UKCPVS Stakeholder meeting 2024

Tuesday 16th January 2024

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AHDB
NIAB
BBSRC bioscience for the future

A multi-locus strategy for durable yellow rust resistance in wheat, in the face of a rapidly changing pathogen landscape

DSV
Lantmännen Lantbruk
syngenta
Limagrain United Kingdom
R·A·G·T SEEDS
Sejet plant breeding
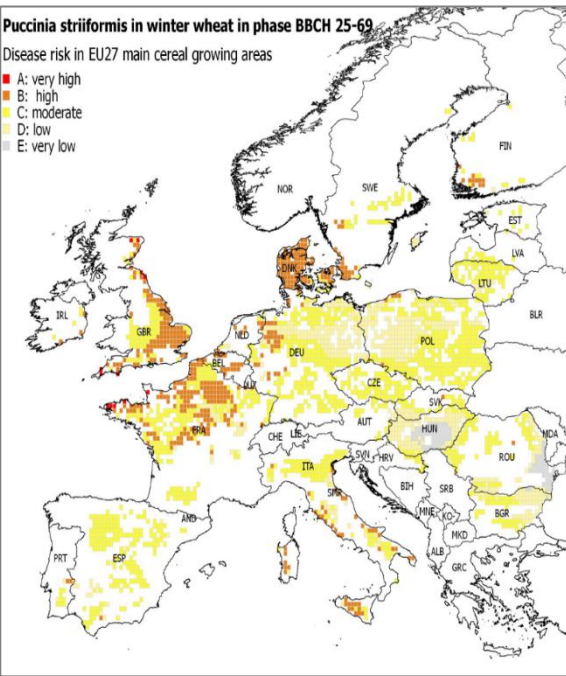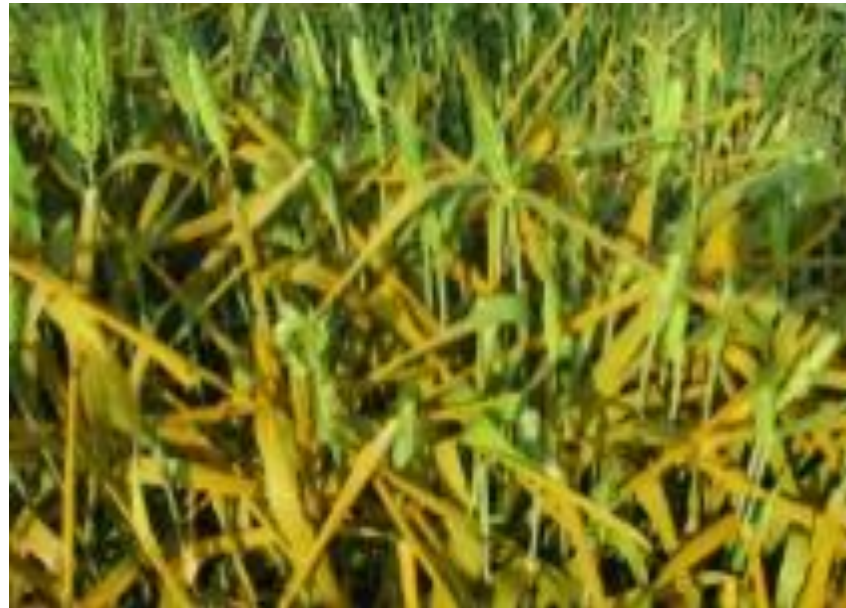**KWS**

Dr Lesley A. Boyd, NIAB

Start date: September 2018
End date: September 2023

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Multi-partner project with UK and European wheat breeders supported by BBSRC & AHDB

Puccinia striiformis in winter wheat in phase BBCH 25-69
Disease risk in EU27 main cereal growing areas
A: very high
B: high
C: moderate
D: low
E: very low

Wheat yellow rust occurrence across Europe

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Lantmännen Lantbruk

Limagrain United Kingdom

R·A·G·T SEEDS

Sejet plant breeding

syngenta

The problem:

1) Yellow rust of wheat is an annual problem, controlled through a management scheme of fungicide application and variety resistance.

2) Breeding for resistance has relied on major sources of resistance that are usually race-specific.

Application timing

17th April

10th April

4th April

Consequence of late fungicide application (courtesy of BASF)

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Overall Aim

Characterise yellow rust resistance in UK and Northern European wheat germplasm to identify the most effective Field Resistance across years and locations.

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Genome-Wide Association Study (GWAS)

A new GWAS panel of 458 wheat varieties was assembled and genotyped using the Breeders 35K SNP array.

The panel has been screened for yellow rust resistance across multi-sites in the UK, France, Germany, Denmark and Sweden.

Field trials undertaken in 2018, 2019, 2020, 2021 and 2022.

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• **2018 – 2022 yellow rust field trials**

Data transformation:
ORD – 1 to 9 scale
PCT – 1 to 100
LOG – Log10

Field trials
✓ 88 Field scores
✓ 237 Traits

Company	Name, Country	2018	2019	2020	2021	2022
Lantmannen	Svalöv, Sweden		X (2)	X (2)	X (1)	X (1)
	Bjertorp					X (1)
Limagrain	Osgodby, Lincolnshire, UK		X (2)			
	Rothwell, Lincolnshire, UK	X (3)	X (2)	X (3)	X (1)	X (1)
	Woolpit, UK				X (2)	X (1)
Sejet	Sejet, Denmark		X (2)	X (2)	X (1)	X (2)
	Fyn, Denmark		X (2)	X (2)	X (1)	
RAGT	Ickleton, UK	X (2)	X (1)	X (2)	X (2)	X (2)
	Lille, France		X (1)	X (2)	X (2)	X (1)
DSV	Wardington, UK		X (2)	X (2)	X (2)	X (1)
	Asendorf, Germany		X (1)	X (2)	X (1)	
KWS	Wetze , Germany		X (2)	X (1)	X (1)	X (2)
	Thriplow, UK	X (2)	X (1)	X (3)	X (2)	X (2)
Syngenta	Whittlesford, UK		X (1)	X (2)		X (2)
	Chartres, France			X (3)	X (2)	X (2)

Percentage yellow rust infection in 2022 field trials
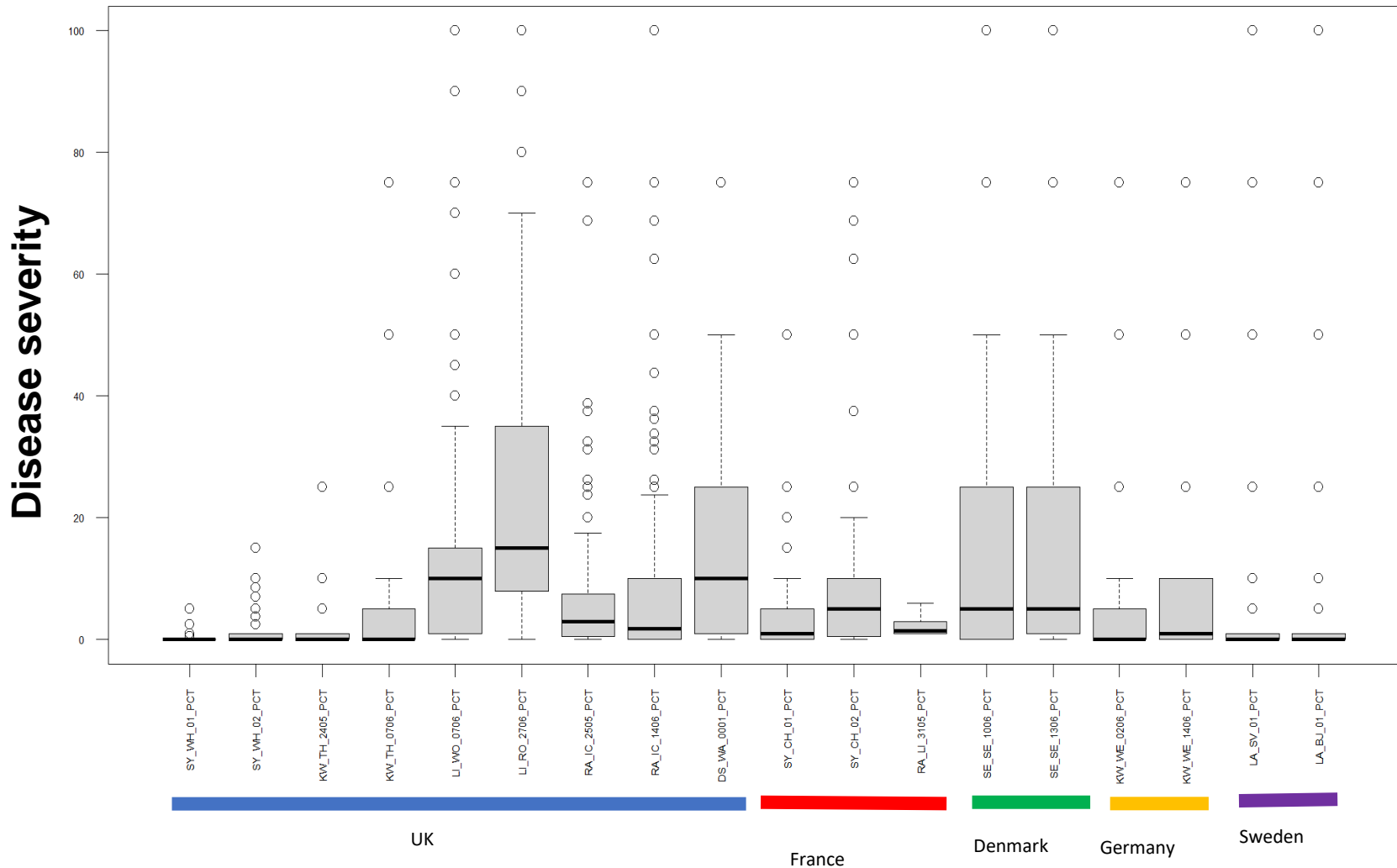
Correlations between 2022 field data

Correlations between phenotypic data suggests differing pathogen populations in Sweden from France, Germany and Denmark. Population in the UK more variable.
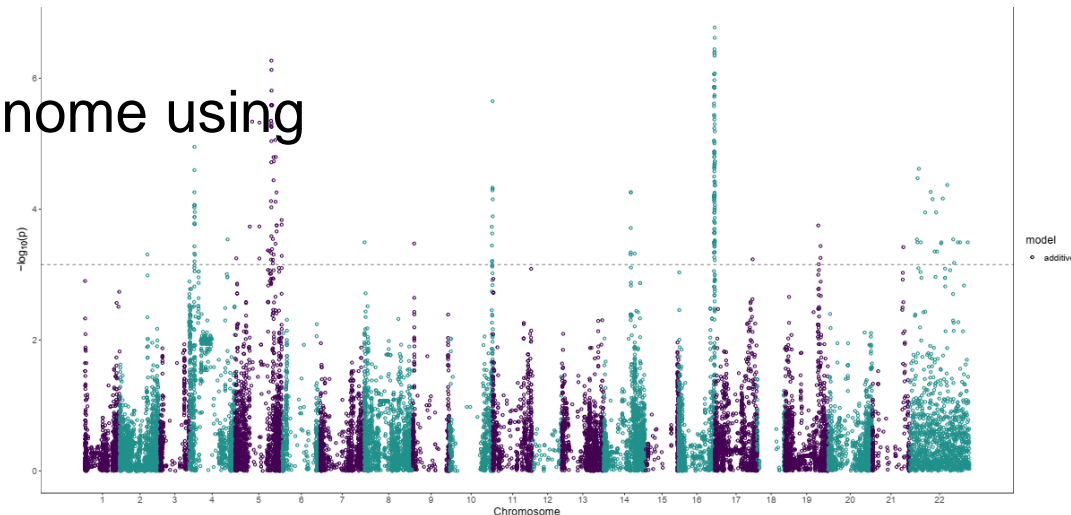
Genome Wide Association Study - GWAS

GWAS looks for associations between variation in the resistance phenotype and regions of the wheat genome, linking resistance phenotypes to specific alleles at defined marker locations:

- Genotyped with the Axion 35K Breeder's SNP array

- 19703 SNP were identified and used in a GWAS analyse

- Mapped markers to wheat genome using Chinese Spring RefSeq 2.0

- 1016 SNPs were unmapped

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Genome-Wide Association Study (GWAS)

Analysis of data sets initially used a False Discovery Rate (FDR) q-value = 0.02, but was reanalysed with a more stringent cutoff of q = 0.05

An MTA was called as real if it had a significance score above threshold > 3.0 at one site/year, or if a score > 1.5 was found in more than one location and/or season.

42 MTA were identified, of which 29 were present in most of the wheat varieties of the panel, i.e., > 90%, indicating that these yellow rust resistance loci have become fixed in UK and Northern European winter wheat germplasm.

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Genome-Wide Association Study (GWAS)

Four MTAs occurred at a low frequency (less than 25%): were 1A011 (18.5%), 2B153 (17.4%), 3B004 (21.8%) and UN004 (6.25%).

Another six MTAs of potential values were: 2A647 (43.8%), 2D999 (30.7%), 3A010 (57.4%), 3A241 (61.1%), 4B605 (38.4%) and 6A612 (43.1%).

The MTA on chromosome 2A – 2A019 – was effective in 2018 and 2019 but lost in 2020. *Aegilops ventricosa* translocation confirmed with *Yr17* KASP markers.

Breeder Friendly Formats:

Reporting the presence/absence of the resistance allele in each wheat variety provides the breeders with a picture of the genetic diversity within their winter wheat germplasm.

Marker	AX-951278	AX-949447	AX-945430	AX-946713	AX-948480	AX-949268	AX-948317	AX-947050	AX-945242	AX-947625	AX-951291	AX-952472	AX-949432	AX-944274	AX-949512	AX-946023
Chr	13	16	22	5	22	10	14	22	5	1	8	5	22	21	19	22
Pos	616.9824	609.5646	263	48.04196	605	737.5044	478.9317	437	290.6789	3.256233	4.206356	154.9853	688	548.9161	730.4316	316
QTL	5A617	6A610	UN004	2B048	NEW_18	4A738	5B478	NEW_31	2B290	1A003	3B004	2B154	UN025	7D548	7A730	NEW_25
QTL_YH	5A607	6A612	UN004	2B051	NEW_18	4A713	NA	NEW_31	2B298	1A011	3B004	2B153	UN025	7D548	7A730	NEW_25
Score-Thre	10.31	11.29	8.64	6.92	6.28	5.59	6.06	4.3	12.67	4.01	3.78	3.77	5.92	5.27	4.97	3.23
Min of Eff	-24.5677	-21.0339	-30.6012	0.288054	-41.2732	-14.5178	-24.2981	0.230903	0.338886	-15.8452	0.21652	-25.0102	-21.923	0.143275	-26.3816	-6.87637
Max of Eff	-0.16159	-0.06103	-0.27074	24.00303	-0.21663	-0.15765	-0.4669	25.45069	35.16661	-0.23199	20.54162	-0.16166	-0.14814	23.64984	-0.34339	-1.72677
SNP	A/G	C/G	T/C	A/C	T/C	A/C	T/C	A/G	T/C	A/T	A/G	T/G	T/C	A/C	T/C	T/C
Resistant_	G	G	C	A	C	C	C	A	T	T	A	C	C	A	C	C
Axiom call	A	C	T	C	T	A	T	G	C	A	G	T	T	C	T	T
Resistant_	BB	BB	BB	AA	BB	BB	BB	AA	AA	BB	AA	BB	BB	AA	BB	BB
Susceptibl	2	2	2	0	2	2	2	0	0	2	0	2	2	0	2	2
W1482_GL	R	S	S	R	R	S	R	R	R	S	S	S	S	R	R	R
W1108_DA	R	S	S	R	R	R	R	R	R	S	S	S	R	R	R	R
W1858_SA	R	S	S	R	R	R	R	R	R	S	S	S	R	R	R	R
W1830_RO	R	R	S	R	R	R	R	R	R	S	S	R	R	R	R	R
W0533_M	R	R	S	R	R	R	R	R	R	S	S	S	R	R	R	R
U112943_	R	S	S	R	R	R	R	R	R	S	S	S	R	S	R	R
X0090_AM	R	R	S	R	R	R	R	R	R	R	S	S	R	R	R	R
Synthetic_	S	S	S	R	R	R	R	R	R	S	S	S	S	R	R	R
X0090_AM	R	R	S	R	R	R	R	R	R	R	S	S	R	R	R	R
W0067N_	R	R	S	R	R	R	R	R	R	R	S	S	R	R	R	R
S0017_MA	S	S	S	R	R	R	R	R	R	S	R	R	R	R	R	R
X0069_SKY	R	S	S	R	R	S	R	R	R	S	S	S	R	R	R	R
UKNOWN	R	S	S	R	R	S	R	R	R	S	S	S	R	R	R	R
X0058_RG	R	S	S	R	R	S	R	R	R	S	S	S	R	R	R	R

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Validation of MTAs in bi-parental mapping populations

- A number of bi-parental populations were developed by the Breeders

- These populations were screened for yellow rust resistance in the field between 2018 to 2022

- The populations were genotyped by the breeders

- Initial mapping was undertaken by the breeders

Bi-parental mapping:

- **RAGT - Reflection x Dickens:**
  - 2A019, 2B153, 3A010, 5B697, 6A612

- **RAGT – Gladiator x Vuka**
  - 2A019, 3B004

- **Sejet – Revelation x Solstice**
  - 2A087, 1B545, 4B605, 5A685

- **DSV – Mallet x Solstice**
  - 1A011, UN004

- **DSV – Crusoe x Solstice:**
  - UN004

- **Syngenta – Graham x Solstice**
  - 6A612

- **Syngenta – Flanders x Solstice**
  - 2B153
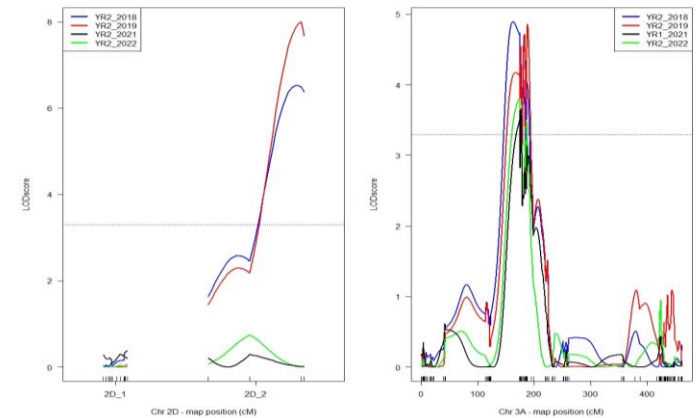
YELLOWHAMMER
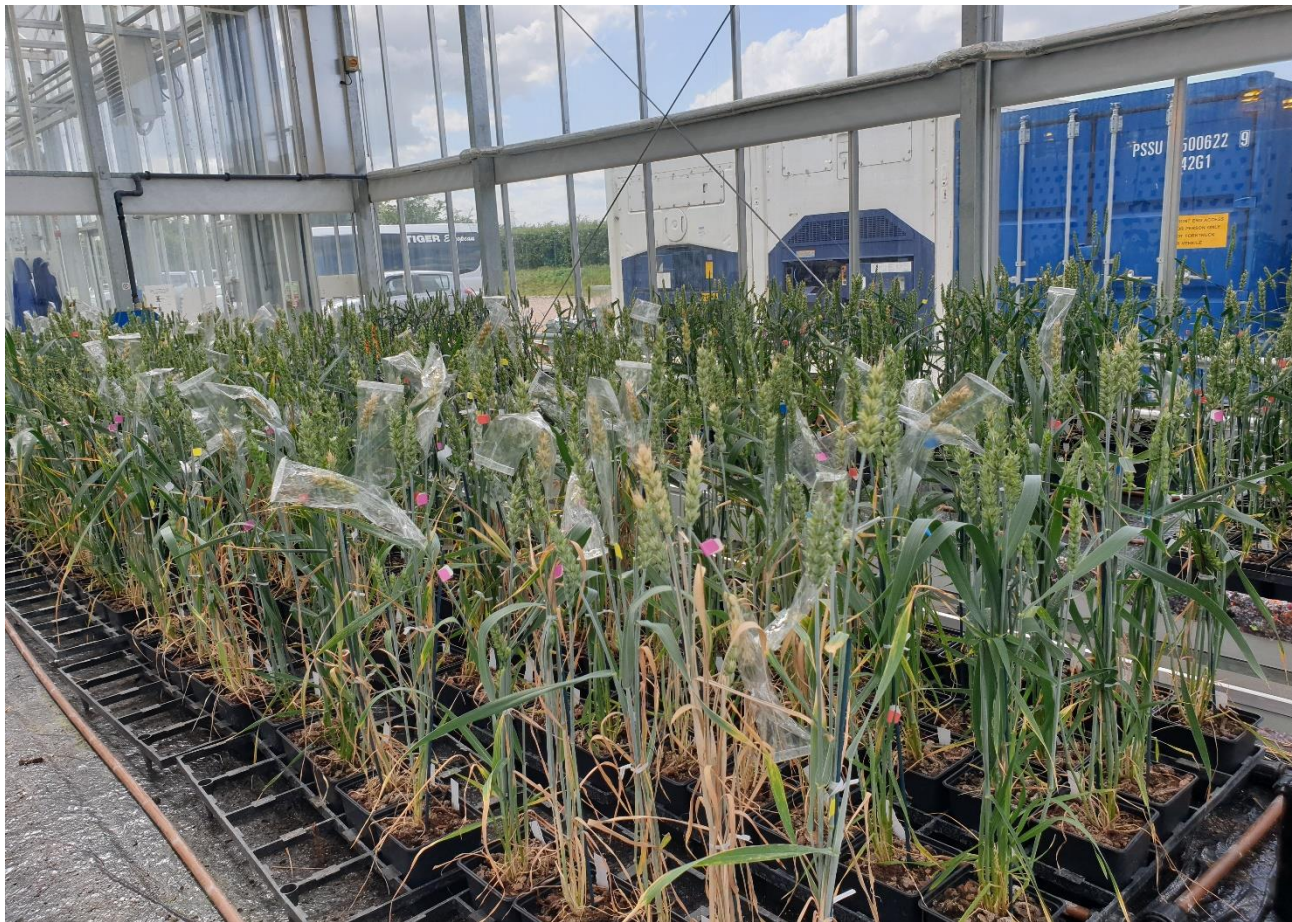
Microphenotyping and differential gene expression analysis

NILs have been developed by Limagrain and RAGT:

• Limagrain NILs had different combinations of HITs 1 (2A019), 2 (6A612) and 4/5 (2D999)

• RAGT NILs with and without HIT 2 (6A612)

• RAGT NILs with and without Yr15

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NIAB glasshouse experiment
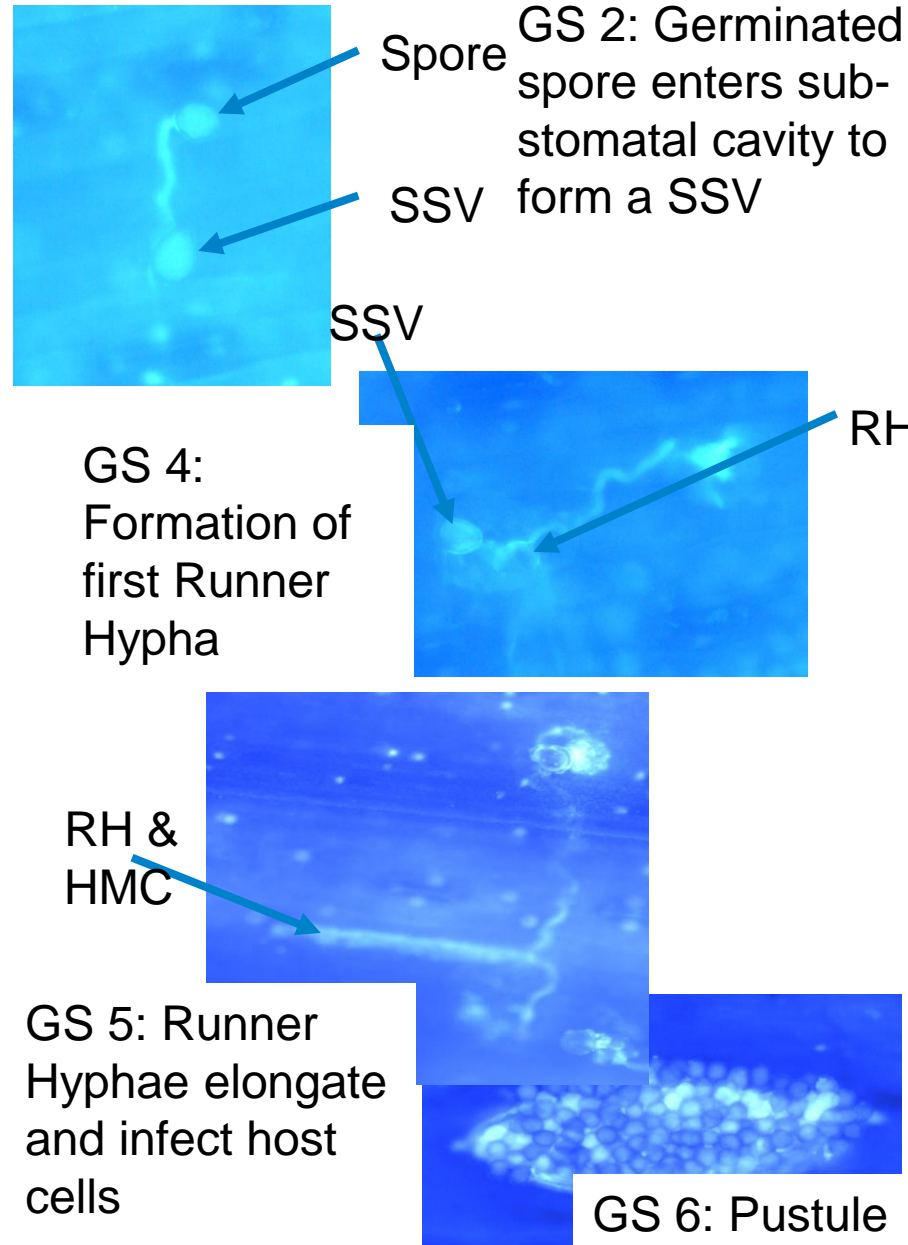using isolate 19/501 from Bennington/Zyatt

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Micro-phenotyping protocol:

Yellow rust growth stages (GS):
1. Germinated spore
2. Germ tube enters stomata and forms a Sub-Stomatal Vesicle (SSV)
3. Formation of Infection Hyphae and Haustorial Mother Cells (HMC)
4. Formation of first Runner Hypha (RH)
5. Runner Hyphae elongate and infect additional host cells
6. Formation of pustule

Spore

SSV

GS 2: Germinated spore enters sub-stomatal cavity to form a SSV

SSV

RH

GS 4: Formation of first Runner Hypha

RH & HMC

GS 5: Runner Hyphae elongate and infect host cells

GS 6: Pustule

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Microphenotyping and differential gene expression analysis

Limagrain NILs had different combinations of HITs 1 (2A019), 2 (6A612) and 4/5 (2D999)

RRR (HIT 1; **HIT 4/5**; HIT 2) Field score of 1
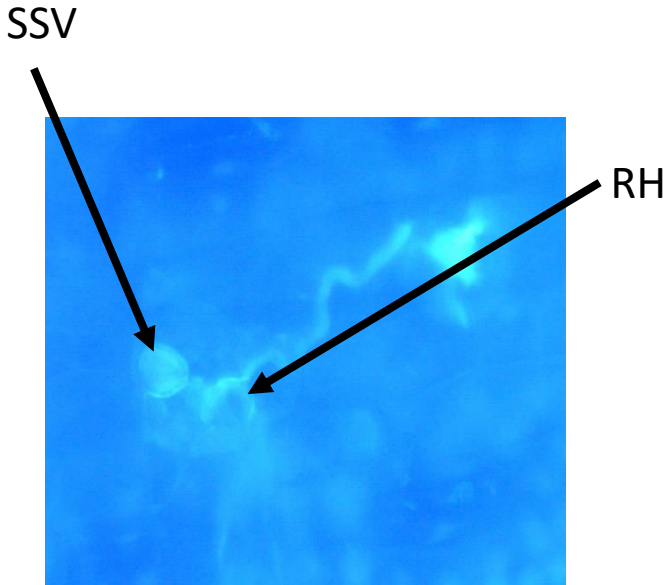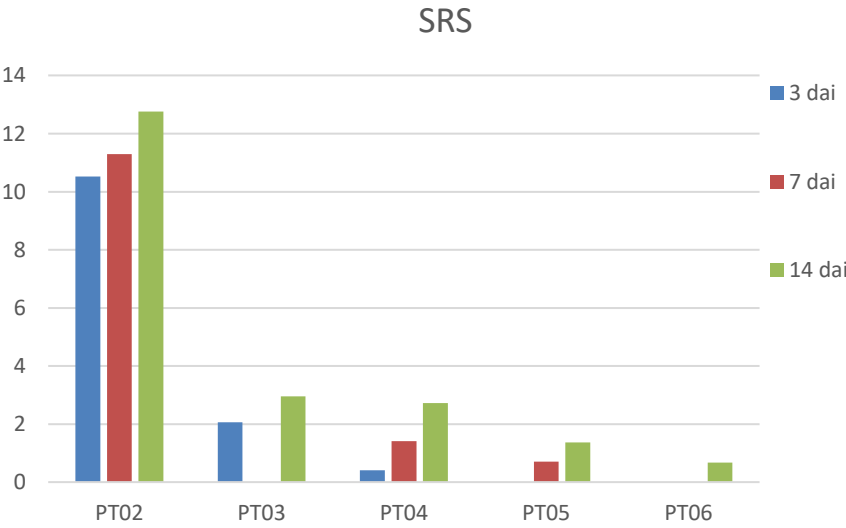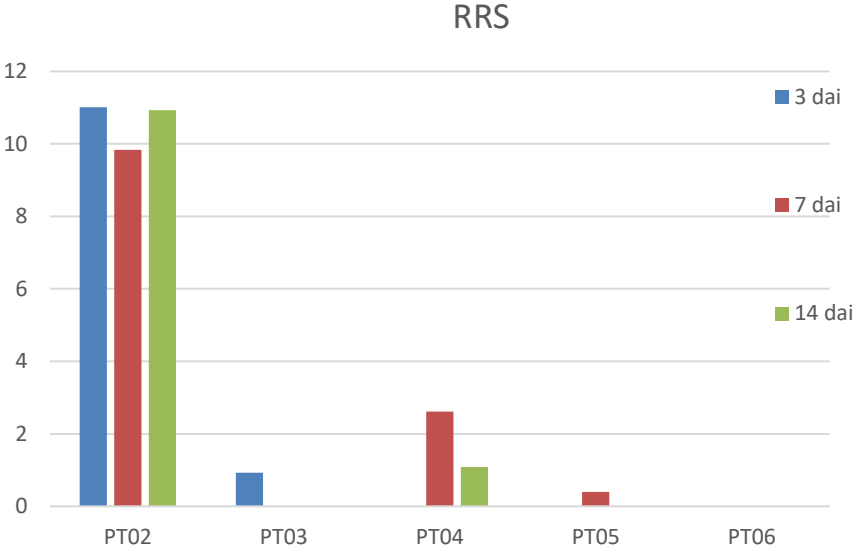
RRS (HIT1; **HIT 4/5**) Field score of 6

SRR (**HIT 4/5**; HIT 2) Field score of 4

SRS (**HIT 4/5**) Field score of 7

KASP markers confirmed HITs 1 & 2 were segregating but HIT 4/5 was fixed.
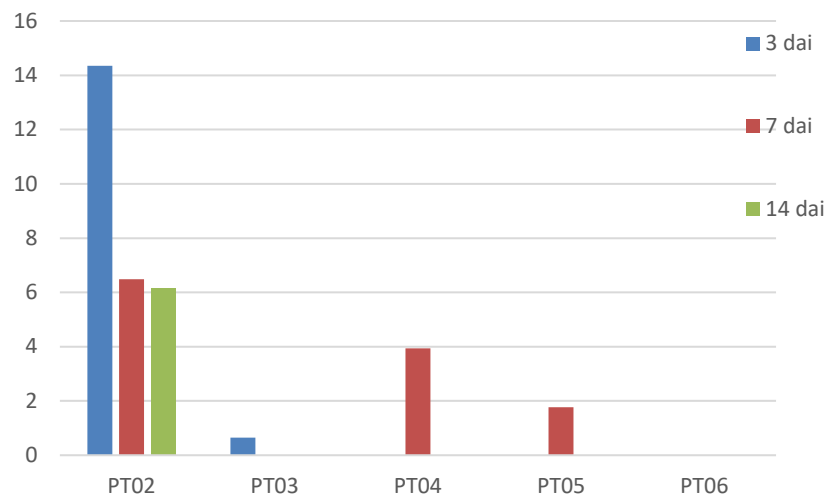
Compare RRS vs SRS – HIT 1 (2A019)

RRS

SRS

SSV

RH

Growth stage 4:
Formation of first Runner
Hypha

HIT 1 appears to
primarily stop yellow
rust development at
stage 4

Limagrain
United Kingdom

Compare SRR vs SRS – HIT 2.1 (6A612.1)

SRR

Legend: 3 dai, 7 dai, 14 dai

X-axis: PT02, PT03, PT04, PT05, PT06

SRS

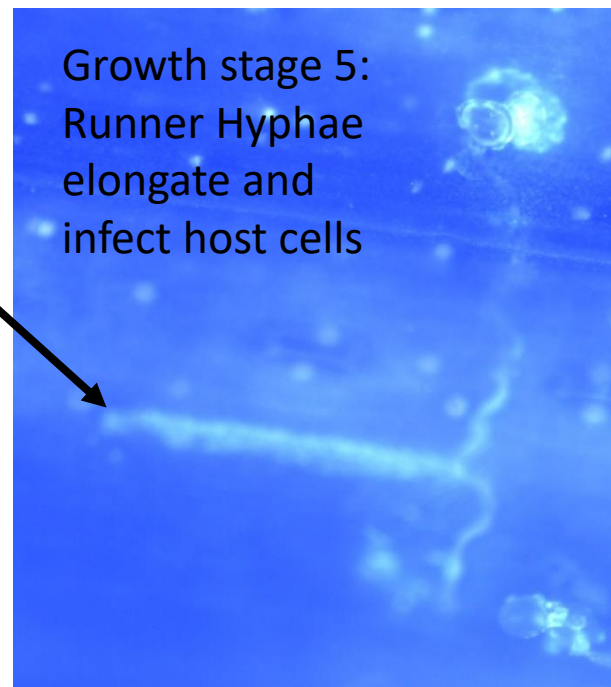Legend: 3 dai, 7 dai, 14 dai

X-axis: PT02, PT03, PT04, PT05, PT06
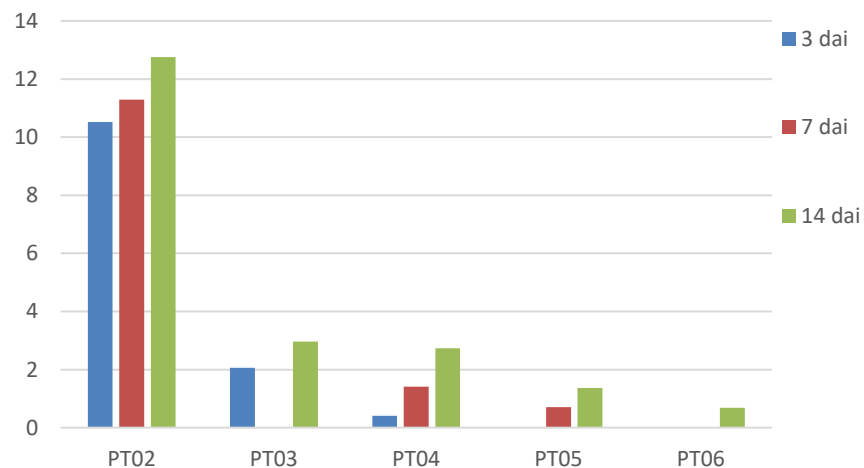
Growth stage 5: Runner Hyphae elongate and infect host cells

RH & HMC

HIT 2.1 appears to prevent pustule formation, but permits runner elongation

Limagrain
United Kingdom

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Microphenotyping

19PD1020.016.028 – HIT 2.2

19PD1020.016.050 – susceptible

KASP markers indicated that HIT 2 in the RAGT NILs was not the same genetic region as in the Limagrain NILs.
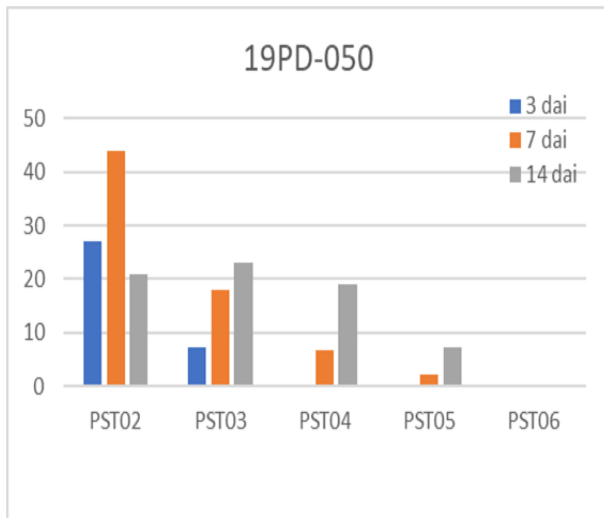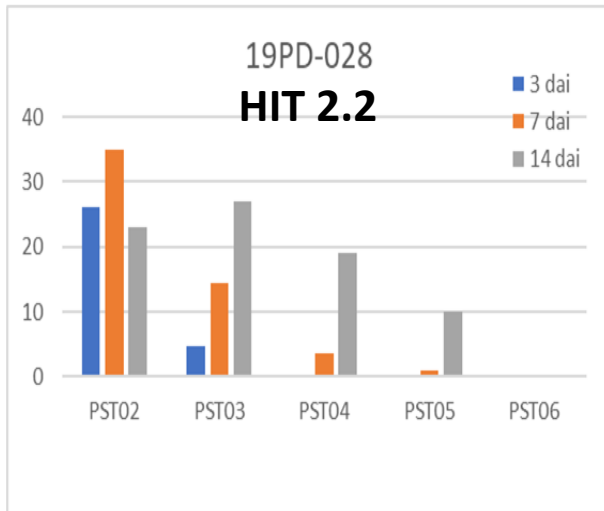

19PD.028 resistant HIT 2.2
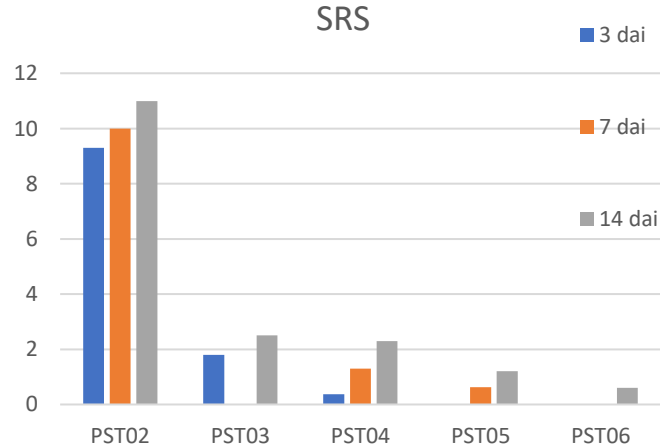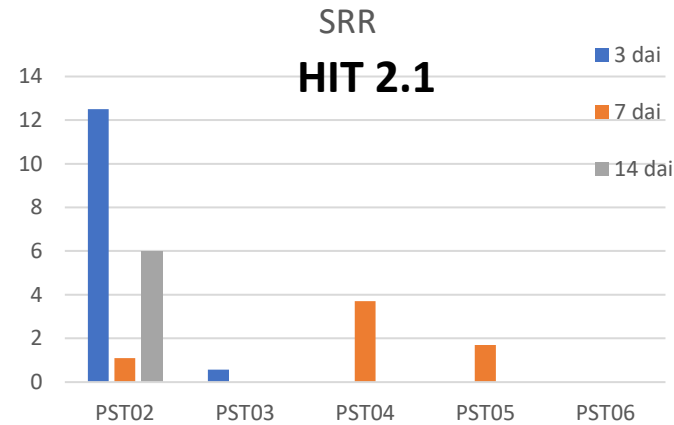
19PD.050 susceptible

The microphenotypes of HIT 2.1 and HIT 2.2 also differ.

Comparison of NILs with and without HIT2.2

19PD-028

HIT 2.2

SRR

HIT 2.1

19PD-050

SRS

The NILs with and without HIT2.2 did not differ in terms of the rate of *Pst* development. Similar percentages of germinated spores had reach each of the developmental stages at 3, 7 and 14 dai in the two NILs.

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Microphenotyping

18PD1040.003.007 – Yr15

18PD1040.003.004 - susceptible

HIT Yr15

Comparison of NILs with and without Yr15

18PD_007

18PD_004

The NILs with and without Yr15 did not differ in terms of the rate of *Pst* development. Similar percentages of germinated spores had reach each of the developmental stages at 3, 7 and 14 dai in the two NILs.
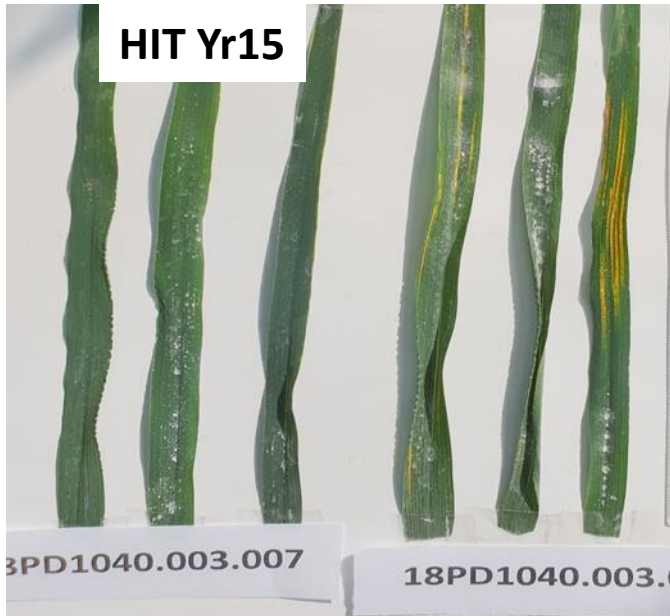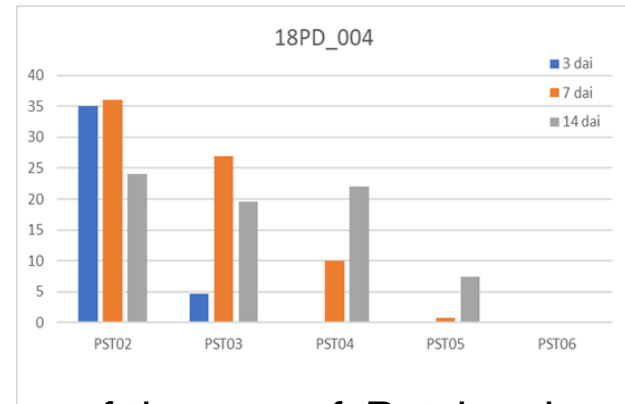
RNAseq analysis of wheat gene expression - *Yr15*
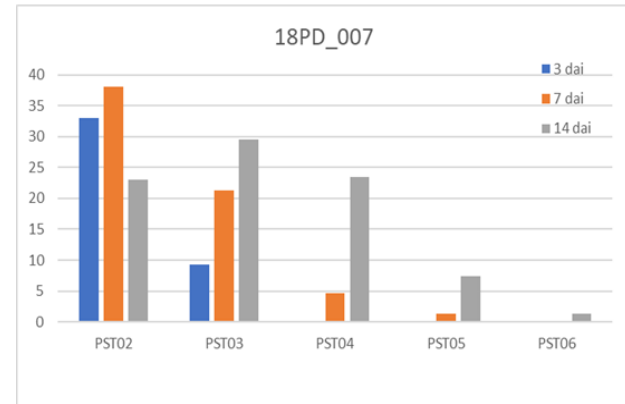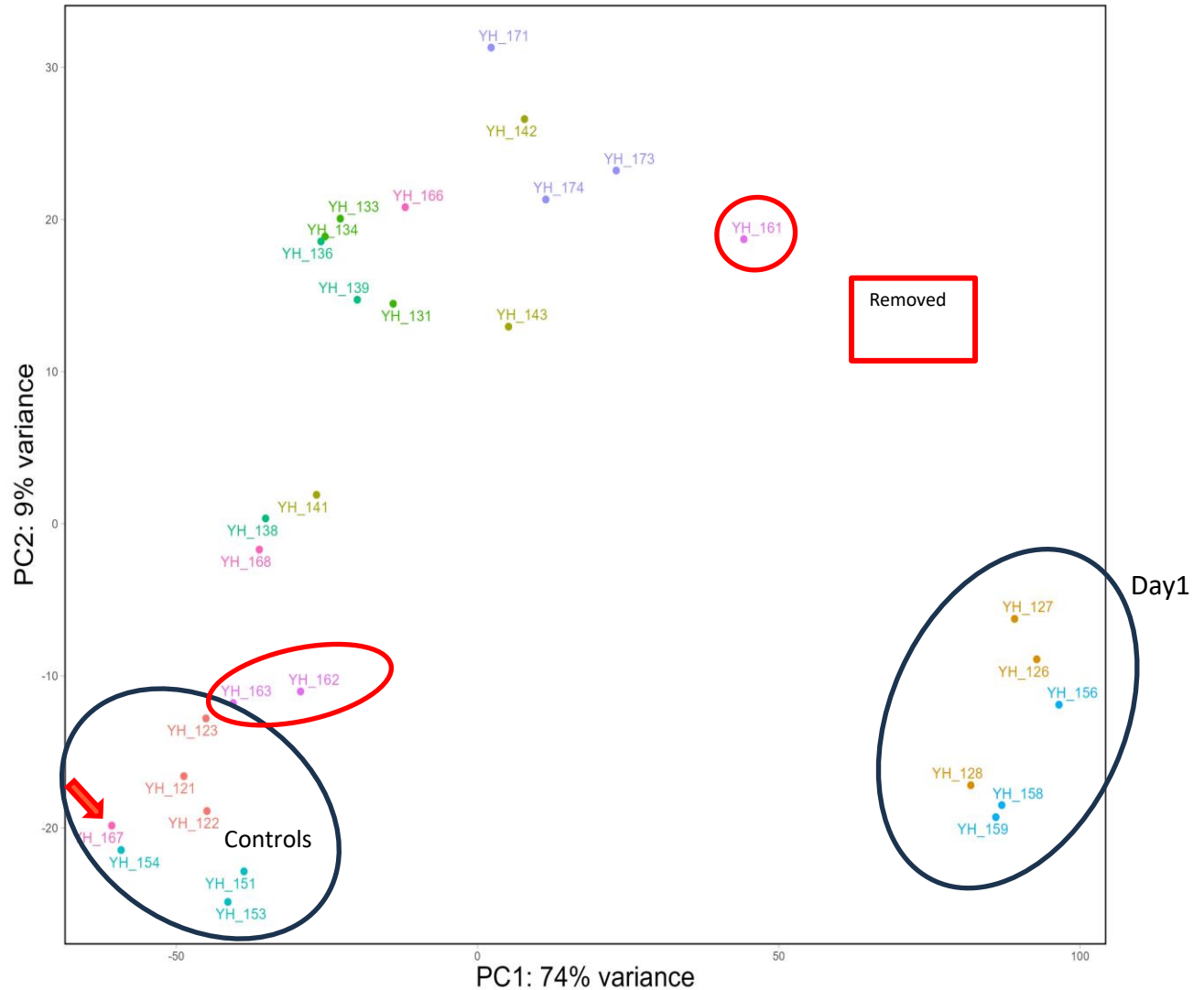
Relationship between libraries after cleaning

Sample	Line	Treatment	Condition
YH_121	P18_4	control	Yr15_neg
YH_122	P18_4	control	Yr15_neg
YH_123	P18_4	control	Yr15_neg
YH_126	P18_4	D1	Yr15_neg
YH_127	P18_4	D1	Yr15_neg
YH_128	P18_4	D1	Yr15_neg
YH_136	P18_4	D7	Yr15_neg
YH_138	P18_4	D7	Yr15_neg
YH_139	P18_4	D7	Yr15_neg
YH_141	P18_4	D14	Yr15_neg
YH_142	P18_4	D14	Yr15_neg
YH_143	P18_4	D14	Yr15_neg
YH_151	P18_7	control	Yr15+
YH_153	P18_7	control	Yr15+
YH_154	P18_7	control	Yr15+
YH_156	P18_7	D1	Yr15+
YH_158	P18_7	D1	Yr15+
YH_159	P18_7	D1	Yr15+
YH_166	P18_7	D7	Yr15+
YH_168	P18_7	D7	Yr15+
YH_171	P18_7	D14	Yr15+
YH_173	P18_7	D14	Yr15+
YH_174	P18_7	D14	Yr15+
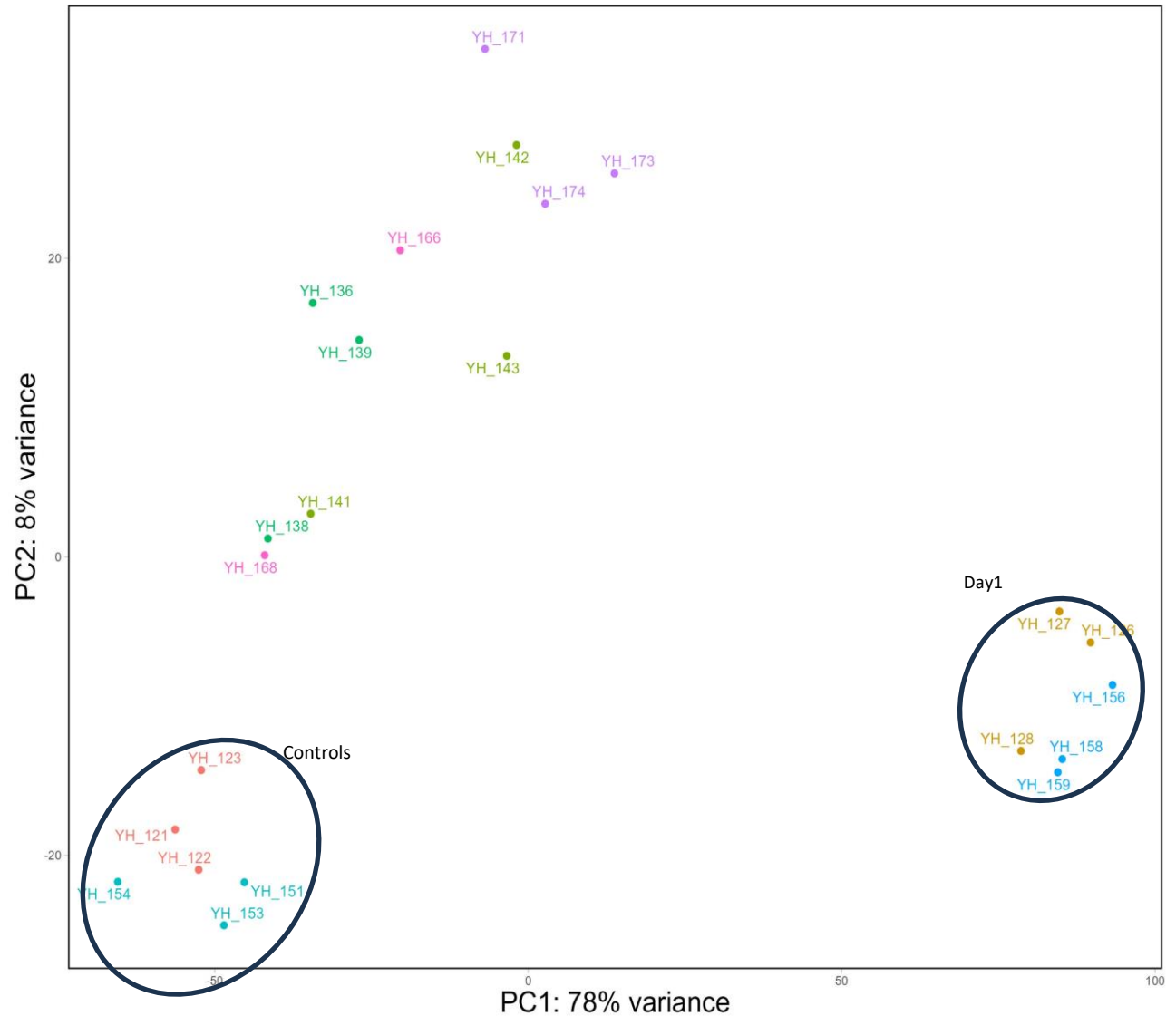
Removed all 3 dai libraries

## Cloning of the wheat *Yr15* resistance gene sheds light on the plant tandem kinase-pseudokinase family

Valentina Klymiuk[1,2], Elitsur Yaniv[1,2,3,4], Lin Huang[1,2,5], Dina Raats[1,2,6], Andrii Fatiukha[1,2], Shisheng Chen[7], Lihua Feng[1,2], Zeev Frenkel[1], Tamar Krugman[1], Gabriel Lidzbarsky[1], Wei Chang[3,4], Marko J. Jääskeläinen[3,4], Christian Schudoma[6], Lars Paulin[3], Pia Laine[3], Harbans Bariana[8], Hanan Sela[1,9], Kamran Saleem[10], Chris Khadgi Sørensen[10], Mogens S. Hovmøller[10], Assaf Distelfeld[1,11], Boulos Chalhoub[12], Jorge Dubcovsky[7,13], Abraham B. Korol[1,2], Alan H. Schulman[3,4,14] & Tzion Fahima[1,2]

Evaluation of marker-assisted selection for the stripe rust resistance gene *Yr15*, introgressed from wild emmer wheat

Elitsur Yaniv · Dina Raats · Yefim Ronin · Abraham B. Korol · Adriana Grama · Harbans Bariana · Jorge Dubcovsky · Alan H. Schulman · Tzion Fahima
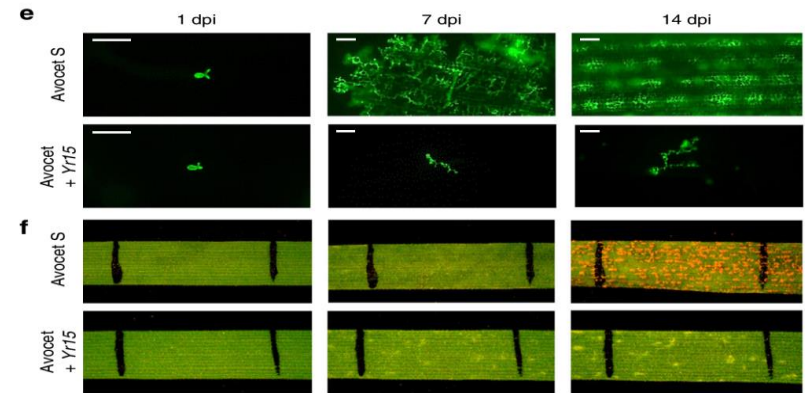
✓ *Yr15* was introgressed into hexaploidy wheat from a wild emmer wheat
✓ It was mapped to the short arm of chromosome 1B
✓ Major, seedling expressed R-gene
✓ Encode a tandem kinase-pseudokinase designated as WTK1
✓ YH GWAS panel - *Yr15*+ was identified in homozygosis in 10 lines (2.3%)

# Preliminary RNAseq analysis of wheat gene expression - *Yr15*

Top 50 DEG

Sample	Line	Treatment	Condition
YH_121	P18_4	control	Yr15_neg
YH_122	P18_4	control	Yr15_neg
YH_123	P18_4	control	Yr15_neg
YH_126	P18_4	D1	Yr15_neg
YH_127	P18_4	D1	Yr15_neg
YH_128	P18_4	D1	Yr15_neg
YH_136	P18_4	D7	Yr15_neg
YH_138	P18_4	D7	Yr15_neg
YH_139	P18_4	D7	Yr15_neg
YH_141	P18_4	D14	Yr15_neg
YH_142	P18_4	D14	Yr15_neg
YH_143	P18_4	D14	Yr15_neg

Sample	Line	Treatment	Condition
YH_151	P18_7	control	Yr15+
YH_153	P18_7	control	Yr15+
YH_154	P18_7	control	Yr15+
YH_156	P18_7	D1	Yr15+
YH_158	P18_7	D1	Yr15+
YH_159	P18_7	D1	Yr15+
YH_166	P18_7	D7	Yr15+
YH_168	P18_7	D7	Yr15+
YH_171	P18_7	D14	Yr15+
YH_173	P18_7	D14	Yr15+
YH_174	P18_7	D14	Yr15+

Yr15 homolog on CS was identified among the Top 15 DEG
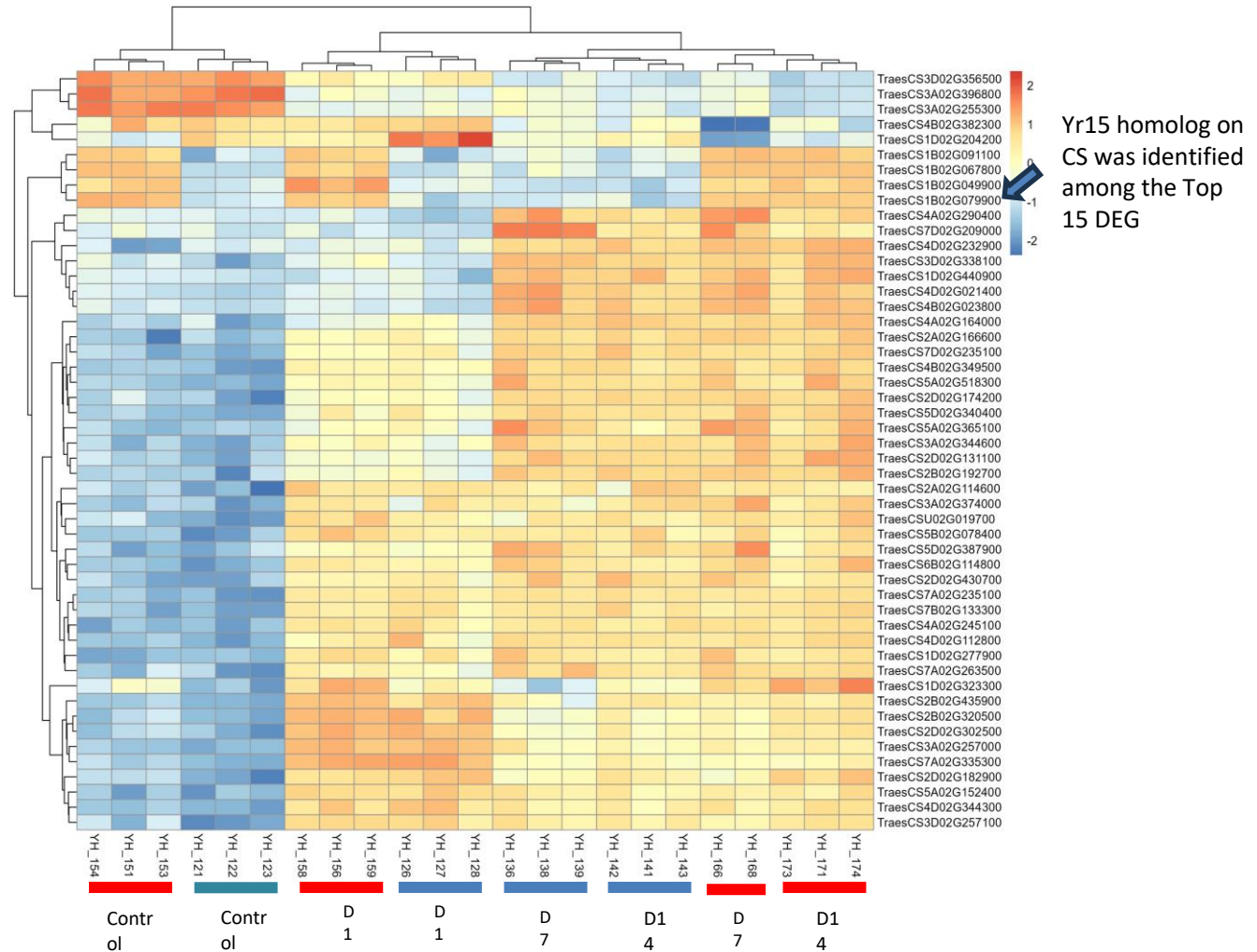
Differential RNAseq data from NILs differing for specific yellow rust resistance loci can be used to identify the gene responsible for the resistance phenotype.
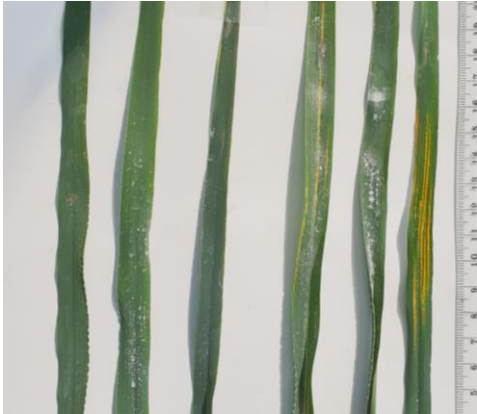
Yr15+   Yr15-

Chrom	Start	End	Annotation
chr1B	56994218	57003660	DIS3-like exonuclease 2
chr1B	57629806	57630250	Heavy metal transport/detoxification protein
chr1B	58540169	58541331	F-box protein
chr1B	58563500	58586329	Receptor-like protein kinase
chr1B	59469710	59475326	Disease resistance protein RPM1
chr1B	59514359	59528030	Receptor-like protein kinase
chr1B	59535857	59540771	Receptor kinase 1
chr1B	61894143	61898608	Interactor of constitutive active ROPs 2, chloroplastic
chr1B	62408881	62427835	Protein kinase-like – WTK1
chr1B	62681663	62681947	Low temperature and salt responsive protein family
chr1B	62719857	62724509	Glycosyltransferase
chr1B	65348022	65353543	Glycosyltransferases

Take home messages:

An in-depth knowledge of the sources of resistance, and the markers that enable their identification in future wheat varieties, coupled with the annual results of the UKCPVS, enables breeders to identify which of these sources of yellow rust resistance have become ineffective.

This in turn enables breeders to combine those yellow rust resistances that are still effective into new wheat varieties.

The identification the gene/s underlying yellow rust resistance loci provide candidates for KASP marker development, improving marker resolution.

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NIAB:

Lesley Boyd
Megan Burt
Tansy Chia
John Connell
Charlotte Nellist
Amelia Hubbard
Keith Gardner
Joachim Nwezeobi
Sarah Holdgate
Lucy James
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Tina Henriksson

AHDB

BBSRC
bioscience for the future

DSV

KWS

Lantmännen
Lantbruk

Limagrain
United Kingdom

R·A·G·T
SEEDS

Sejet
plant breeding

syngenta

Questions