Transgenic and genomics-assisted breeding approaches to improve durable fungal disease resistance in wheat

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Wageningen, NL, September 3, 2015
Narrow basis of our food sources. Wheat is a major contributor

20% of all calories for human nutrition are derived from wheat

Around 10% of potential wheat yield are lost because of pathogens

Oerke, 2010
Disease resistance in wheat: major genes are an important source of resistance

Wheat gene catalogue:

Against leaf rust: 65 genes
Against powdery mildew: 43 genes
Against stem rust: 50 genes
Against stripe rust: 68 genes

http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp
*R* genes (effector-triggered immunity) remain a highly important source of resistance in breeding

**Major resistance (*R*) genes are often not durable:**

Can we find ways to use them more durably?

Can we modify them to make them more broad-spectrum and/or more durable?
Use resistance genes in a more sustainable way

- Pyramiding of genes

- Improving resistance genes based on molecular knowledge: modification of resistance genes

- Multilines: host diversity effects
Make pyramids of major $R$-genes (can also be in the form of cassettes)

The idea is to put more than one gene into the same cultivar

- Theoretically attractive
  - Multiple mutations to virulence in same pathogen strain unlikely
  - If loss of virulence imposes fitness cost, many $R$-genes working together may result in weak pathogen
Wheat powdery mildew caused by *Blumeria graminis* f.sp. *tritici* (Bgt)
Allele pyramiding

Race specific resistance:

<table>
<thead>
<tr>
<th>Wheat differential line</th>
<th>Pm3 allele</th>
<th>mildew isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asosan/8*Chancellor</td>
<td>Pm3a</td>
<td>isolate 1</td>
</tr>
<tr>
<td>Chul/8*Chancellor</td>
<td>Pm3b</td>
<td>isolate 2</td>
</tr>
<tr>
<td>Sonora/8*Chancellor</td>
<td>Pm3c</td>
<td>isolate 3</td>
</tr>
<tr>
<td>Kolibri</td>
<td>Pm3d</td>
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Allele pyramiding:

Expectation:

- Pm3 pyramid e.g. Pm3b + Pm3d

→ strategy for:
- expansion of resistance spectra
- longer durability (if redundancy in recognition)
**Pm3 resistance genes:**

- dominant CC-NBS-LRR genes (Yahiaoui et al., 2004)
- 17 functional (*Pm3a* - *g, Pm3k* - *t*) (Bhullar et al., 2009, 2010)
Pyramiding of $R$ genes / alleles

Cross of differential lines
F1 hybrids – *Pm3* differential lines

**Infection test:** F1 hybrids vs differential lines

Chul/8*CC (Pm3b) × Bgt 97011 (avrPm3b / AvrPm3f)
Chul/8*CC × Bgt 98229 (avrPm3b / AvrPm3f)
M.Amber/8*CC × Bgt 07201 (AvrPm3b / avrPm3f)

=> *Pm3f* resistance is suppressed in F1 hybrids
Gene dosage is unlikely to be related to suppression

*Pm3* alleles show dominant inheritance, *i.e.* heterozygous gene dosage does not affect the intensity of resistance reaction and does not explain resistance gene suppression.

Homozygous lines would be very informative: genetically stable material for further molecular analysis of the suppression effect.
Wheat lines containing transgenic *Pm3* alleles:

Chul (landrace from Asia)

Bobwhite S26
Pyramiding strategy: combine two different alleles in the same genotype by crossing of transgenic lines

Cross of transgenic lines
(different insertion site)

- Cross breeding of transgenic Bobwhite SH9826 lines:
  - $Pm3a_{HA}$
  - $Pm3b, b_{HA}, b_{myc}$
  - $Pm3c_{HA}$
  - $Pm3d_{HA}$
  - $Pm3f_{HA}$

(Brunner et al., 2012)

- Stable expression over multiple generations

- 3 independent crosses per combination
Infection test: double homozygous vs parental lines

Double homozygous lines: additivity of gene function...

Bgt 95.9 Asosan
(avrPm3a / AvrPm3d)

Bgt 07298
(AvrPm3a / avrPm3d)
Double homozygous lines: but not always additive!

**Infection test:** double homozygous vs sister lines

<table>
<thead>
<tr>
<th>Bgt isolate 07230</th>
<th>$Pm3b$</th>
<th>$Pm3b \times Pm3f$</th>
<th>$Pm3f$-HA</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<th>Bgt isolate 97011</th>
<th>$Pm3b$</th>
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<th>$Pm3f$-HA</th>
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<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
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→ field trials 2014-2018
**Double homozygous lines:**

Protein analysis

**Western Blot:** double homozygous vs sisterlines

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<table>
<thead>
<tr>
<th>cross</th>
<th>Pm3b&lt;sub&gt;myc&lt;/sub&gt;&lt;sup&gt;f&lt;sub&gt;HA&lt;/sub&gt;&lt;/sup&gt;</th>
<th>sister line Pm3&lt;sub&gt;b&lt;sub&gt;myc&lt;/sub&gt;&lt;/sub&gt;&lt;sup&gt;(Δf&lt;sub&gt;HA&lt;/sub&gt;)&lt;/sup&gt;</th>
<th>sister line Pm3&lt;sub&gt;(Δb&lt;sub&gt;myc&lt;/sub&gt;)&lt;sup&gt;f&lt;sub&gt;HA&lt;/sub&gt;&lt;/sup&gt;</th>
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<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
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</table>

**Pm3b-myc**

- IB: α-myc
- Ponceau

**Pm3f-HA**

- IB: α-HA
- Ponceau

Protein levels can **not** explain suppression

→ Suppression at post-translational level
Mutation in MHD motif:

- Relaxation of nucleotide binding pocket
- Autoactive state (ATP bound) (Tameling et al., 2006; van Ooijen et al., 2008)
- Mimics an activated protein
**N. benthamiana infiltrations:**

**Heterologous system**

- **Pm3f_{D501V} +**
  - GUS -control
- **Pm3f_{D501V} +** hPm3-1B (78% similarity to PM3B)

**Pm3b**

**hPm3-1B**
N. benthamiana infiltrations:
Heterologous system

$Pm3f_{D501V}$ +

$Pm3b$  $hPm3-1B$
(78% similarity to PM3B)

$\rightarrow$ post-translational suppression

$\rightarrow$ *Nicotiana* system recapitulates wheat results
**Co-IP:** *Nicotiana benthamiana* mixed infiltrations

- CaMV-35S promoter
- 2 dpi

PM3B and PM3F form heteromers
Conclusions on *Pm3* suppression

• Suppression can be a limiting factor for resistance pyramiding. This should also be considered for gene cassettes

• Suppression mechanism involves post-translational processes, possibly formation of heteromeric, non-functional protein complexes

• This mechanism may explain many phenomena
  – Loss of resistance in polyploid species *(see next results on Pm8)*
  – Dominance/recessiveness of some *R* genes depending on genetic background

• Suggests possibilities to circumvent suppression
**Pm3 and Pm8:** Suppression of the powdery mildew resistance gene Pm8 derived from rye after introgression into some wheat backgrounds.

- Pm3 and Pm8 are orthologs (Hurni et al. 2013, Plant J.)
Suppression of *Pm8* in wheat

- Not all wheat lines with 1BL.1RS are resistant to powdery mildew:

  - Suppressor is linked with *Pm3* haplotype markers (McIntosh et al., 2011)
  - Some lines with suppressed *Pm8*-mediated resistance are known to carry *Pm3* or have *Pm3*-lines in pedigree

→ Is *Pm3* the suppressor of *Pm8*?
Suppression of \( R \) genes

- Considerable problem in resistance breeding
- Often observed when genes from a lower ploidy level are introduced in a higher one (e.g. also in producing «Synthetic» wheat)
- Might be caused by the polyploid nature of the wheat genome

\[ \Rightarrow \] So far only genetic data but no description of the molecular mechanism
Suppression of *Pm8*-mediated resistance

- The *Pm8* gene and a *Pm3* allele are present in lines suppressing *Pm8*-mediated resistance.

<table>
<thead>
<tr>
<th>Wheat line</th>
<th><em>Pm8</em>-mediated resistance</th>
<th><em>Pm8</em></th>
<th><em>Pm3</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kavkaz/4*Federation</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Benno</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ambassador</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Veery#6</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Veery#5</td>
<td>-</td>
<td>+</td>
<td><em>Pm3_8152</em></td>
</tr>
<tr>
<td>Florida</td>
<td>-</td>
<td>+</td>
<td><em>Pm3CS</em></td>
</tr>
</tbody>
</table>

*+/−: presence/absence of the phenotype or gene

→ Suppression of *Pm8*-mediated resistance is not due to gene absence or mutation

Hurni et al. 2014, Plant J.
Pm8 is suppressed in presence of Pm3CS

- Crossing of a Pm8-translocation line with a Pm3CS line

\[ Pm3CS \rightarrow Pm8 \]

Chinese Spring Kavkaz/4*Federation

F4

<table>
<thead>
<tr>
<th></th>
<th>(ΔPm3CS)/Pm8</th>
<th>Pm3CS/Pm8</th>
<th>Pm3CS/(ΔPm8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bgt 07230</td>
<td></td>
<td></td>
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<tr>
<td>Bgt 07250</td>
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</tbody>
</table>

→ Pm8-mediated resistance is suppressed in lines homozygous for Pm8 and Pm3CS
Do functional *Pm3* alleles suppress *Pm8*?

- Crosses between *Pm8* and *Pm3* (*a*, *b* or *f*) transgenic lines

- Stable transgenic lines used:
  - *Pm8*#59 (Hurni et al., 2013)
  - *Pm3a*#1 (Brunner et al., 2012)
  - *Pm3f*#1 (Brunner et al., 2012)
  - *Pm3b*_{HA} (Stirnweis et al., submitted)

- All lines showed race-specific powdery mildew resistance over several generations

- Selection for sister and double homozygous lines by PCR markers in F3 generation
*Pm8* is suppressed in double homozygous line

- Cross *ubi:Pm8-myc* with *ubi:Pm3b-HA*
- Infection test with F4 individuals

\[
\begin{array}{ccc}
Pm8/(\Delta Pm3b) & Pm8/Pm3b & (\Delta Pm8)/Pm3b \\
Bgt AK3-11 & & \text{AvrPm8/avrPm3b} \\
Bgt 98229 & \text{AvrPm8/avrPm3b} & \\
Bgt 10004 & \text{avrPm8/AvrPm3b} & \\
\end{array}
\]

→ *Pm8*-mediated resistance is suppressed in the double homozygous line *Pm8/Pm3b*, but dependent on the pathogen race used!
Conclusions on *Pm8* and allele pyramiding

- The *Pm8* gene is present in suppressed translocation lines along with a *Pm3* allele
- *Pm3* is the dominant suppressor of *Pm8*

→ A post-translational mechanism is involved in suppression
Negative interference of NB-LRR proteins: Outlook and lessons learnt for classical breeding

- Possible suppression effects should be identified before the lengthy breeding process starts (classical or transgenic): If genes are cloned, the Nicotiana assay gives a rapid answer.

- Understanding the molecular basis of suppression in the LRR domain: make modifications in genes to avoid it?

- Gene pyramids might have to be studied more before making them...
PM3 activity project: towards artificial resistance genes

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

published in the focus issue “Translational research” of Molecular Plant-Microbe Interactions, Vol. 27, No.3, 2014
Virulence analysis of our *Bgt* isolate collection:

Enhanced *Pm3* signalling responsible for extended resistance spectrum
• Mutation in MHD motif:

→ relaxation of nucleotide binding pocket
→ autoactive state (ATP bound) (Tameling et al., 2006; van Ooijen et al., 2008)

→ mimics an activated protein

"Pm3^{HR}"
Results:

*Pm3* alleles with extended or narrow spectrum

6 dpi

\[ Pm3c^{HR} \]

\[ Pm3b^{HR} \]
Results:

Pm3 alleles with extended or narrow spectrum

Stirnweis et al., MPMI, 2014
**Results:**

*Pm3c vs Pm3b*

Pm3c with two amino acid changes has become a stronger allele.

=> Pm3c with two amino acid changes has become a stronger allele.
Results:

**Pm3a vs Pm3f**

A. L456P/Y458H enhances activity of PM3F without causing autoactivity

B. P456L/H458Y reduces activity of PM3A

Stirnweis et al., MPMI, 2013
Results:  

**Pm3a** vs **Pm3f** transient assay in wheat

L456P/Y458H: > expansion of PM3F resistance spectrum  
> without unspecific resistance activation  
> explains all the *Pm3a*-ARC effect
Conclusions

Resistance specificity

= Recognition specificity + Activation efficiency

• ARC2 loop is key regulator of ‘molecular switch’ in CC-NBS-LRR
• Minimally invasive resistance optimization (TALEN, CRISPR-Cas)
• Beneficial effect? Temperature insensitivity?!
• “a general blueprint through which nucleotide binding–leucine-rich repeat genes against diverse pathogens could be enhanced” (McDowell et al., MPMI, 2014)
We have recently started a new series of field trials for 2014-2018:

Pyramiding of *Pm3* alleles

Testing a new *Pm3* allele

Agronomically relevant resistance can only be determined with confidence in the field.

Establishment of a Protected site for field trials by the Swiss Government and Parliament starting in 2014

www.protectedsite.ch
Conclusions

Resistance genes: more efficient use in future breeding?

• Resistance genes should be used in a more sustainable way: this seems to be possible, particularly when transgenic/cisgenic approaches are included

• There was probably not enough time in evolution to come up with all possible interesting variants: Artificial resistance genes based on knowledge of natural diversity can be envisaged