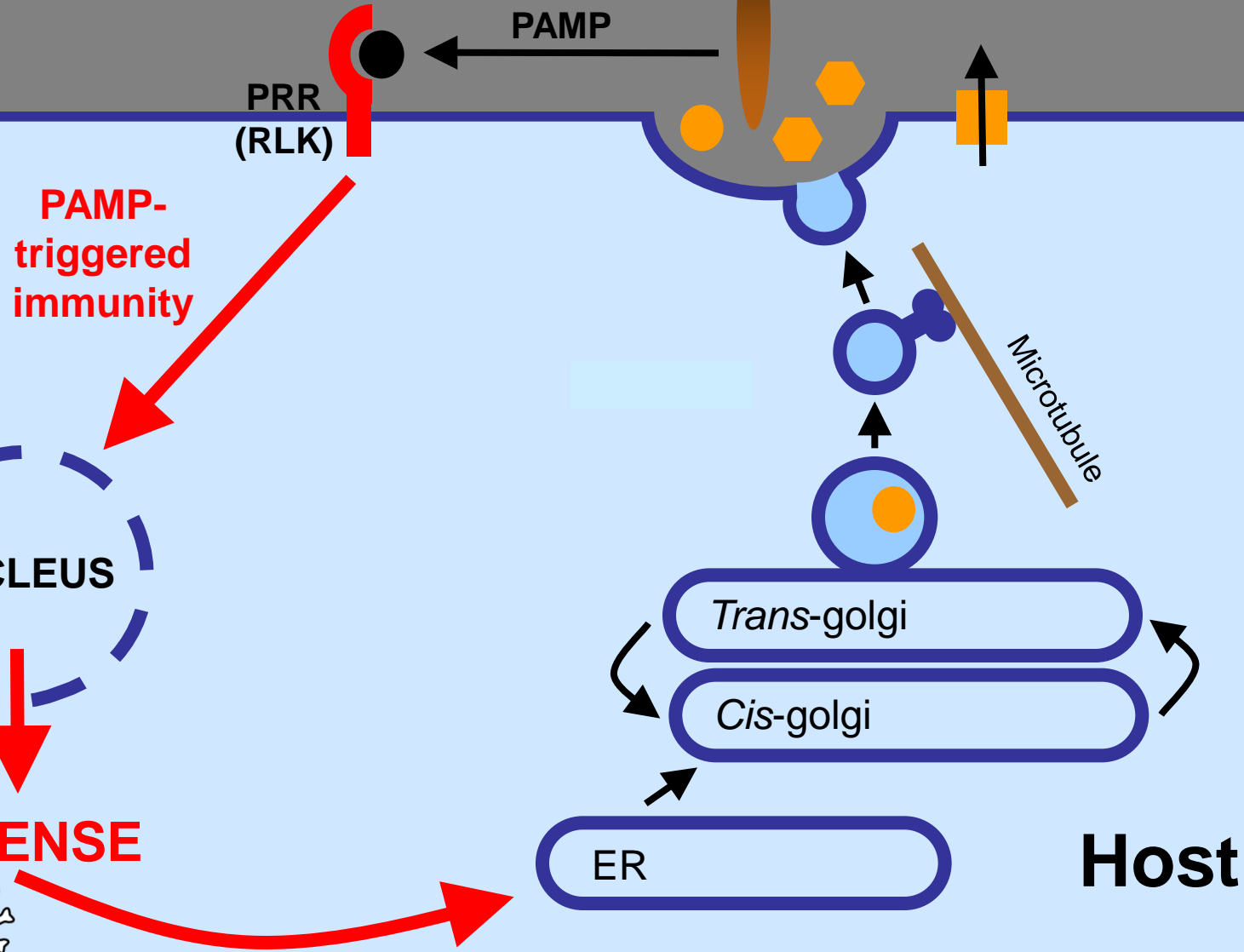


# **DURES***Trit* - Functional characterisation and validation of nonhost components in *Triticeae* species for durable resistance against fungal diseases.

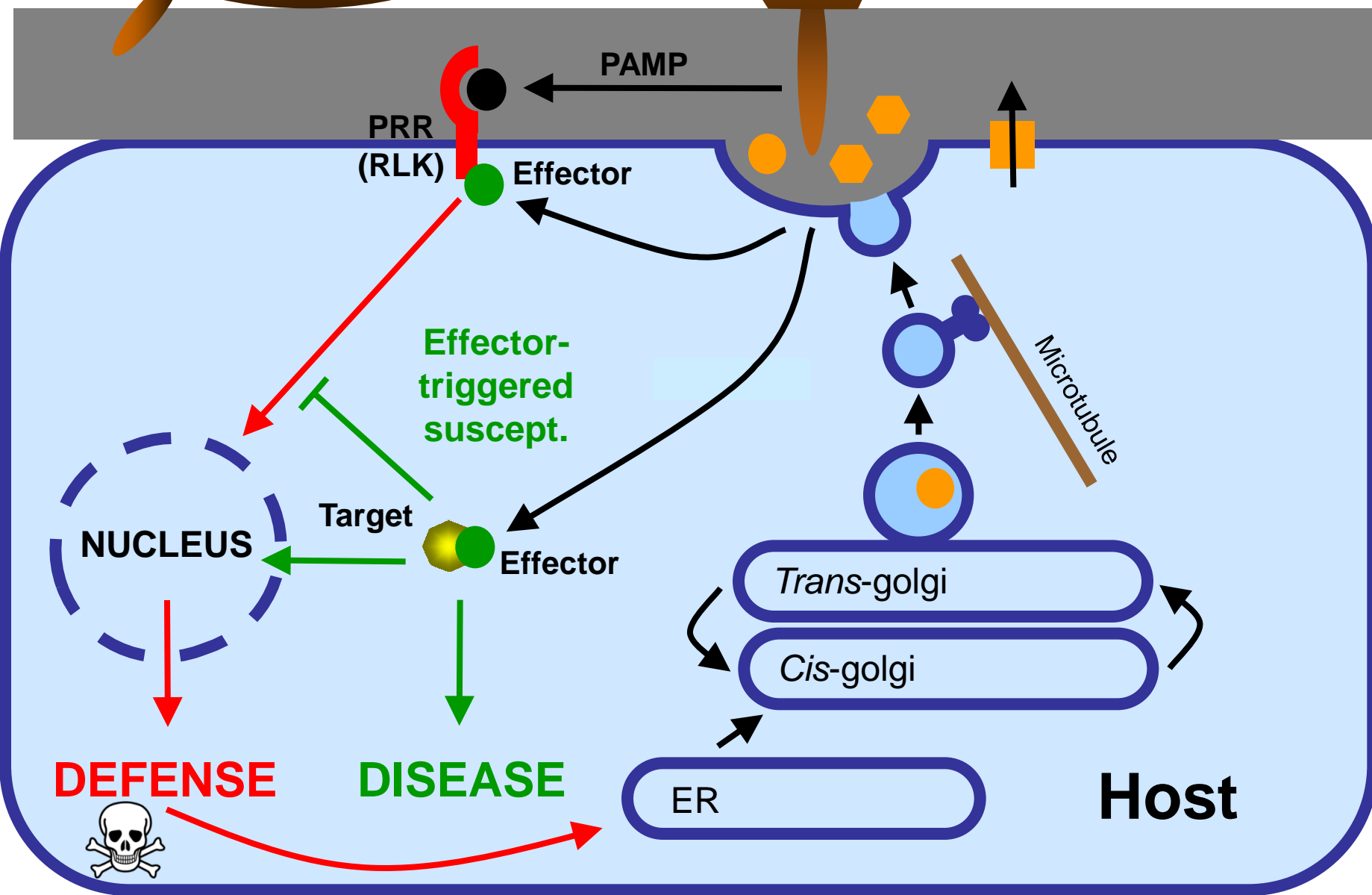
Patrick Schweizer

2nd Grant-holders Workshop  
Lisbon, May 2015

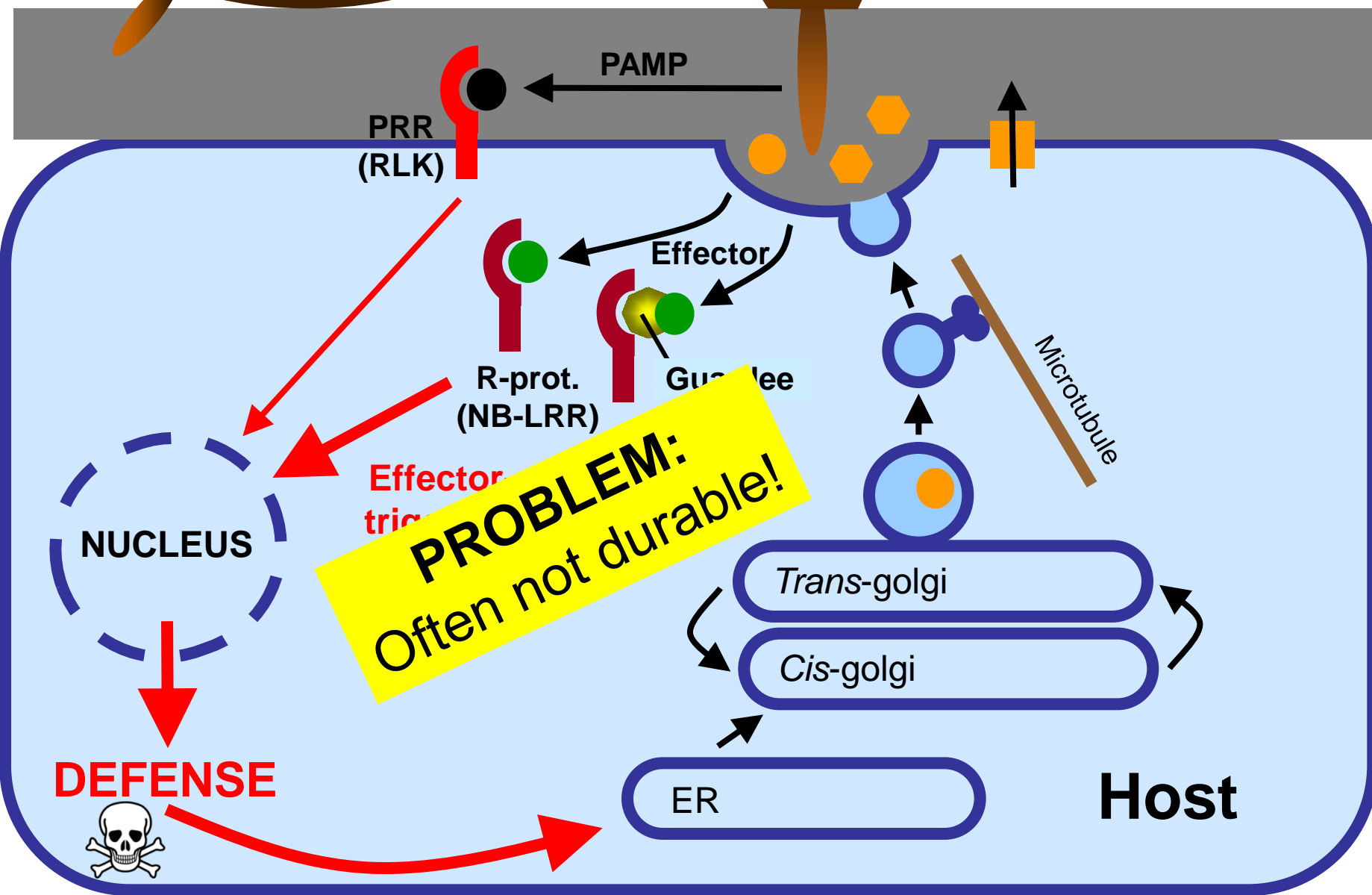
**Pathogen**



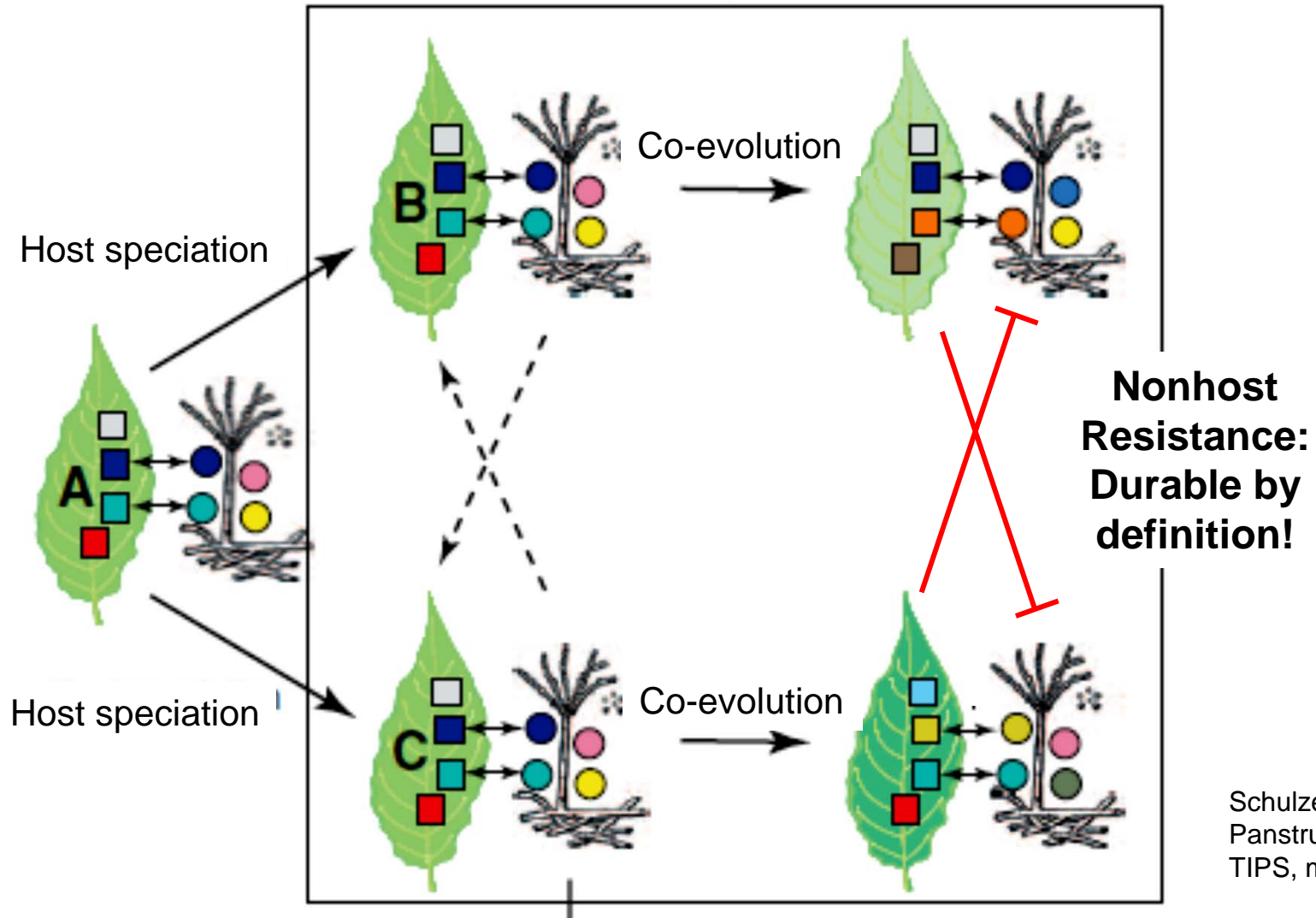
# Pathogen



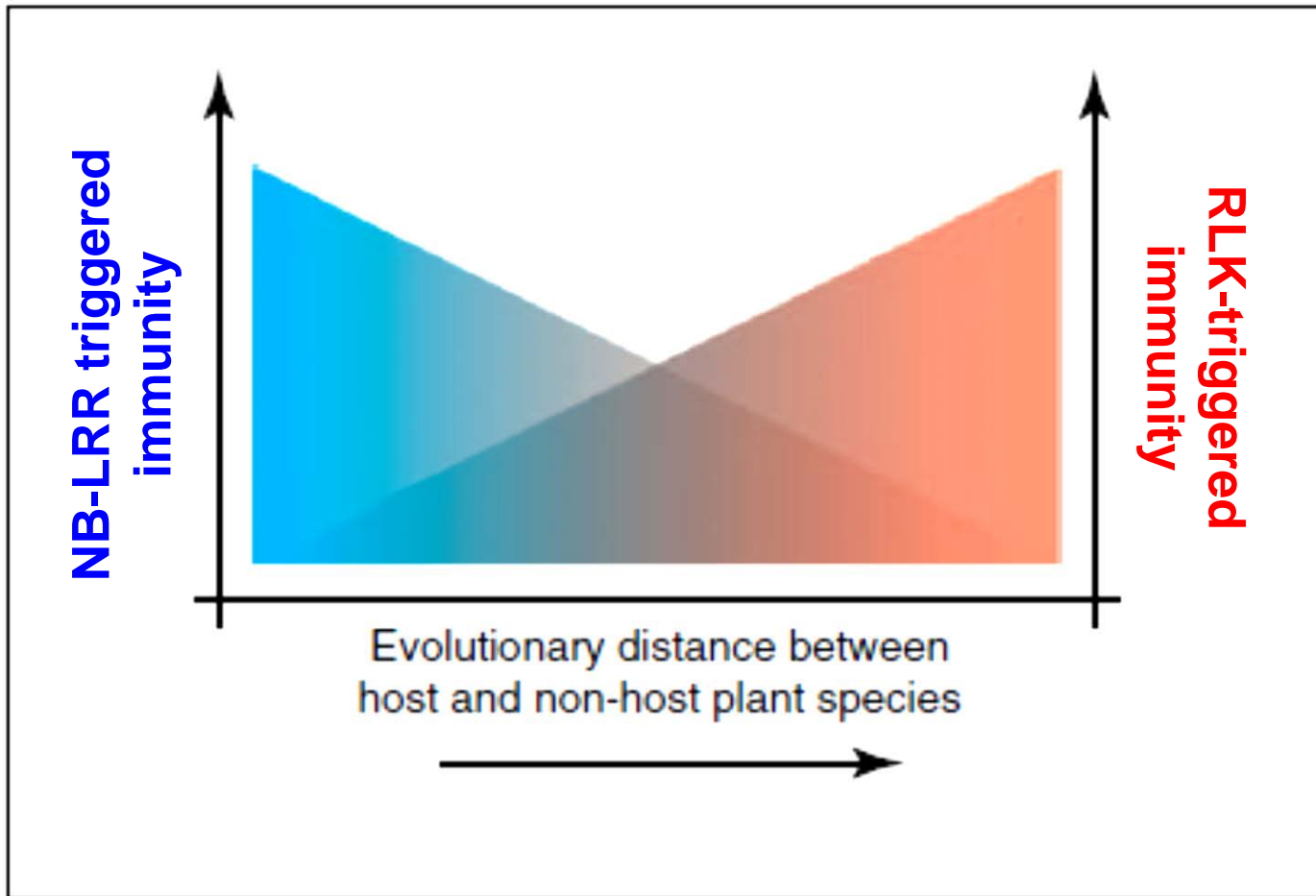
# Pathogen



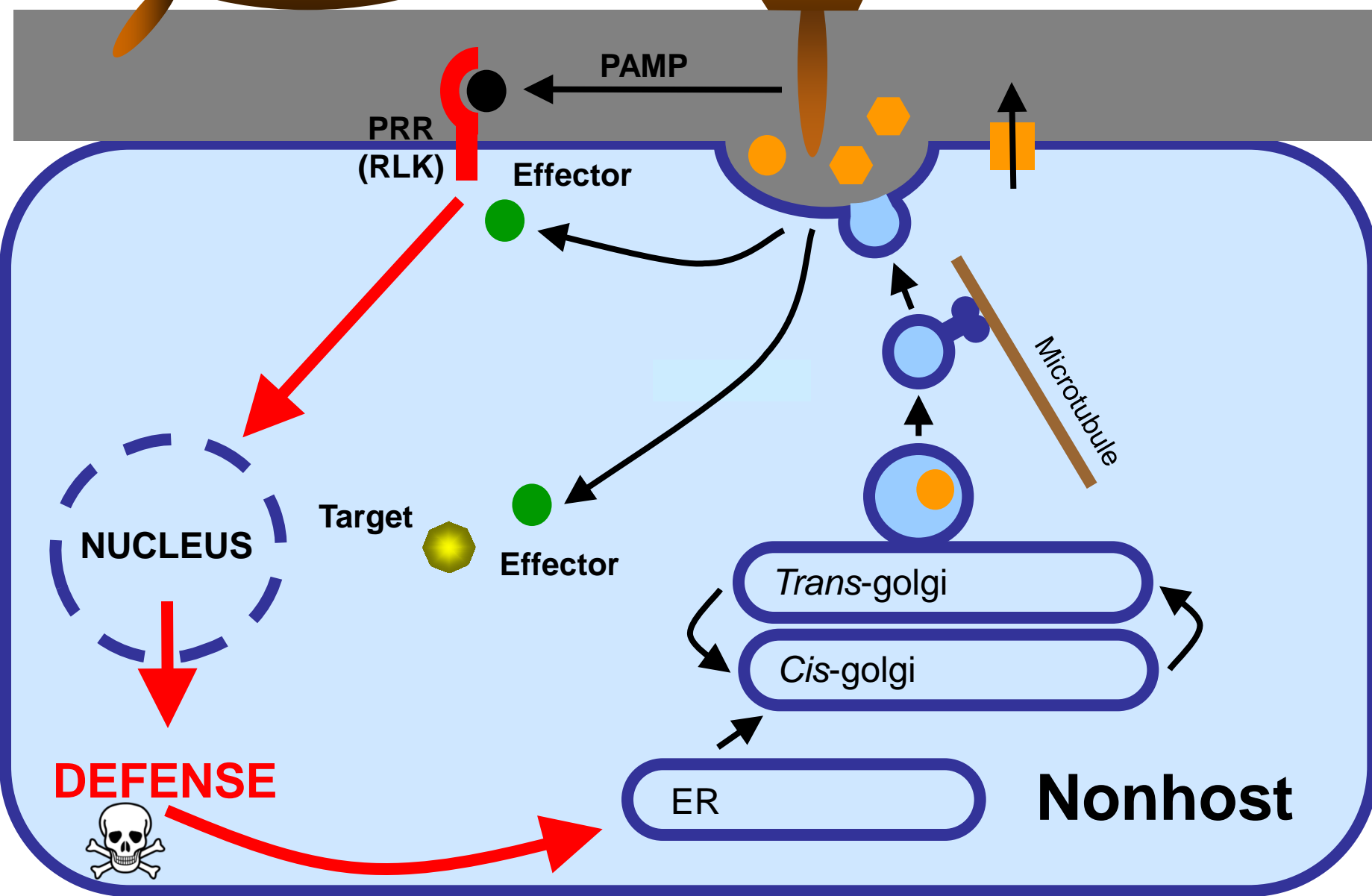
# Nonhost resistance – following host speciation and plant/pathogen co-evolution...



# Nonhost resistance – likely triggering mechanisms



**Pathogen**



## Main objectives of DURESTrit:

- Important components of nonhost resistance (NHR) in *Triticeae* crops to pathogenic fungi?
- Can we exploit NHR for conferring durable resistance to pathogens?
- Special emphasis on pathogen-recognition receptors (RLKs) because of previous RNAi results (GABI-nonhost and GABI-phenome projects)



Adapted and non-adapted powdery mildew fungi  
(*Blumeria graminis*) attacking *Triticeae* crop species  
barley and wheat – A model system for NHR



## Matching funds...



### **Functional Characterization and Validation of Nonhost Components in *Triticeae* Species for Durable Resistance against Fungal Diseases (DURESTrit)**

**P1:** IPK Gatersleben (Patrick Schweizer (coord.), Jochen Kumlehn, Nils Stein)

**P2:** JKI (Brigitte Ruge-Wehling)

**P3:** RWTH Aachen (Ralph Panstruga, Ulrich Schaffrath)

**P4:** University Wageningen (Rients Niks)

**P5:** NIAB Cambridge (Lesley Boyd, Emma Wallington)

**P6:** Imperial College London (Pietro Spanu)

### **Host Targets of Fungal Effectors as Keys to Durable Disease Resistance**

**P7:** USDA-ARS / ISU Ames (Roger Wise, Roger Innes, Dan Nettleton)

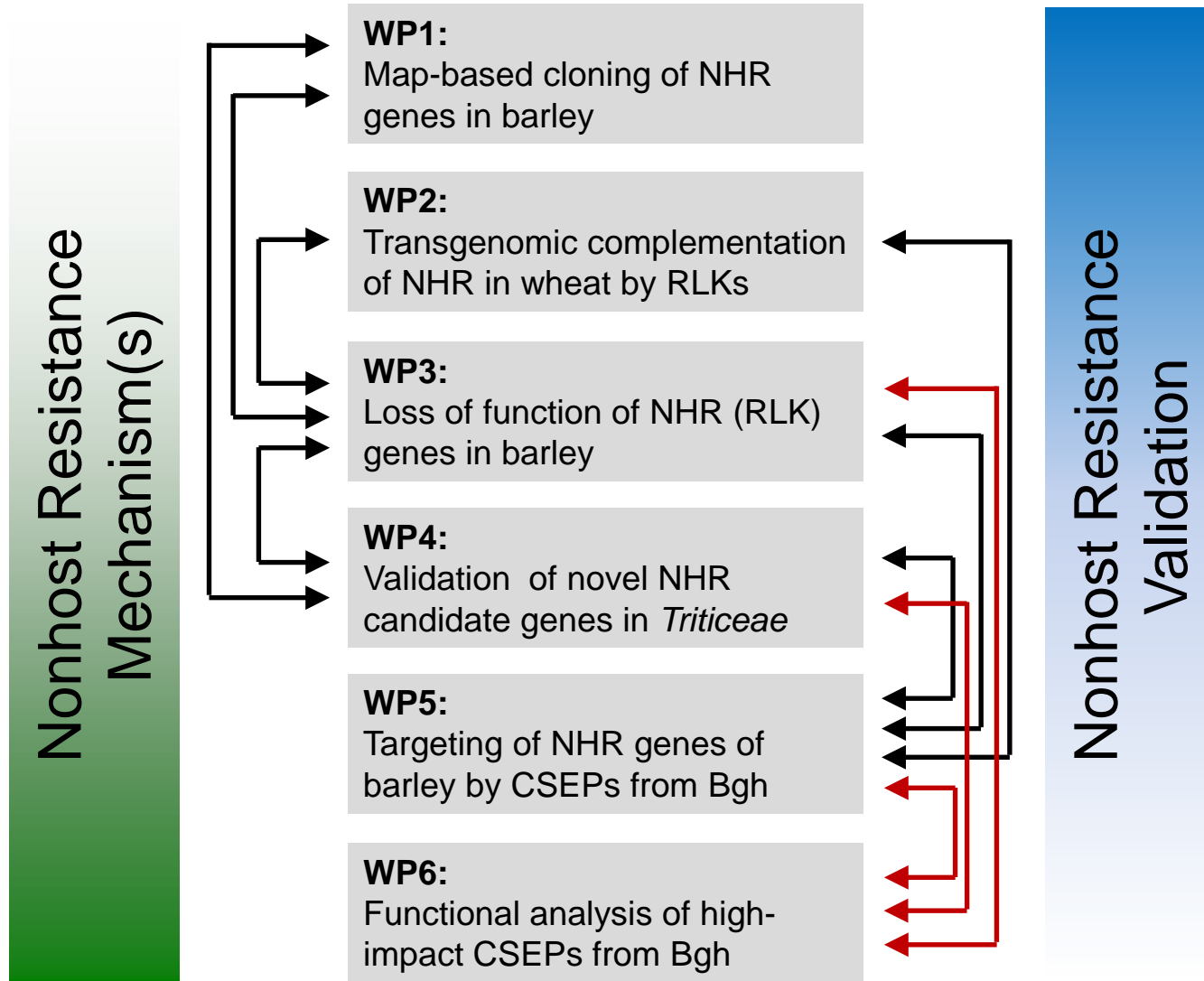
**P8:** Cornell University (Adam Bogdanove)

#### **Further PI's:**

Fredy Altpeter, Univ. of Florida

Adah Leshem, ISU

Jacquelyn Jackson, Tuskegee



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

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

# Background data and progress so far...

## **WP1:**

Map-based cloning of NHR  
genes in barley

# Barley and its relatives

Autogamous

Diploid ( $2n=2x=14$ )

Genome size: 5.1 Gbp

>80% repetitive DNA

3 gene pools

## Primary gene pool

*H. vulgare* L. ssp *vulgare*

*H. vulgare* L. ssp *spontaneum*

## Secondary gene pool

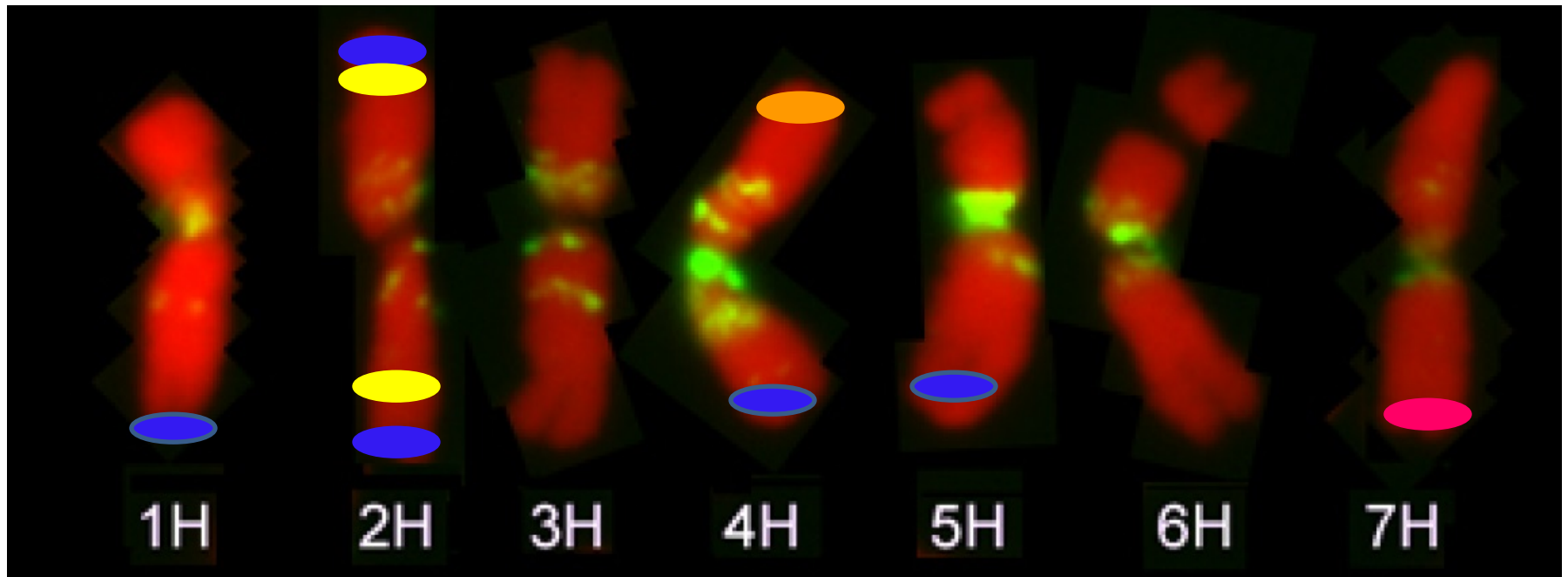
*H. bulbosum* L.

## Tertiary gene pool

~ 30 species



# Fungal Resistance Genes Transferred from *H. bulbosum* to Barley



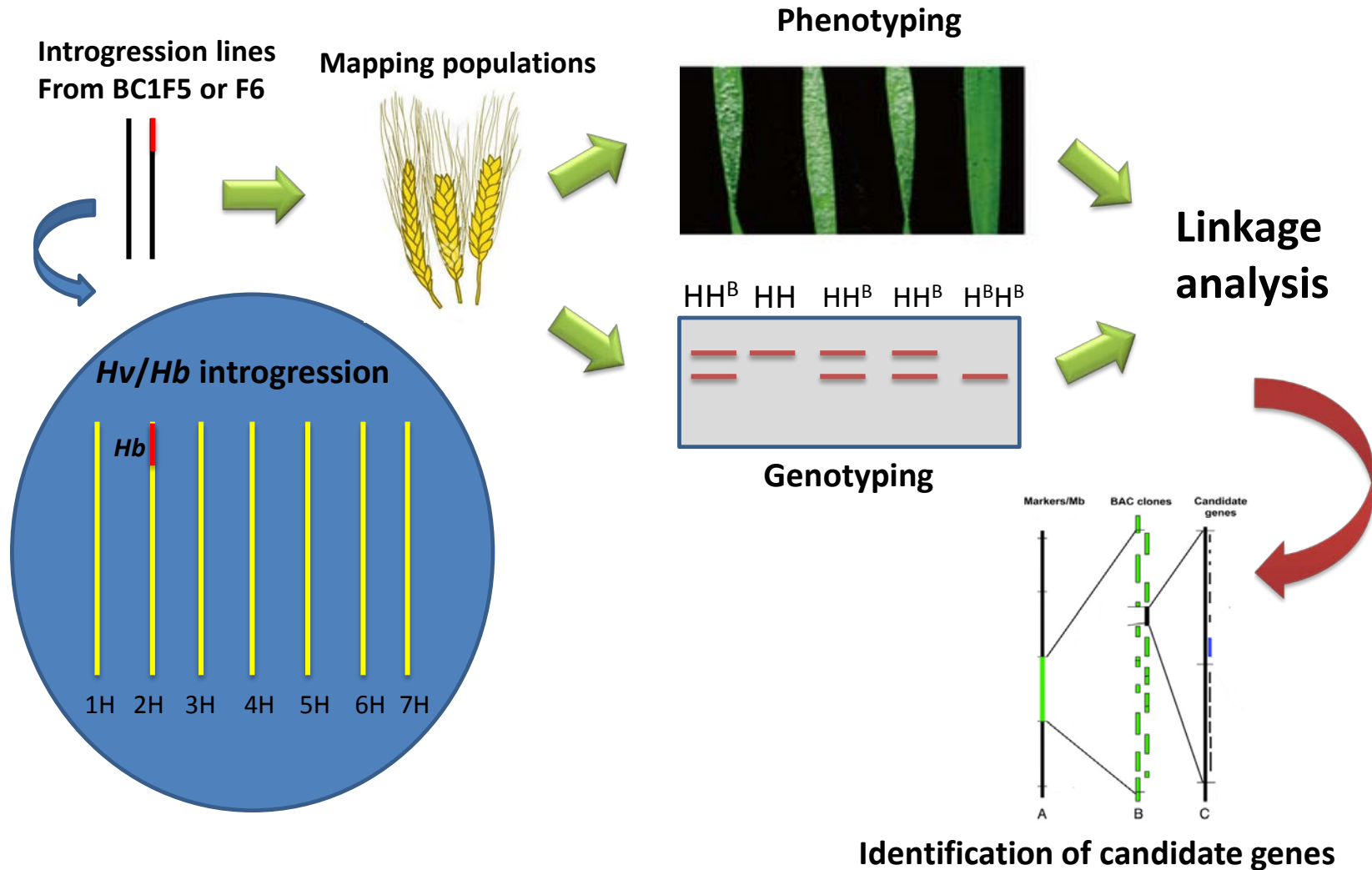
Leaf Rust

Powdery Mildew

Scald

Stem Rust

# Fine-mapping pipeline of *H. bulbosum* introgression lines





Recombination event →

BC1F6

Backcrossed

F7

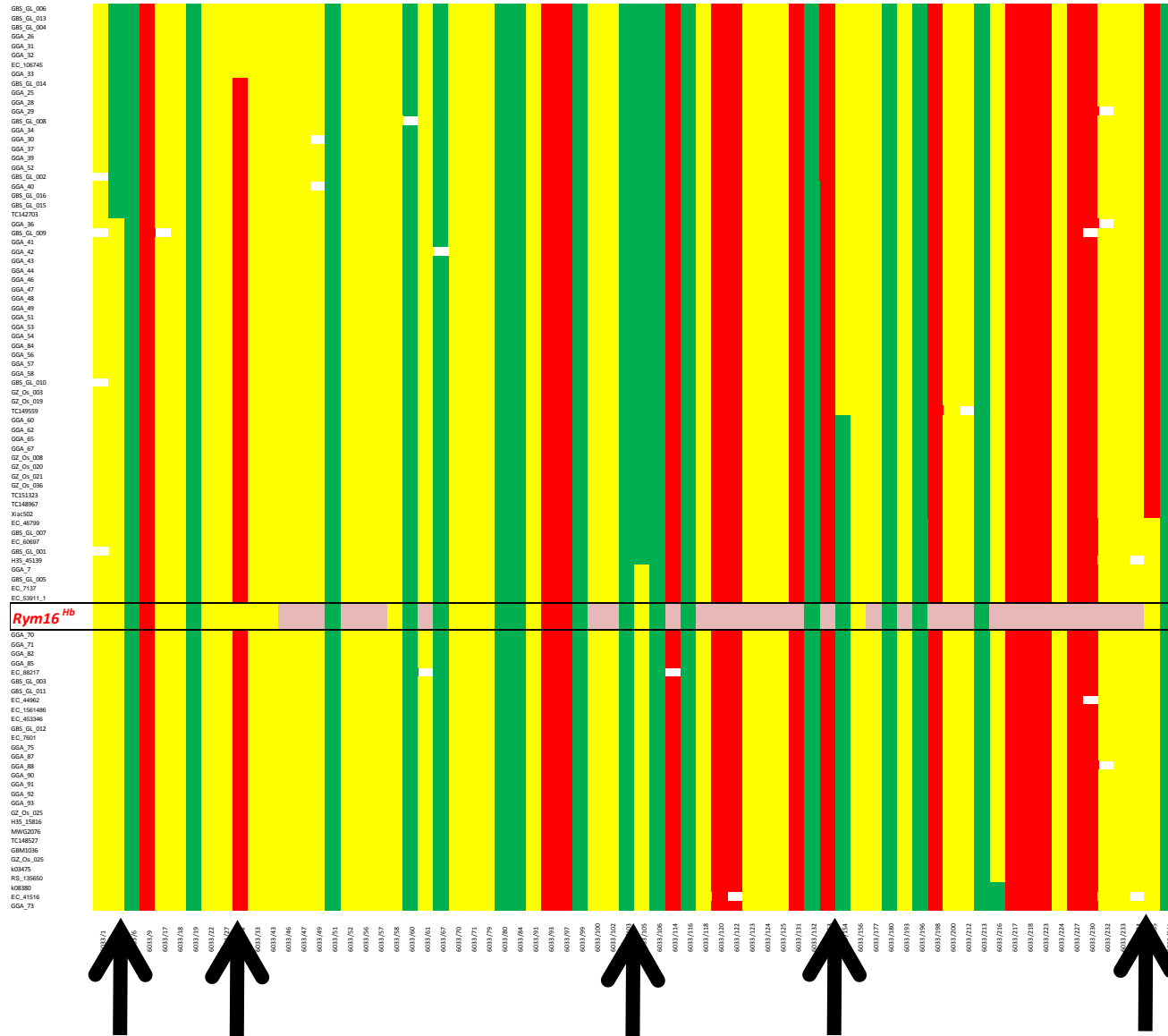
Selfed

| Mbp | Genotype ID | CM2    | M5     | M6     | CM5    | CM6   | CM8    | CM9  | CM11   | CM15   | CM18   | CM19   |
|-----|-------------|--------|--------|--------|--------|-------|--------|------|--------|--------|--------|--------|
|     |             | 3.6605 | 5.0902 | 5.5081 | 6.7295 | 7.125 | 9.5579 | 10.7 | 12.508 | 18.894 | 22.154 | 24.788 |
|     | 4176/16-1   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/16-2   | A      | A      | A      | A      | A     | A      | A    | A      | B      | B      | B      |
|     | 4176/16-3   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/16-5   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/16-6   | H      | H      | H      | H      | H     | H      | H    | H      | B      | B      | B      |
|     | 4176/16-7   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/16-8   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/16-9   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/16-10  | H      | H      | H      | H      | H     | H      | H    | H      | B      | B      | B      |
|     | 4176/26-1   | A      | A      | A      | A      | A     | A      | A    | A      | B      | B      | B      |
|     | 4176/26-2   | H      | H      | H      | H      | H     | H      | A    | A      | B      | B      | B      |
|     | 4176/26-4   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/26-5   | H      | H      | H      | H      | H     | H      | H    | H      | B      | B      | B      |
|     | 4176/26-6   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/26-7   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/26-8   | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 4176/26-10  | H      | H      | H      | H      | H     | H      | H    | H      | B      | B      | B      |
|     | 5216/4-1    | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/4-2    | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 5216/4-3    | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/4-4    | A      | A      | A      | A      | A     | A      | B    | B      | B      | B      | B      |
|     | 5216/4-5    | A      | A      | A      | A      | A     | A      | B    | B      | B      | B      | B      |
|     | 5216/4-6    | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/4-7    | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 5216/4-8    | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/4-9    | A      | A      | A      | A      | A     | A      | B    | B      | B      | B      | B      |
|     | 5216/4-10   | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/6-1    | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 5216/6-2    | B      | B      | B      | B      | B     | B      | B    | B      | B      | B      | B      |
|     | 5216/6-3    | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/6-4    | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/6-5    | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/6-6    | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |
|     | 5216/6-10   | H      | H      | H      | H      | H     | H      | B    | B      | B      | B      | B      |

|   |                                |
|---|--------------------------------|
| A | Homozygous for Introgression   |
| B | Without introgression          |
| H | Heterozygous for introgression |

2HS  
Intro-  
gression

# F7 population from WB5/117

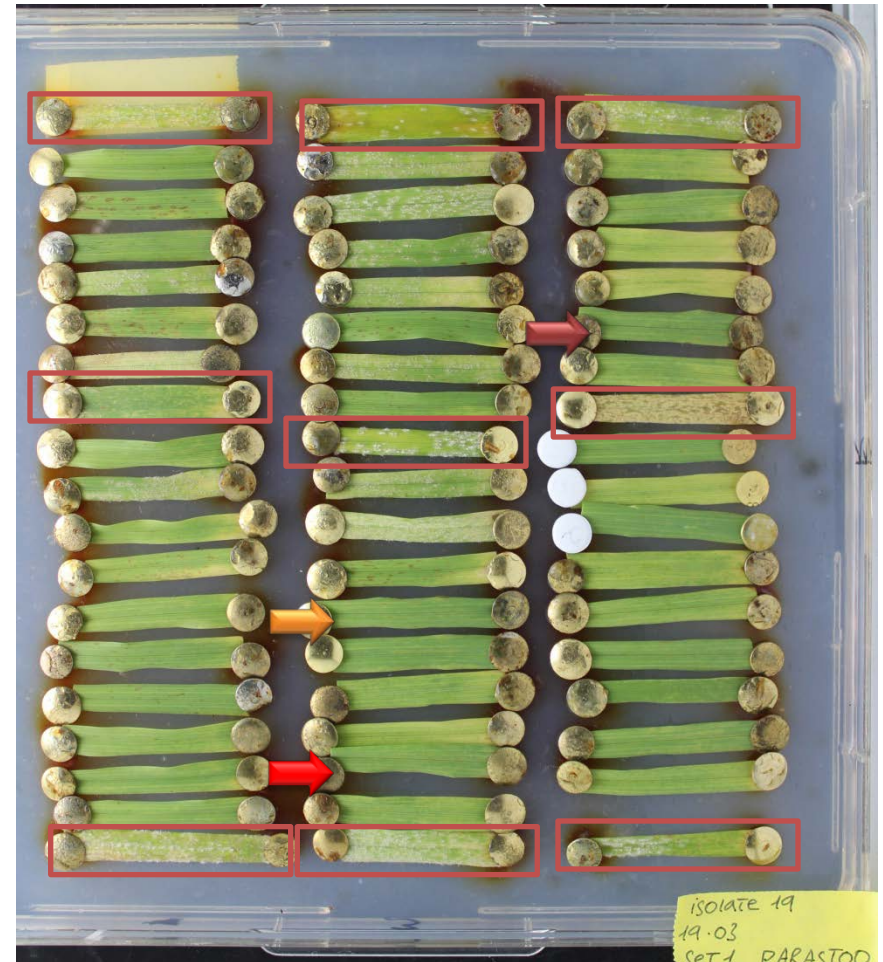


2HL  
Intro-  
gression

# Result of DLA with isolate 19.

| Sheet1/set1    |                |                |
|----------------|----------------|----------------|
| Golden promise | Ingrid         | Golden promise |
| 4176/16_41     | 4176/26_45     | 5216/6_47      |
| 4176/16_42     | 4176/26_46     | 5216/6_48      |
| 4176/16_43     | 4176/26_47     | 5216/6_49      |
| 4176/16_44     | 4176/26_48     | 5216/6_50      |
| 4176/16_45     | 4176/26_49     | 5216/6_51      |
| 4176/16_46     | 4176/26_50     | 5216/6_52      |
| Borwina        |                | Ingrid         |
| 4176/16_47     | 4176/26_51     | 5216/4_41      |
|                | Golden promise |                |
| 4176/16_48     | 4176/26_52     | 5216/4_42      |
| 4176/16_49     | 4176/26_53     | 5216/4_43      |
| 4176/16_50     | 4176/26_54     | 5216/4_44      |
| 4176/16_51     | 5216/6_41      | 5216/4_45      |
| 4176/16_52     | 5216/6_42      | 5216/4_46      |
| 4176/26_41     | 5216/6_43      | 5216/4_47      |
| 4176/26_42     | 5216/6_44      | 5216/4_48      |
| 4176/26_43     | 5216/6_45      | 5216/4_49      |
| 4176/26_44     | 5216/6_46      |                |
| Golden promise | Borwina        | Golden promise |

Second leaves



7dpi

- **SusPtrit**: Experimental line with exceptional susceptibility to several non-adapted **rusts**
- Mapping population was available:



- Accumulation of susceptibility genes to wheat **powdery mildew** (Bgt) → **SusBgtSC** and **SusBgtDC** (Aghnoum & Niks, 2010)



# QTLs mapped for NHR to *Bgt* in SusPtrit x Vada

## ■ QTL mapping: MapQTL software

- first for each replicate, and then for the average of data

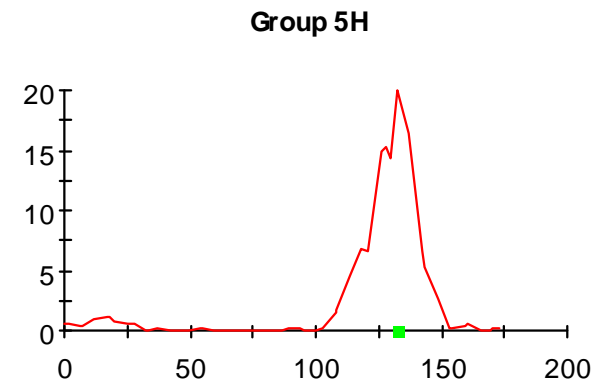
## ■ On **Vada x SusPtrit**:

- **Large-effect QTL** on 5H, very consistent over the reps;



- Same peak marker as Jafary *et al.*, (2008) barley-*Puccinia persistens* interaction LOD 3.88

- Other QTL on 2H, LOD 4.9



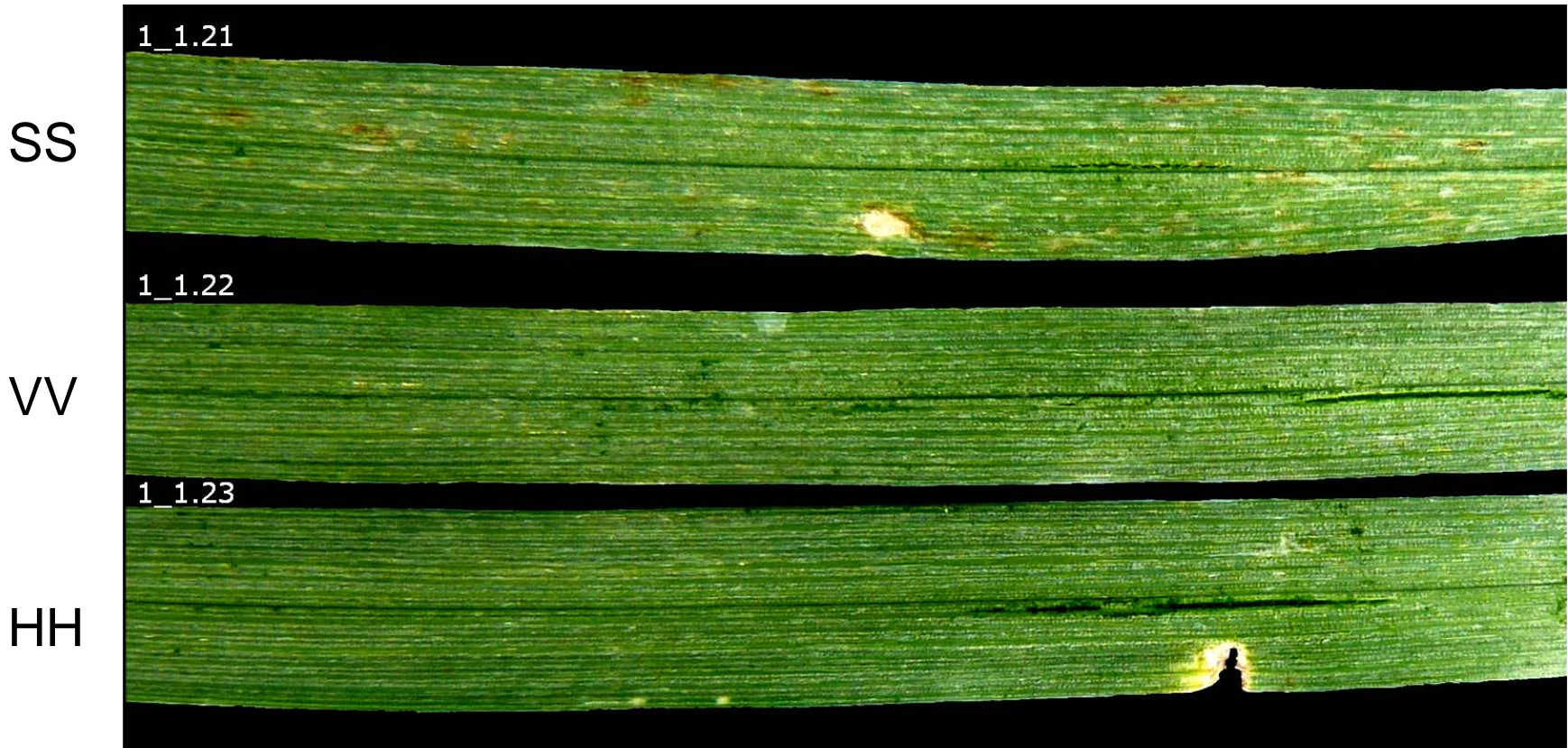
**Bgt: VxS LOD 19.9**

Explained phenotypic effect:  
> 40%

***“Rbgtq1”***



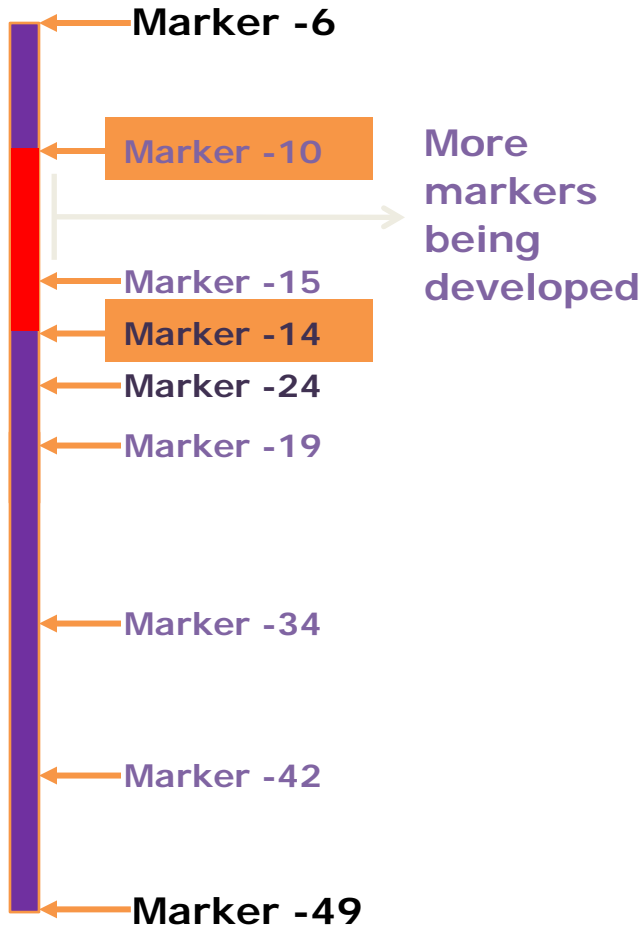
# QTL fine mapping for NHR to *Bgt* on chr. 5HL



SS → SusPtrit (Susc)  
 VV → Vada (Res)  
 HH → heterocygous (Res)

|        | markers |      |      |      |      | score |
|--------|---------|------|------|------|------|-------|
|        | M-10    | M-15 | M-14 | M-24 | M-19 |       |
| 1_1.21 | SS      | SS   | SS   | SS   | SS   | 5.0   |
| 1_1.22 | SS      | VV   | VV   | VV   | VV   | 2.0   |
| 1_1.23 | SS      | HH   | HH   | HH   | HH   | 2.0   |

## *Rbgtq1*: fine-mapping so far



- The QTL region was narrowed down to the interval between markers -10 and -14.
- Distance:
  - Consensus map: 0.32 cM
  - Morex map: 0.14 cM
- New markers are being developed in the region: synteny with rice and Brachypodium

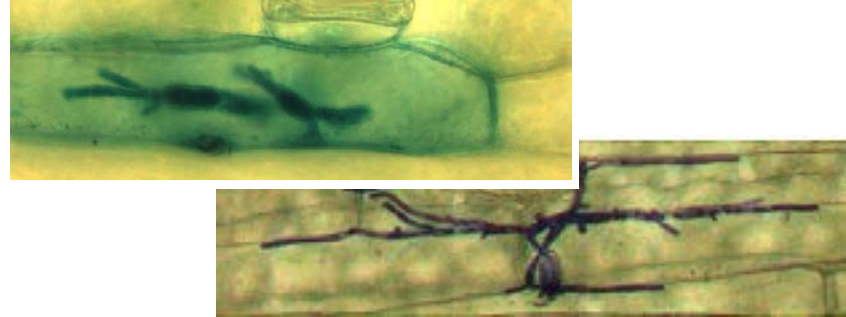
## **WP2:**

Transgenomic complementation  
of NHR in wheat by RLKs

## **WP3:**

Loss of function of NHR (RLK)  
genes in barley

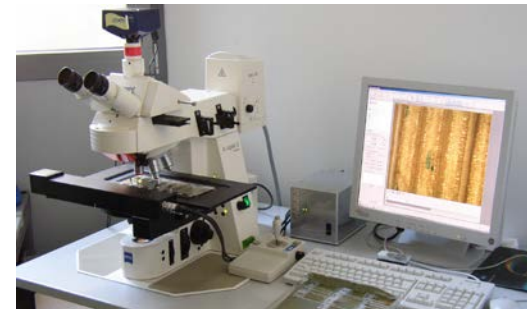




Challenge with *B. graminis*



HT cloning and bombardment



Automated microscopy

Quantitative interaction phenotype:

SI = susceptibility index ( $\Sigma$  susceptible cells/ $\Sigma$  GUS-cells)

HI = haustorium index ( $\Sigma$  haustoria/ $\Sigma$  GUS-cells)

Colony size =  $\Sigma$  of segmentewd hyphae pixels

# The GABI-nonhost TIGS screening:

**Screen:** Susceptibility of barley to *Bgt*  
**Input:** Approx. 750 RNAi constructs  
**Targets:** Mostly transcriptionally up-regulated transcripts

| RNAi Target           | Description (Blast X) <sup>b</sup> | Nonhost-resistance <sup>c</sup> |                | PTI <sup>d</sup>       |                | <i>Mla9</i> -resistance <sup>e</sup> |                |
|-----------------------|------------------------------------|---------------------------------|----------------|------------------------|----------------|--------------------------------------|----------------|
|                       |                                    | Mean ± SE <sup>f</sup>          | p <sup>g</sup> | Mean ± SE <sup>h</sup> | p <sup>i</sup> | Mean ± SE <sup>f</sup>               | p <sup>g</sup> |
| Pos. ctr <sup>j</sup> |                                    | 4.84 ± 0.57                     | 0.0001         | -3.74 ± 0.263          | <0.0001        | 8.86 ± 0.57                          | <0.0001        |
| Neg. ctr <sup>k</sup> |                                    | 0.10 ± 0.04                     |                | 0.00                   |                | 0.33 ± 0.22                          |                |
| <i>Rnr1</i>           | Nonclathrin coat protein γ 1       | <b>1.08 ± 0.33</b>              | <b>0.007</b>   | -0.14 ± 0.14           | 0.0675         | 0.68 ± 0.20                          | 0.088          |
| <i>Rnr2</i>           | BAH domain protein                 | <b>0.35 ± 0.17</b>              | <b>0.029</b>   | -0.15 ± 0.23           | 0.1769         | 0.42 ± 0.18                          | 0.249          |
| <i>Rnr4</i>           | Endo-1.4-beta-glucanase            | <b>0.56 ± 0.18</b>              | <b>0.005</b>   | 0.16 ± 0.37            | 0.1067         | 0.51 ± 0.25                          | 0.211          |
| <i>Rnr5</i>           | ARM Repeat protein                 | <b>0.35 ± 0.19</b>              | <b>0.007</b>   | <b>-1.18 ± 0.22</b>    | <b>0.0024</b>  | 0.40 ± 0.25                          | 0.402          |
| <i>Rnr6</i>           | Cellulose-synthase like D2         | <b>1.29 ± 0.49</b>              | <b>0.036</b>   | <b>1.08 ± 0.14</b>     | <b>0.0002</b>  | <b>3.81 ± 0.70</b>                   | <b>0.0003</b>  |
| <i>Rnr7</i>           | EF Hand protein                    | <b>1.15 ± 0.43</b>              | <b>0.048</b>   | -0.06 ± 0.18           | 0.0643         | 0.32 ± 0.14                          | 0.289          |
| <i>Rnr8</i>           | Receptor-like kinase               | <b>0.74 ± 0.32</b>              | <b>0.044</b>   | -0.42 ± 0.28           | 0.4673         | 0.34 ± 0.15                          | 0.333          |
| <i>Rnr9</i>           | Subtilisin-like protein            | <b>0.50 ± 0.23</b>              | <b>0.009</b>   | -0.65 ± 0.40           | 0.2853         | 0.09 ± 0.09                          | 0.33           |
| <i>Rnr10</i>          | Stomatin-like protein              | <b>0.54 ± 0.30</b>              | <b>0.044</b>   | <b>-0.87 ± 0.25</b>    | <b>0.0444</b>  | 0.10 ± 0.10                          | 0.377          |

**Rnr1-10** = **R**equired for **n**onhost **r**esistance gene 1-10

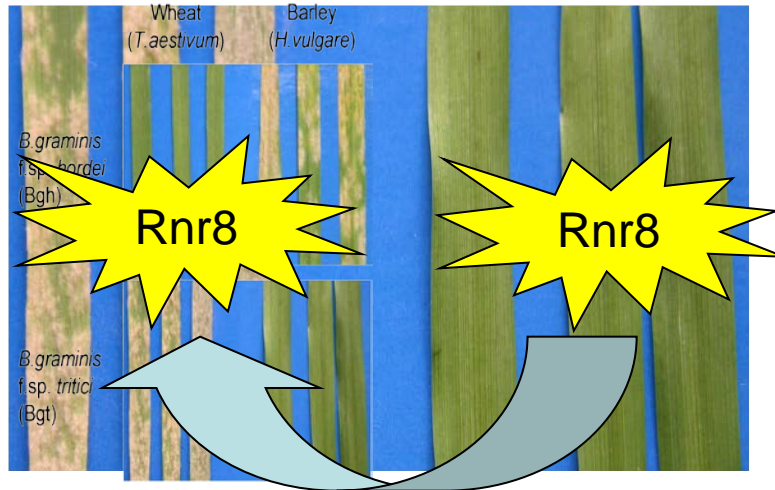
Wheat  
(*T.aestivum*)

Barley  
(*H.vulgare*)

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

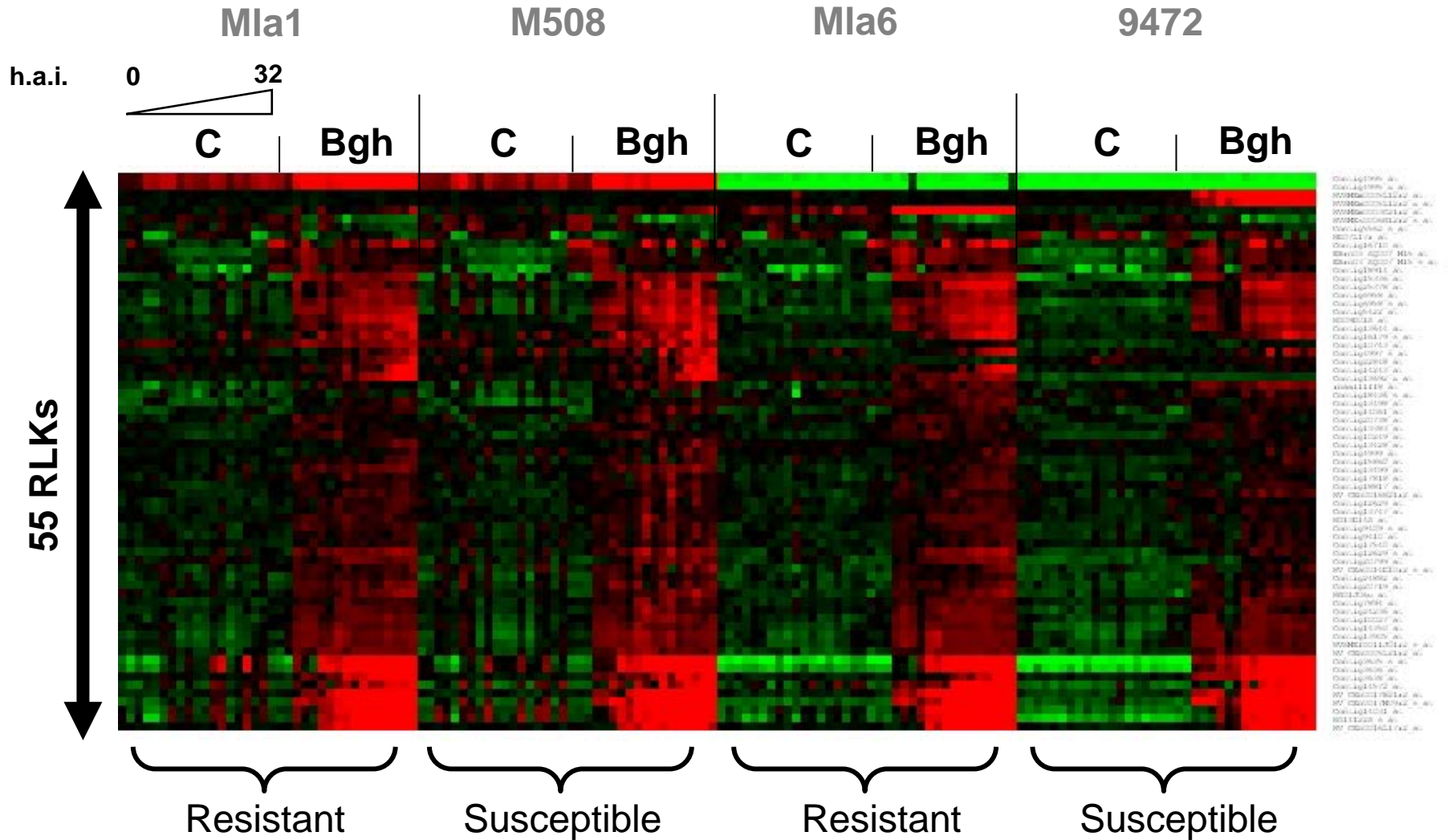


*B.graminis*  
f.sp. *tritici*  
(Bgt)



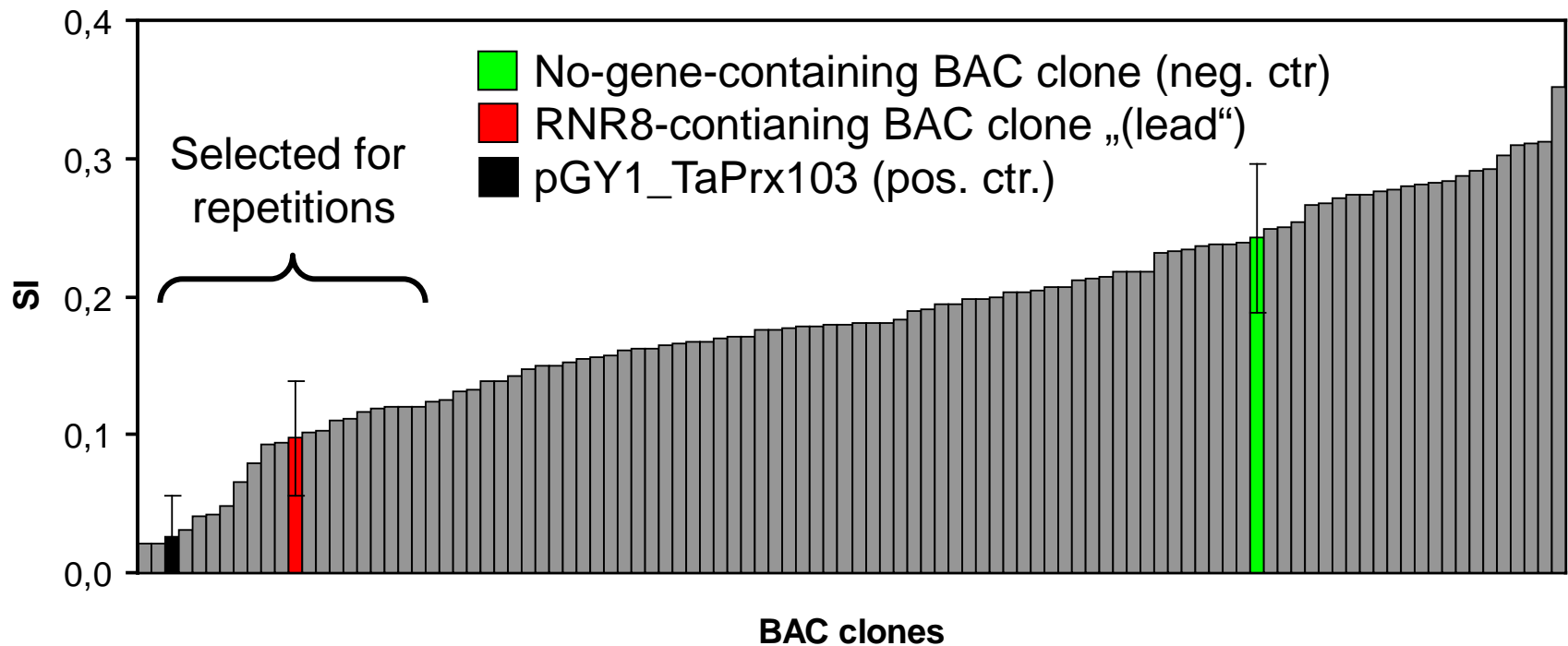
Engineering of „NHR“ into  
wheat by transgenomic  
complementation of barley  
*Rnr8* encoding the  
LRR/malectin-RLK  
HvLEMK1

# More RLK candidates (BB10 expression data):



# Trangenomic BAC complementation by bombardment in wheat cv. Kanzler:

1st round screening:



➔ 10/20 BAC clones significantly protected wheat  
from *Bgt* attack (5 independent experiments)

# HiSeq of „active“ BAC's:

**BlastX:**

Serine/  
Threonine  
RLK

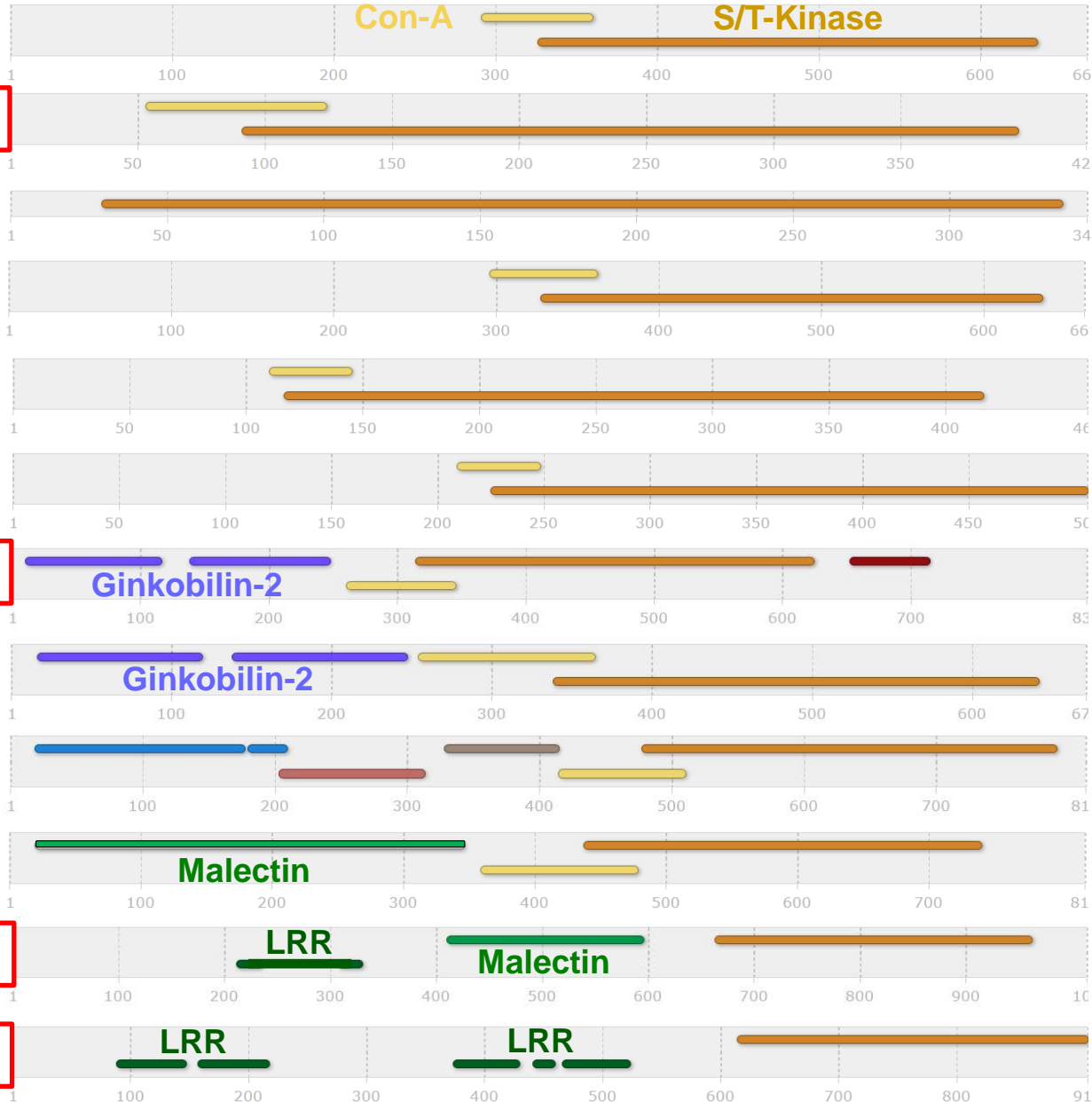
Cysteine-  
rich RLK

S-locus  
RLK-like

Feronia-like

LRR/Malectin  
(*HvLEMK1*)

LRR RLK





# The genes for DURES *Trit*.

| U35_gene Nr. | Gene name for DURESTrit | BAC effect in wheat | RLK subclone effect wheat | TIGS effect Bgh in barley | TIGS effect Bgt in barley | Regul_PM (BB10) | Regul_PM_EPI (IPK) | For DURESTrit | BlastX NCBI       |
|--------------|-------------------------|---------------------|---------------------------|---------------------------|---------------------------|-----------------|--------------------|---------------|-------------------|
| 16133        | RLK_3Hcluster_2_3_compl | RES                 | RES                       | SUS                       | NS                        | UP              | NS                 | YES           | TAK               |
| 19434        | RLK_7_compl             | RES                 | RES                       | RES                       | n.a.                      | UP              | UP                 | YES           | LRR-RLK           |
| 18640        | RLK_10_compl            | RES                 | n.a.                      | RES                       | SUS                       | UP              | UP                 | YES           | Cysteine rich RLK |
| 2893         | Rnr8 (HvLEMK1)          | RES                 | RES                       | NS                        | SUS                       | UP              | UP                 | YES           | LRR/malectin-RLK  |
| 16135        | RLK_3Hcluster_1_compl   | RES                 | NS                        | RES                       | NS                        | UP              | NS                 | NO            | TAK               |
| 16131        | RLK_3Hcluster_3_compl   | RES                 | n.a.                      | SUS                       | NS                        | Not present     | NS                 | NO            | TAK               |
| 23916        | RLK_3Hcluster_4_compl   | RES                 | NS                        | n.a.                      | n.a.                      | NS              | UP                 | NO            | LRK               |
| 23916        | RLK_3Hcluster_5_compl   | RES                 | n.a.                      | n.a.                      | n.a.                      | NS              | UP                 | NO            | LRK               |
| 16132        | RLK_3Hcluster_6_compl   | RES                 | n.a.                      | SUS                       | NS                        | UP              | NS                 | NO            | TAK               |
| 5178         | RLK_8_compl             | RES                 | n.a.                      | n.a.                      | n.a.                      | UP              | UP                 | NO            | Cysteine rich RLK |
| 5179         | RLK_9_compl             | RES                 | n.a.                      | NS                        | NS                        | UP              | UP                 | NO            | Cysteine rich RLK |
| 26561        | RLK_11_compl            | RES                 | NS                        | NS                        | NS                        | UP              | UP                 | NO            | S-locus RLK-like  |
| 1716         | RLK_12_compl            | RES                 | NS                        | RES                       | NS                        | NS              | NS                 | NO            | FERONIA RLK-like  |
| 46214        | RLK_13_compl            | RES                 | n.a.                      | n.a.                      | n.a.                      | Not present     | NS                 | NO            | Cysteine rich RLK |
| 43306        | RLK_14_compl            | RES                 | n.a.                      | RES                       | NS                        | UP              | Not present        | NO            | No match          |

→ Converging evidence from transient expression,  
TIGS and transcript regulation

## *RLK*-silenced barley

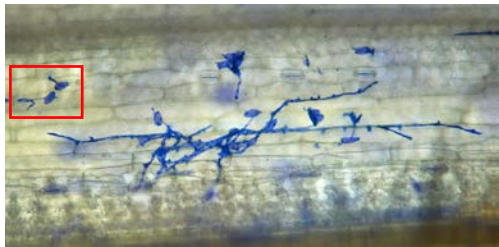
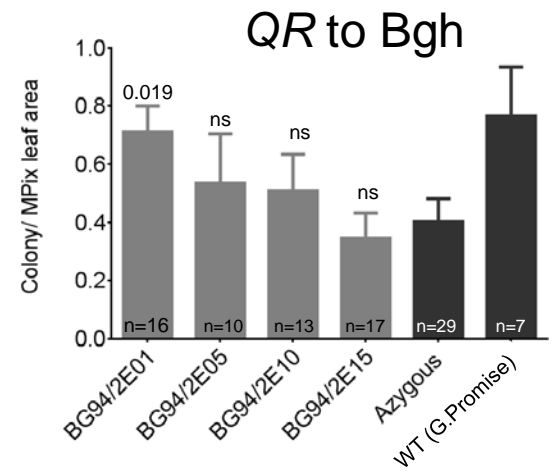
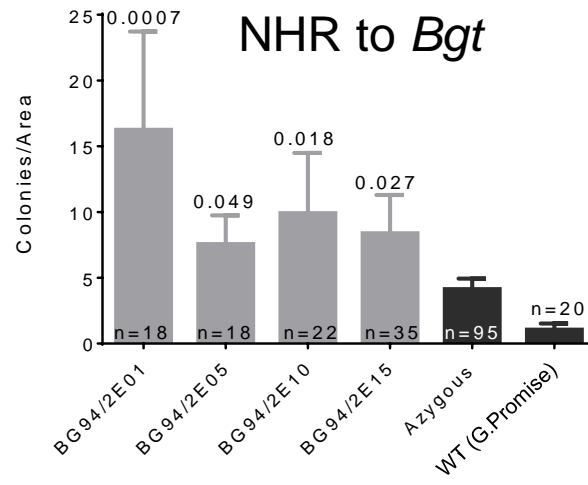
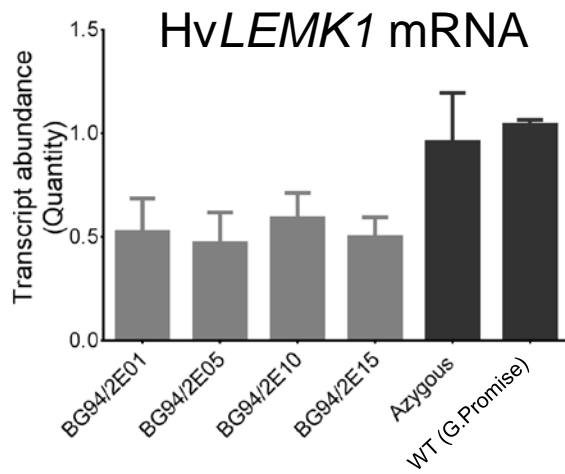
- HvLEMK1 → 24 events with intact RNAi cassette
- RLK\_3Hcluster\_2\_3\_compl → 18 events with intact RNAi cassette
- RLK\_7\_compl → 0 events with intact RNAi cassette
- RLK\_10\_compl → 21 events with intact RNAi cassette

## Barley *RLK*-expressing wheat

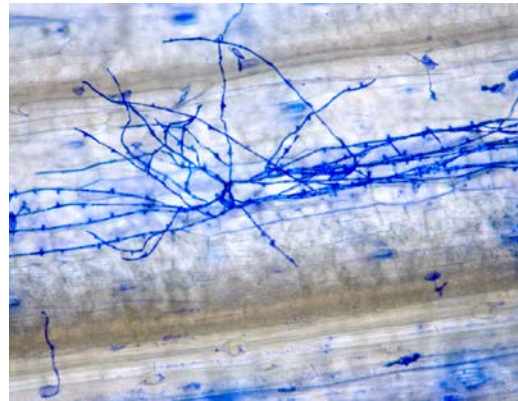
HvLEMK1 → We are growing the first generation of transgenic plants in “Fielder” background

- Fielder is highly susceptible to Bgt
- 38 T0 plantlets gone into jiffies at beginning of March, plus 3 non-transformed controls
- Plants Sampled for RNA extractions and mildew inoculation.
- Data not ready yet (April 2015)
- The three other HvRLKs → Full-CDS cDNA cloning successful

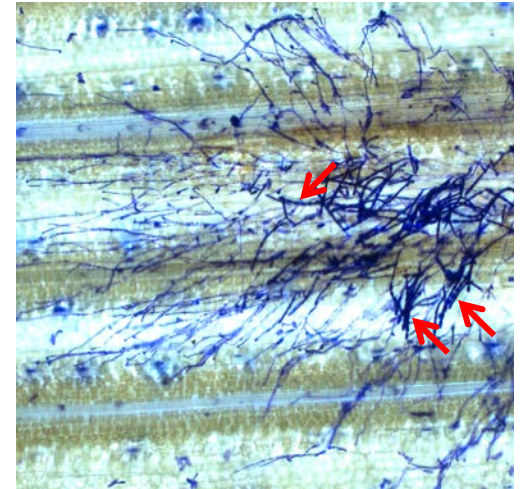




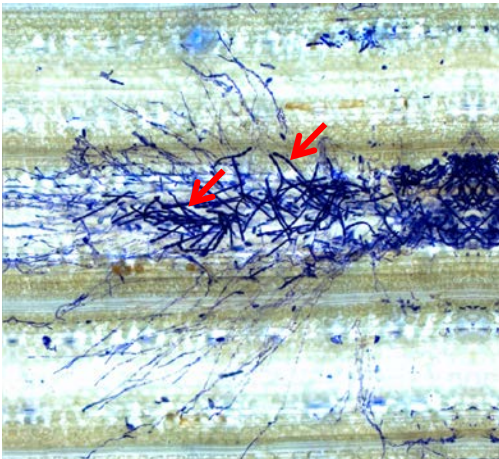
WT (G.Promise)



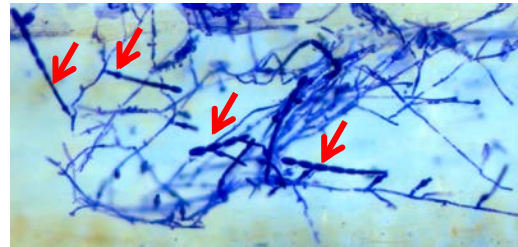
BG94/2E05



BG94/2E15



BG94/2E01



BG94/2E10

RNAi of HvLEMK1  
in barley

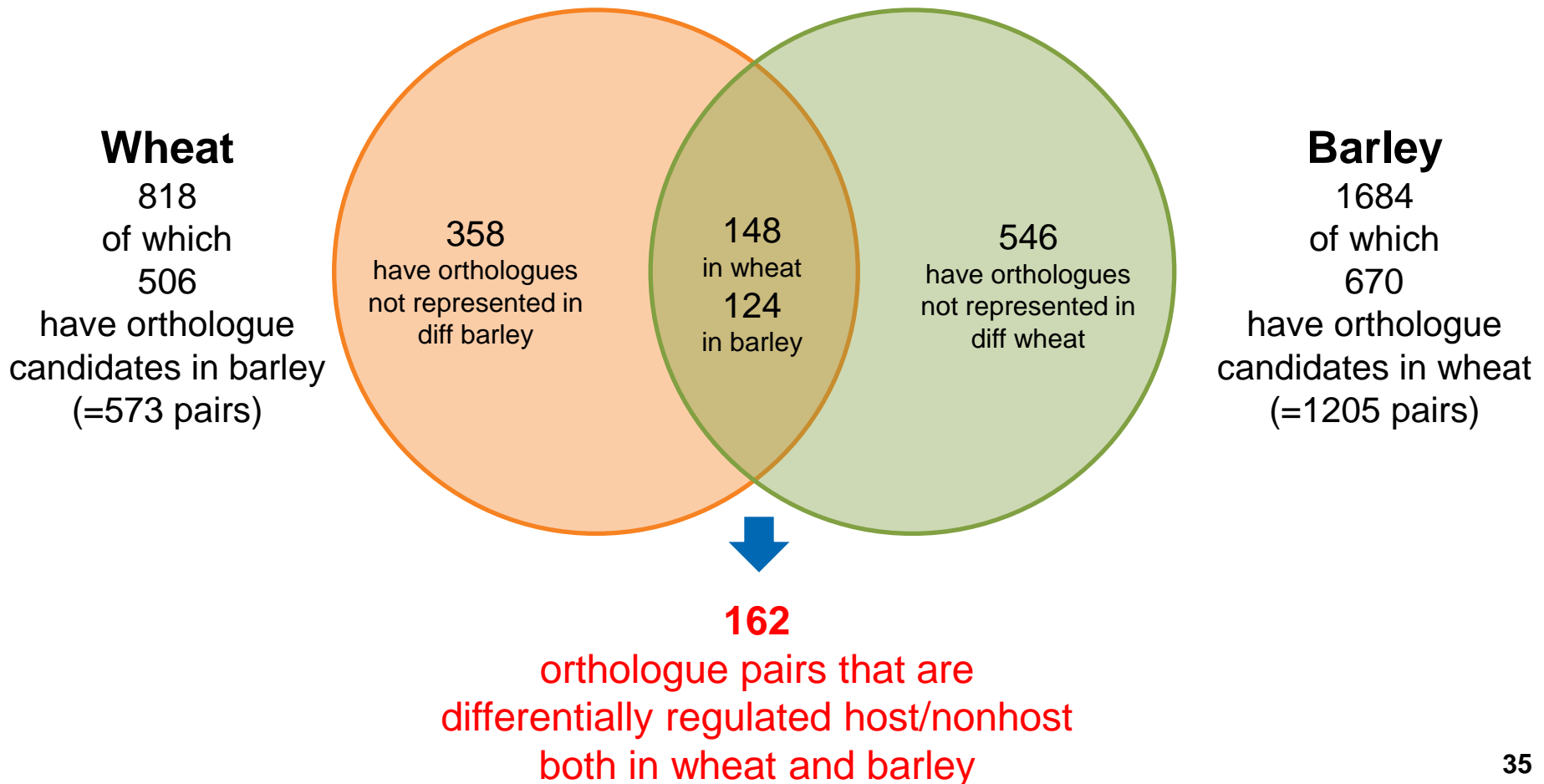
## **WP4:**

Validation of novel NHR  
candidate genes in *Triticeae*

# Prioritisation of candidate genes for new TIGS screen

Results from **TritNONHOST** consortium available:

- barley and wheat orthologous transcripts differentially regulated upon attack by host and nonhost powdery mildew isolates



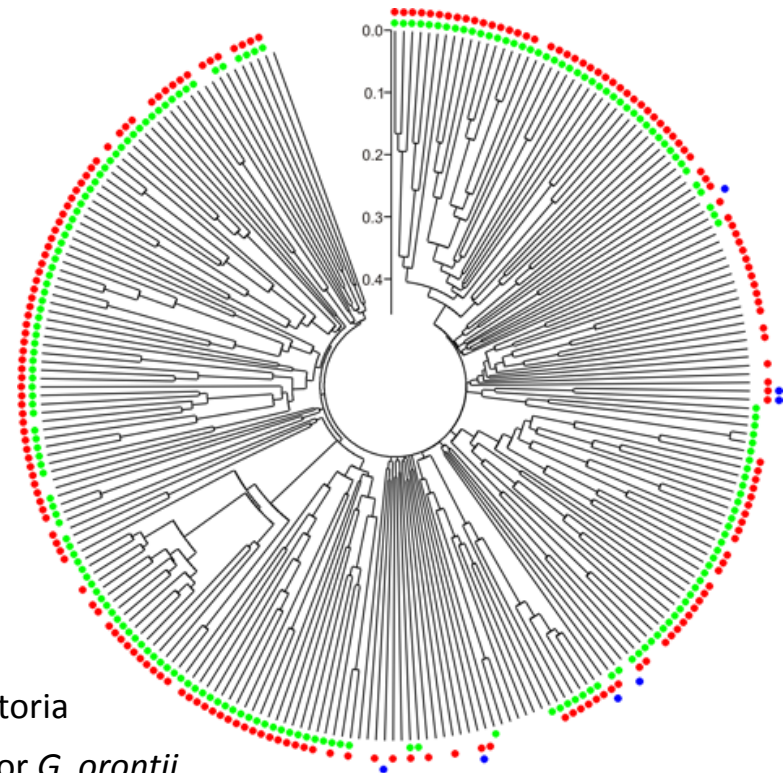
## **WP5:**

Targeting of NHR genes of  
barley by CSEPs from Bgh

# Candidates for **S**ecreted **E**ffector **P**roteins (CSEPs)

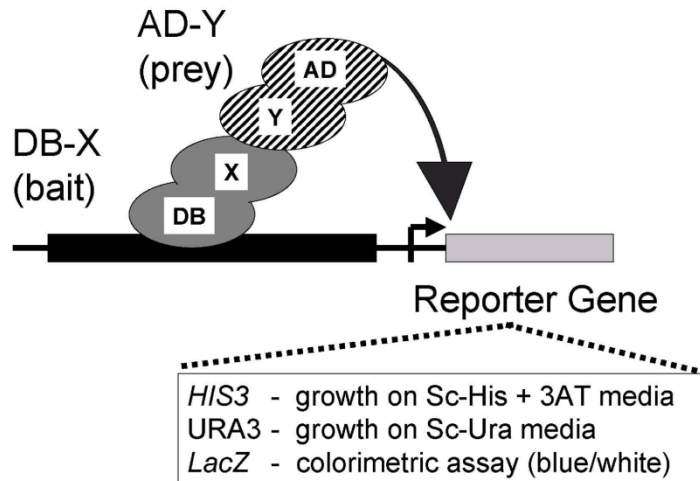
- >500 CSEPs in *Blumeria graminis* f.sp. *hordei*
- only 16 similar to CSEPs from *Golovinomyces orontii* and *Erysiphe pisi*
- >7% of the genome
- clustering in 72 families
- many of them have a YxC motif (FxC, WxC)

- containing YxC motif
- overrepresented in haustoria
- conserved in *E. pisi* and/or *G. orontii*



## Interactors of >500 CSEPs:

### I: „Classical“ Y2H screen



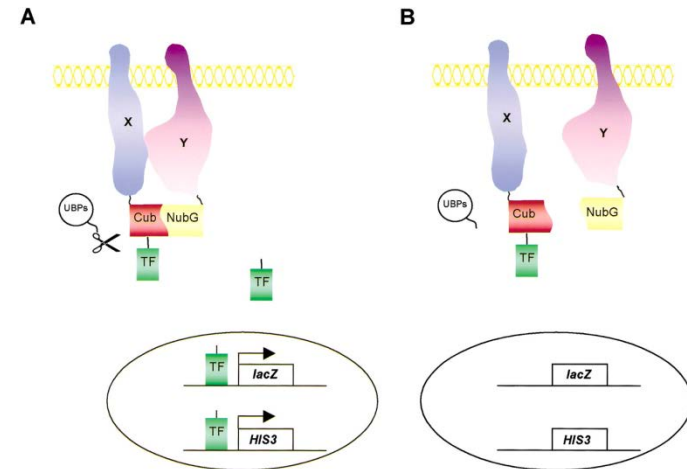
#### advantages:

- Fast and robust

#### disadvantages:

- Not well suited for membrane-localized proteins
- Requirement for domain-screens

### II: Split-ubiquitin Y2H screen



#### advantages:

- RLKs localized at the plasma membrane
- only the artificial transcriptional activator must enter the nucleus
- 3 different reporter systems

#### disadvantages:

- only the recognition of interaction on the cytosolic site



## Summary:

- All DURES *Trit* partners have started research
  - All staff hired
  - Work with NSF-funded partners coordinated
  - Plant materials being built up successfully
  - >500 synthetic CSEP DNAs received
  - Yeast-2-hybrid screens have started
- 
- No TALEN mutants in barley yet
  - CA close to signatory round

## **Thanks for slides to:**

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# Key expertise and interaction among partners for major deliverables:

