

DURES*Trit* - Functional characterisation
and validation of nonhost components in
Triticeae species for durable resistance
against fungal diseases.

1st Grant-holders Workshop
Rome June 12/13, 2014

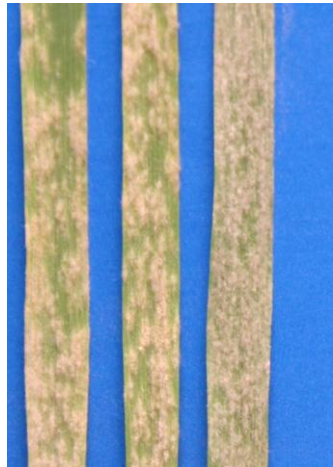
Wheat
(*T.aestivum*)

Barley
(*H.vulgare*)

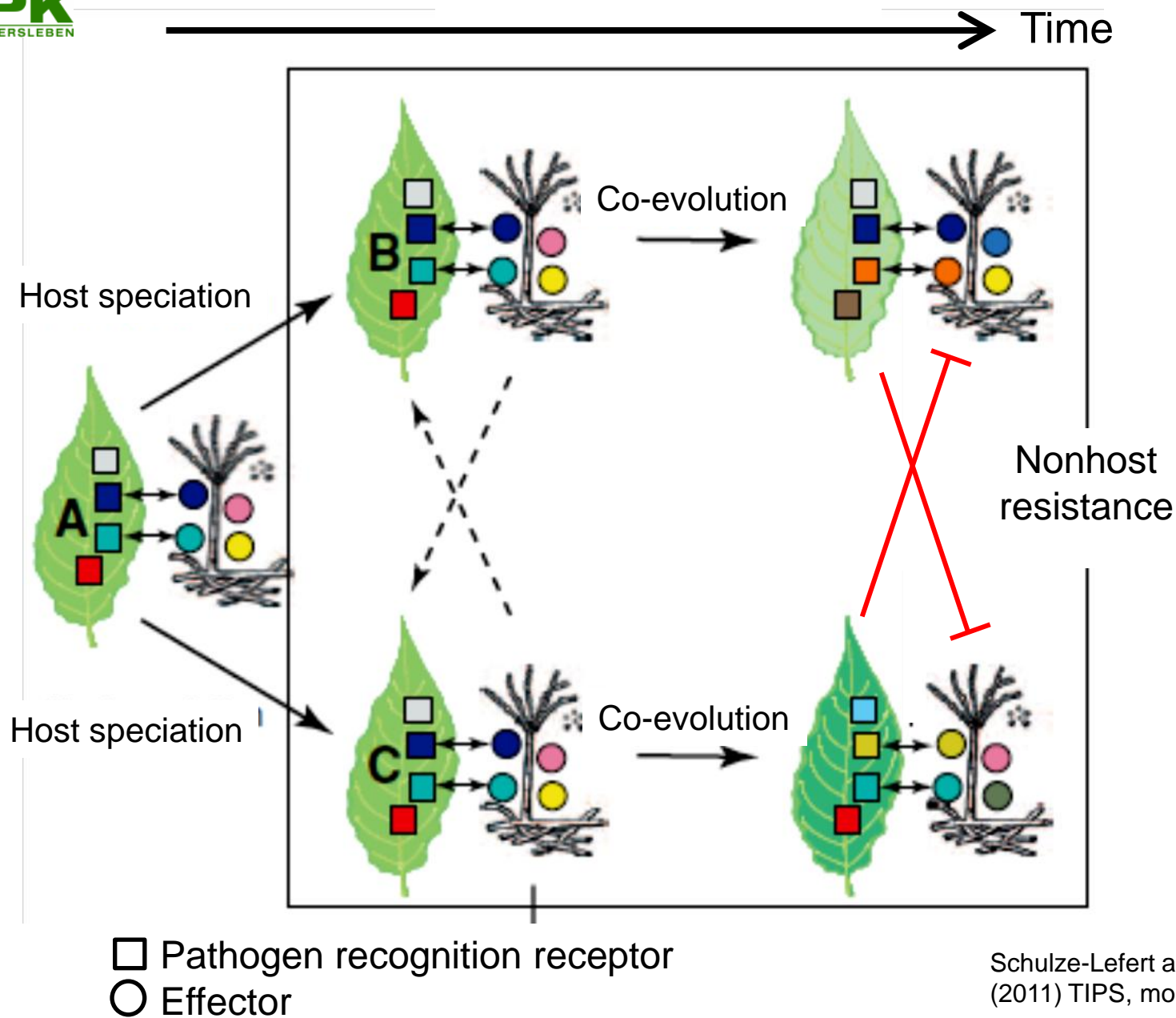
B.graminis
f.sp. *hordei*
(Bgh)

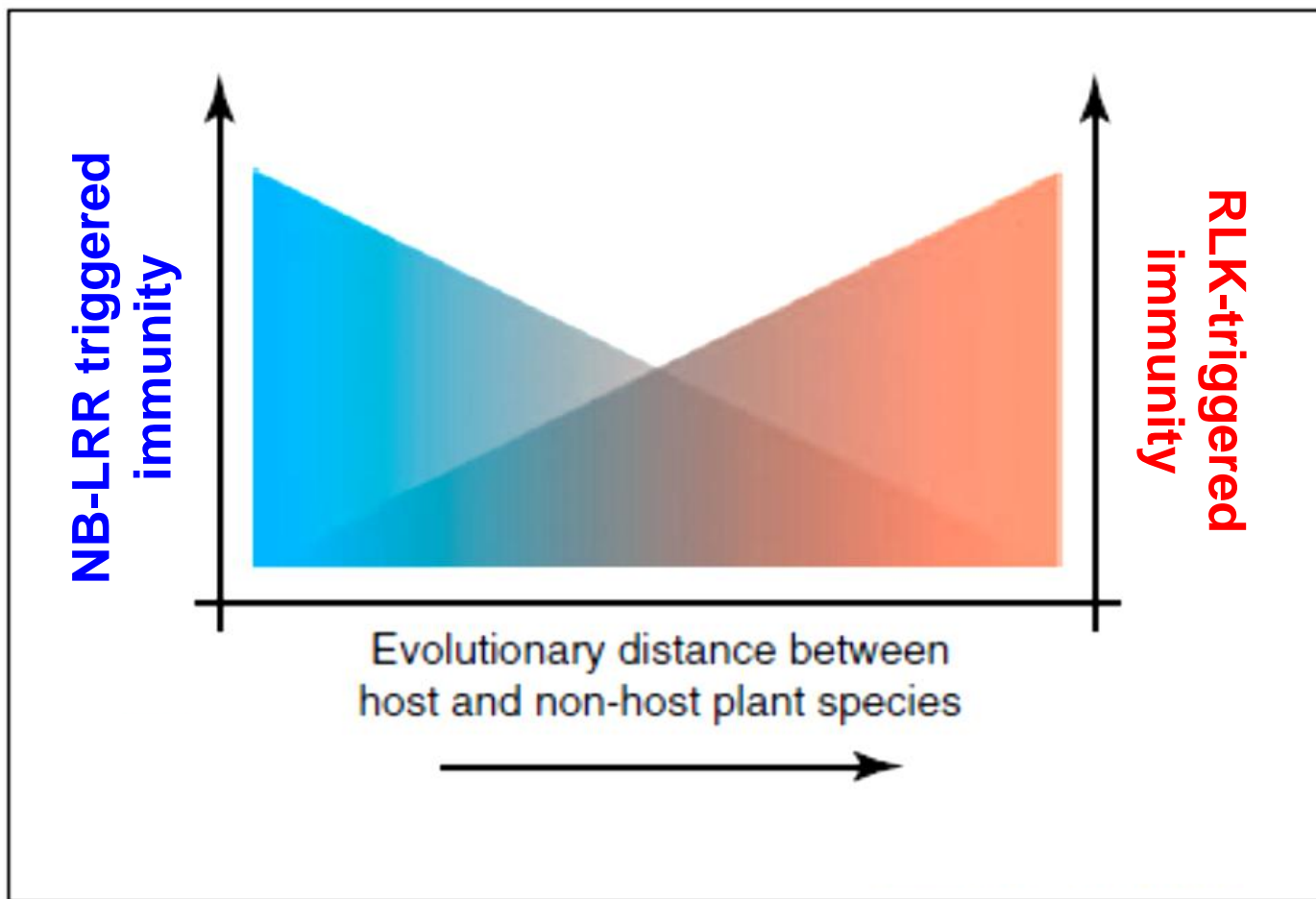


B.graminis
f.sp. *tritici*
(Bgt)



Nonhost resistance
(NHR) of wheat and
barley to non-adapted
powdery mildew fungi





The BIG questions:

- What are the important components of nonhost resistance (NHR) in *Triticeae* crops?
- Can we exploit NHR for conferring durable resistance to pathogens?

Matching funds...



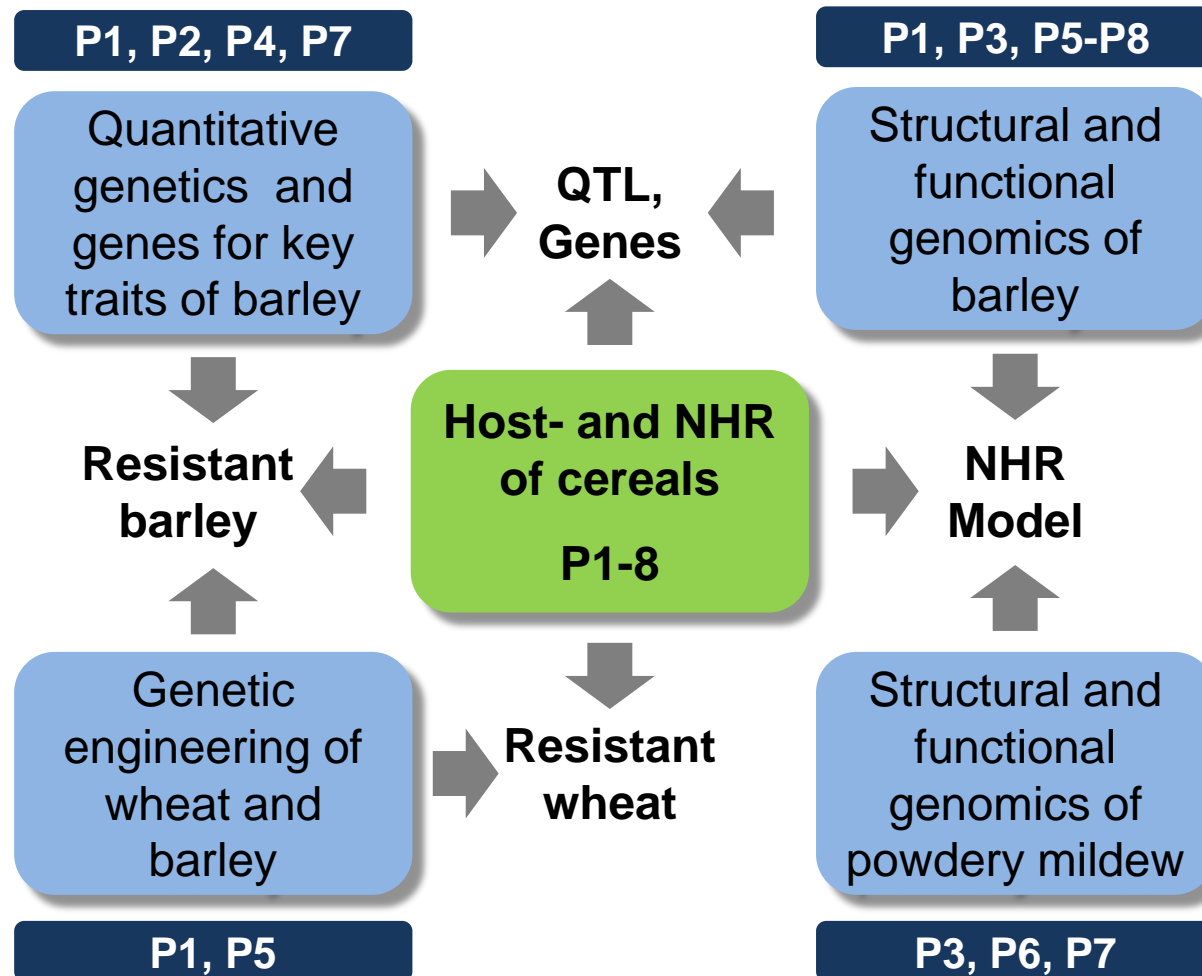
Functional Characterization and Validation of Nonhost Components in Triticea species for Durable Resistance against Fungal Diseases (DURESTrit)

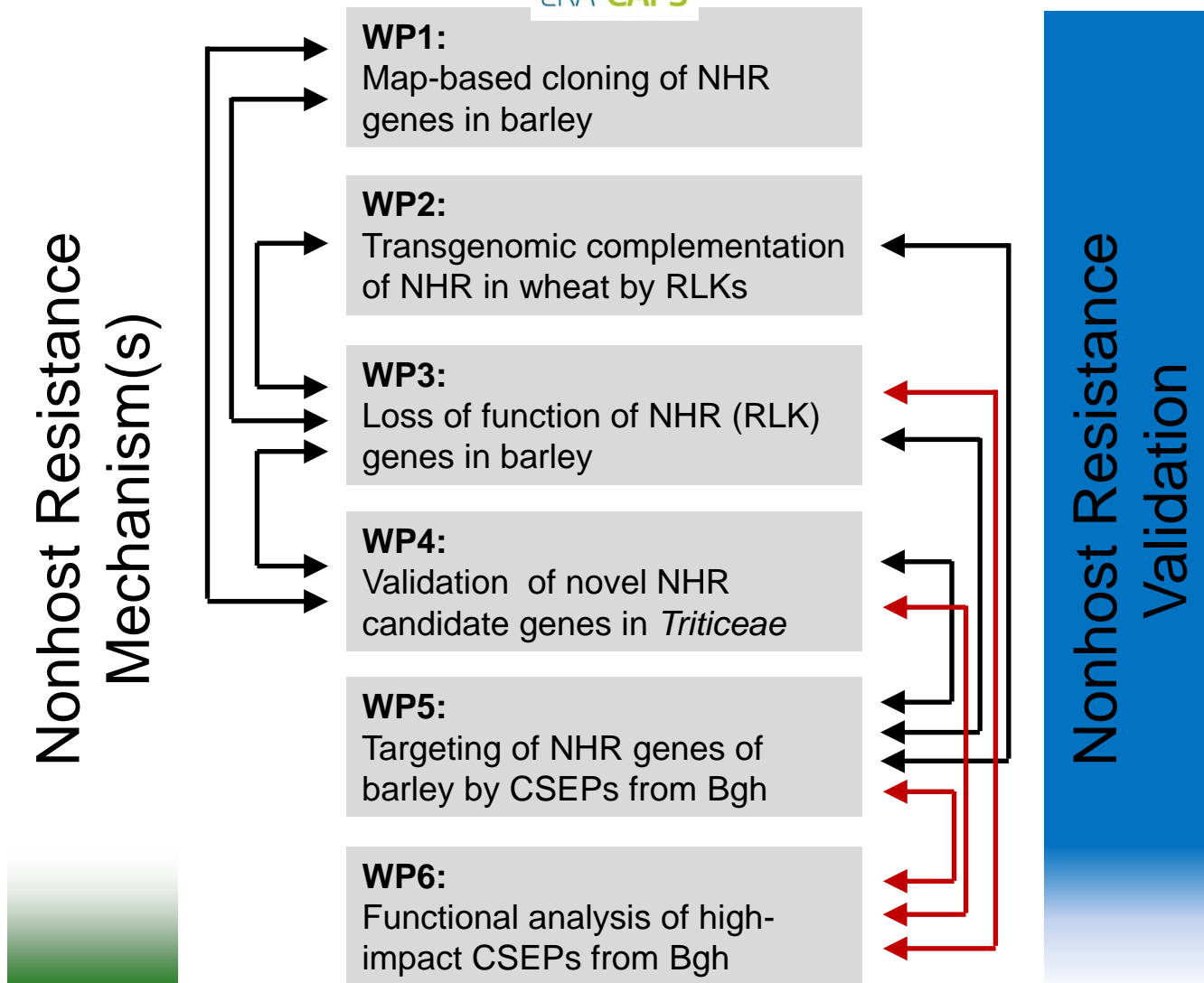
Patrick Schweizer, IPK, Germany (P1)
Jochen Kumlehn, IPK, Germany (P1b)
Nils Stein, IPK, Germany (P1c)
Pietro Spanu, Imperial College, UK (P6)
Brigitte Ruge-Wehling, JKI, Germany (P2)
Rients Niks, Wageningen, Netherlands (P4)
Ralph Panstruga, Aachen, Germany (P3)
Ulrich Schaffrath, Aachen, Germany (P3b)
Lesley Boyd, John Innes Centre, UK (P5)
Emma Wallington, NAIB, UK (P5b)

Host Targets of Fungal Effectors as Keys to Durable Disease Resistance

Roger Wise, USDA-ARS / ISU (P7)
Dan Nettleton, ISU (P7b)
Adam Bogdanove, Cornell (P8)
Roger Innes, Indiana
Fredy Altpeier, Univ. of Florida
Adah Leshem, ISU
Jacquelyn Jackson, Tuskegee

Key expertise and interaction among partners for major deliverables:





Black arrows: Interactions between ERA-CAPS-funded WPs.

Purple arrows: Interactions between ERA-CAPS and NSF-funded WPs

DURES *Trit*: Cherry picking from previous projects



GABI-nonhost
(2003-2006)



GABI-phenome
(2008-2011)



ERA-PG
TritNONHOST
(2009-2013)



Blumeria Haustoria -
Barley interactome
(2010-2013)

Barley TIGS screen:
Rnr1-10 for NHR to
Bgt (*Rnr8*=RLK)

Barley TIGS screen:
Further RLKs for
NHR and QR

(RNAi) & wt plants:
NHR-regulated
transcripts & vali-
dated *Rnr* genes

Bgh HIGS screen:
BECs required for
infection of barley

ERA-CAPS project DURES *Trit*

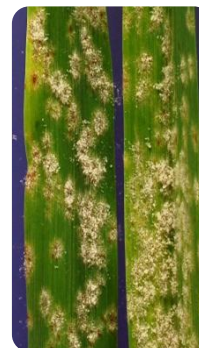
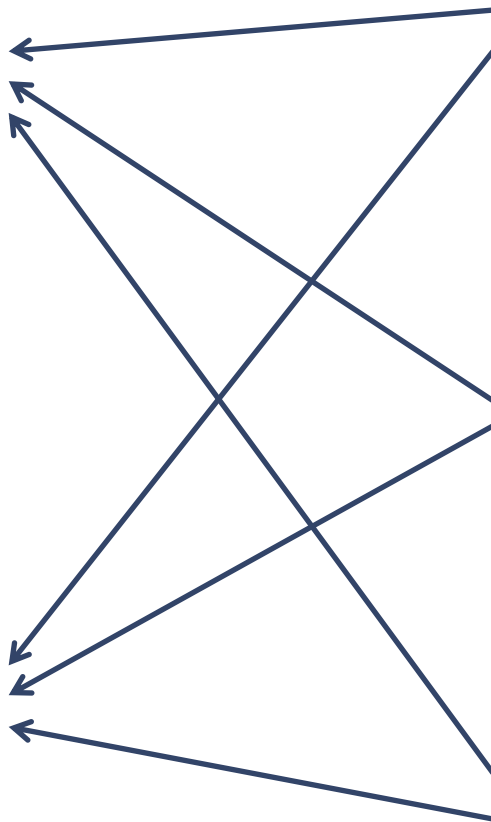
Transcriptome analysis in TritNONHOST



Wheat



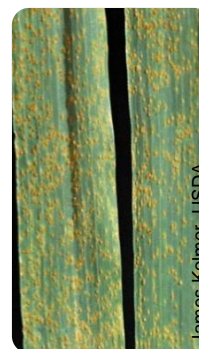
Barley



Powdery mildew

Blumeria graminis f. sp. *tritici*
host: wheat, nonhost: barley

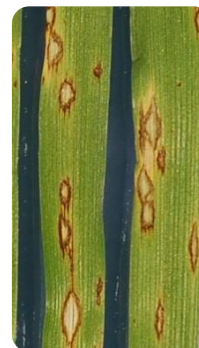
Blumeria graminis f. sp. *hordei*
host: barley, nonhost: wheat



Rust

Puccinia triticina
host: wheat, nonhost: barley

Puccinia hordei
host: barley, nonhost: wheat



Blast

Magnaporthe oryzae
host: wheat and barley

Magnaporthe grisea
nonhost: wheat and barley

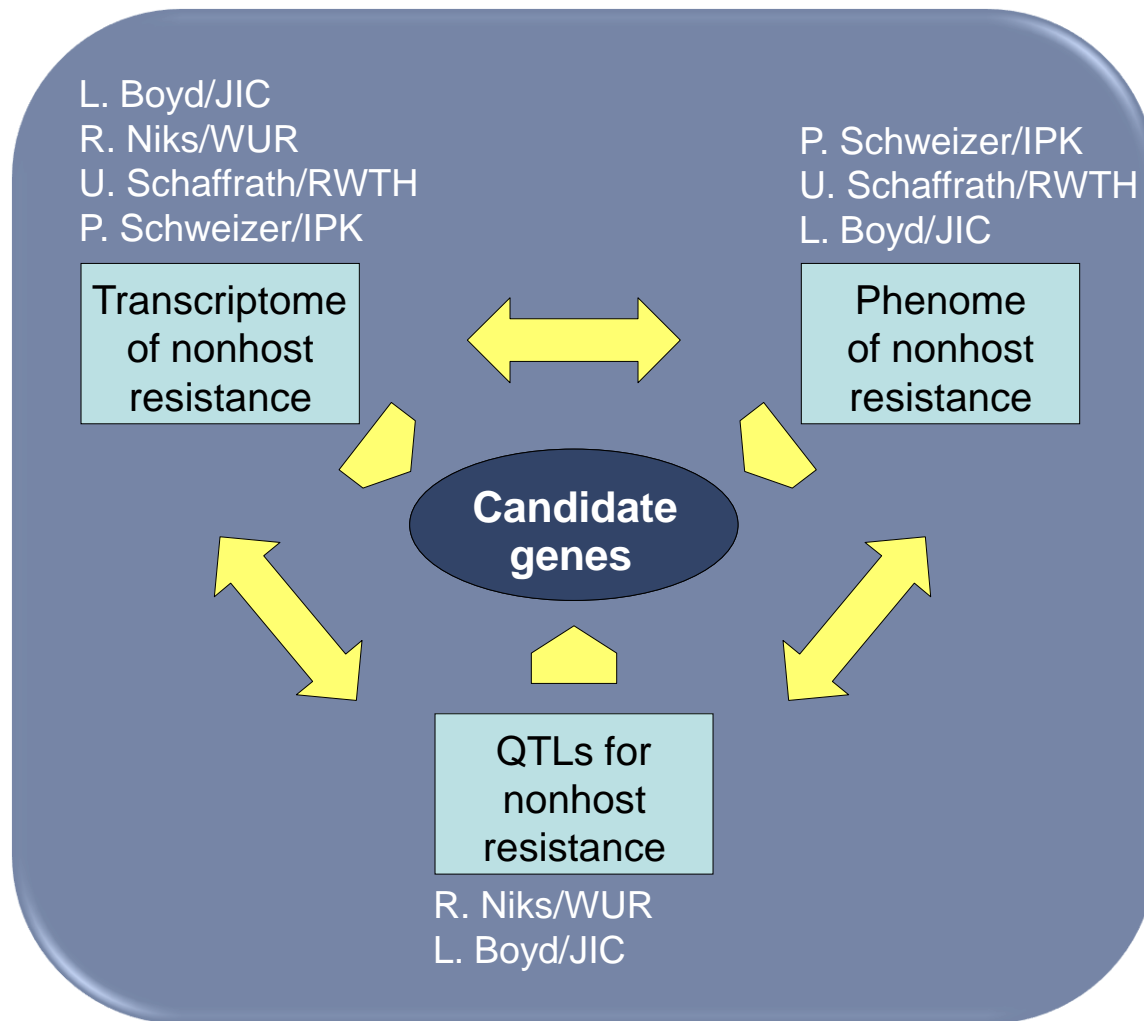
Motivation & Objectives of TritNONHOST



Wheat



Barley



WP1:

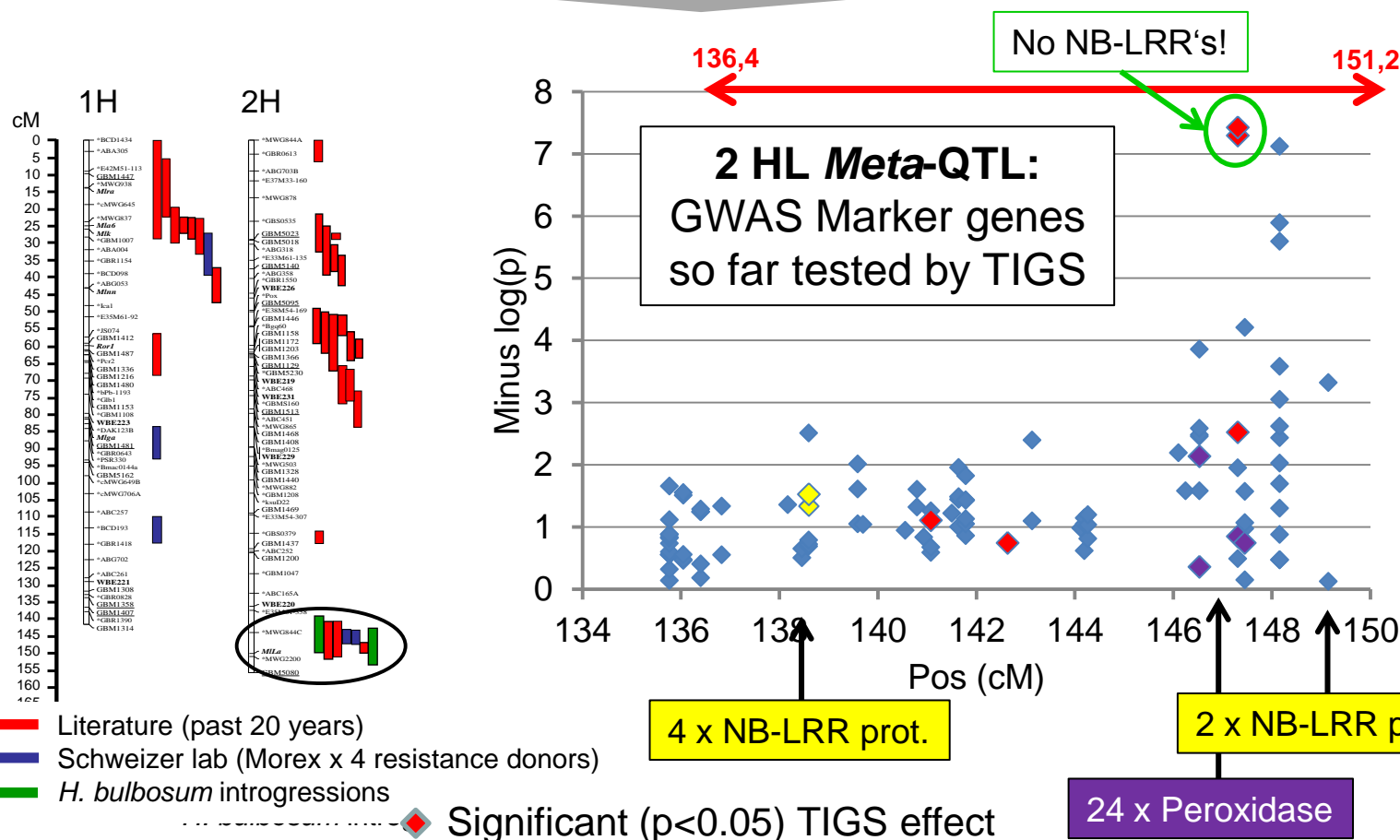
Map-based cloning of NHR genes in barley

Meta-QTL
mapping
(integrated)

GWAS 9K
iSelect
106 genes

Seq. capture
(POPSEQ)
535 genes

RNAi (TIGS) of
96 prioritized
genes (in progr.)



WP2:

Transgenomic complementation
of NHR in wheat by RLKs

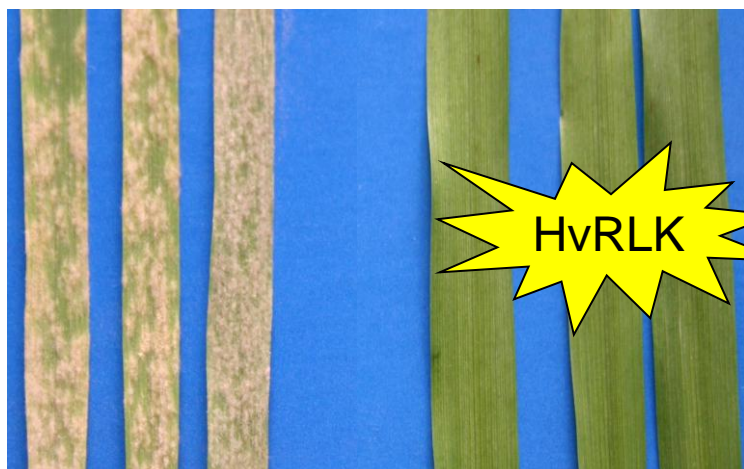
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Engineering of NH-like
resistance into wheat
by transgenomic
complementation of
barley BACs containing
RLKs?

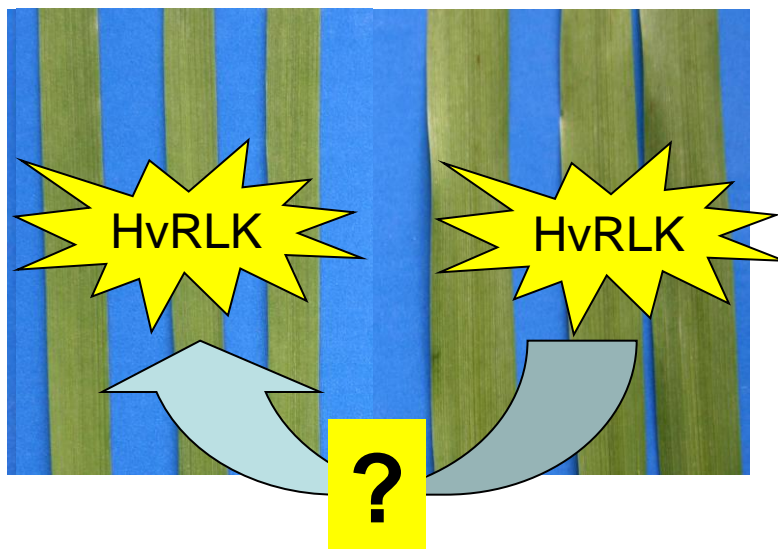
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Engineering of NH-like
resistance into wheat
by transgenomic
complementation of
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RLKs?

Barley RLK candidates from BAC complementation and TIGS screens for validation in DURESTrit:

Barley gene	Function (BlastX)	Relative Bgt infection (%)	Evidence from
None (control BAC)	n.a.	BAC: 100	
None (empty hairpin)	n.a.	Hairpin: 0	
HvRLK_2_3H_compl	TAK kinase-like	BAC: 51,0* Subclone: 53,8**	Transient expression of BAC and BAC-subclone in wheat
HvRLK_7_compl	LRR receptor-like kinase	BAC: 62,9* Subclone: 67,2**	Transient expression of BAC and BAC-subclone in wheat
HvRLK_10_compl	Cystein rich repeat (DUF26) kinase	BAC: 66,9* Hairpin: 6,6*	Transient expr. of BAC in wheat and transient RNAi in barley
RNR8	LRR receptor-like kinase	BAC: 62,8** Subclone: 46,4*** Hairpin: 7,9*	Transient expression of BAC and BAC-subclone in wheat, plus transient and stable RNAi in barley

WP3:

Loss of function of NHR (RLK)
genes in barley

Transgenic loss-of-function barley:

	Barley transgenic genotypes (GT)							
Transgene	GT1	GT2	GT3	GT4	GT5	GT6	GT7	GT8
RNR8 TALEN	+			+				
RNR8 RNAi		+						
TaRNR8 transgenomic compl.			+	+				
HvRLK_10_compl TALEN					+			+
HvRLK_10_compl RNAi						+		
(TaRLK_10_compl) transgenomic compl.							+	+



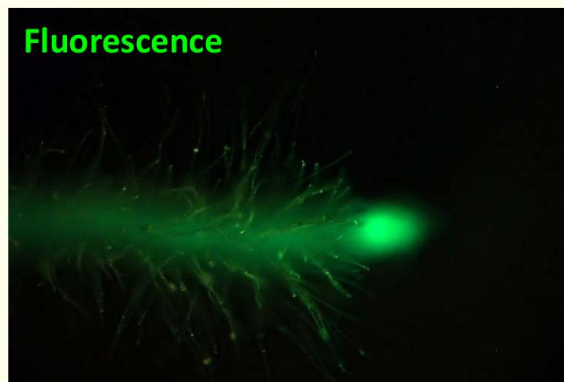
Already available/in progress at IPK

- Plant development
- Responses to adapted (Bgh) and non-adapted (Bgt) PM
- Optional: Responses to other pathogens

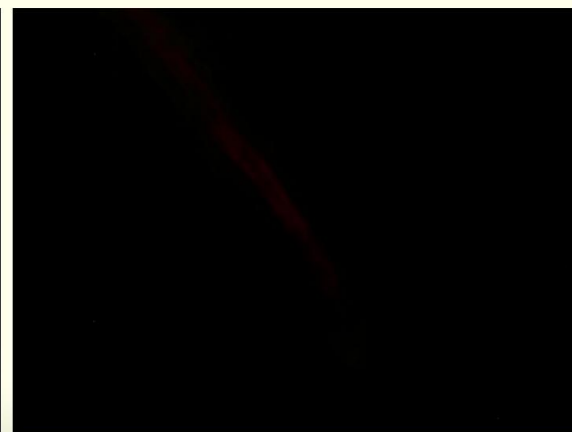
Identification and characterization of GFP knock-out plants

Maia Gurushidze

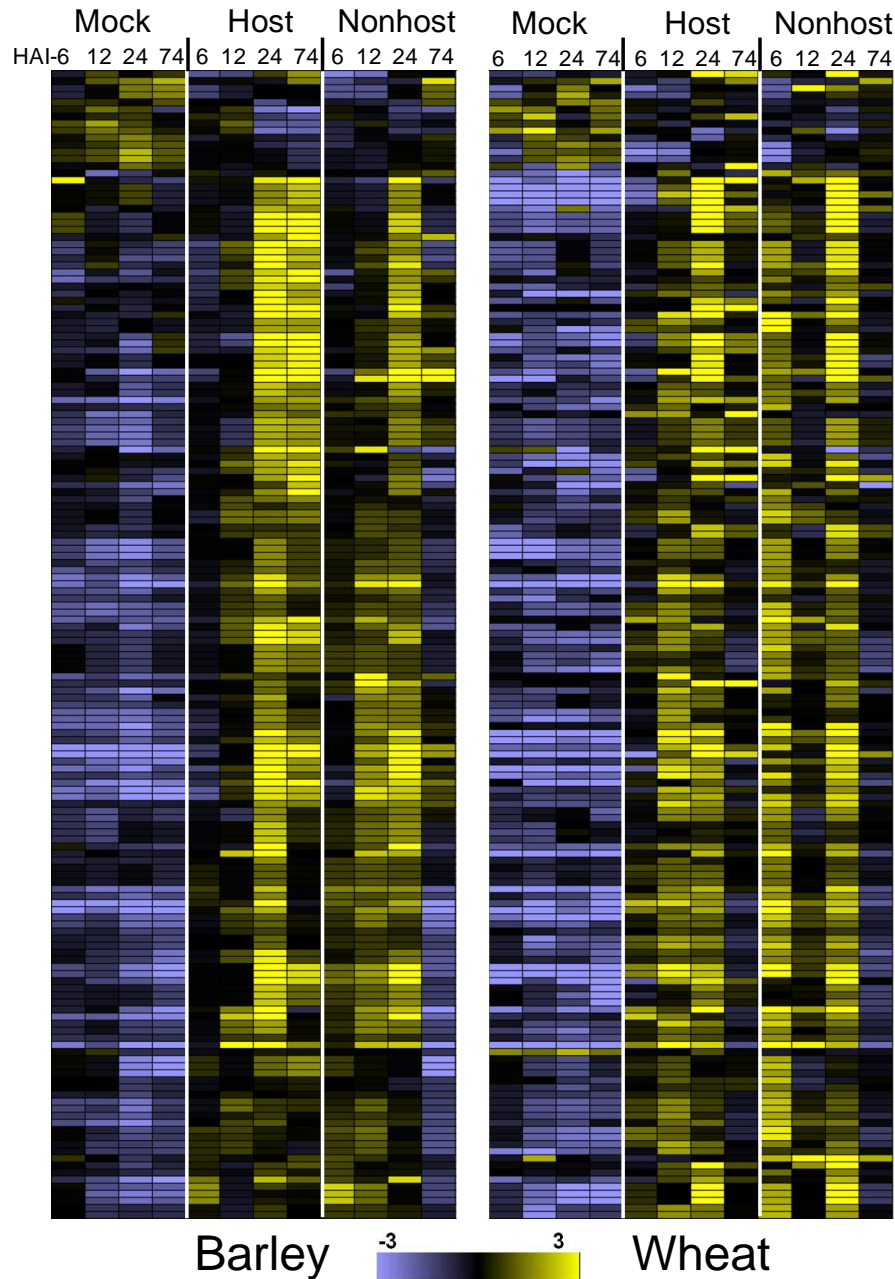
GFP transgenic line
(positive control)



GFP transgenic line
re-transformed
with *GFP*-TALENs



WP4:
Validation of novel NHR
candidate genes in *Triticeae*



162 Ortholog-matched barley and wheat genes differentially regulated host/NH:

→ Co-regulation in wheat and barley!

→ „Food“ for a VIGS-based gene-discovery approach

WP5:

Targeting of NHR genes of
barley by CSEPs from Bgh

DURESTrit

- Establish Gateway-compatible **library of ca. 500 *Bgh* effector candidates** (gene synthesis – in collaboration with Pietro Spanu) → general resource
- Test interactions between effector candidates and selected „nonhost defence proteins“ (receptor-like kinases etc.) in **yeast two-hybrid** and **yeast split ubiquitin** assays (alternative: library screens)
- Validate interactions by Bimolecular Fluorescence complementation (**BiFC**), overexpression and Host-Induced Gene Silencing (**HIGS**)
- Characterize protein-protein interaction by **site-directed mutagenesis**

WP6:

Functional analysis of high-impact CSEPs from Bgh

BSMV-VIGS of BEC1019

HOR11358 (*Mla9*)

- 1st leaf w/BSMV
- *Bgh* 5874 (*avr_{a9}*)
- Phenotype 3rd leaf
- 4 replications

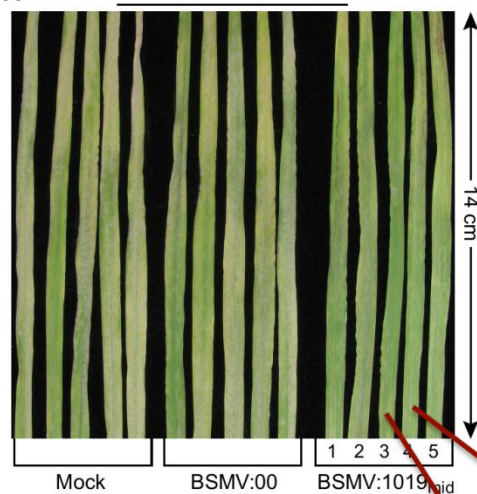


Ehren Whigham

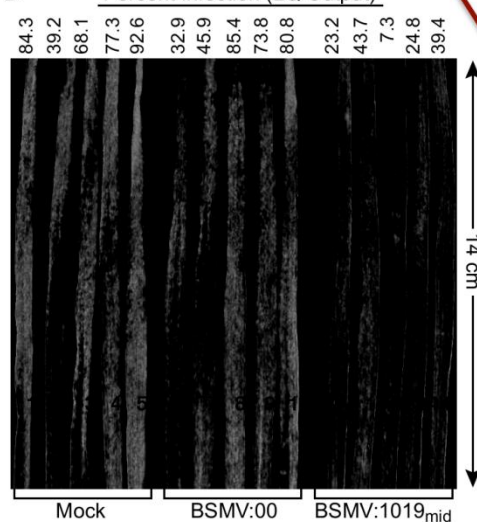


Divya Mistry

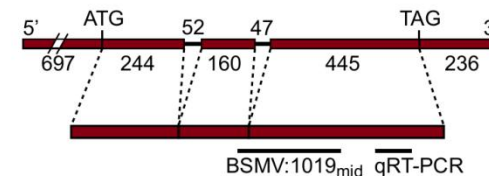
A HOR 11358 Third Leaves



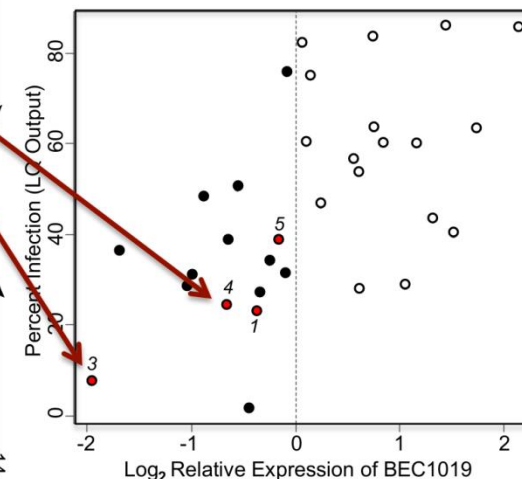
B Percent Infection (LQ Output)



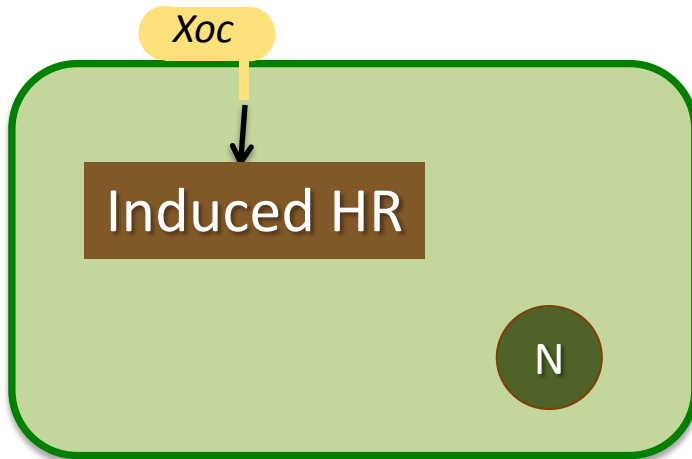
C



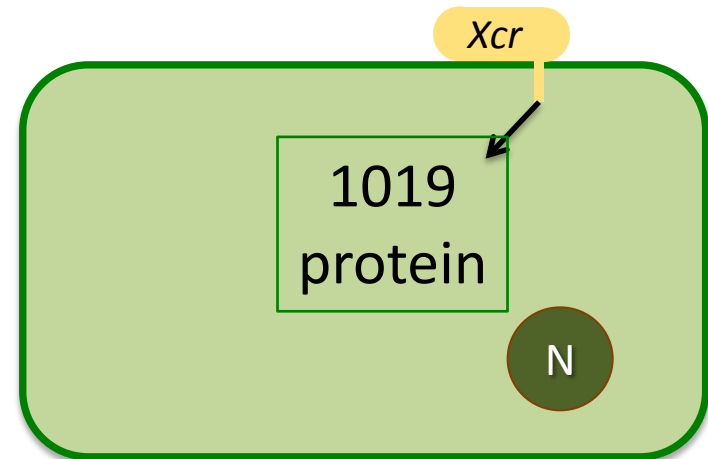
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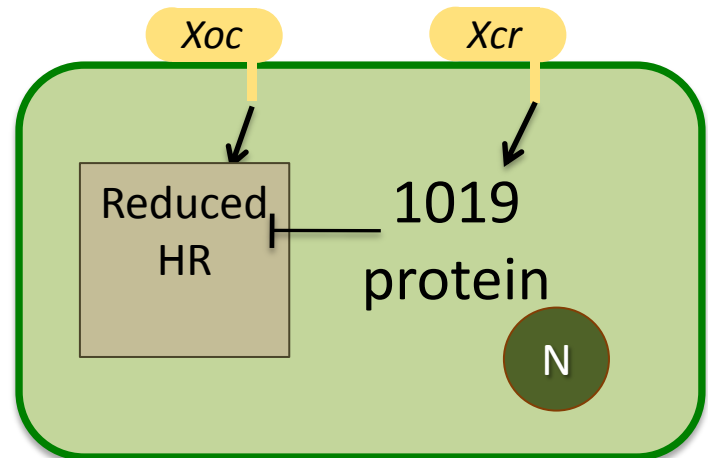
Xanthomonas T3S Delivery of BEC1019



Xoc induces HR-like symptoms
(on barley)

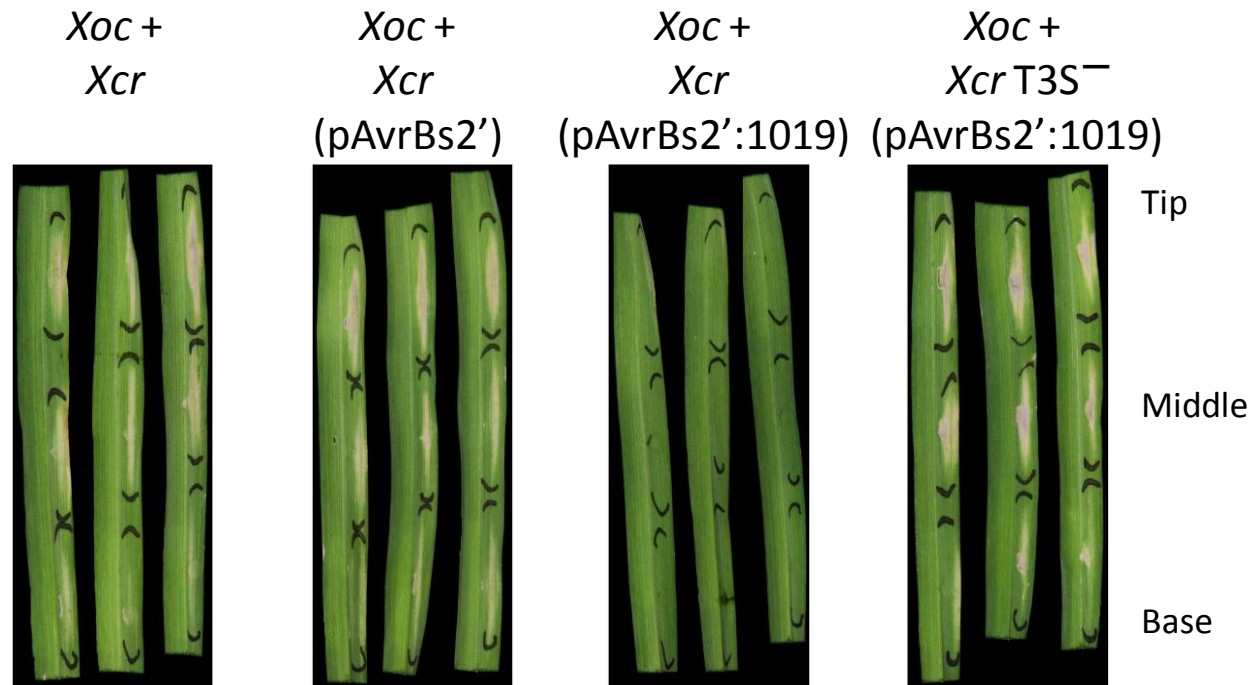


Xcr T3S delivers BEC 1019 gene product;
No observable phenotype



Reduced HR-like symptoms caused by *Xoc*

BEC1019 Suppresses *Xoc*-Induced Cell Death



- Host Genotype: CI 16151 (*Mla6*)
- *Xoc* (OD 0.02) co-infiltrated with *Xcr* (OD 0.4) via syringe

Shan Qi

Expected outputs:

- Map-based cloning of 1-2 major QTL on chromosome 2H for NHR in barley derived from the near nonhost species *H. bulbosum* plus 1-2 QTL from NHR-compromised experimental barley lines (IPK, JKI, WUR)
- Introgression of three genes encoding NHR-mediating receptor-like kinases (RLKs) from barley into transgenic wheat leading to information about their roles in plant developmental as well as biotic stress resistance (NIAB, IPK)
- Further validation of the proposed role of these plus additional RLK candidates by RNAi and TALEN-mediated mutagenesis in transgenic barley attacked by Bgh and Bgt (IPK)
- Discovery of additional components of NHR in barley by transient gene silencing (RWTH, IPK, WUR)
- Discovery of molecular interactions of Bgh effector-like proteins and host targets in barley, and functional data obtained by transient gene silencing in both plant and pathogen (ICL, RWTH, ISU, CU)

Thank you!