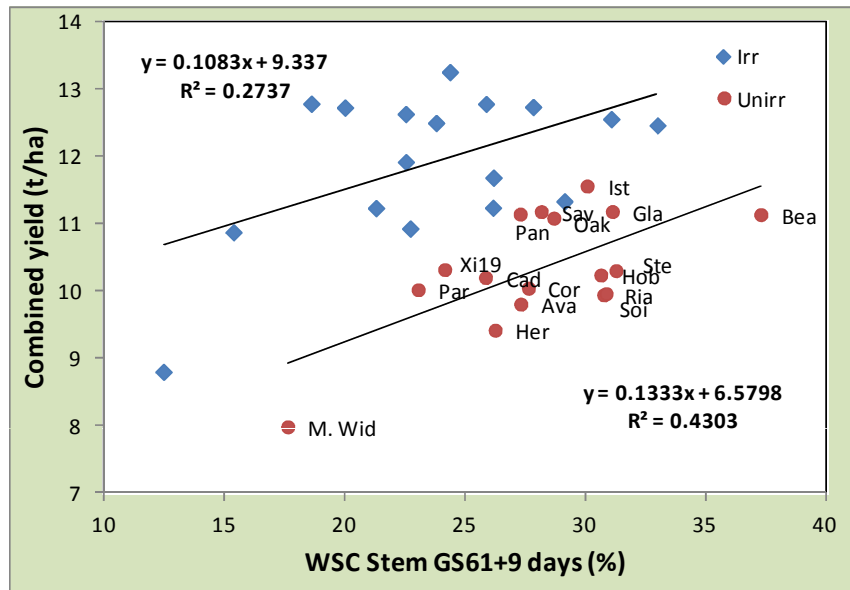
Grain yield responses to irrigation



Sutton Bonington 2010-11



Grain yield versus stem WSC reserves



Sutton Bonington 2010-11

WGIN 2 (9.2 QTL Detection)

2010-11 and 2011-12 expts

- Rialto x Savannah DH population for phenotyping for yield physiological traits (94 lines and 2 parents)
- 2 sites: Nottingham - irrigated & unirrigated; JIC - unirrigated
- Target traits
 - ^{13}C Δ grain
 - senescence kinetics
 - canopy temperature
 - 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



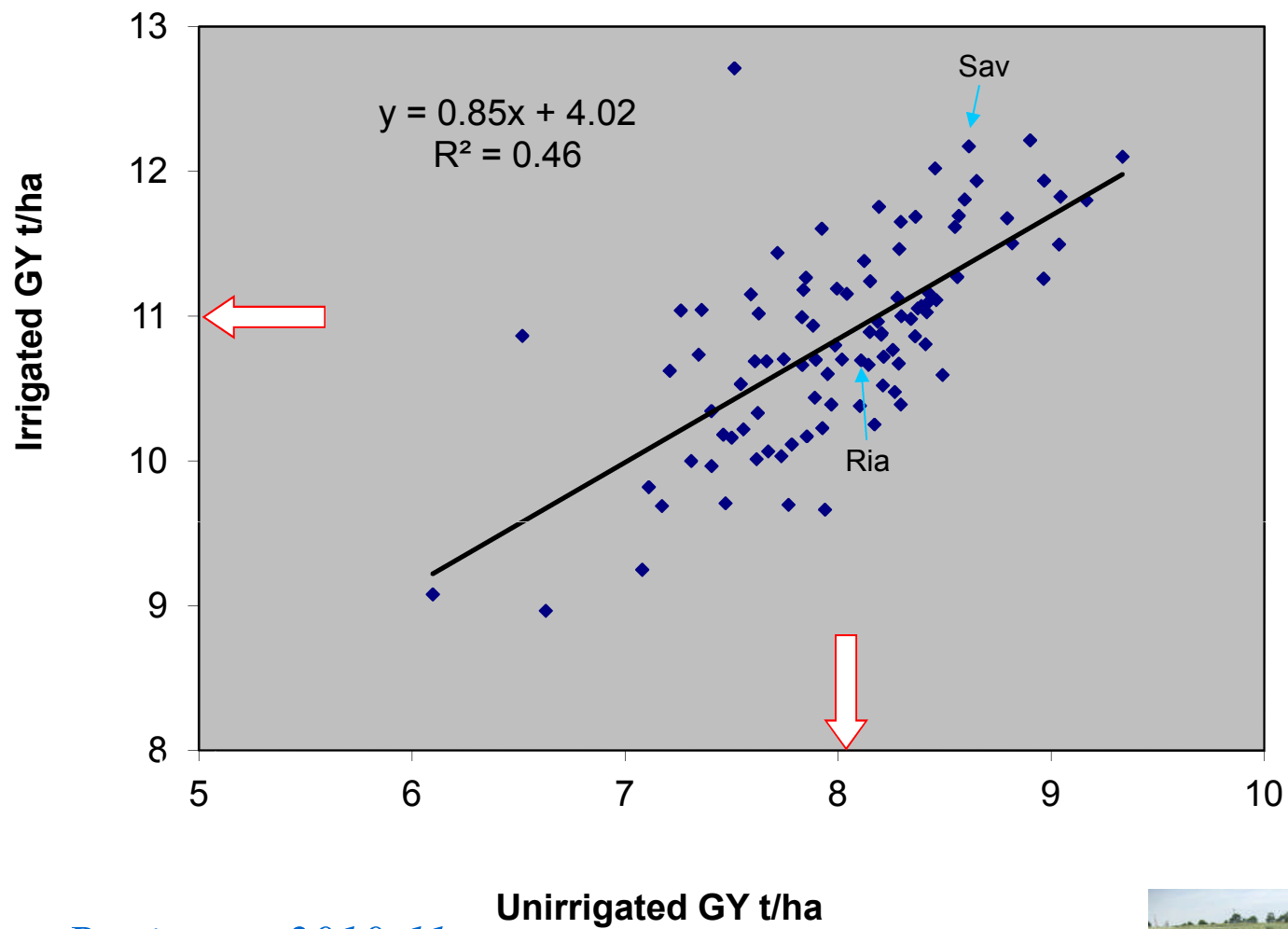
L2



L39



L47



Sutton Bonington 2010-11



WGIN 2 (9.3 Develop new DH pop)

	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.

- Candidate F1(s) made at JIC crosses to maize informed by data analysis from LK0986 project
- F1 crossed with maize
- 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

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.

Acknowledgments:

Nottingham

J. DeSilva

J. Alcock

M. Tovey

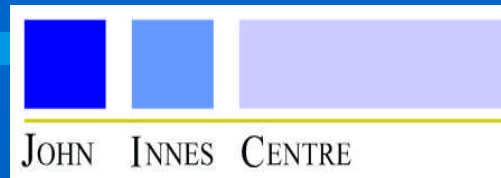


JIC

J. Snape

S. Griffiths

S. Orford



Resistance to Take-all Resistance to Septoria

Richard Gutteridge
Kostya Kanyuka
Kim Hammond-Kosack



WGIN MM@RRes
11th Nov 2011

Take-all disease of wheat

- *Gaeumannomyces graminis*
var. *tritici* (**Ggt**)
 - ascomycete soil borne fungus
 - related to rice blast fungus
Magnaporthe oryzae (previously
M. grisea)



Severely infected take-all plant

Take-all disease – soil-borne fungus

In 1st wheats - no disease problem



A major problem
for 2nd / 3rd wheat crops

2nd wheat syndrome



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

The risk of take-all is largely dependent on the amount of fungal
inoculum in the soil at the time of sowing

Objective 10 - Talk Outline

- **Hexaploid wheat – Watkins / Gediflux collections**
Diploid wheat
 - field evaluation to identify potentially resistance genotypes
- **Diploid wheat**
 - mapping populations
 - introgression to *T. aestivum*
- **Hexaploid wheat – inoculum build-up in 1st wheats – 2010**
 - results so far / current activities

Watkins Hexaploid wheat collection

Take-all assessments

- **2008 and 2009 field season – one plot / genotype – established from 45 seeds**
- **823 lines from the collection (both years – 740 lines)**



3rd wheat situation

Watkins / Gediflux Hexaploid wheat collections

Take-all assessments

3rd wheat situation

2008 and 2009 field season – one plot / genotype –
established from 45 seeds

823 lines Watkins + **60 lines** Gediflux collection

Method - Discarded all lines more susceptible than Hereward

2010 field season - one plot / genotype

504 Watkins lines and **36 Gediflux lines**

5 blocks of 8 controls + 20 Hereward control plots.

2011 field season - one plot / genotype

490 Watkins lines and **36 Gediflux lines**

Watkins / Gediflux Hexaploid wheat collections

Take-all assessments

3rd wheat situation

Field experiment 2012: one plot / genotype

104 Watkins lines, 36 Gediflux lines

5 blocks of 8 controls, 20 Hereward plots.

Sown October 2011.

Plus the most resistant 144 lines Watkins lines have gone into the wheat pre-breeding LoLa field trials - 5 reps / genotype

30% of Watkins lines (248 lines) remaining after 4 years of trialling

Protocol change

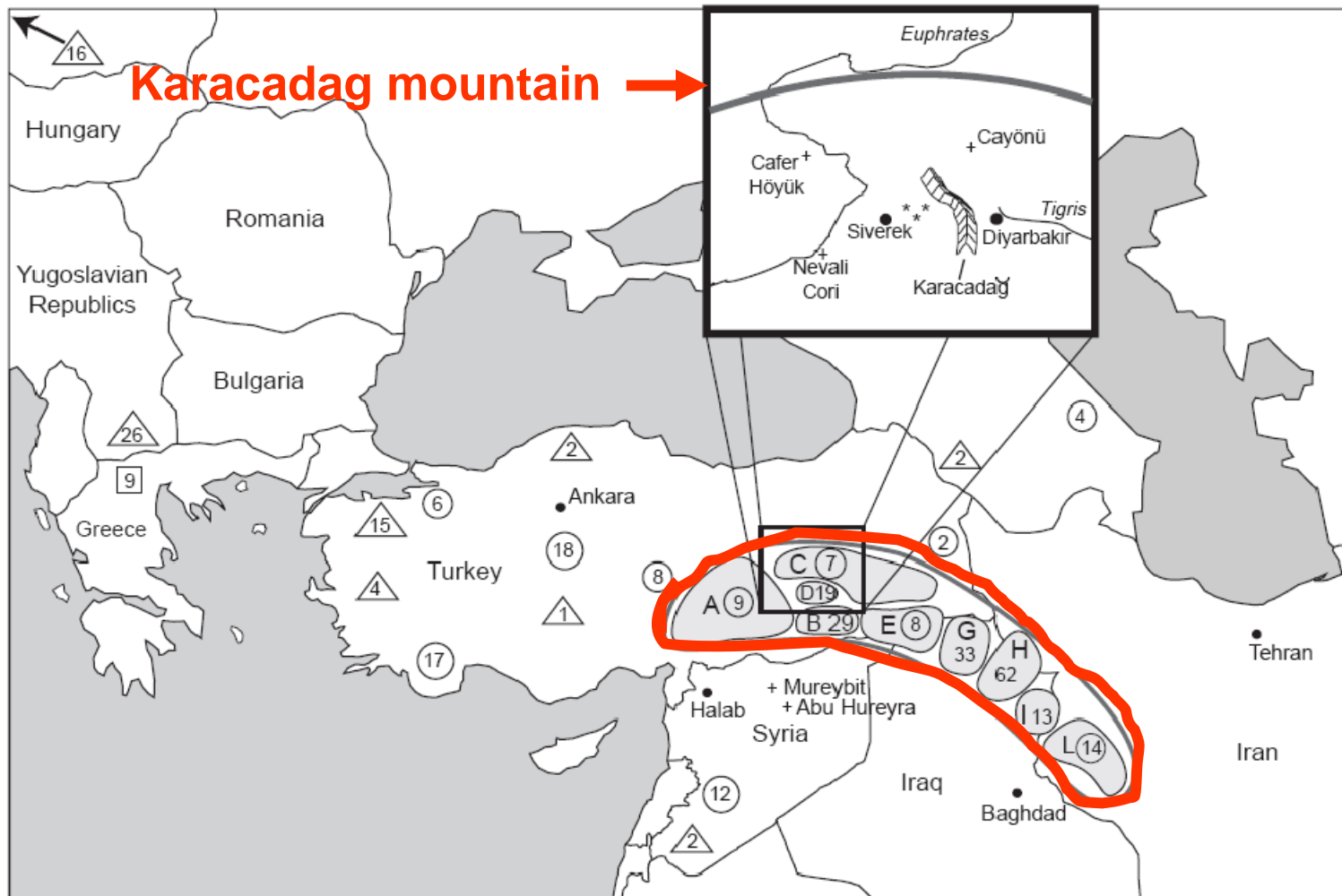
2011 - hand sowing 60 seeds per plot (3 rows x 50cm)

2012 - combined drilling 200 seeds (4 rows x 1m)

Objective 10 - Talk Outline

- Hexaploid wheat – Watkins / Gediflux collections
Diploid wheat
 - field evaluation to identify potentially resistance genotypes
- Diploid wheat – *T. monococcum*
 - mapping populations
 - introgression to *T. aestivum*
- Hexaploid wheat – inoculum build-up in 1st wheats – 2010
 - results so far / current activities

T. monococcum (AA genome) domesticated from *T. boeoticum*



Karacadağ mountain →

— Limits of Fertile Crescent

* Sampling of Karacadağ lines

+ Archeological site

A–L: areas of wild *T. m. boeoticum* sampling in the Fertile Crescent

○ *T. m. boeoticum*

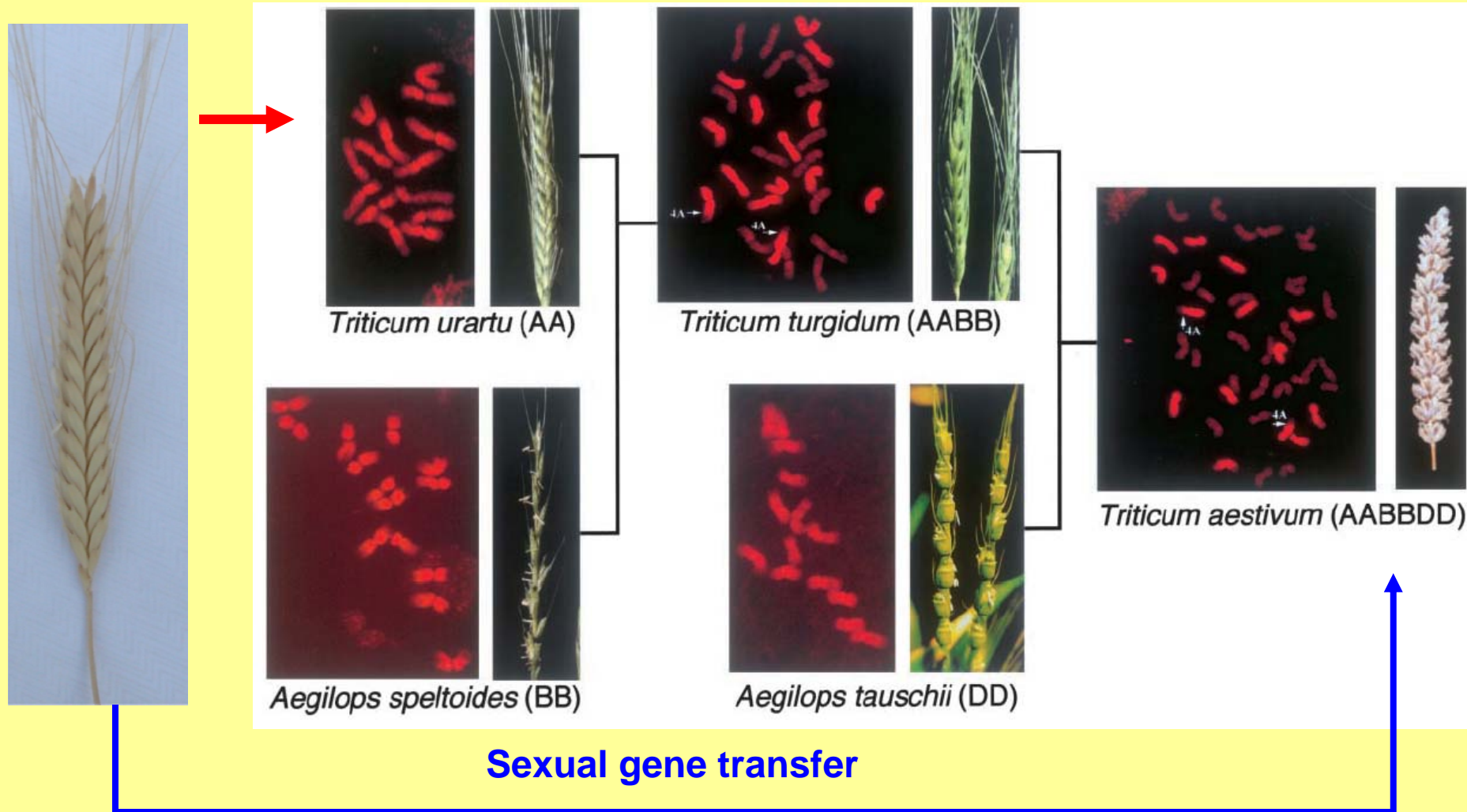
△ *T. m. monococcum*

□ *T. m. aegilopoides*

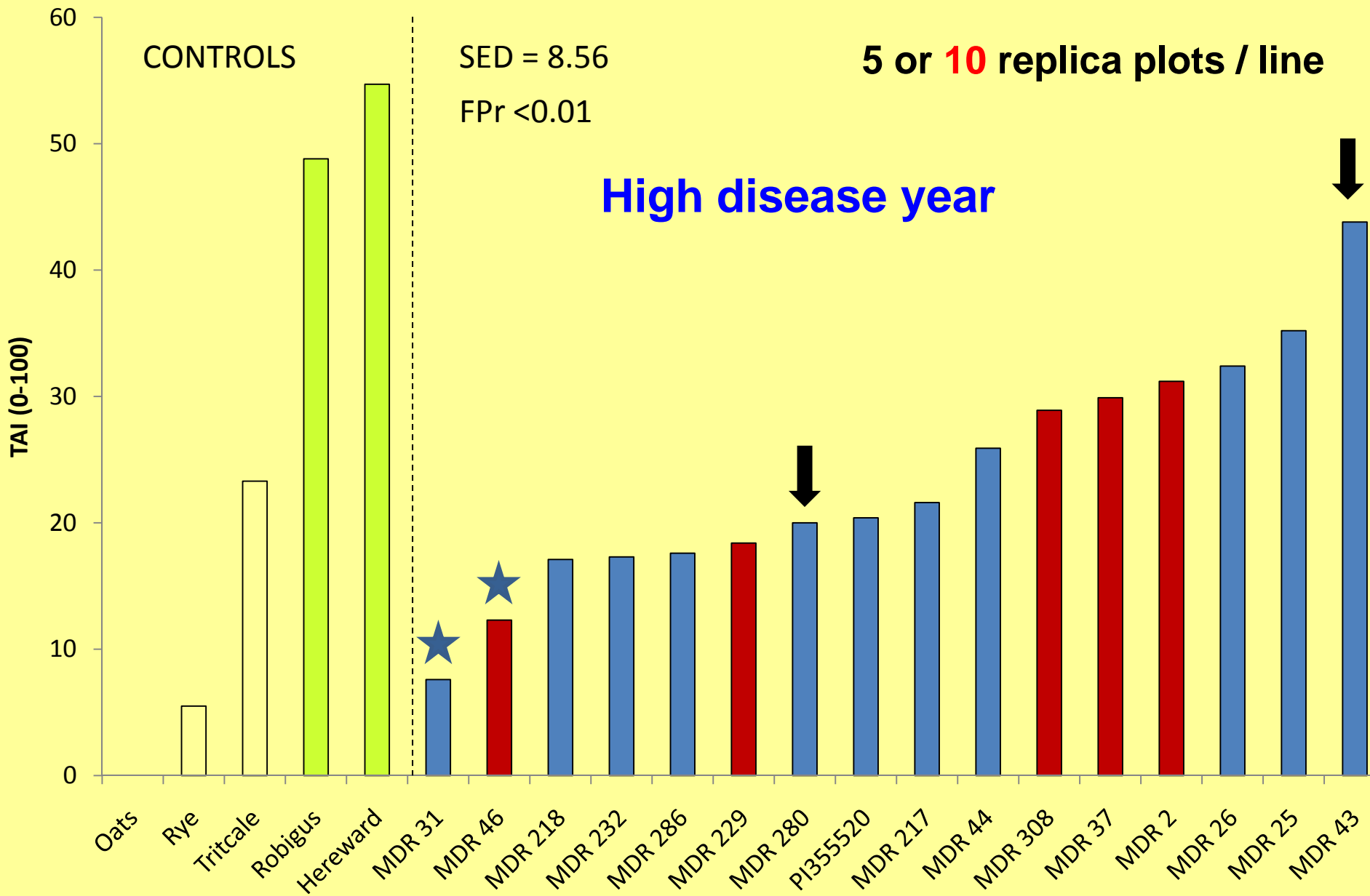
(with number of samples)

Origin of bread wheat

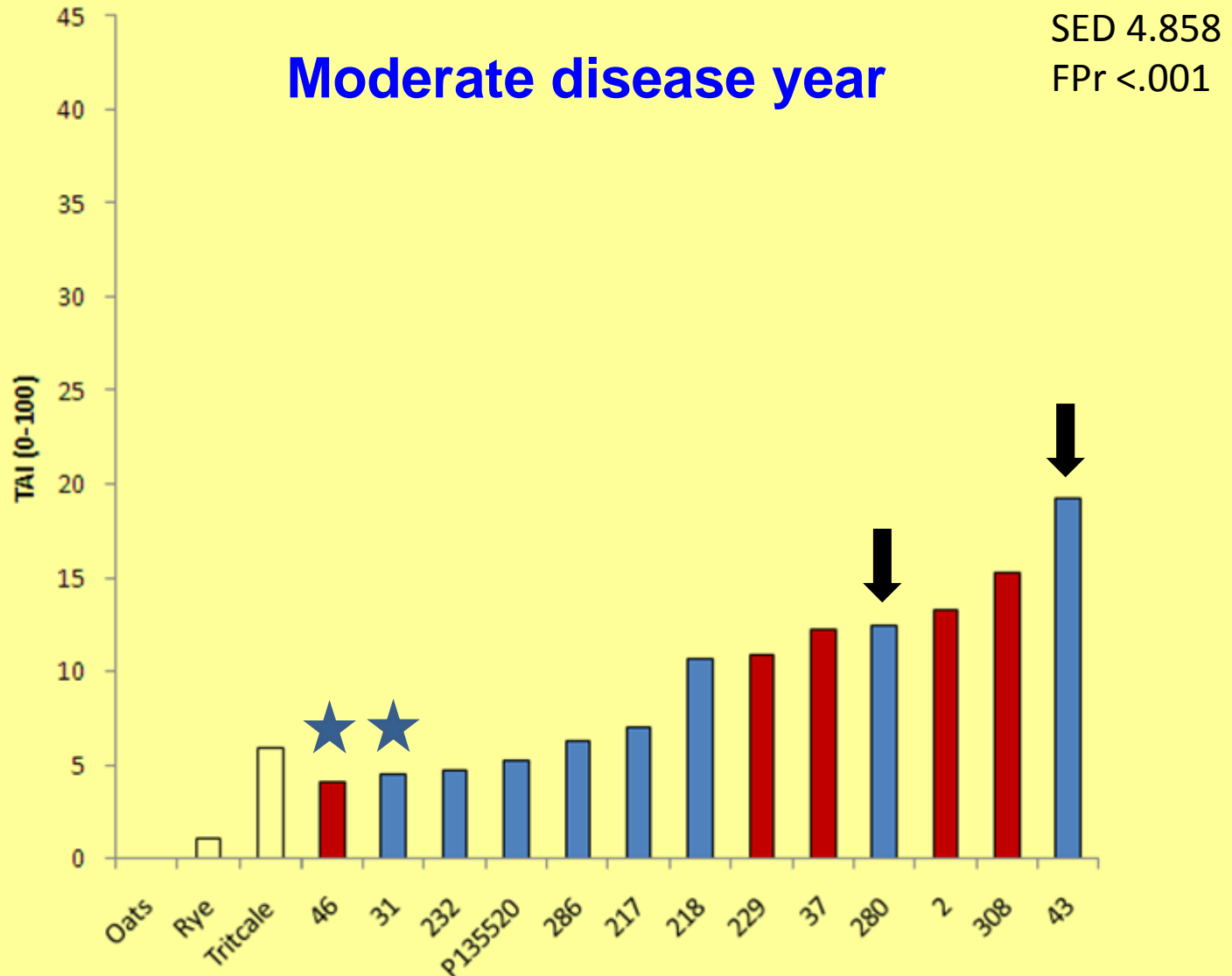
T. monococcum A^mA^m



Diploid wheat field experiment 2008

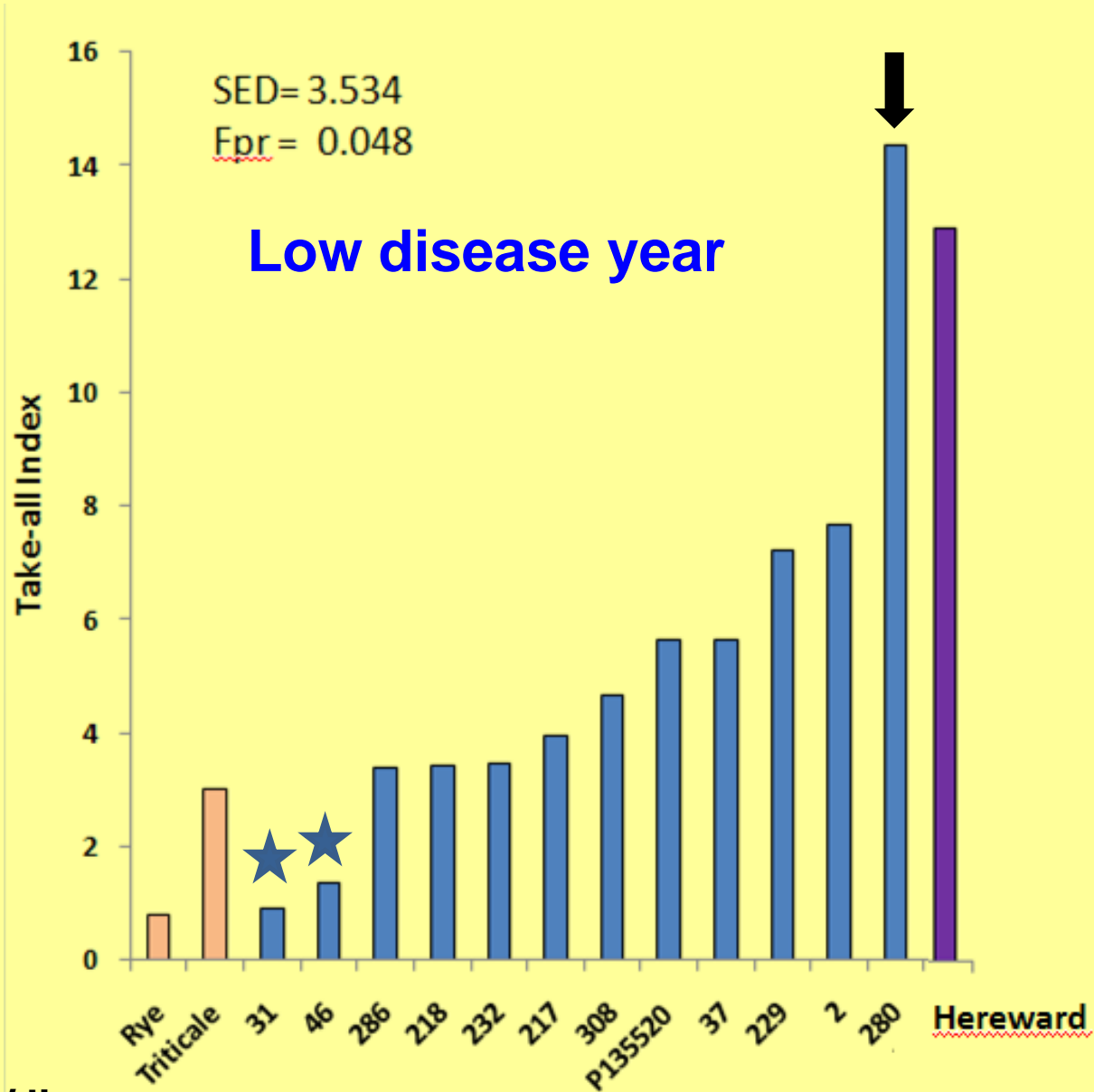


Diploid wheat field experiment **2010**

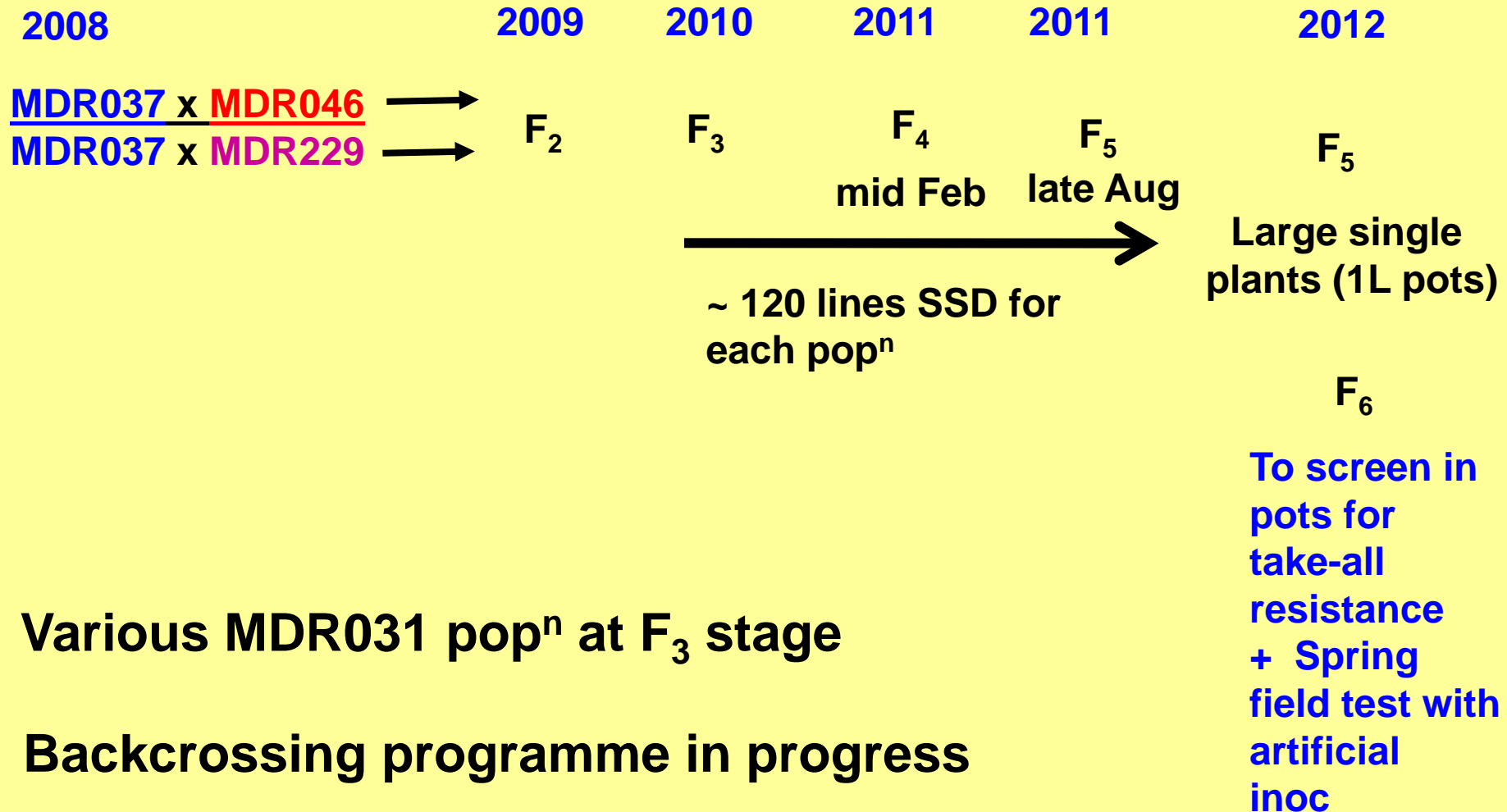


10 replica plots / line

Diploid wheat field experiment 2011



T. monococcum - Glasshouse crosses



T. monococcum* introgression to *T. aestivum

New strategy

1. Graham Moore (JIC)

New *T. aestivum* sources

Paragon	- <i>ph1</i> mutant	and wild-type
Chinese Spring	- <i>ph1</i> mutant	and wild-type

**Received 10 seed from JIC in July 2011
and are currently multiplying up the stock**

Objective 10 - Talk Outline

- Hexaploid wheat – Watkins / Gediflux collections
Diploid wheat

- field evaluation to identify potentially resistance genotypes

- Diploid wheat – *T. monococcum*
 - mapping populations
 - introgression to *T. aestivum*

- Hexaploid wheat – inoculum build-up in 1st wheats – 2010 / 2011

Results: no inoculum build up in 2010 due to the very dry winter / spring

2011 – more promising - 203 A x C DH , 3 rep exp – cores taken from all plots + entire trial over-sown with cv Oakley

Objective 11

T. monococcum – Introgression of the *TmStb1* locus conferring resistance to *Septoria tritici* blotch into hexaploid wheat

5 years of field assessment of resistance

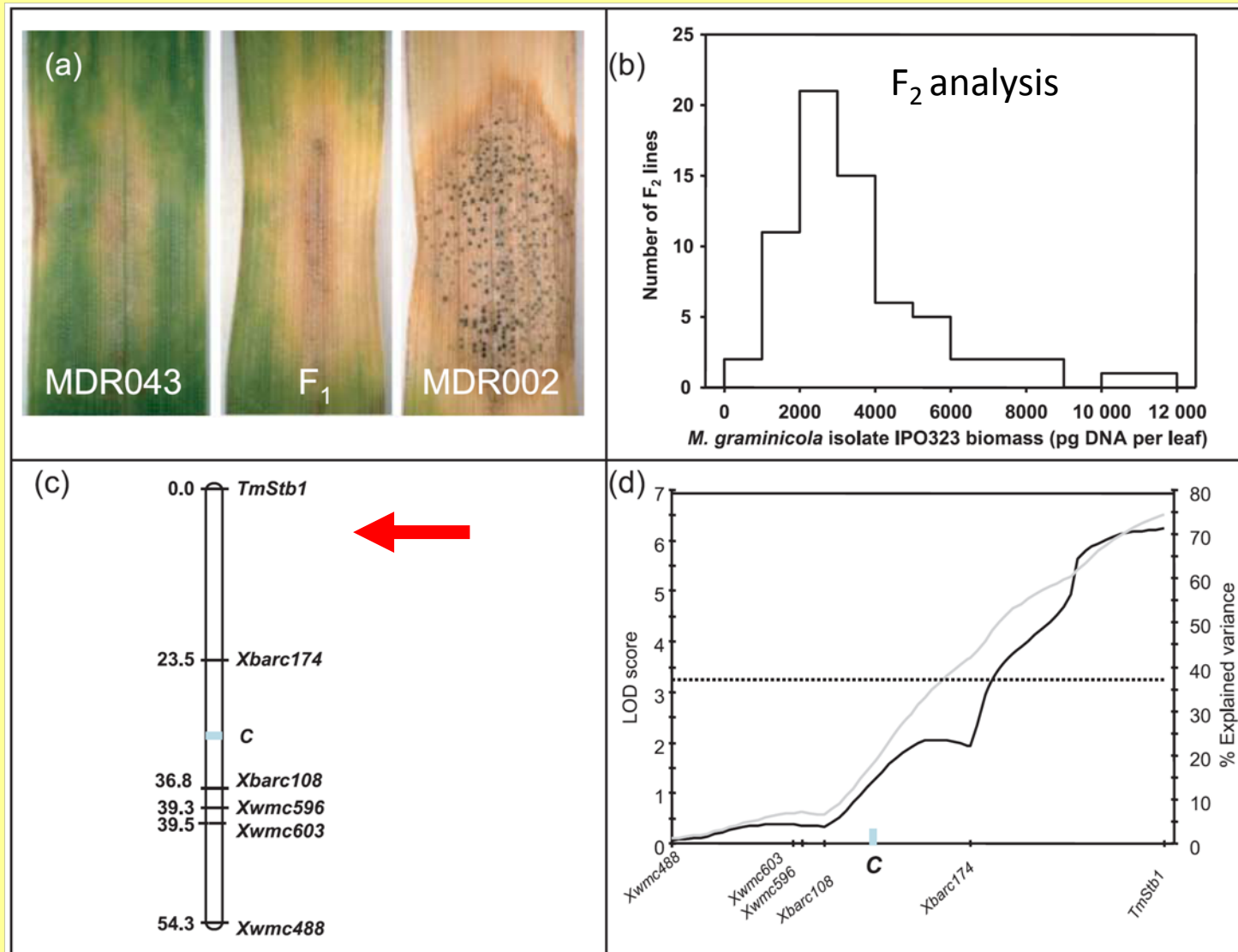
Hexaploid wheat



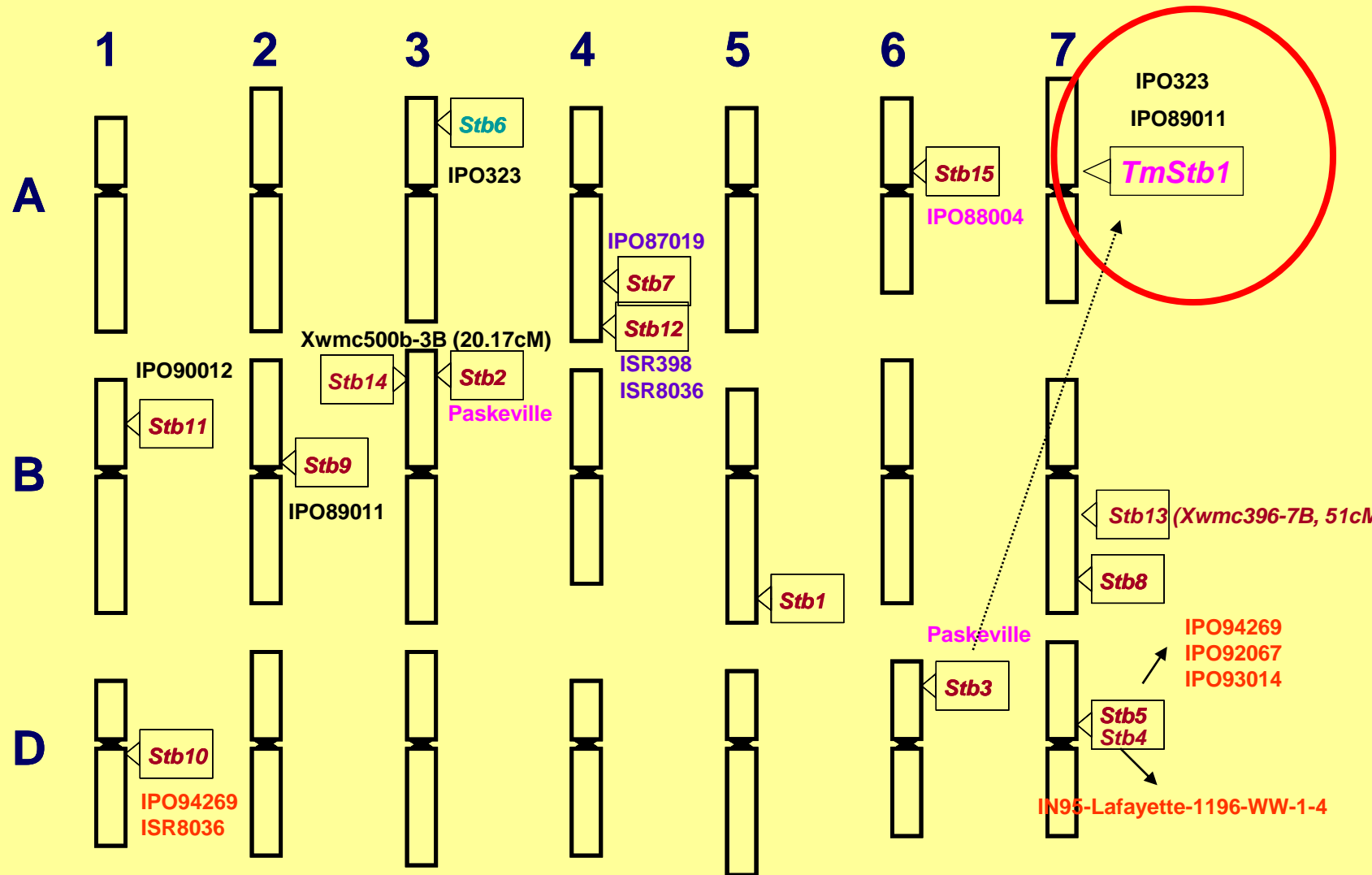
Triticum monococcum
- diploid wheat AA



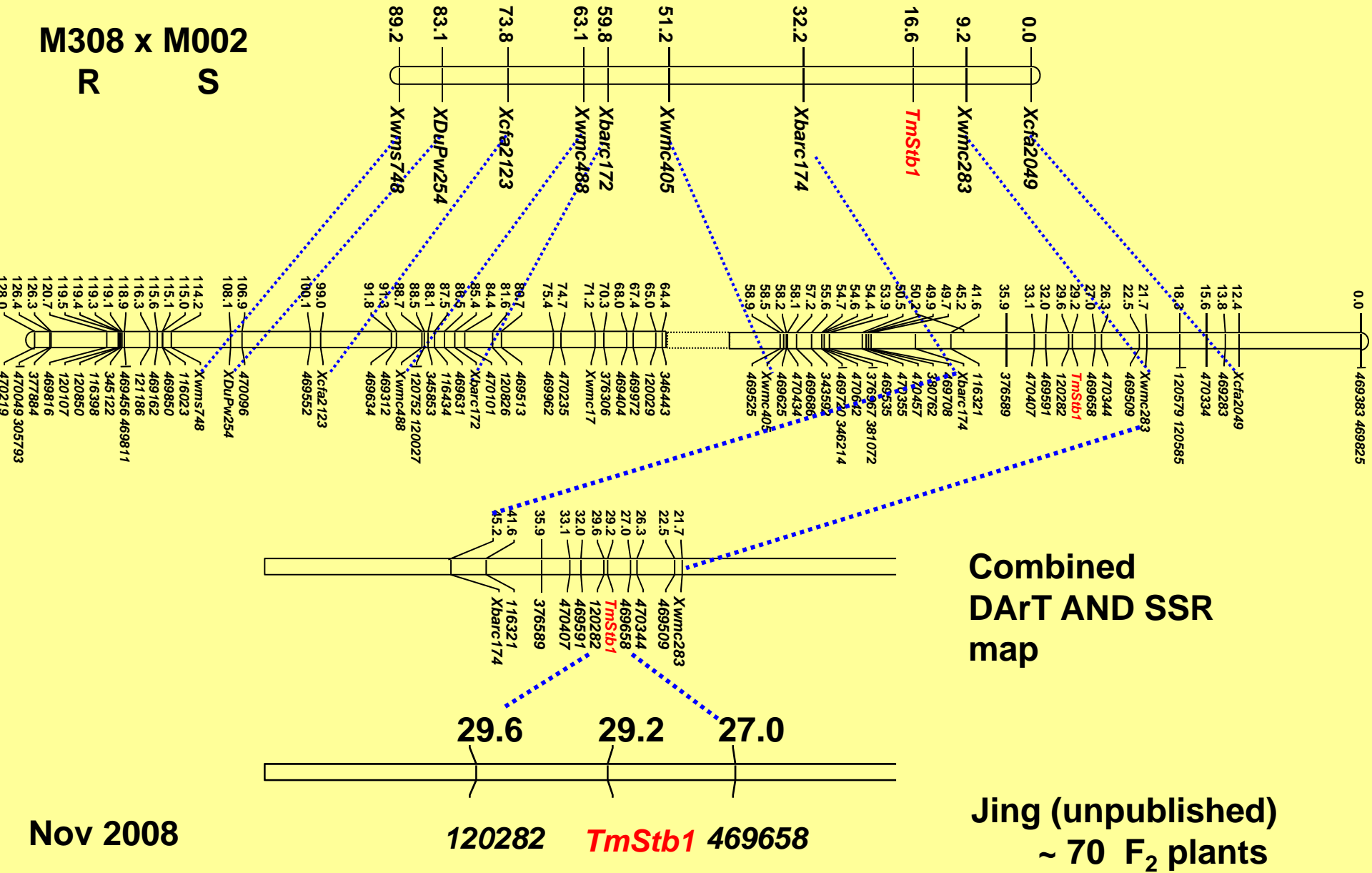
Resistance conferred the *TmStb1* locus on 7A^m



Wheat *Stb* genes for resistance to *Septoria tritici* blotch

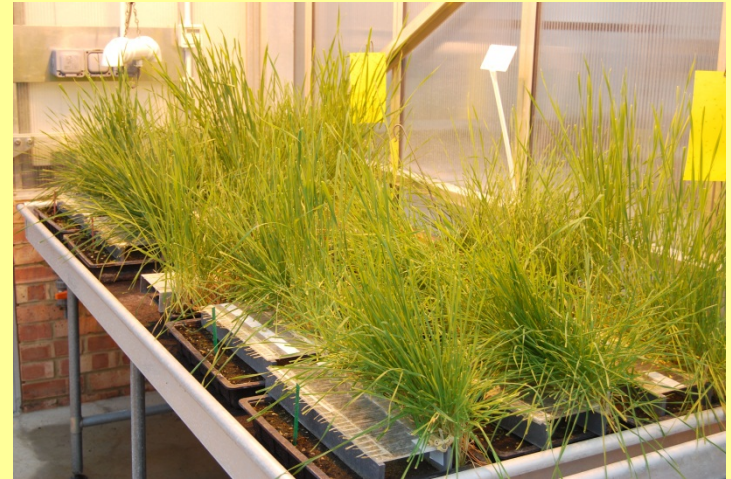


Fine-mapping of *TmStb1* locus on 7A^m

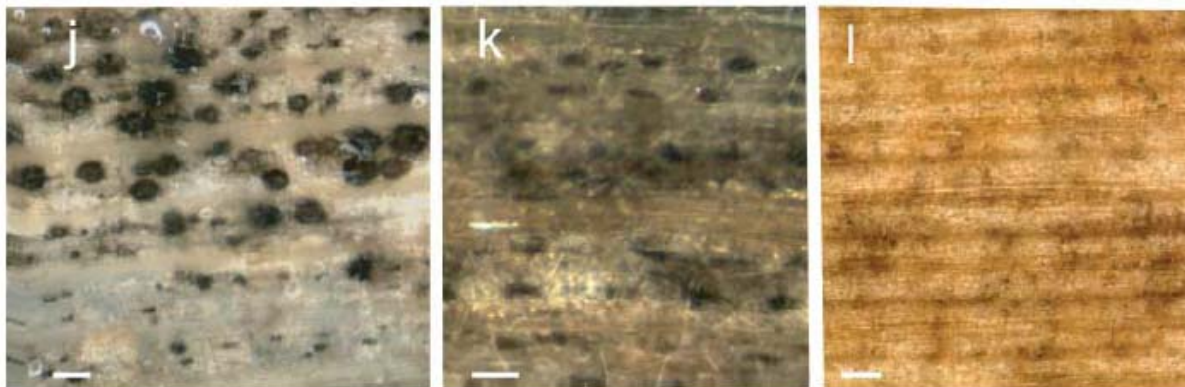
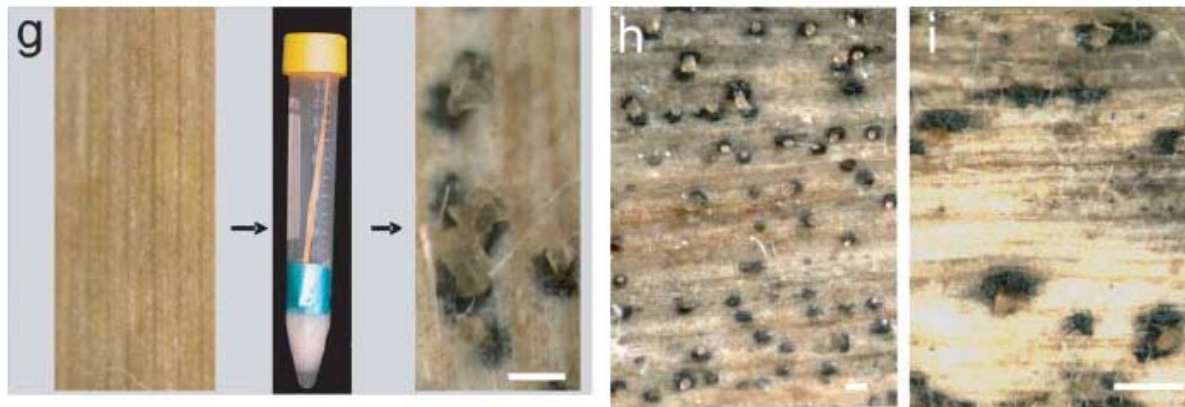
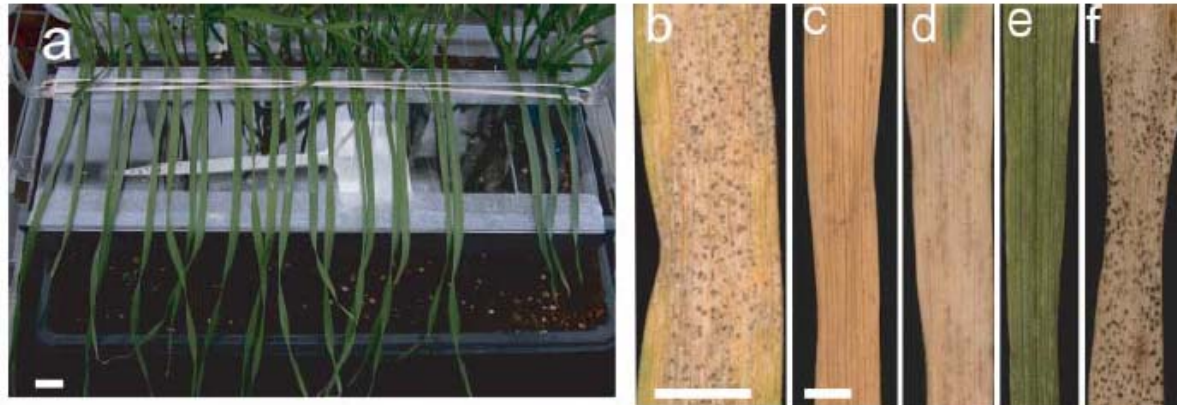


Screening *Triticum monococcum* MDR002 x MDR308 population for resistance to Septoria isolate IPO323

- Screened **411 F₂ plants** (2 leaves per plant).
- Selected **214 F₂ plants** for further study. All these were grown to maturity and the majority produced F₃ seed.
- Selected **106 F₃ families** for re-screening.
- So far screened **79 F₃ families**.
- So far confirmed 49 F₂s as resistant and 25 as susceptible.



Screening for Septoria resistance in *T. monococcum*



30 to 35 days
post
inoculation

Score pycnidia
development
3 – 4 days later

Jing et al. (2008)
New Phytologist

Predicted F₂ phenotypes

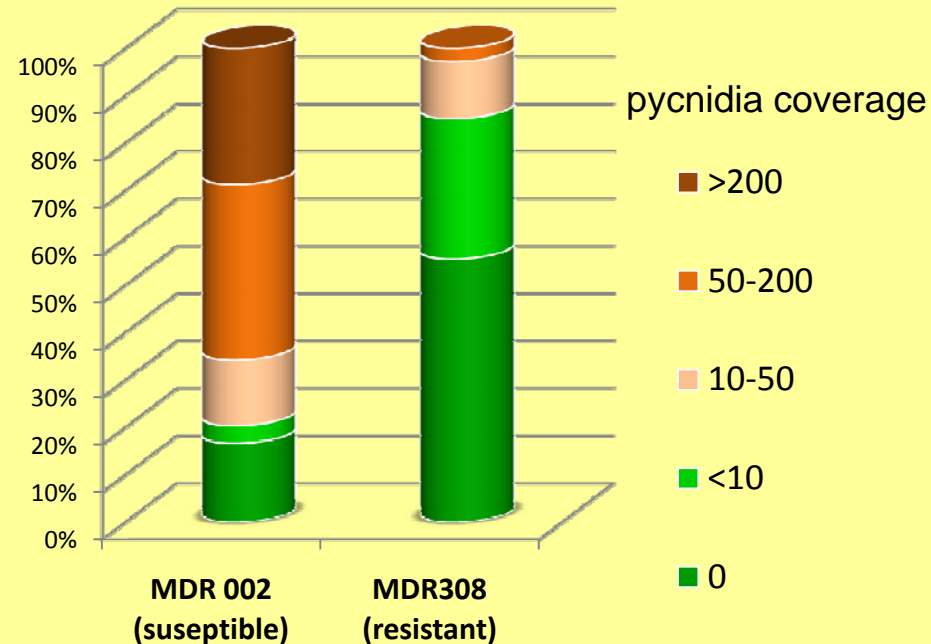
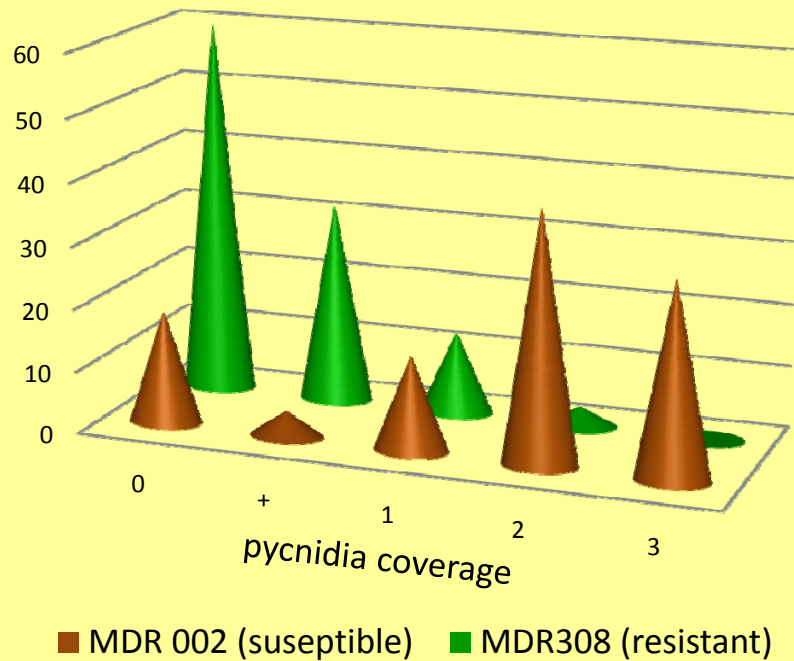
- **30 F₂s – R – possibly homozygous for *TmStb1***
- **19 F₂s – R – possibly heterozygous at the *TmStb1* locus**
- **25 F₂s – possible homozygous S**
- **5 F₂s – unscorable**

Note : we selected in the F₂ generation individual plants with the most easily scoreable R and S phenotypes, so this would skew the genetic ratio obtained in the F₃ generation

F₂ to F₃ phenotype relationships were good (but not a perfect match)

Difficulties encountered during *Tm* - *Stb* phenotyping

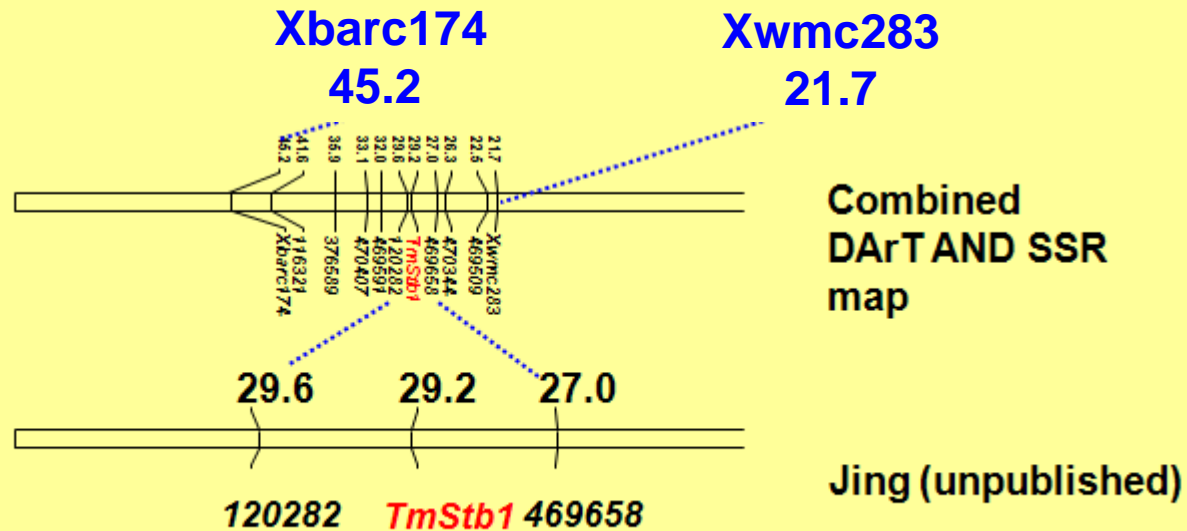
- **Resistant MDR308** and susceptible **MDR002** lines were included as controls in each resistance test
- In total screened 108 individual plants for each of the parental genotypes



Scored - Day 30 to Day 35 post high humidity treatment

Fine Mapping and Bulk Segregant Analysis

M308 x M002
R S



F_3 : 30 Resistant lines and 25 Susceptible lines

1. Assess each for presence / absence of flanking SSR markers
2. Identify closer markers – which type?
3. Make bulks of 10 lines x 2 per phenotype
4. Apply a NGS strategy
5. Define a very small segment for *Ta* introgression via *ph1* crossing



Many thanks to



RRes Farm staff

Richard Gutteridge

Vanessa McMillan (HGCA-BBSRC)

Kostya Kanyuka

Steve Freeman (WGIN casual)

Gail Canning (BBSRC-Pre-breeding LoLa)

Summer students 2011

- root sampling – seed harvest

Martha Jones (Pre-breeding LoLa)

David Franklin (Pre-breeding LoLa)

Leanne Freeman (WGIN)