



Molecular approaches towards breeding for durable resistance

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Natural genetic resistance: an economical and ecological strategy for disease reduction:

General IPM Principles

- ▶ **Measures for prevention and/or suppression of harmful organisms: point (1)**
- ▶ **Non-chemical methods to be preferred: point (4)**
- ▶ **Target-specificity and minimization of side effects: point (5)**

Genetic resources for resistance breeding

Major genes (single genes with a high activity concerning resistance)

Minor genes (mostly quantitative trait loci, acting partially and additively)

Pyramided genes and combined QTL in naturally occurring genotypes






Durable resistance

Resistance is defined as „durable“ when it remains effective in cultivars that are widely grown for long periods and in environments favorable to the disease (Johnson 1983).

There are single, major resistance genes which are durable, but these are exceptions

Durable resistance is mostly polygenic and caused by genes acting quantitatively



Strategies towards achieving durable resistance:

“Good practice in resistance breeding”

- ▶ **only combinations/pyramids of resistance genes**
- ▶ **combine additively acting QTL**
- ▶ **optimize the use of the genetic resources and do not rest! (see the Ug99 stem rust example in wheat)**

Outline

- 1. Can major genes be used in a more effective and more durable way?**
 - pyramiding (classical and transgenic)
 - improvement of genes *in vitro* by mutagenesis/ modification
 - multilines (classical and transgenic)
- 2. How to approach partially acting resistance genes at the molecular level**

Classical and transgenic approaches based on additive actions

Major resistance (R) genes: the example of wheat

Many genes have been overcome by the pathogens, a non-sustainable use.

Wheat gene catalogue (<http://wheat.pw.usda.gov/ggpages/wgc/2003/>):

Against leaf rust: 65 genes

Against powdery mildew: 36 genes

Against stem rust: 50 genes

Against stripe rust: 68 genes

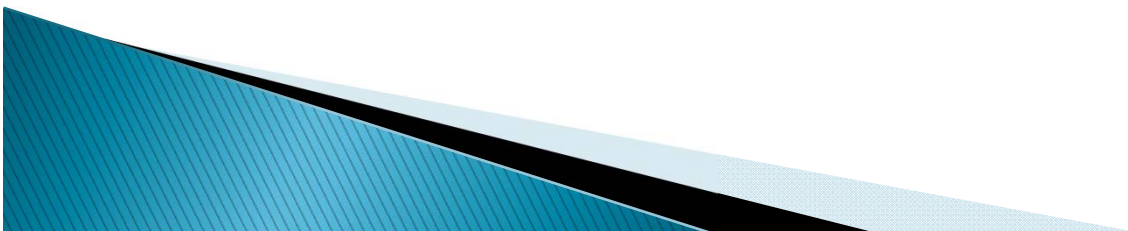


Problems with major genes in agricultural applications

Pathogens can adapt, *i.e.* there is a strong selection for virulent genotypes

R genes are frequently only useful for a few years and then overcome by the pathogen

Non-sustainable use of resistance resources and germplasm



7 alleles of the *Pm3* powdery mildew resistance gene in elite wheat material (*Pm3a – g*)



**Transgenic ways of a more sustainable use of
Pm3 alleles?**

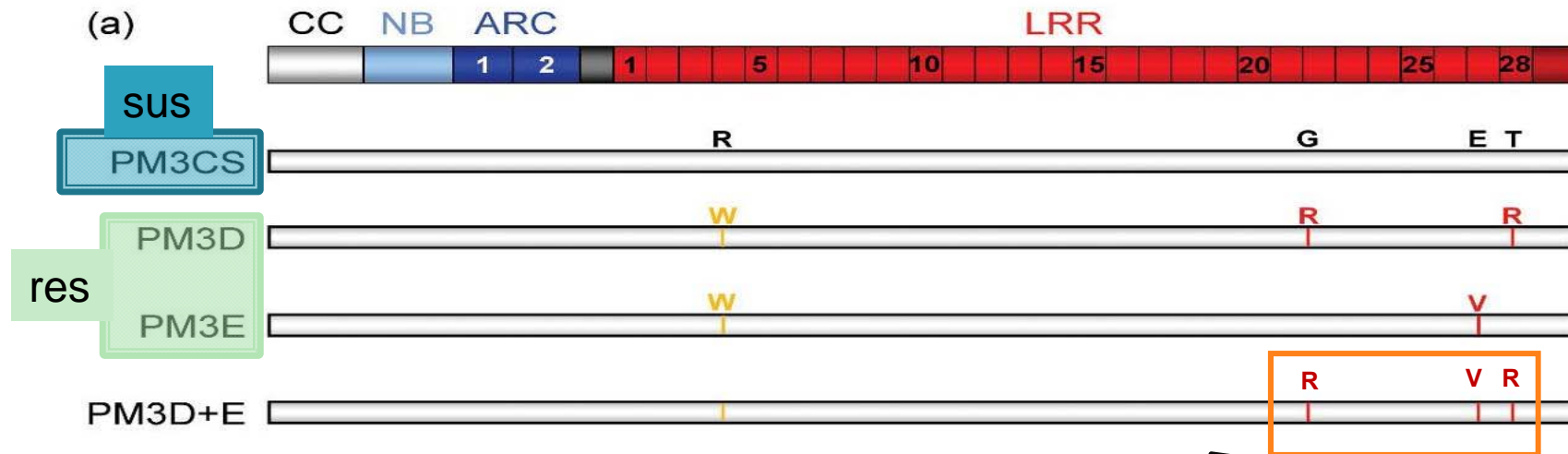
Strategy I:

**Rational design of new alleles with broader
specificity based on the molecular
understanding of protein function ?**



Rational design of new alleles with broader specificity based on the molecular understanding of protein function?

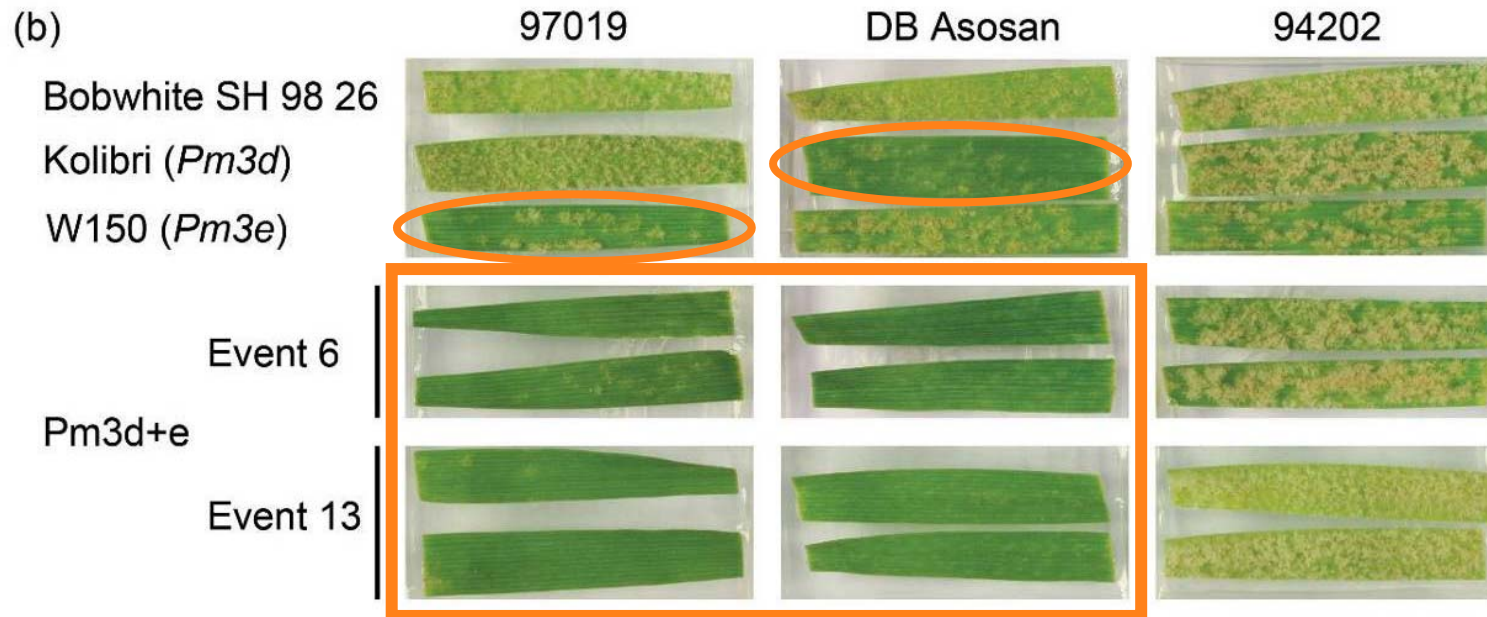
Intragenic allele pyramiding of *Pm3d* and *Pm3e*



Does the hybrid PM3D+E gene has the specificities of both parental alleles?

Intragenic allele pyramiding of *Pm3d* and *Pm3e*

Stable wheat transformation with *Pm3d+e*:



Pm3d+e has dual resistance specificity

Artificial resistance genes?

Natural allelic diversity certainly provides interesting leads to create new resistance genes

But:

Efficient screening systems are needed and not yet available in grasses

To effectively design new alleles, the interactions with corresponding pathogen molecules must be understood

Transgenic ways of a more sustainable use of *Pm3* alleles?

Strategy II:

Pyramiding (combining) alleles of major R genes in the same genotype/cultivar

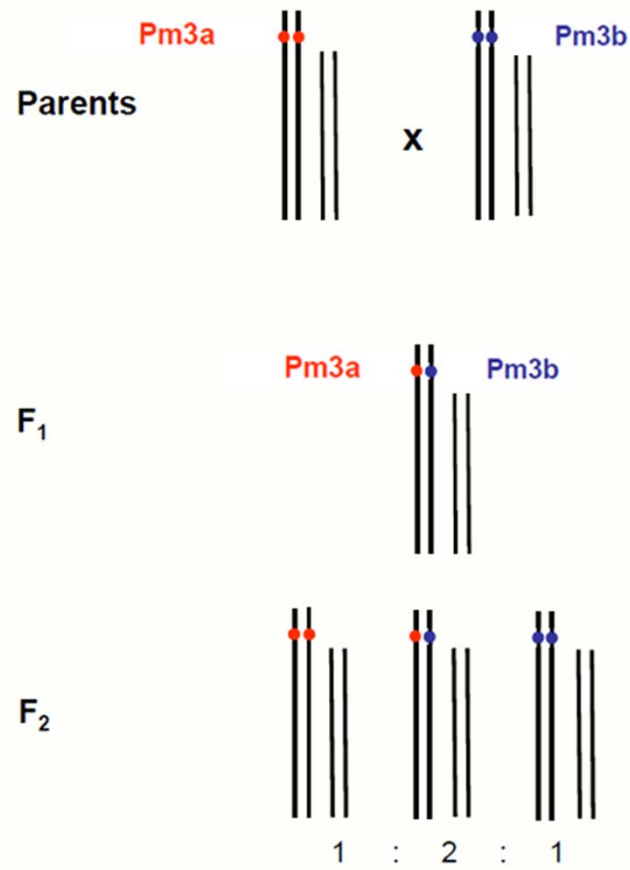
Rationale behind the strategy:

Multiple mutations to virulence in same pathogen strain unlikely

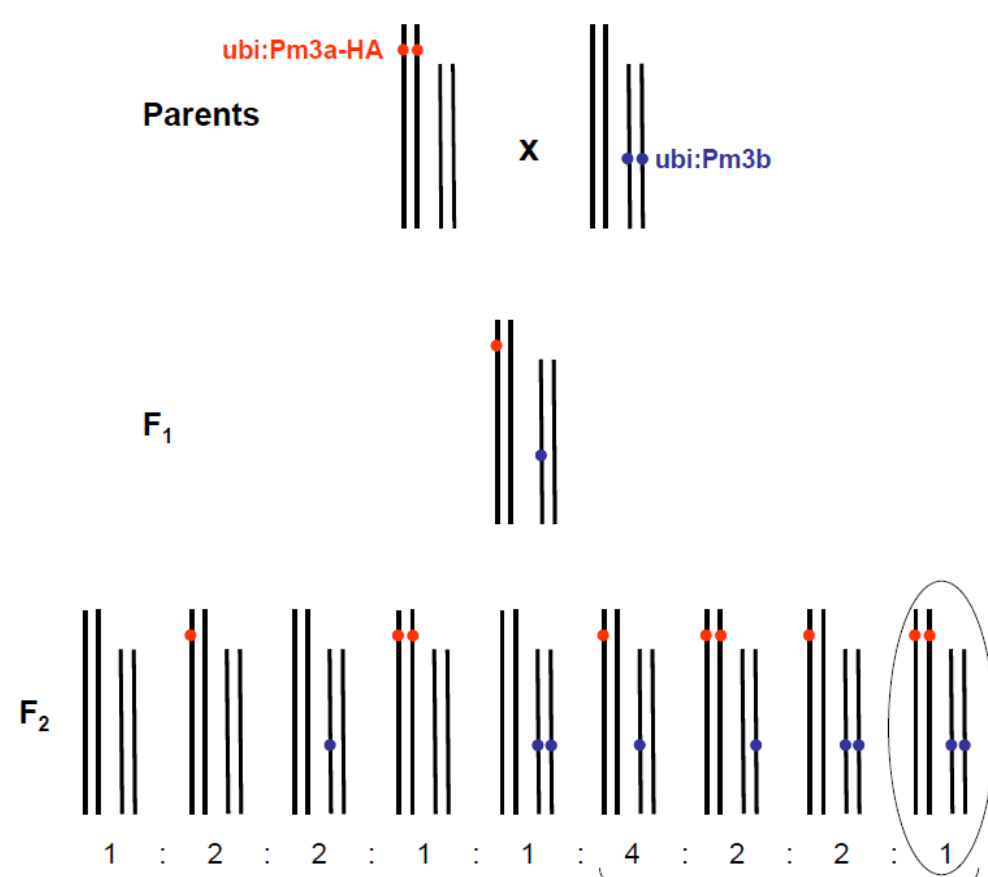
If loss of virulence imposes fitness cost, many *R*-genes working together may cripple pathogen

Pyramiding of *R* gene alleles

Cross of donor lines (true alleles)



Cross of transgenic lines (different insertion sites)



Pyramiding of R gene alleles

- Cross breeding of transgenic lines:

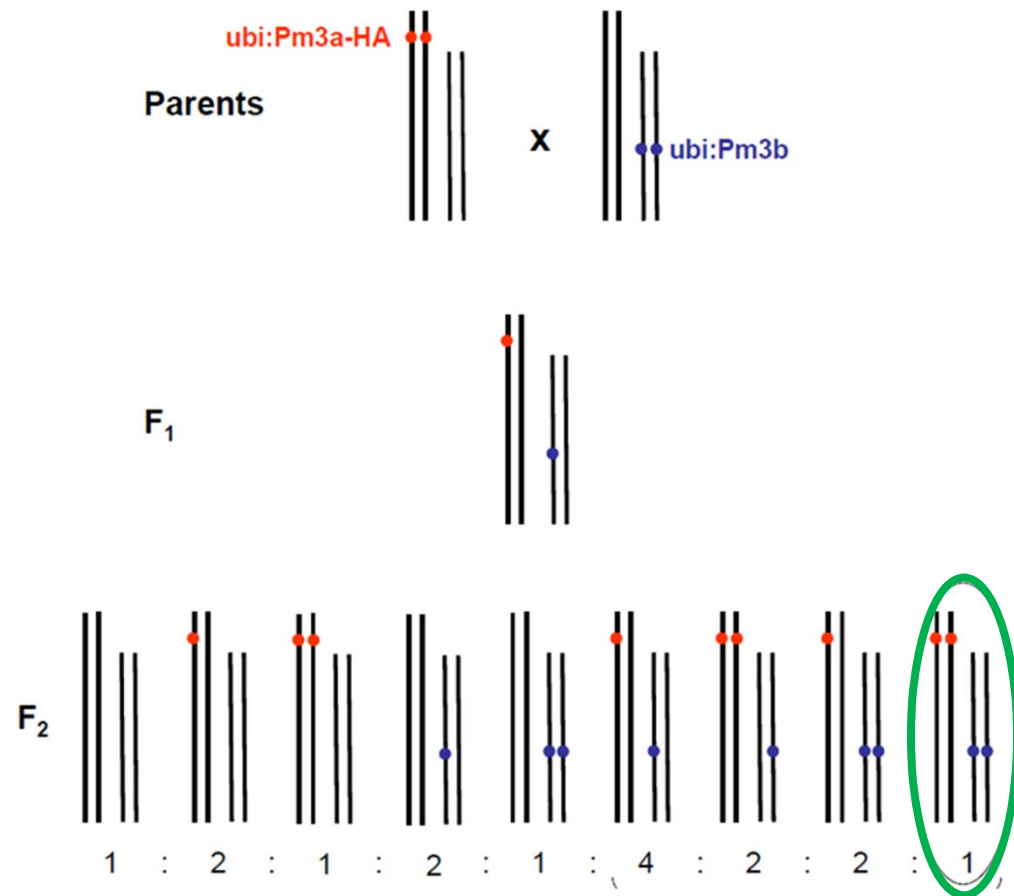
- *Pm3a-HA*
- *Pm3b*, *b-HA*, *b-myc*
- *Pm3c-HA*
- *Pm3d-HA*
- *Pm3f-HA*

- 3 independent crosses per combination






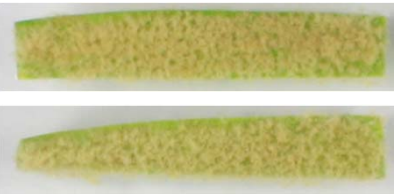
- Analysis of segregation in F3 / F4 with dominant markers

Cross of transgenic lines

(different insertion sites)



Double homozygous (pyramided) lines

	Pm3a-HA	Pm3a + Pm3d	Pm3d-HA
Bgt isolate 95.9 Asosan			
Bgt isolate 07298			

**Transgenic ways of a more sustainable use of
Pm3 alleles?**

Strategy III:

**Multilines with different *Pm3* resistance alleles
in a completely identical genetic background?**

Transgenic resistance genes in wheat: a large field trial in Switzerland 2008-2010



Field site 2009

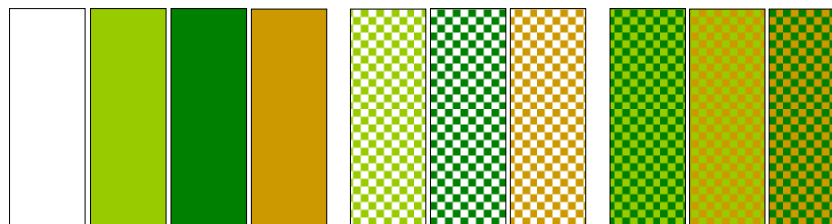
Approaches towards extension of usefulness of the major *Pm3* resistance gene

A) *Pm3* overexpression



- field resistance against powdery mildew
- possible pleiotropic phenotypes (“costs of overexpression”)

B) The multiline strategy

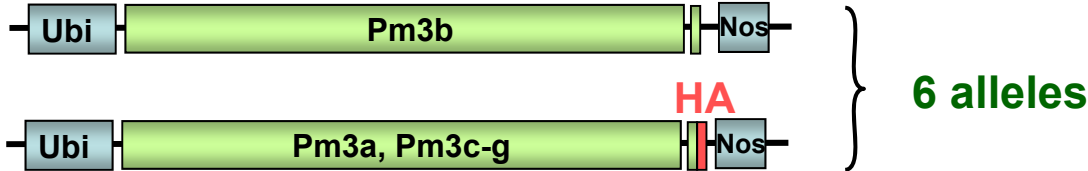


Mixtures of transgenic lines carrying different *Pm3* alleles (real isogenic lines)

- a long term strategy for field resistance against powdery mildew?

Transgenic *Pm3* wheat lines

Transformation of spring wheat Bobwhite SH 98 26



Year	Lines											
	Pm3a		Pm3b				Pm3c		Pm3d	Pm3f		Pm3g
	#1	#2	#1	#2	#3	#4	#1	#2	#1	#2	#1	
2008			█	█	█	█			█			
2009	█	█	█	█	█	█	█		█	█		█
2010	█	█	█	█	█	█	█	█		█	█	█



Functional analysis

The function of the transgenes was confirmed by **leaf segment infection tests** and **spontaneous infections in the greenhouse**.



Sb #4



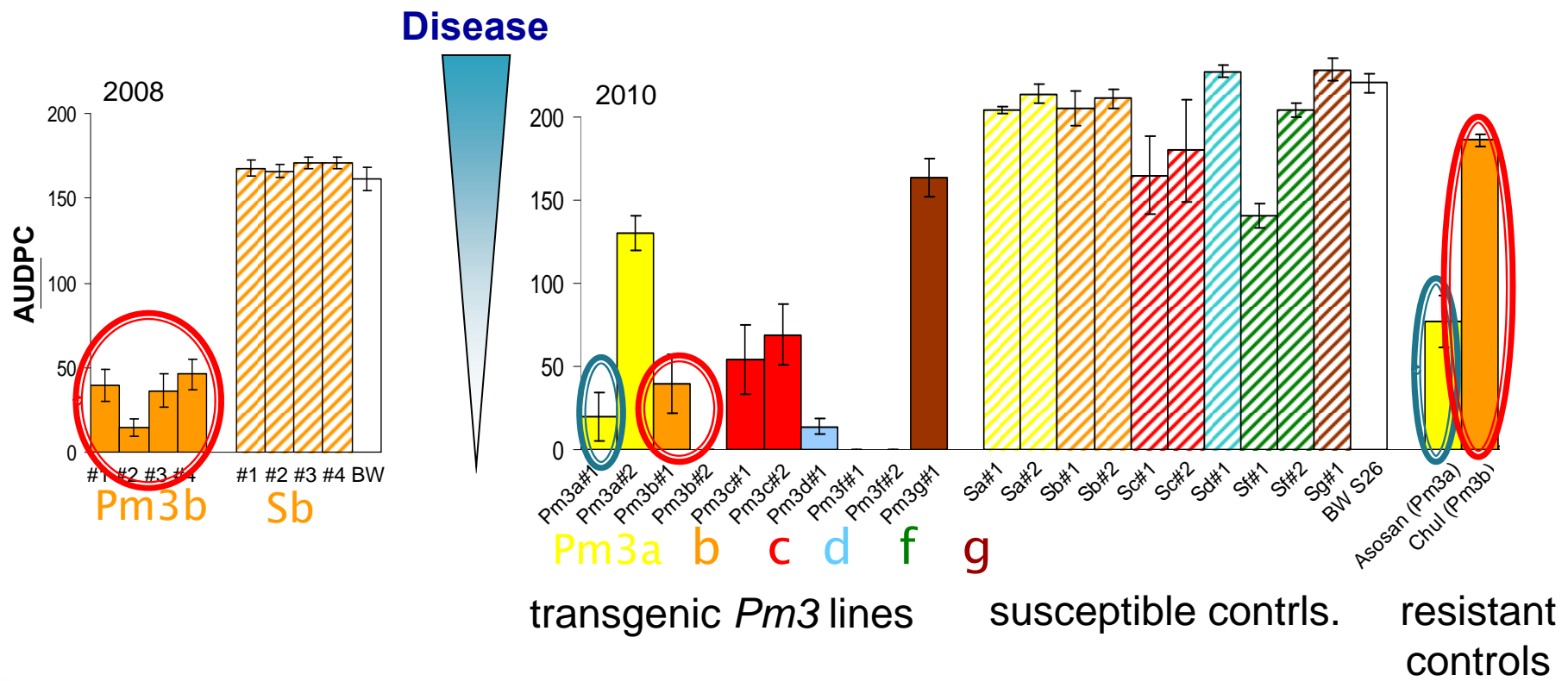
Pm3b #4



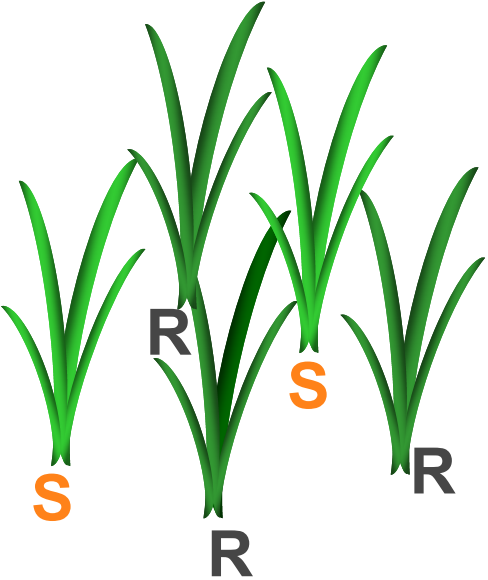
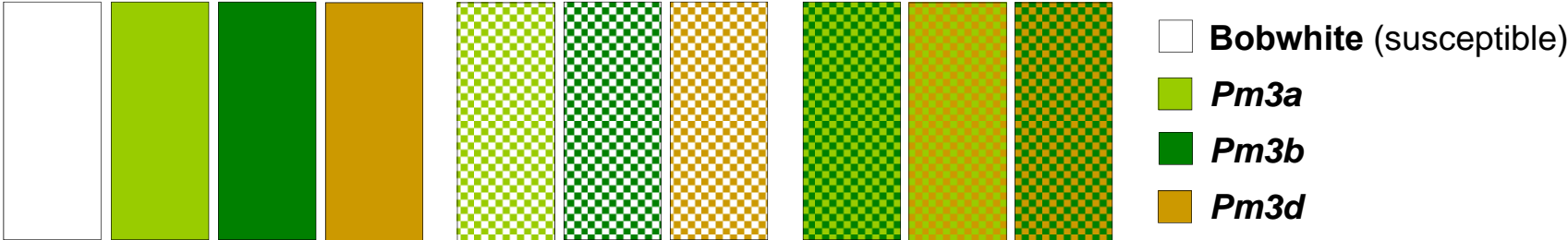
Sb #4

Pm3b #4

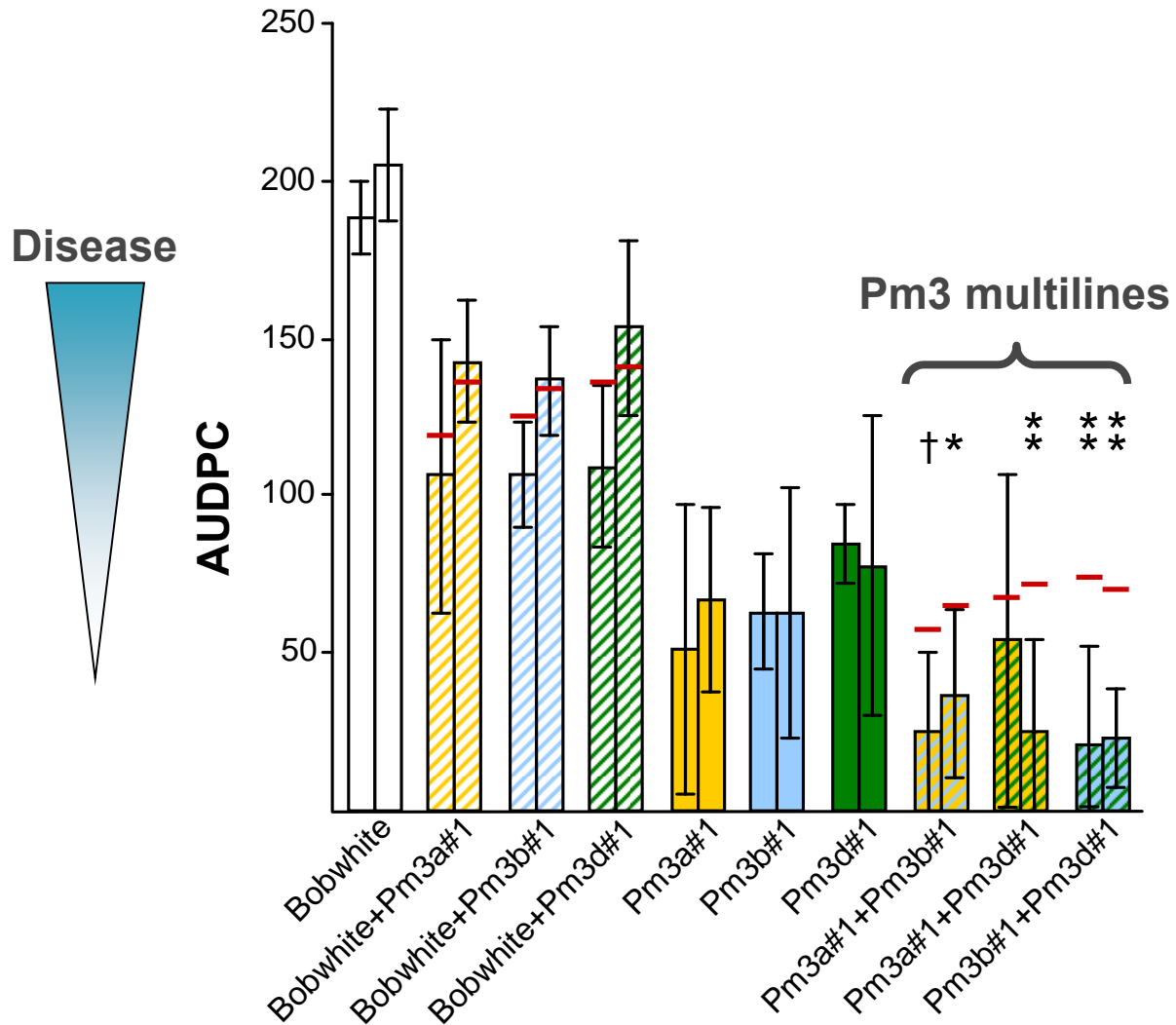
Pm3 lines were resistant in the field



Multilines with different *Pm3* resistance alleles in a completely identical genetic background?



Mixtures are more resistant than the components



How to approach partially acting resistance genes at the molecular level



cv. 'Arina': Swiss winter wheat, susceptible



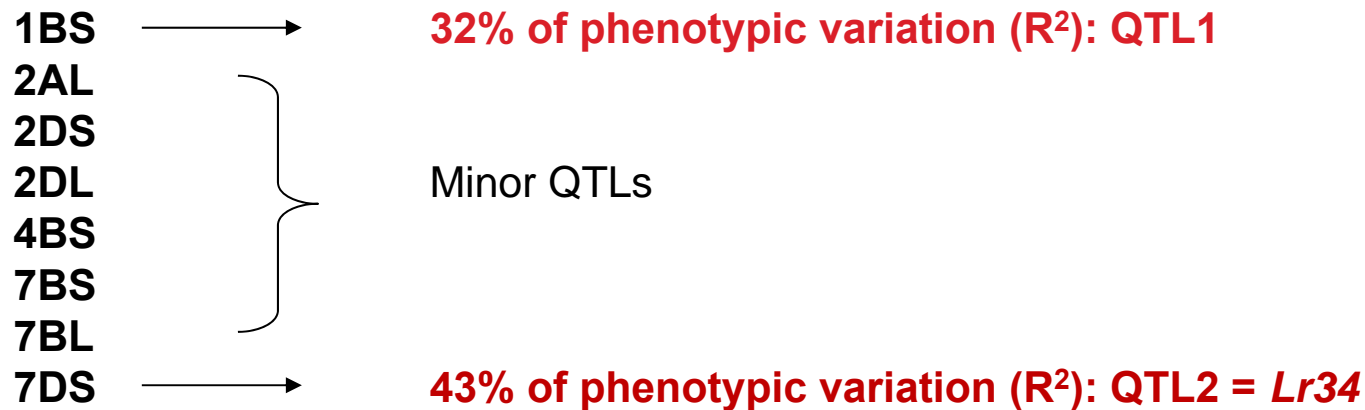
cv. 'Forno': Swiss winter wheat, durably resistant

Durable leaf rust resistance in the Swiss cultivar 'Forno'

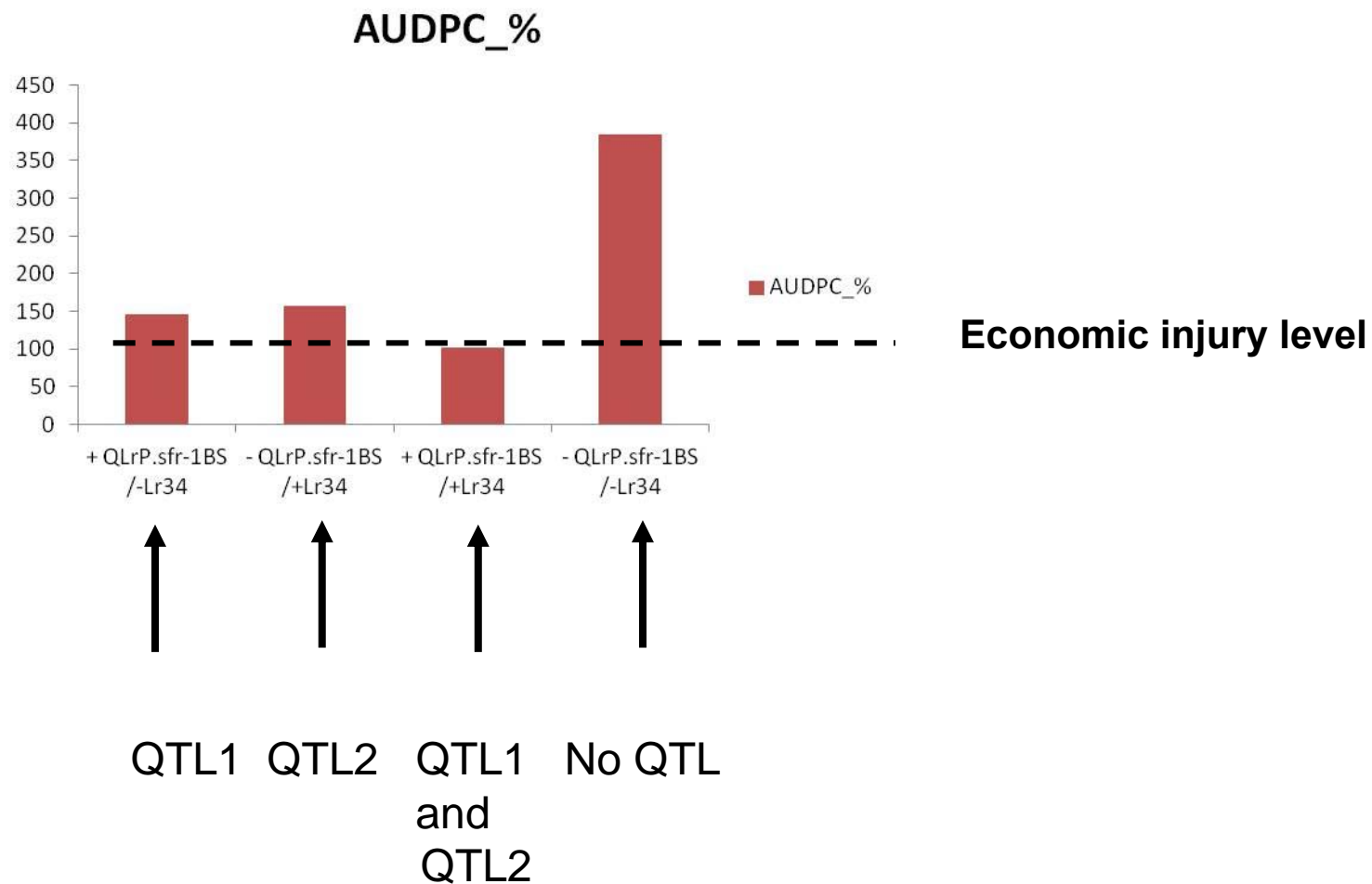
Analysis of the genetic basis of this durable resistance based on linkage analysis and QTL mapping

Dissection of the quantitative and durable leaf rust resistance

QTL analysis revealed **8 chromosomal regions** contributing to the quantitative and durable leaf rust resistance in population of 240 recombinant inbred line population derived from a cross “Arina” x “Forno”, with two major loci:



Effects of two genes (*QLrP-sfr-1BS* and *Lr34*, QTL1 and QTL2) on leaf rust development in a mapping population of recombinant inbred lines: additive gene effects



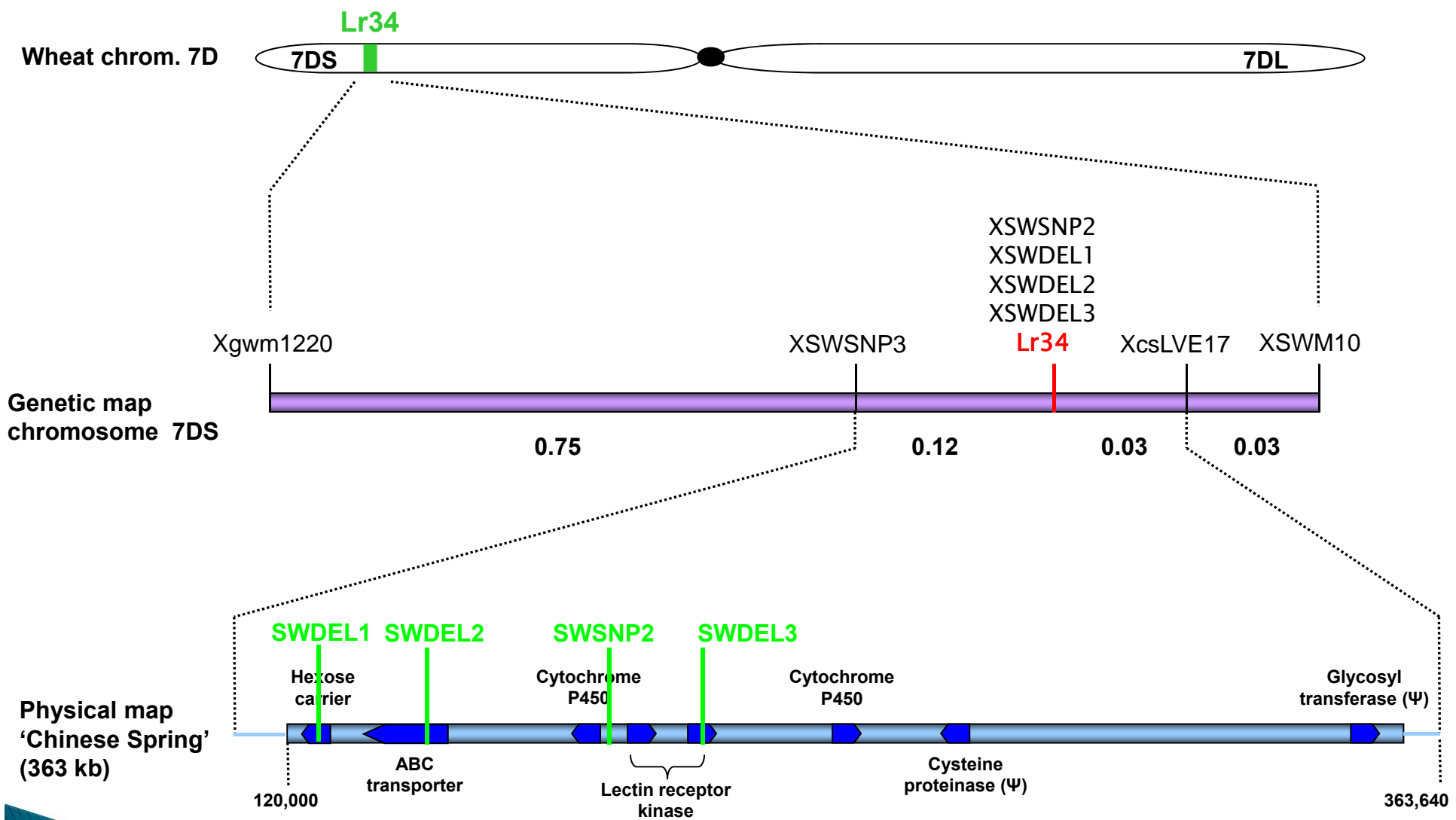
Lr34 (=QTL2): a durable leaf rust resistance gene



***Lr34* is a durable disease resistance gene**

- **Has been effective against leaf and stripe rust for more than 100 years in the field, certainly for 40 years at large scale**
- **Confers partial resistance**
- **Prolongs the latency period and reduces the production of spores (slow-rusting gene)**
- **Is not race specific**

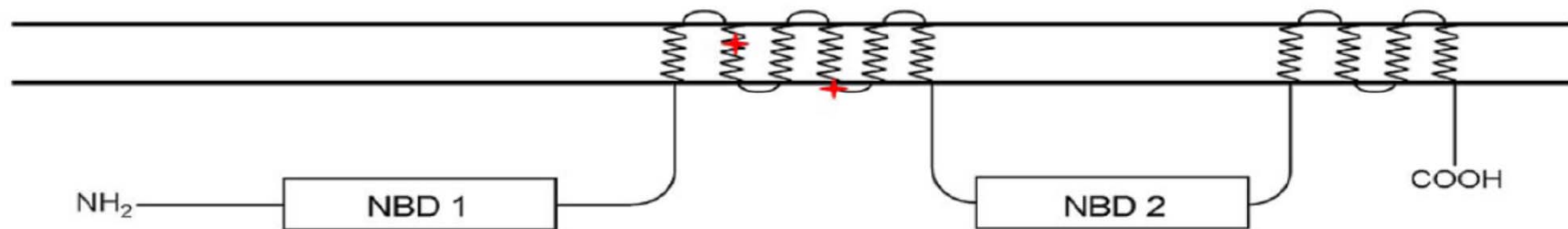
Map-based cloning of the *Lr34* gene



Eight open reading frames within a 363 kb target interval

The molecular basis of the resistance effect of the *Lr34* gene: *Lr34* encodes a putative ABC transporter (ABCG or PDR) protein

Schematic structure of LR34 in the plant membrane:

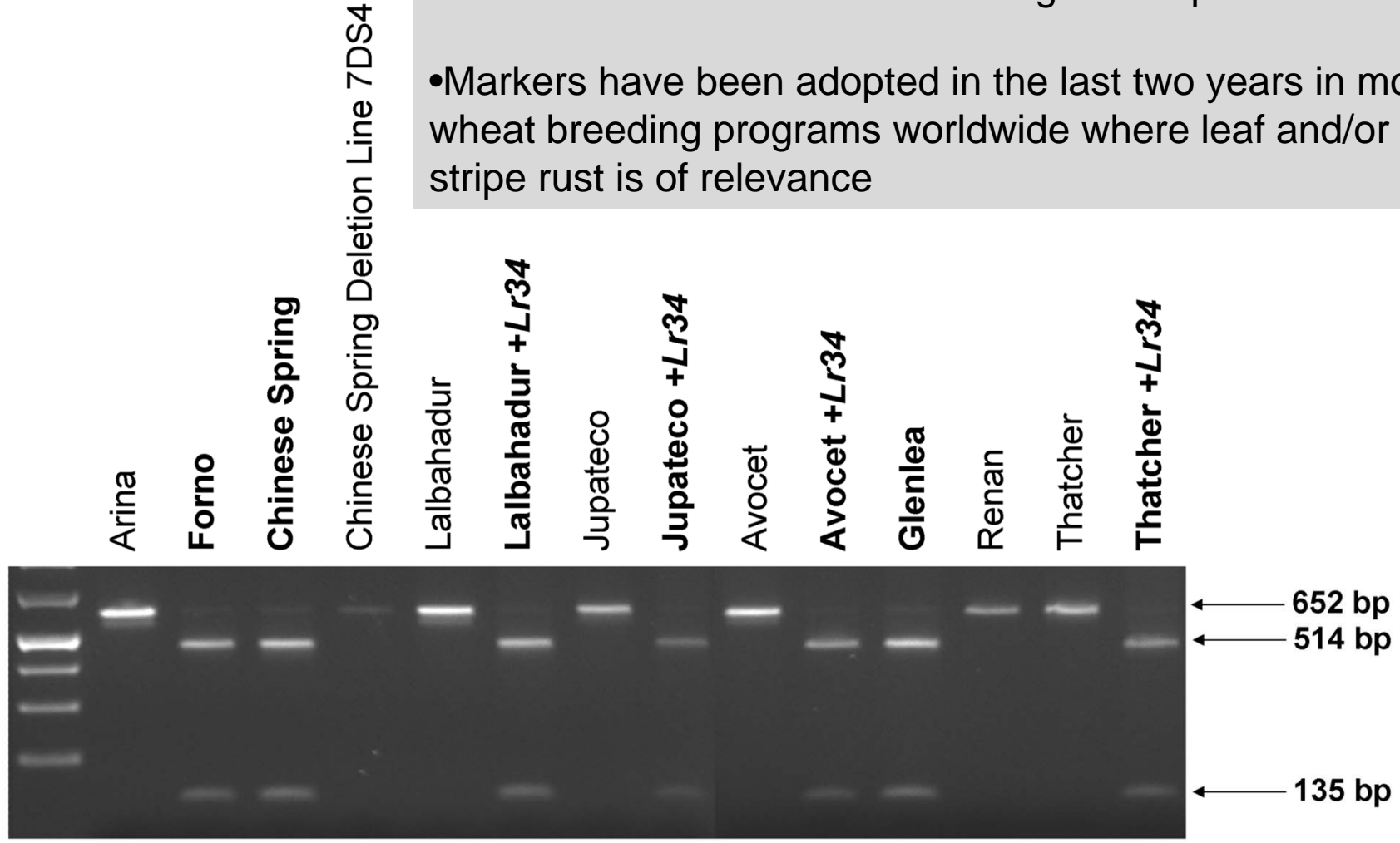


Resistant and susceptible allele differ by only two amino acid polymorphisms

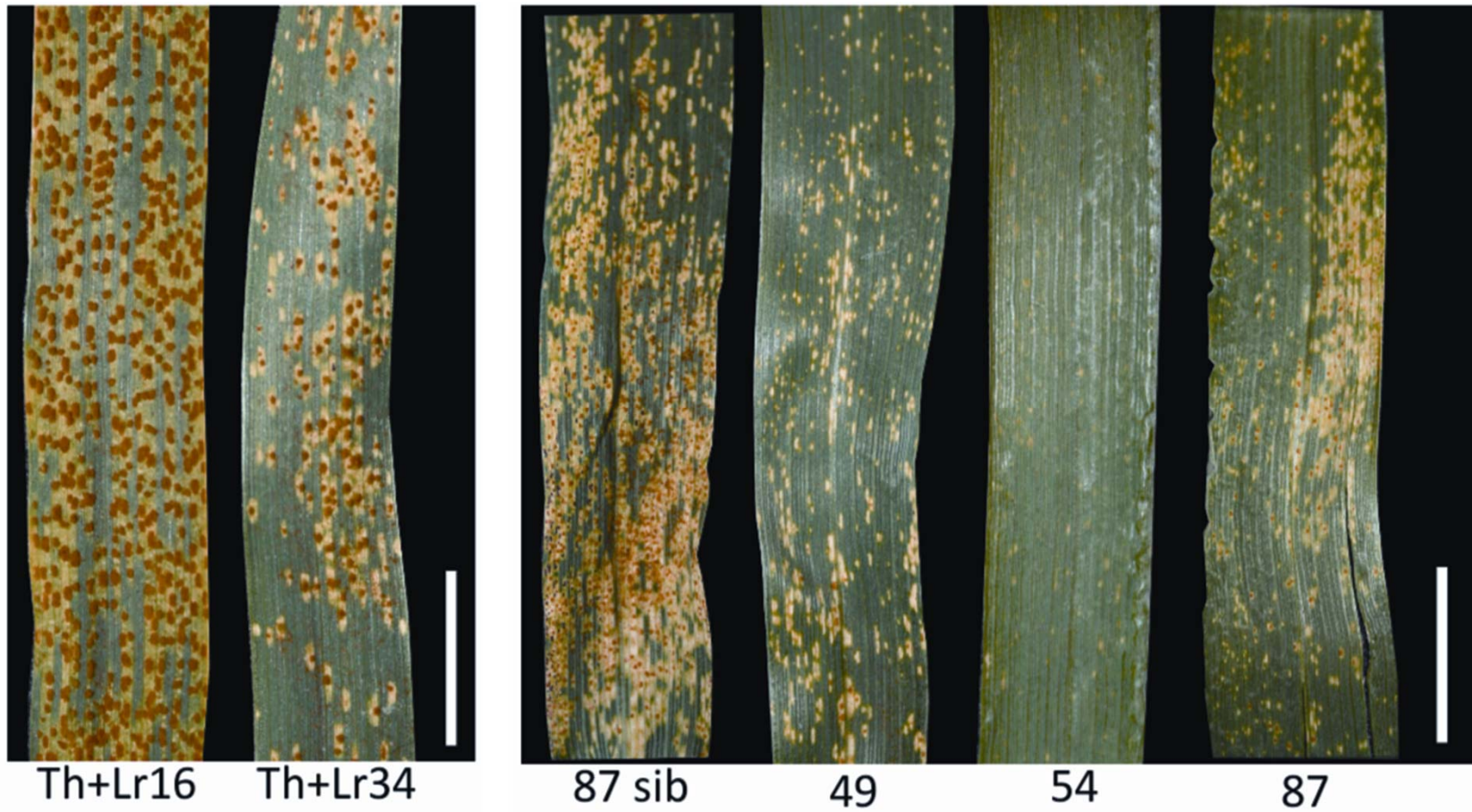


Development and application of molecular markers for the *Lr34* gene:

- Perfect markers derived from the gene sequence
- Markers have been adopted in the last two years in most wheat breeding programs worldwide where leaf and/or stripe rust is of relevance

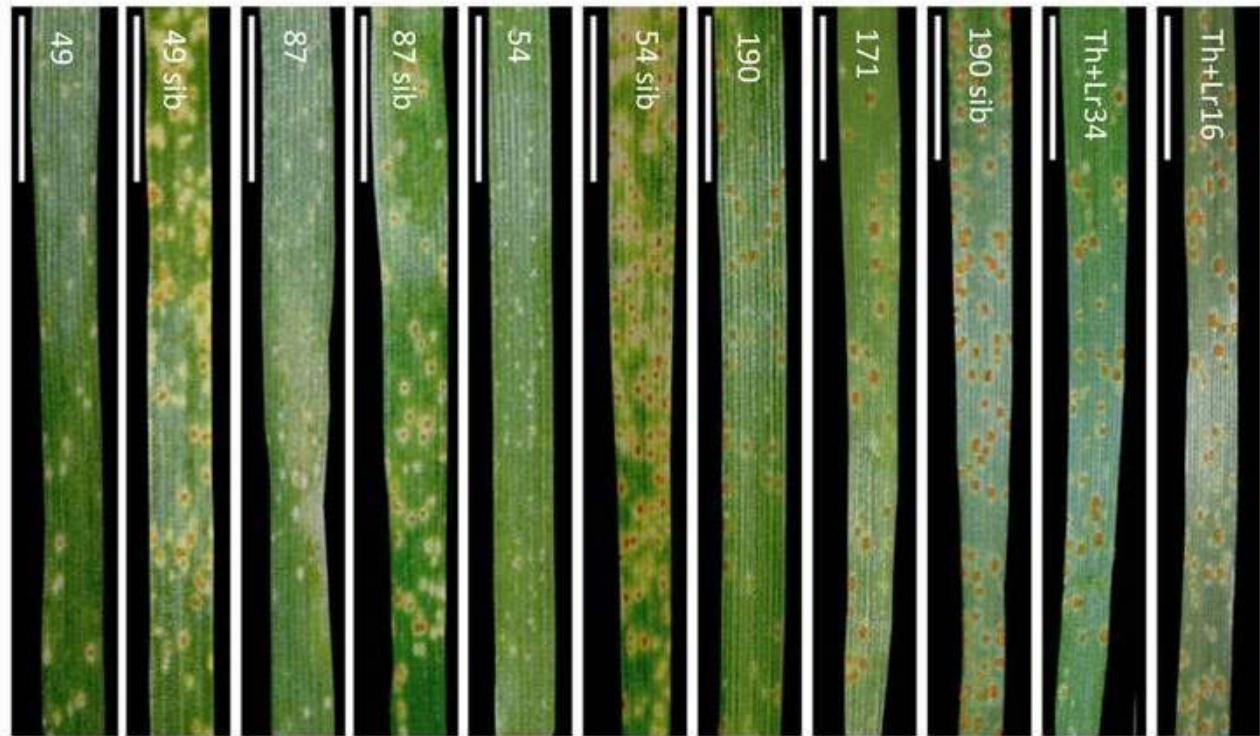
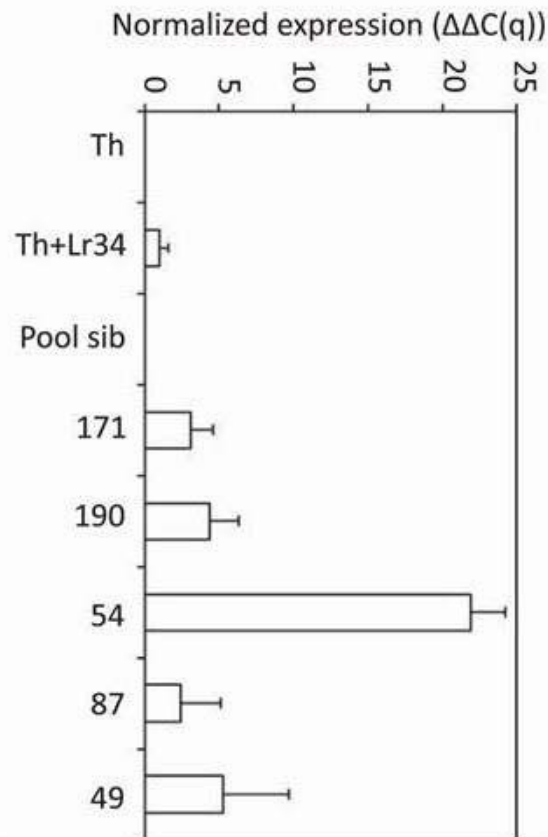


Functional studies of the *Lr34* gene in transgenic wheat: flag leaf of adult plant



Risk, Selter et al. 2012
Plant Biotech. J.

Genetic background of transgenic wheat plays an important role: seedling resistance at 20°C



→ Additive interaction(s) with other gene(s)

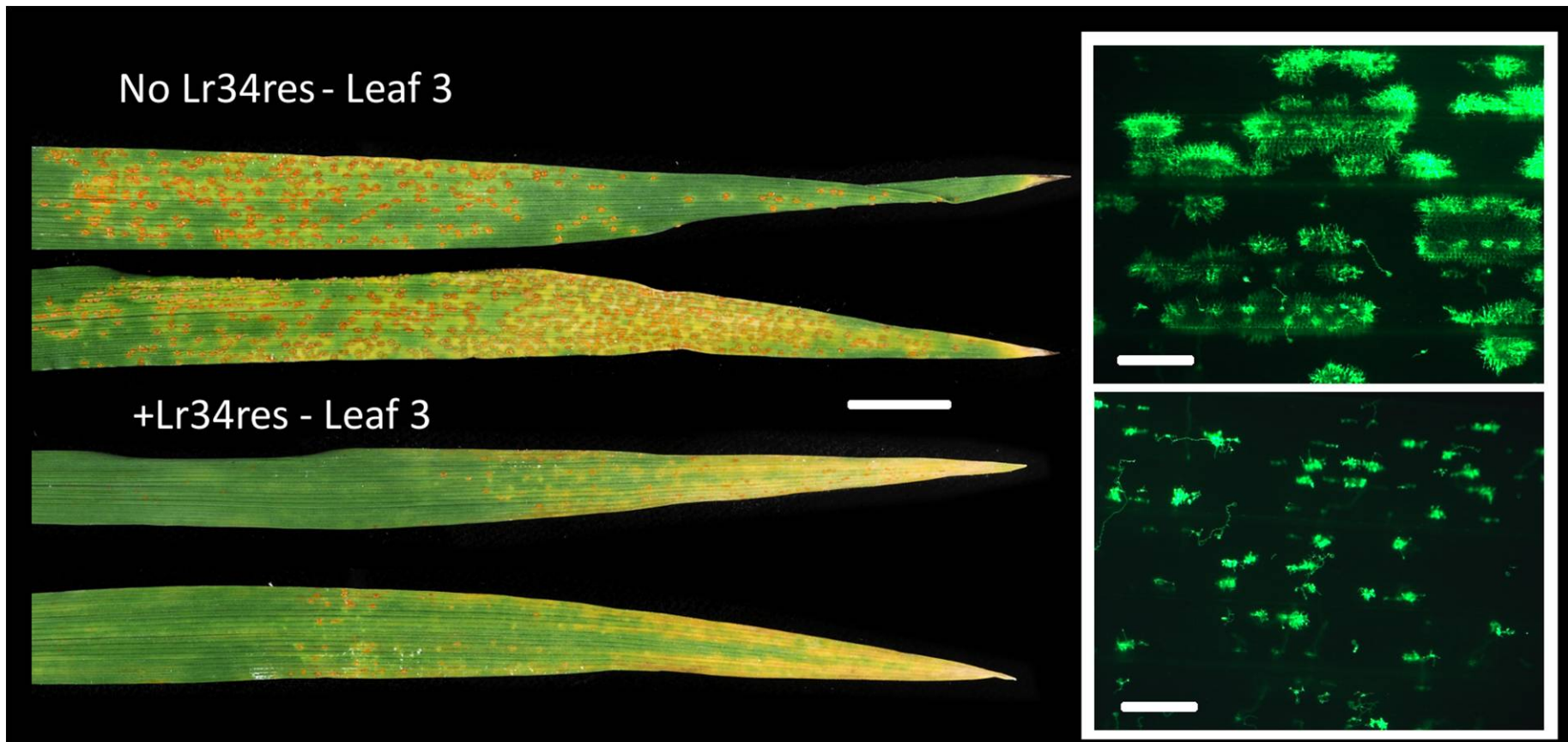
Risk, Selter et al. 2012
Plant Biotech. J.

Conclusions from transgenic wheat lines with *Lr34*

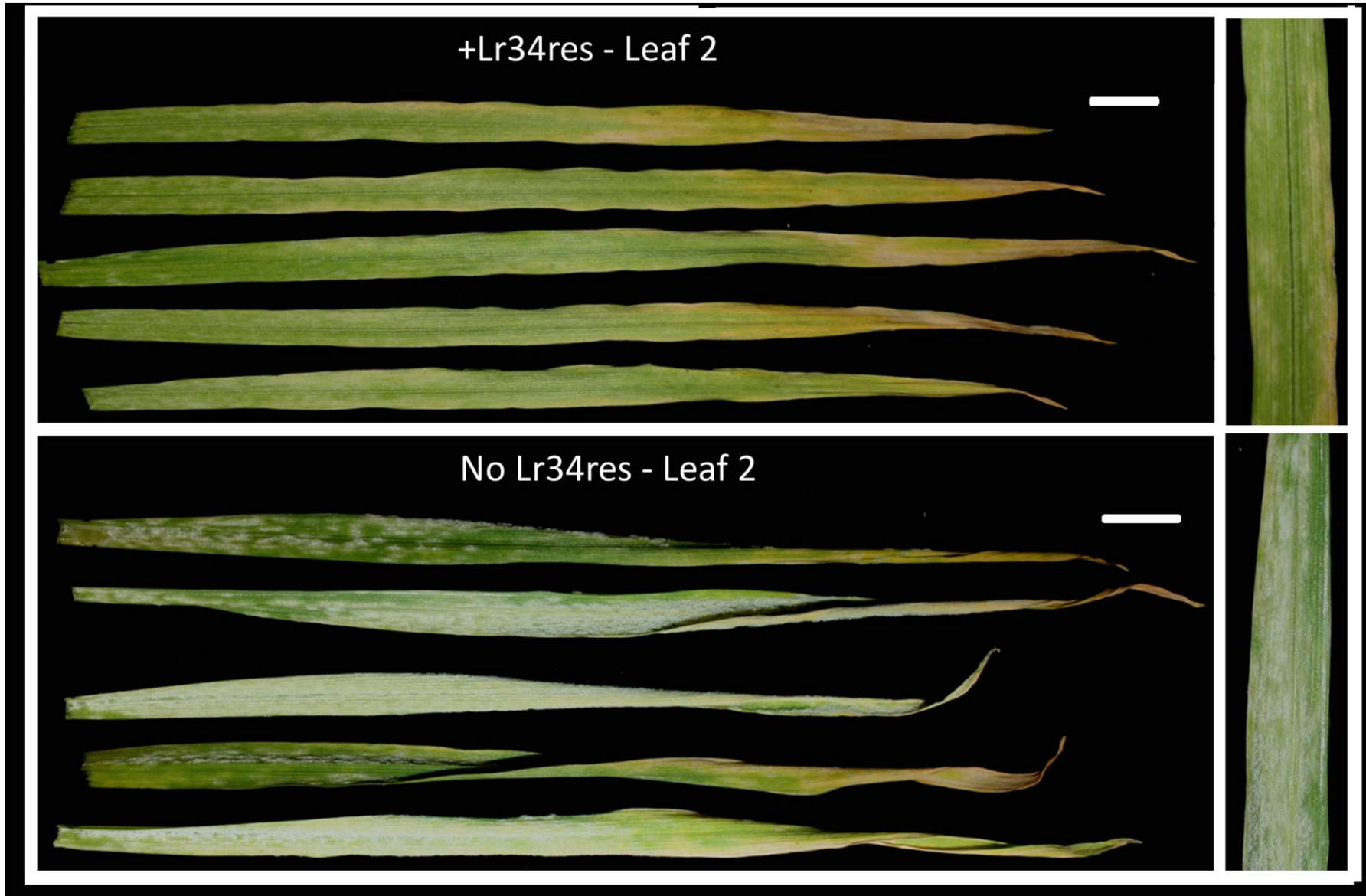
- The transgene is fully functional against leaf rust
- The genetic background can result in improved resistance: one should broadly cross the most active transgene into a broad set of breeding material (...normal breeding procedure...)
- Combination with other durable resistance genes in a cassette or by crossing seems a good strategy



Heterologous expression of *Lr34* provides multi-pathogen resistance in barley seedlings



Heterologous expression of *Lr34* provides multi-pathogen resistance in barley seedlings: powdery mildew



Conclusion from the heterologous barley system:

All components needed for resistance are also present in barley (substrate for transport, signal transduction chain, response or priming mechanisms...)

Generally true for grasses?

Interesting new type of resistance to further explore and improve (by reduction of negative side effects)



Transgenic use of R genes and QTL provide new approaches for a more sustainable and efficient use of genetic resources for resistance

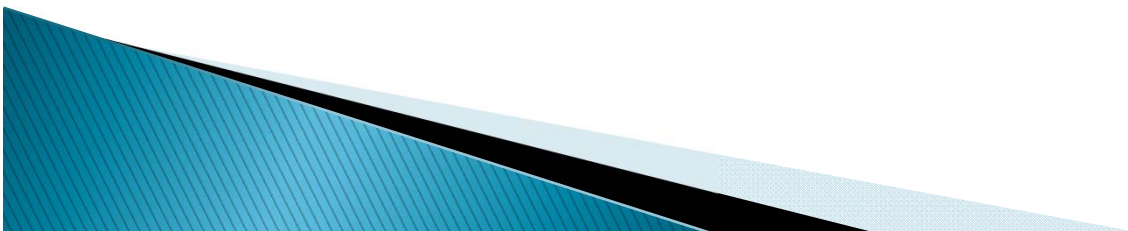
Evidently, such work is very difficult in the current regulatory environment



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Resistance breeding: outlook

- Resistance breeding is a continuous task
- Innovative use of existing resistance resources is imperative for a sustainable application
- There are new options on the horizon towards artificial, new resistance genes based on improved molecular understanding of disease resistance



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