EfectaWheat
An Effector- and Genomics-Assisted Pipeline for Necrotrophic Pathogen Resistance Breeding in Wheat

James Cockram, NIAB, Cambridge, UK

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Project Partners

Partner	Name	Institute	Total
1	James Cockram, Ian Mackay, 1xPDRA	NIAB, UK	3
2	Judith Turner, 1x tech	Fera, UK	2
3	Lise Jørgensen, Annemarie Justesen, 2x tech	AARHUS, Denmark	4
4	Morten Lillemo, 1`x PDRA	NMBU, Norway	2
5	Lorenz Hartl, Volker Mohler 1xPRDA, 1x Tech	LfL, Germany	4
6	Richard Oliver, Caroline Moffat, KC Chun, 1x tech	Curtin Uni, Australia*	4

** Self-funded*

Project background

EfectaWheat Project duration: 3 years

EfectaWheat Target crop: bread wheat

EfectaWheat Target trait: Leaf spot group (LSG) necrotrophic pathogens

LSG pathogens

LSG necrotrophic pathogens are economically important in EU:

- *Pyrenophora tritici-repentis* (*Ptr,* tan spot; TS)
    - *Parastagonospora nodorum* (*Pn*, cause of Septoria nodorum blotch; SNB)
        - *Zymoseptoria tritici* (*Zt,* Septoria tritici blotch; STB)

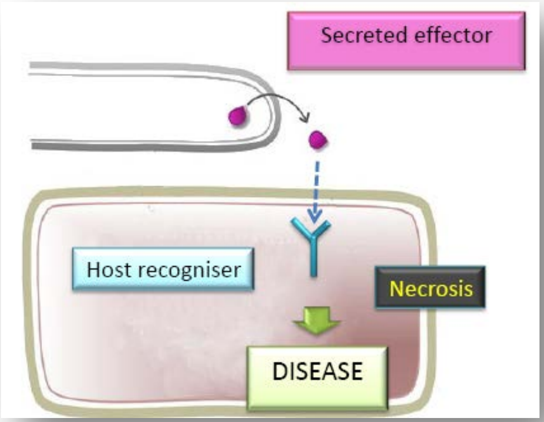
Pathogen-host interactions characterised by:

- *Quantitative resistance*
    - *Multiple (weak) QTLs*
        - *Isolate variation*

Effectors

LSG pathogens produces necrotrophic effectors that mediate host cell death, promoting fungal sporulation.

The discovery of effectors has revolutionised disease resistance breeding, allowing complex genetic resistance mechanisms to be broken down into constituent parts.



Naïve model

Interaction between necrotrophic effector and product of dominant host sensitivity gene leads to disease.

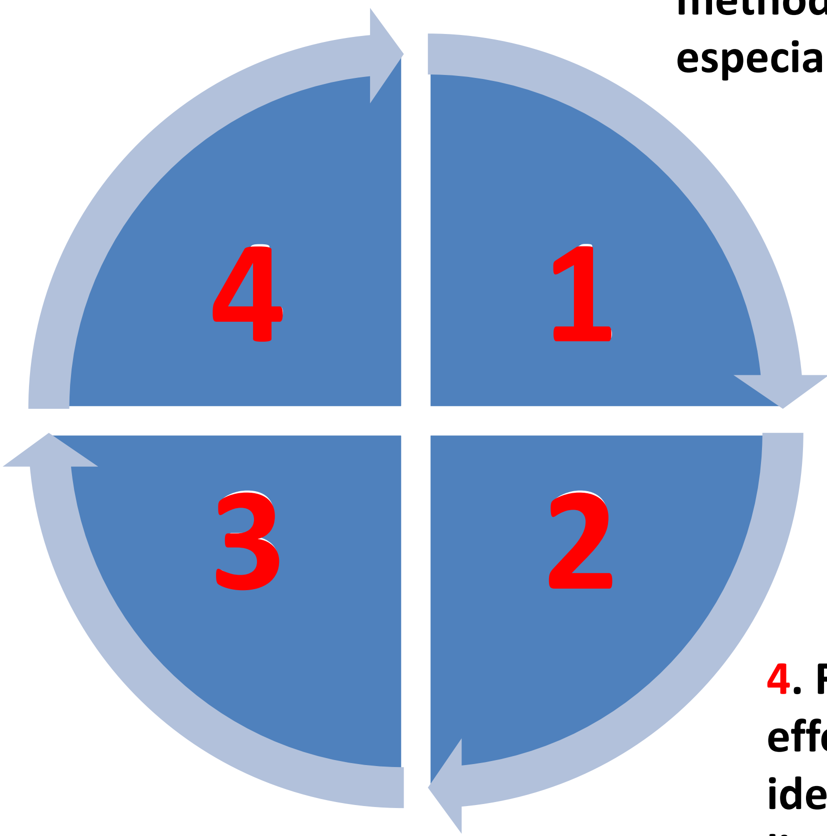
Multiple effectors and receptors in each disease

The more matching receptors and recognisers, the more virulence.

Project Aims

- Establish relative impact of LSG pathogens in target EU countries using molecular approaches.
- Identify new *Ptr* and *Pn* effectors
- Determine the effector profile of *Pn* and *Ptr* isolates and the sensitivity of cultivars to effectors
- Screen LSG effectors against high-resolution wheat germplasm resources.
- Resolve LSG effector and field resistance QTLs at high-resolution
- Deliver tools (effectors and markers) for LSG resistance breeding to EU breeders and researchers.

Summary of work flows



1. Survey of LSG prevalence (UK, DEU, NOR, DNK) using molecular approaches (qPCR). Traditional methods to diagnose LSG can be unreliable, especially in mixed infection

2. Effector characterisation and identification via (i) pathogen resequencing, (ii) effector knockout lines and biochemical purification of culture filtrates. Protein production via *E. coli* shuffle strains.

3. Effector-based phenotyping, single isolate virulence profiling; multi-site multi-year field pathotesting

4. Fine-scale QTL mapping, cross-comparison of effector sensitivity and disease QTLs, identification of ‘instant NILs’ from MAGIC lines for further investigation.

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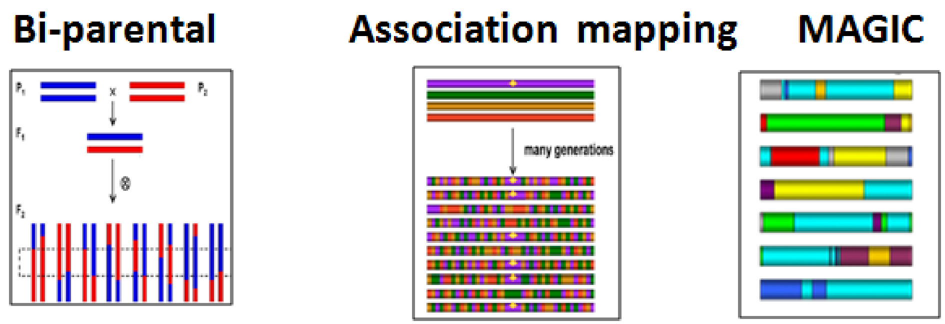
Wheat germpasm and markers

Advances in wheat genetic markers and mapping resources and approaches are well timed to allow rapid progress in rapidly characterising effector and adult plant resistance loci.

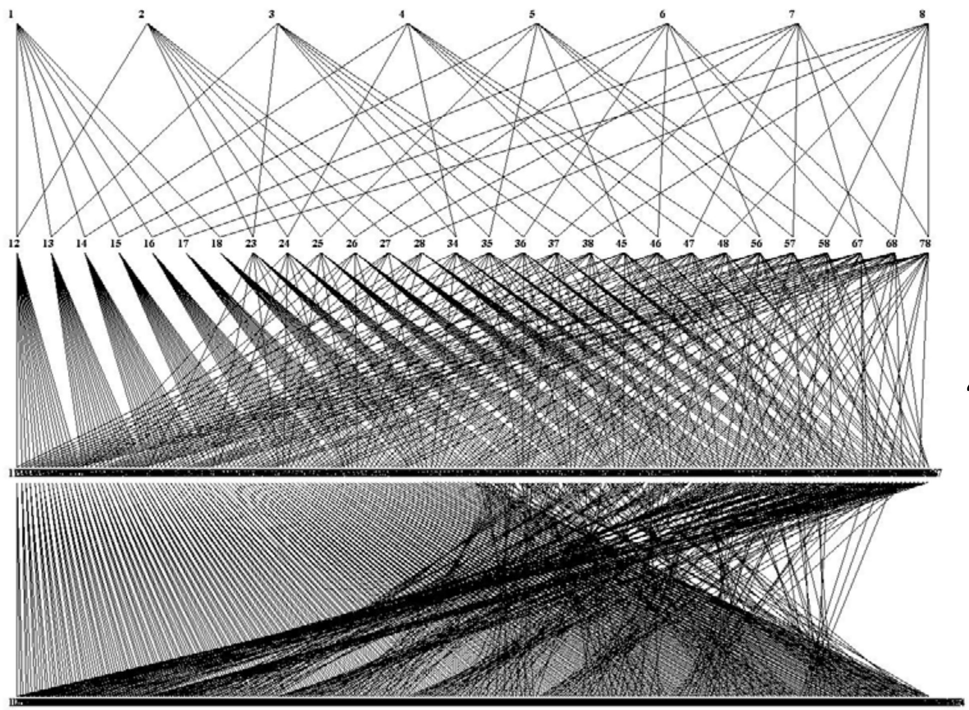


	Bi-parental	Association mapping	MAGIC
Allele:	low	high	med
Structure:	low	high	low
Recomb:	low	high	med

Red = good Blue = bad

Wheat MAGIC

Multi-parent Advanced Generation Inter Cross



28 F1 crosses

4-way: 210 crosses (all poss crosses between unrelated F1s)

8-way: 315 possible crosses

1,000 RILs.

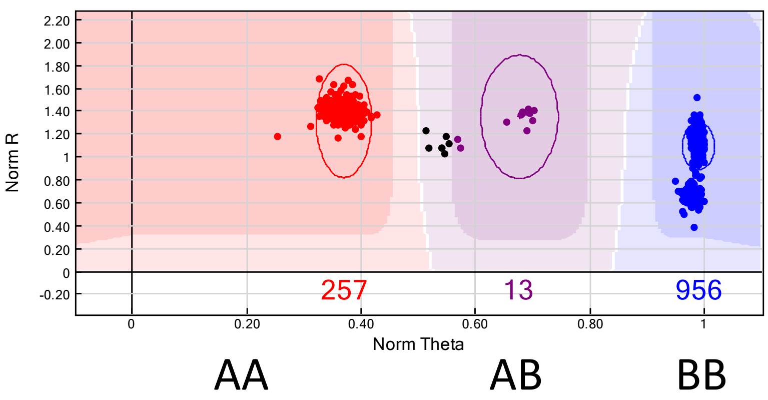
(Utilize residual heterozygosity to catalogue 'instant NILs' across the genome)

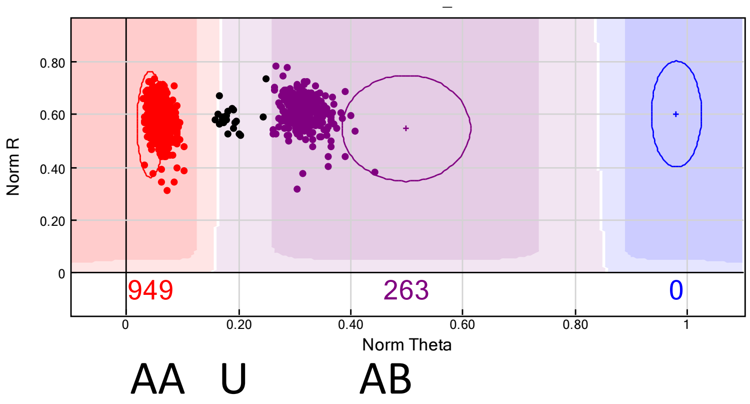
Wheat genetic Markers

Large numbers of genetic markers now available for wheat.

We have used Illumina 90k SNP chip for MAGIC and AM panels.

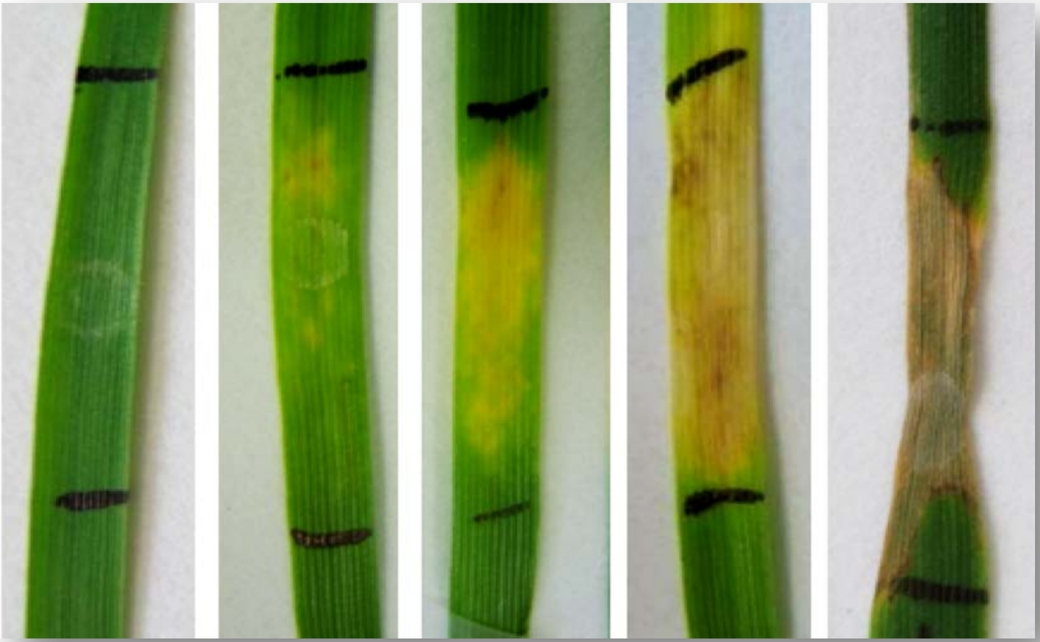
Manual scoring to increase the number of SNPs and the quality of the SNP calls.





ToxA vs AM: proof of concept

ToxA – *Tsn1* (PK-NBS-LRR) (Faris et al 2010)



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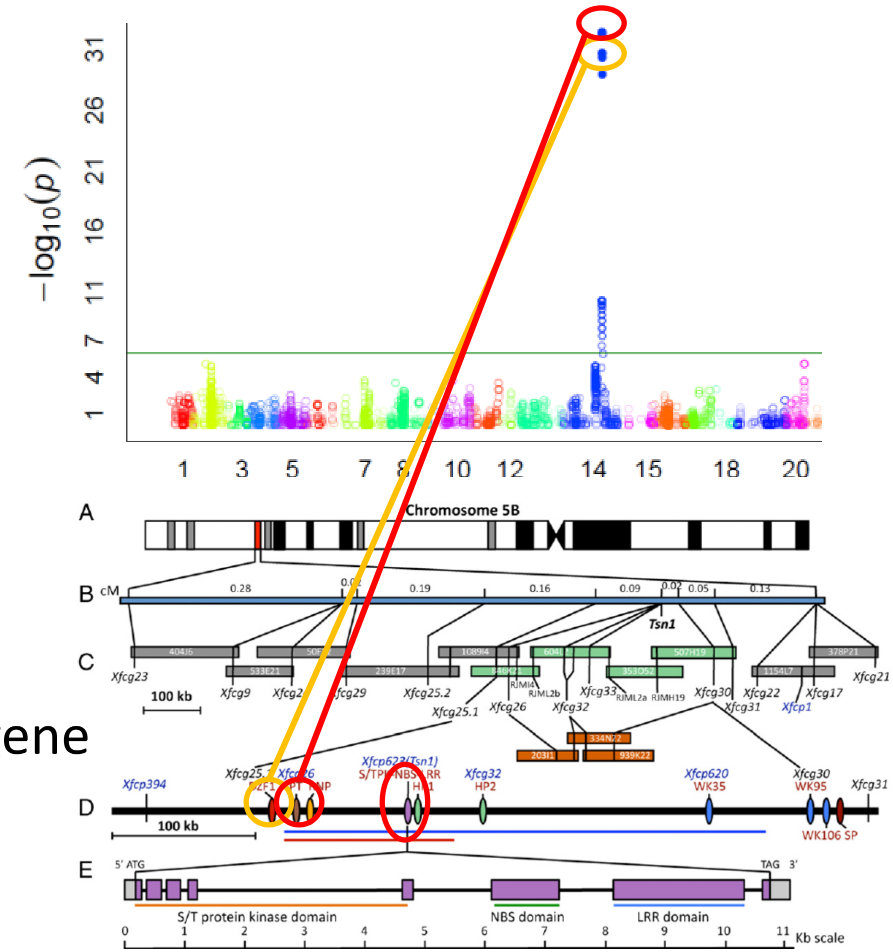
Effectors vs AM: proof of conept

Tsn1 locus (5BL) conferring sensitivity to ToxA has been cloned (Faris et al. 2010)…

500 lines, 6 weeks

~8,000 lines → 10 gene interval. ~8 years.



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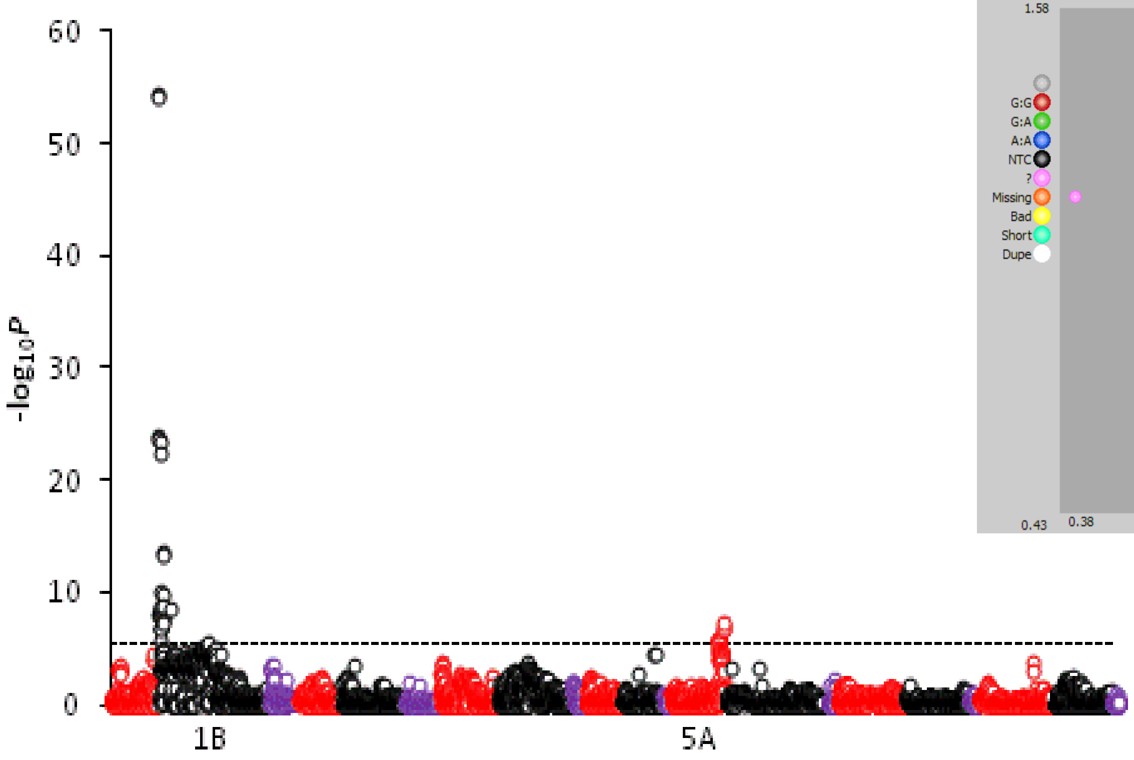
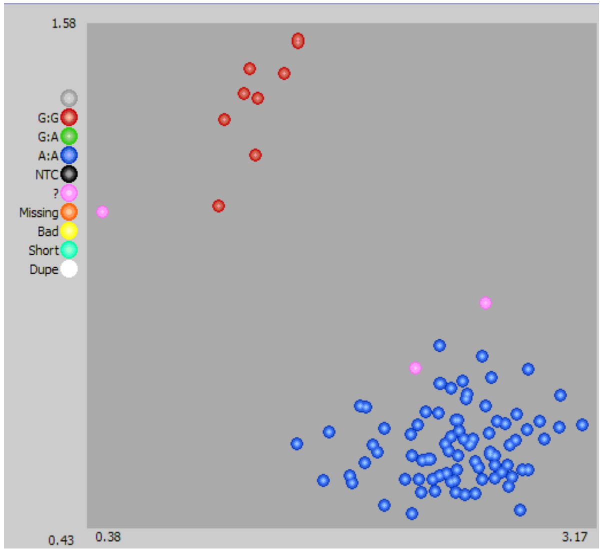
Effectors vs MAGIC: proof of conept

Snn1 locus (1B) conferring sensitivity to SnTox1





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Effectors vs MAGIC: proof of conept

Summary

• Investigate the extent to which Mendelisation of the infection process by identification and deployment of effectors can dissect LSG susceptibility/resistance in EU wheat

• Use residual heterozygosity identified in MAGIC lines to Mendelise LSG Adult plant resistance

• Provide knowledge to aid informed selection for LSG resistance, via effector identification, genetic markers and ultimately, genome editing approaches.

DFG Deutsche Forschungsgemeinschaft

DASTI

The Research Council of Norway

BBSRC
bioscience for the future

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Reduction in losses due to Effector delivery

Vleeshouwers and Oliver MPMI 2014

Total losses to SNB and Tan spot in Australia $300m
In WA $250m

100,000 doses of ToxA, 1 & 3 delivered to breeders 2009 to date
Area sown to Tsn1 wheat in WA reduced
30% to 16%
by 700,000 ha
Average losses 0.3 tonne/ha or 10%
200,000 tonnes = **$50m**

Known SNB and Tan Spot Effect

Effector	Species	Recogniser		Function
ToxA	Tan spot	Tsn1	5BL	NBS-LRR-PK
ToxB	Tan spot	Tsc2	2BS	?
ToxC	Tan spot	Tsc1	2AS	?
Tox1	SNB	Snn1	1BS	WAK
Tox3	SNB	Snn3	5BS	?

Evidence for 15+ effector/recognisers pairs in bo

Wheat leaf blotch disease status

SNB	Used to be major disease in NW Europe – declined since 1970s Rarely seen in UK (Fera), Rothamsted Broadbalk Common in France, Norway, SE USA, Poland, Ukraine Needs heavy sporadic rainfall, stubble retention
Tan spot	3rd commonest disease (after STB and PM) in UK major in Denmark, Germany, Ukraine Needs warmer climate, stubble retention, baseline resistant to fungicides
STB	Re-emergent in SE Australia Dominant in EU Needs regular light rainfall

Modelling Disease from Knowledge of Effector Sensitivities in Cultivars

Presence of Tsn1, Snn1 and Snn3 determined

Known unknowns

Three (and other) effectors may be present in some/all pathogen isolates

Effectors vary in activity (ToxA; Tox1 and Tox3; Tan 2012)
Effector genes vary in expression (Friesen 2011)
Recogniser genes vary in sensitivity (Snn3 and Snn1; Tan/Faris)

Interactions between effectors (ToxA and 1 with Tox3)
Effector responses modulated by undiscovered means

Parents: 8 recent UK varieties, selected in partnership with breeders

Variety	Reason for inclusion
Alchemy	Yield, disease resistance, soft feed type, breeding parent
Brompton	1BL/1RS, hard feed type, OWBM resistance
Claire	Slow apical development, soft biscuit/distilling type
Hereward	High quality benchmark Gp1 bread making type
Rialto	1BL/1RS, Gp2 moderate bread making type
Robigus	High yielding, soft biscuit/distilling type, OWBM resistance
Soissons	Early flowering Gp2 bread making type
Xi19	Facultative, high quality Gp1 bread making type