Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

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Site-directed mutagenesis of candidate susceptibility genes to achieve aphid and disease resistance in barley

6th Plant Genomics & Gene Editing Congress: Europe, Rotterdam, the Netherlands 14-15 May 2018

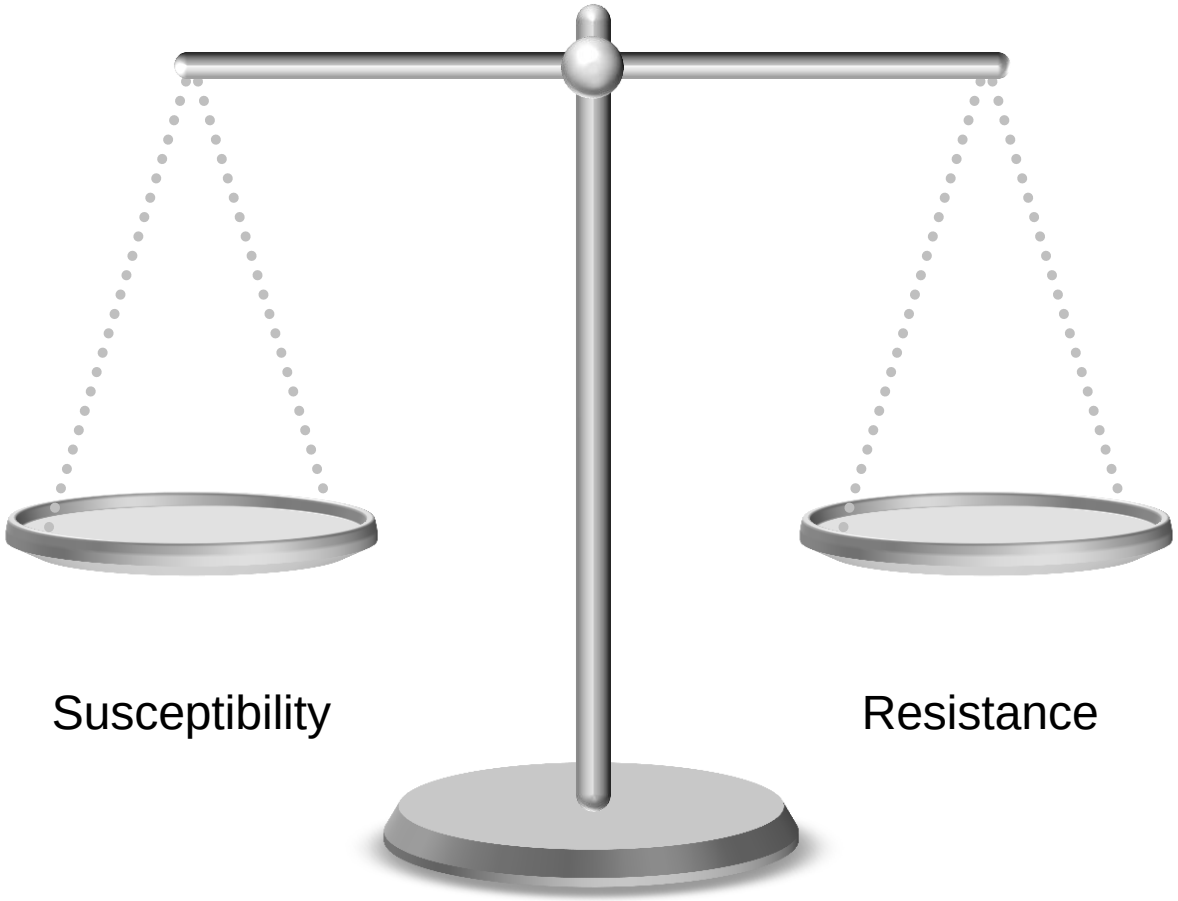
Deeper and deeper understanding of plant defense and plant - pest/disease interactions

along with

Better and better methods to breed with precision

Organisms manipulate their hosts to their own advantage!

Resistance breeding emphasis has so far been placed on introducing resistance genes, now on knock-out of susceptibility genes



Susceptibility gene knock-out by mutation

Type of mutation	Position	Pros and cons
Natural	Random gene and site	- Rare
Induced by chemicals or radiation	Random gene and site	- Too many mutations in each plant
Site-directed	Specific gene and site	+ Single, deliberate

Development of site-directed mutation techniques

ODM Oligonucleotide Dependent Mutagenesis

ZFN Zink Finger Nucleases

TALEN Transcription Activator-Like Effector Nucleases

CRISPR-Cas Clustered Regularly Interspaced Short Palindromic Repeats

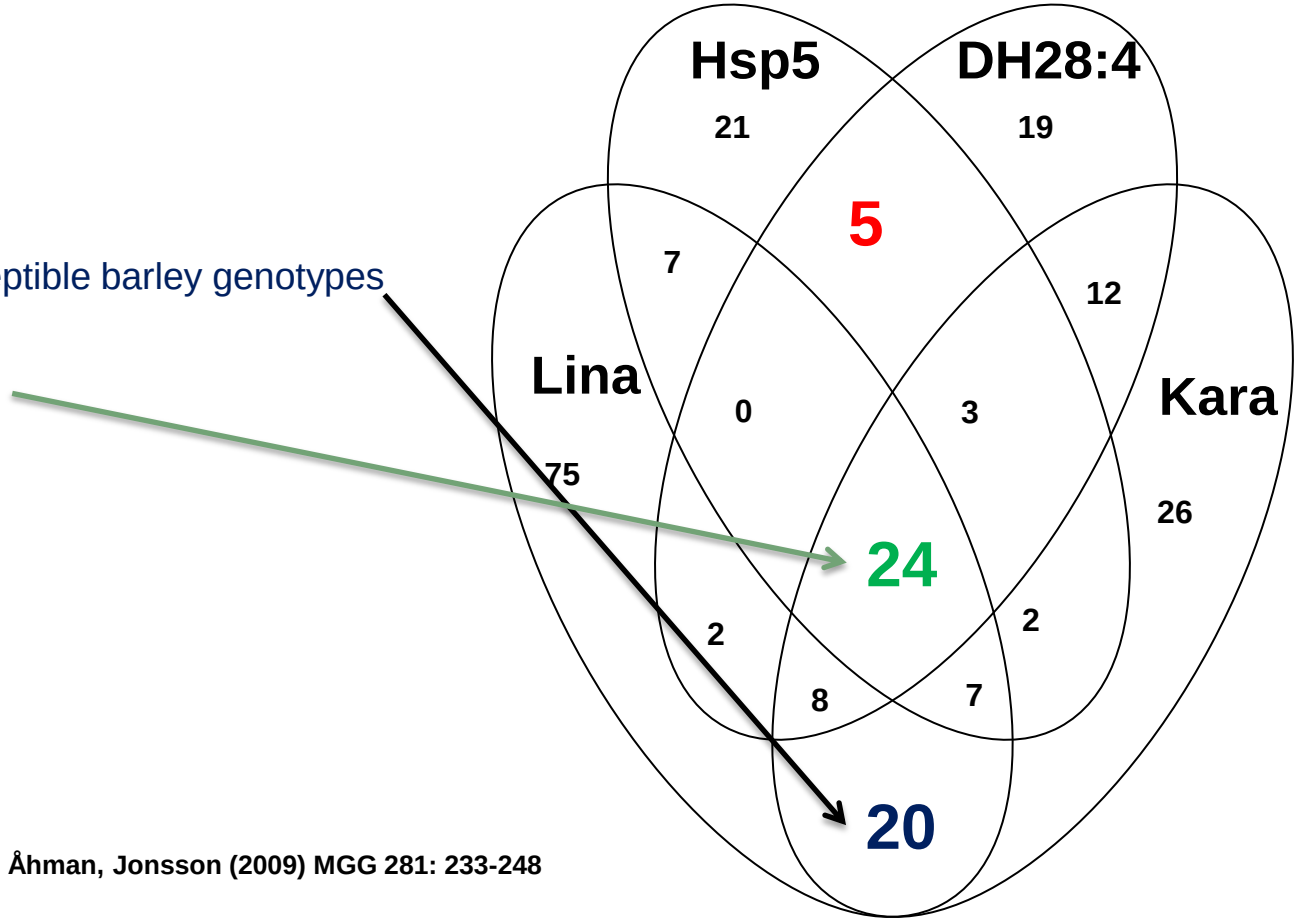
Aphids


Photo: Sate Al-Abbasi

- Ca 4700 species
- Ca 450 species on crop plants
- 95% of the species feed on one or a few related plants
- All are phloem feeders
- Founder female gives rise to a clonal colony of wingless aphids
- Many aphid/host plant interactions are now being studied at the molecular level

Genes differentially induced by *Rhopalosiphum padi* on two susceptible and two partially resistant barley genotypes as determined in a microarray

- Induced only in susceptible barley genotypes
- Induced in all plants



PlantComMistra

Delp, Gradin, Åhman, Jonsson (2009) MGG 281: 233-248

Table 4 Sequences induced specifically in susceptible lines

Barley1 contig no.	Putative function	Log2 ratio[a]	
		Kara	Lina
Contig12046_at	PR1 *Zea Mays*	4.13	2.99
Contig1636_at	*β*-1,3-Glucanase III *Hordeum vulgare*	1.51	1.08
Contig6519_at	Pathogen-induced protein WIR1A *Triticum aestivum*	3.46	3.71
Contig2306_s_at	MeJA-inducable lipoxygenase 2 *Hordeum vulgare*	1.71	1.58
Contig12753_at	Putative ABC transporter *Oryza sativa*	1.93	1.63
Contig9662_at	Hexose transporter-like protein Arabidopsis	1.18	1.27
Contig12724_at	Putative flavanone 3-hydroxylase *Oryza sativa*	1.28	2.38
Contig10860_at	Oxalate oxidase *Oryza sativa*	1.44	2.21
Contig10167_at	WRKY7 transcription factor *Oryza sativa*	1.20	1.54
Contig10168_at	WRKY7 transcription factor *Oryza sativa*	1.27	1.63
Contig11653_s_at	Ca-binding EF-hand protein *Oryza sativa*	1.56	1.36
Contig6170_s_at	Helix-loop-helix protein *Oryza sativa*	1.79	1.61
Contig11773_at	No similarity	2.48	2.04
EBro04_SQ003_G13_at	No similarity	1.34	2.57

[a] Average of all nine pair-wise comparisons for each line

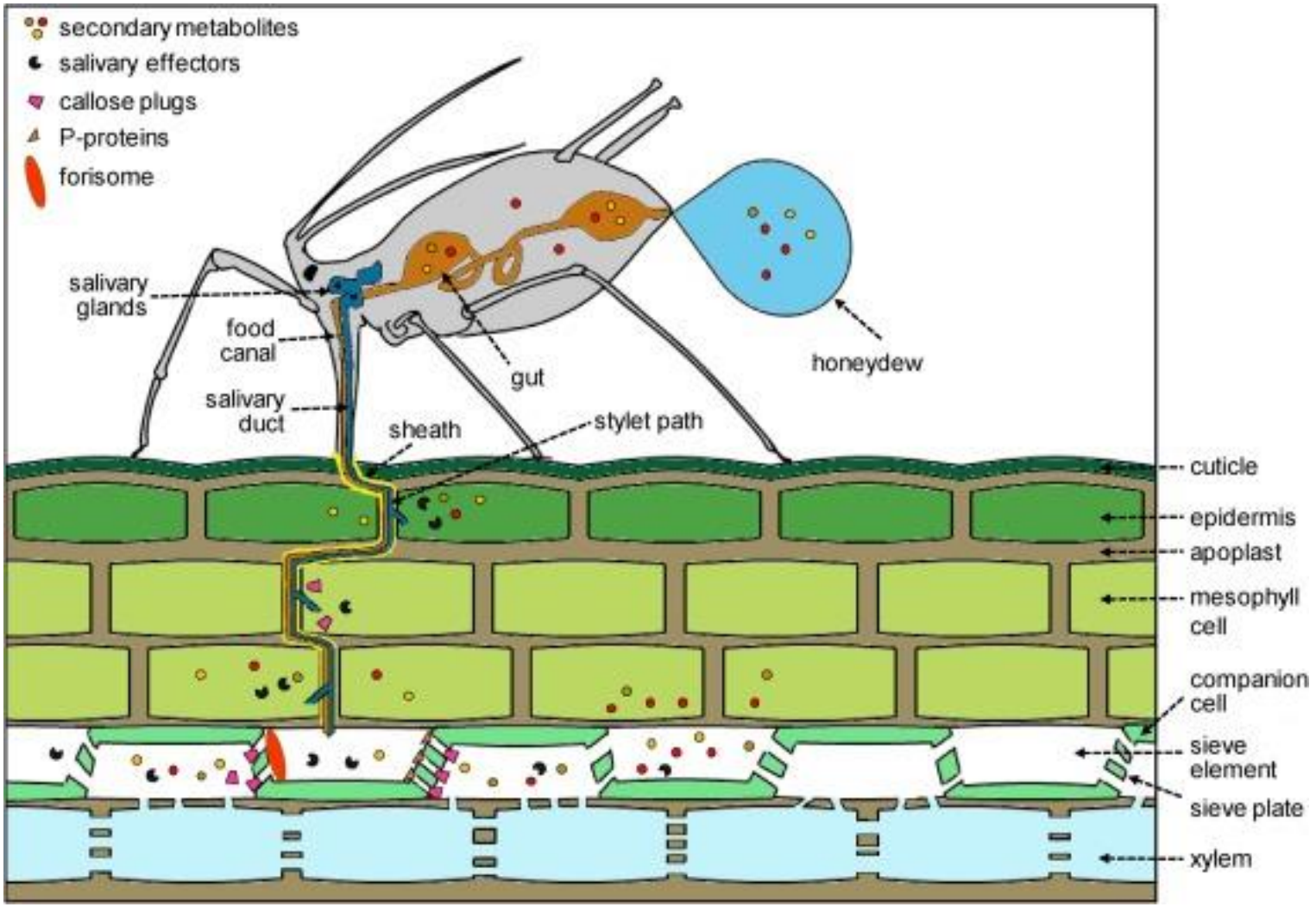


Figure from Nalam, Louis, Shah in Plant Science (2018)

Plant defense against aphids, the pest extraordinaire

Rationale for certain β-1,3-glucanases to be candidates for plant susceptibility to aphids, *R. padi* in particular

- β-1,3-glucanases break down callose
- *R. padi* feeding is not causing much build up of callose in sieve elements (Saheed et al. 2009)
- *R. padi* induces certain glucanase genes more in susceptible than in resistant barley genotypes (Delp et al. 2009; Mehrabi et al. 2016)
- Glucanase genes are upregulated more in susceptible than resistant wheat lines by *Schizaphis graminum* (Reddy et al. 2013), but the reverse is true for *Diuraphis noxia* (van der Westhuizen et al. 1998, 2002) and another study of *R. padi* with just one barley genotype of each kind (Forslund et al. 2000)
- Turnip mosaic virus suppresses callose deposition, induced by its vector *Myzus persicae* (Casteel et al. 2015)

Two **β-1,3-**glucanase genes targeted by CRISPR/Cas9

- Constructs developed by Mark Smedley at John Innes centre and transformed by Agrobacterium into cv. Golden Promise in Wendy Harwood's lab
- Each of two constructs targeted a conserved sequence in both genes and one unique sequence for one of the genes
- Mutation frequencies are determined initially by high-resolution capillary electrophoresis of DNA fragments
- One base insertion is the most common mutation in generation T0
- Mutation frequencies in transgenic T0 are 41 and 25% (n= 39 & 36; 3 targets each)
- T1s are often chimeric
- Homozygous double- and single-gene mutants, without CRISPR construct, will be tested for *R. padi* performance

Defense against pathogens with different life styles: biotrophs necrotrophs

Powdery mildew

Pyrenophora teres f. teres (Ptt)

Photos: Växtskyddscentralen

Mechanical damage facilitates attack by necrotrophic fungi:

Necrosis area (mm^2) at infection site

Cultivar	Pin-damaged		Non-damaged
Barke	116.6	>	31.9
Pirkka	74.9	>	20.6

Pyrenophora teres

Saccharin induces: resistance to

but???

Powdery mildew

Pyrenophora teres f. teres (Ptt)

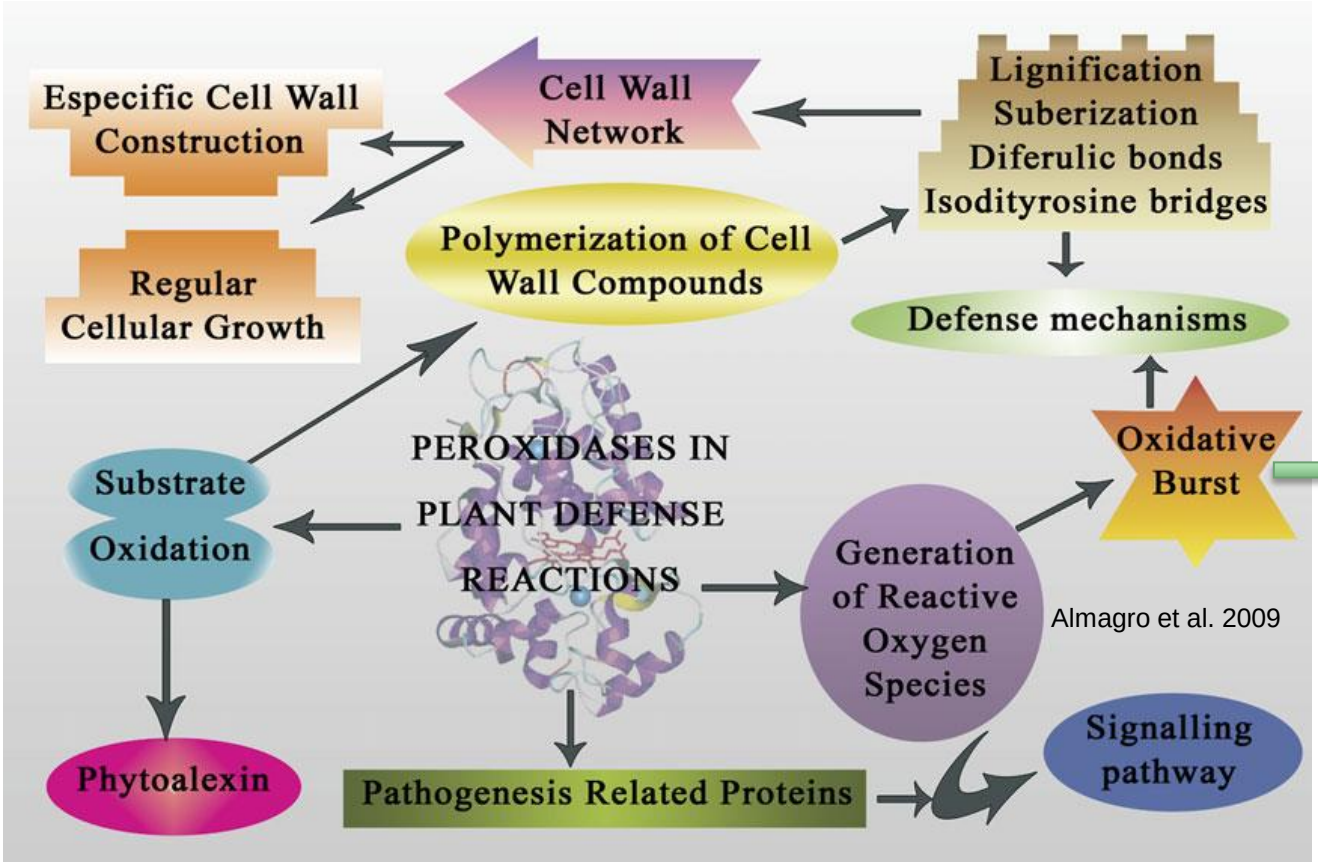
Photos: Växtskyddscentralen

Saccharin induces increased susceptibility to *Pyrenophora teres*

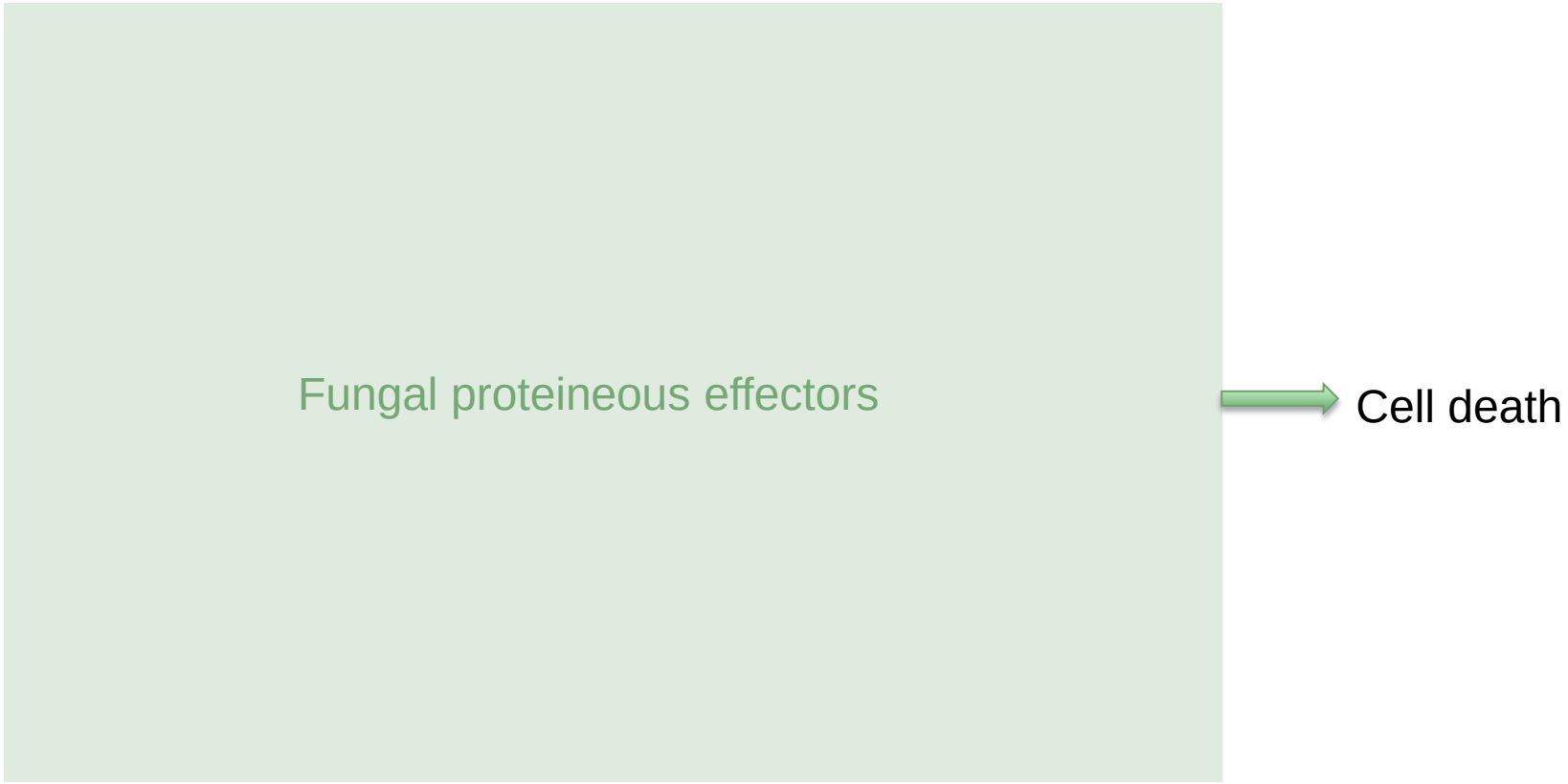
Necrosis area (mm^2) at infection site

Cultivar	Water	Saccharin
Barke	26.6	45.1
	4.4	12.2
	31.9	52.8
Pirkka	1.7	8.2
	20.6	24.2
Prior	1.2	6.7

Saccharin induces Peroxidases



In necrotrophic fungi

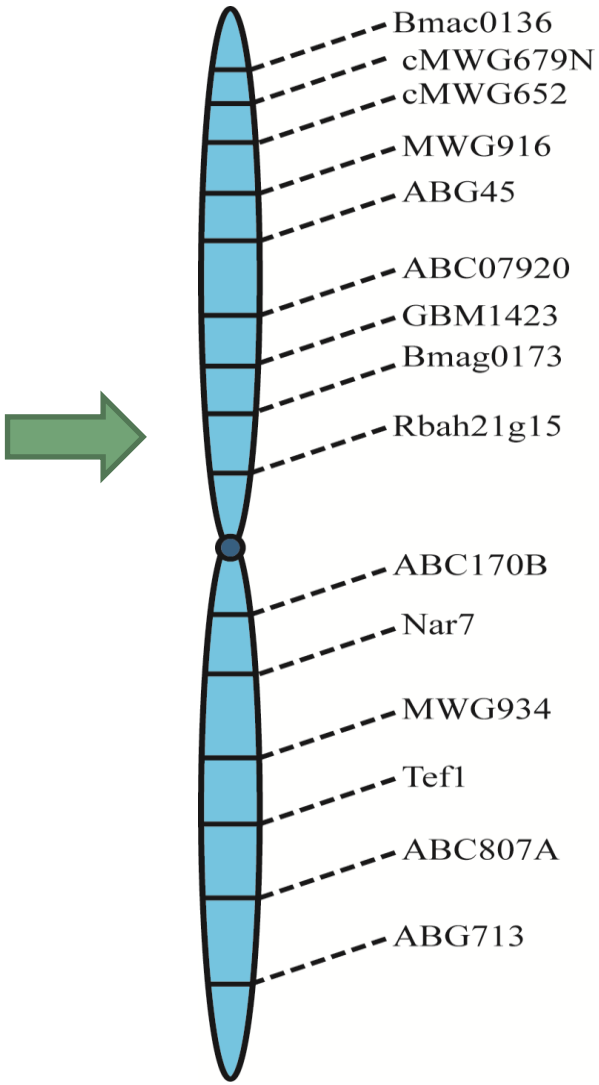


Mapping *Ptt* Resistance

Resistance and susceptibility to *Ptt* have been mapped to chromosome 6H in several mapping populations

Resistant Barley Lines

North America	**Australia**
Chevron	Baudin
Kombar	Tallon
Morex	Kaputar
M120	Pompadour
ND11231	Sterling
NDB112	
Rika	**Ethiopia**
Sm89010	CI9819



Barley Ch. 6H

Cakir *et al.* 2003
Friesen *et al.* 2006
Gupta *et al.* 2010
Liu *et al.* 2010
Ma *et al.* 2004
Manninen *et al.* 2006
Qamar *et al.* 2008
Raman *et al.* 2003
St. Pierre *et al.* 2010

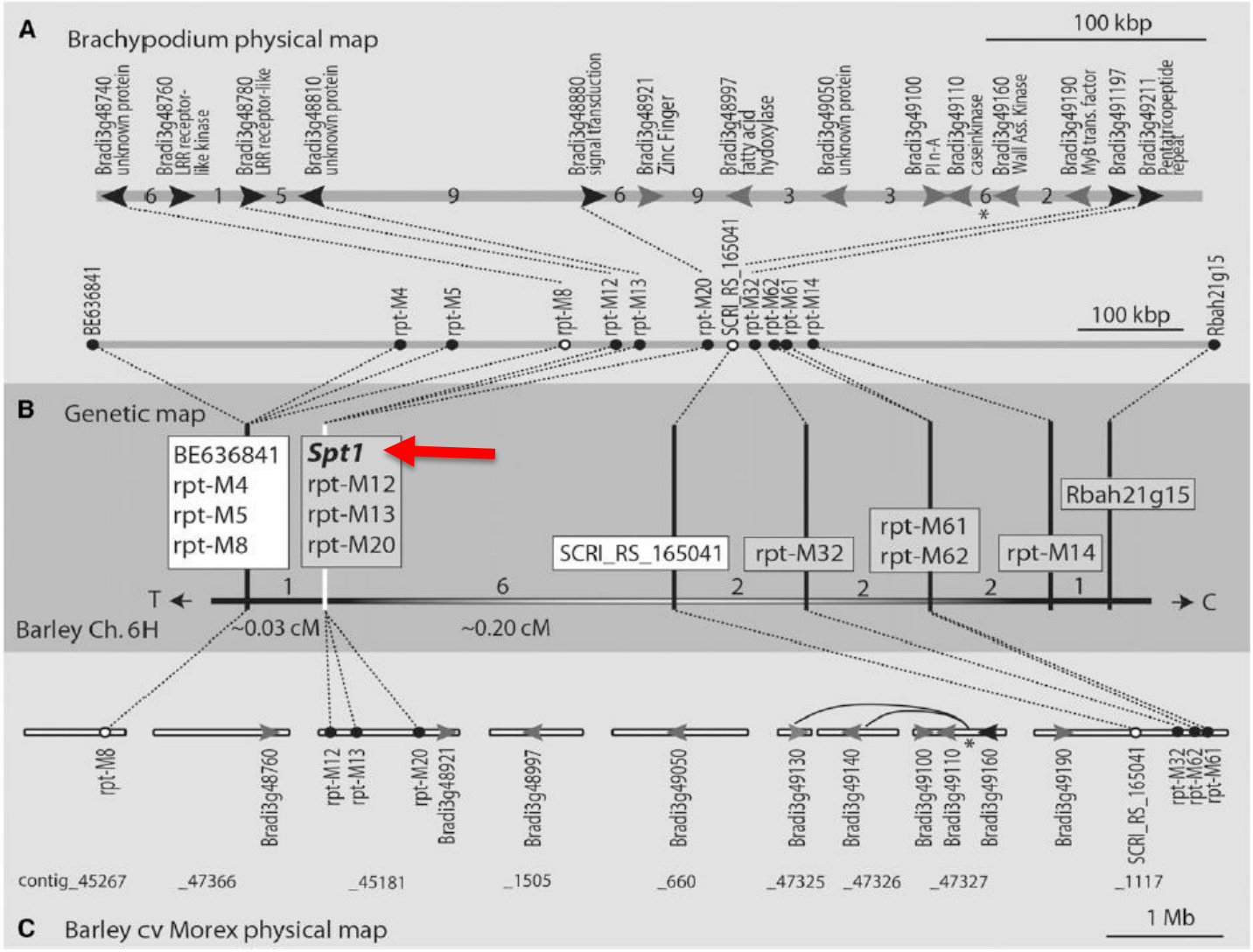
Ptt-isolate specific resistance/susceptibility

- *Ptt* isolates 15A and 6A exhibit differential virulence on barley cultivars Rika and Kombar

- Resistance is recessive (dominant susceptibility)

Abu Qamar et al 2008
Liu et al 2010

High resolution mapping



Richards et al., G3, 2016

Spt1 protein variation

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                    1                                                  50
  Rika Spt.cg2    (1) MANQLKYPMTRNLFFLLSYTAAVVAAPSRPQLSYETRHEHTTVSDYPRPD
Kombar Spt.cg2    (1) MANYLISRFTRNLFFLLTYTAAIVAASSQPQPSYKTTQERITPSNYQLPK
 Morex Spt.cg2    (1) MANHLKRPLVQ-QFFLLTSVAAIVAGPAEPHLSYKIHQHS--SS-YPQPR
 Morex Spt.cg1    (1) MAKYLYGSFAISVCLTLSLVAAITREPSRPEPPSRKISEPFVSYS--PQPQ
     Consensus    (1) MANYLK PFTRNLFFLLSYVAAIVAAPSRPQPSYKTSQE IT S YPQPK
                    51                                                 100
  Rika Spt.cg2   (51) DFANERLYQAYFVIQRFKNIITCDPMNITSTWTGHDICGEKTYAGFHCTA
Kombar Spt.cg2   (51) EFQNERLYQAYLAIQRFKSTITCDPMNITSTWTGHDICGKSTYVGFYCTT
 Morex Spt.cg2   (47) DPPSEQLYHAYFAIQRFKNIITSDPKNVTSTWTGHDICATTSYLGFNCGA
 Morex Spt.cg1   (49) HFPNEGLYKAYLVIQRFKSRISSDPKNITRTWSGHDICGRKSYLGFRCGT
     Consensus   (51) DFPNERLYQAYLVIQRFKSTITSDPMNITSTWTGHDICGKKSYLGFHCTT
                   101                                                 150
  Rika Spt.cg2  (101) LPEYDQNLTITSAVLNGFRLCAPKLQGFVDQLPDLALFQAAANNFGAFDV
Kombar Spt.cg2  (101) PLADNKSLTVTSAILDGFGLCAPKLKGFIDQLPDLALFQAASNNFGAFDF
 Morex Spt.cg2   (97) PHCQASNLTVTSVIFDGFGLCSPMQQDLVDELPDLALFQASSNNFGG-EV
 Morex Spt.cg1   (99) TPDLAKKLTIMAVDFNDFAMSAPMLEGFIDQFPDLVLFQASTNNFGG-GI
     Consensus  (101) PPE AKNLTITSVILNGFGLCAPMLQGFIDQLPDLALFQAASNNFGAFDV
                   151                                                 200
  Rika Spt.cg2  (151) PNLAKLTYIYKLNAGDDQLQQSLSKHRDFGLPVKAVGSVCFGPACAGFDI
Kombar Spt.cg2  (151) PNLAGLTYNYKIETIDTHDAQSLGSN--IDLPTKDLLALCLLRKLICVNT
 Morex Spt.cg2  (146) PILTGLSYMYMLDVHNKQGDQFEEAKS--GTATTHLTQLCLGKNFCFGIR
 Morex Spt.cg1  (148) PPLSSLHYQYKLSIHDDRHDPISENK----------YLLTTIIRVGPTDI
     Consensus  (151) PNLAGLTYNYKLDIHDDQ DQSLEAK   GLPTK L ALCLGKKLAPIDI
                   201                                                 250
  Rika Spt.cg2  (201) GFGSLVQGTPQNRVNRDATNARAILLNYNNLSGPLPANLGLSKLSYLALA
Kombar Spt.cg2  (199) GFASPVQSSLIMGAAAGATDARALLLNSNNLYGPFPANIGFSKLSYLALG
 Morex Spt.cg2  (194) LRPILKPKHP----RHGATSAKALLLNNNQLSESLPANLGLSKVSYLALA
 Morex Spt.cg1  (188) GEPEAVVQTARKG--TNFNIGRSLLLNSNSLSGPLPANLGFSKLRYLALA
     Consensus  (201) GRPSLVQ TP  G   GAT ARALLLNSNNLSGPLPANLGLSKLSYLALA
                   251                                                 300
  Rika Spt.cg2  (251) NNKLTGSIPPSIAHMQDSLLEVLLLNNQLSGCLPNELGMLTKTAVIDAGM
Kombar Spt.cg2  (249) NNKLTGIIPSSIAHMKDSLLEILLLNNQLSGCLPNELGMLTKTAVIDAGM
 Morex Spt.cg2  (240) NNKLTGPIPPSIAQAQDSLLEVVLLNNQLSGCLPNELGMLTKTTVIDAGM
 Morex Spt.cg1  (236) NNKLTGPIPPSIGKMQDSLLEVLLLNNQLSGCVPHELGMLTKTTVIDAGM
     Consensus  (251) NNKLTGPIPPSIAHMQDSLLEVLLLNNQLSGCLPNELGMLTKTTVIDAGM
                   301                                                 350
  Rika Spt.cg2  (301) NQLTGPIPSSFSCLSSVEQLNLAGNRLYGQVPNALCKLAGPTGRLSNLTL
Kombar Spt.cg2  (299) NQLTGPIPSSFSCLSSVEQLNLAGNRLYGQVPDALCKLAGPAGRLANLTL
 Morex Spt.cg2  (290) NQITGPIPSSFSCLGSVEQLNLAGNRLYGQVPDALCKLSWPAGRLANLTL
 Morex Spt.cg1  (286) NQLTGPIPSSFSCLNSVEQLNLGGNRLYGQVPDALCKLAGPAGRLSNLTL
     Consensus  (301) NQLTGPIPSSFSCLSSVEQLNLAGNRLYGQVPDALCKLAGPAGRLANLTL
                   351                                                 400
  Rika Spt.cg2  (351) SGNYFTSVGPACSALIKDGVLDVKHNCIPGFADQRGPAECASFLRQPKTC
Kombar Spt.cg2  (349) SGNYFTSVGPACSALIKDGMLDVKHNCIPGFANQRGPAECASFLSQPKTC
 Morex Spt.cg2  (340) SGNYFTSVGPACAALVKNGVLDVKHNCIPGFANQRGPAECASFLSQPKTC
 Morex Spt.cg1  (336) SGNYFTSVGPACSALIKDGVLDMKYNCIPGMANQRAPAECASFLSQPKTC
     Consensus  (351) SGNYFTSVGPACSALIKDGVLDVKHNCIPGFANQRGPAECASFLSQPKTC
                   401                                                 441
  Rika Spt.cg2  (401) PSASARVACPAADTKTNAAAPEARVAKDYSSYVWYATLHE-
Kombar Spt.cg2  (399) PSASSRVACPAADAKTNAAAPGARVAKDYSSYVTYATLHE-
 Morex Spt.cg2  (390) PSASARVACPAADAQSNAAAPGARVAKDYSSYVWYATLHE-
 Morex Spt.cg1  (386) PAAGARVVCPVVDAKKNAAAPVGRVAPHYSSYVTYATLHE-
     Consensus  (401) PSASARVACPAADAKTNAAAPGARVAKDYSSYVVYATLHE
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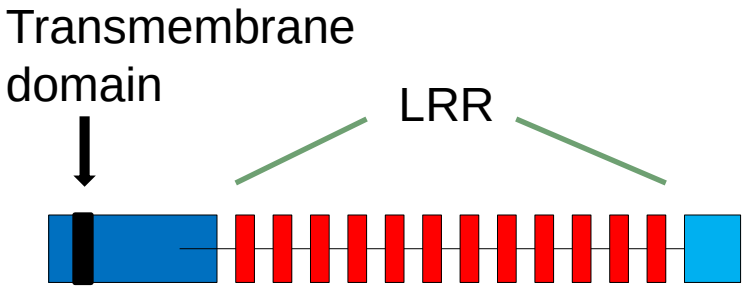
Many resistance genes have this typical structure, in our example it is hypothesized to trigger host susceptibility



Main Goal

Knocking out the candidate gene contributing susceptibility at the 6H region in barley to get a better understanding of this host/pathogen interaction

Long term Goal

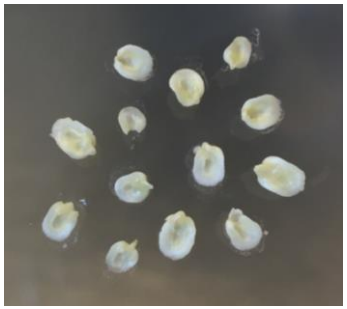
To reduce barley susceptibility to *Ptt*

Rika and Kombar are not compatible with Agrobacterium, thus we use gene gun bombardment

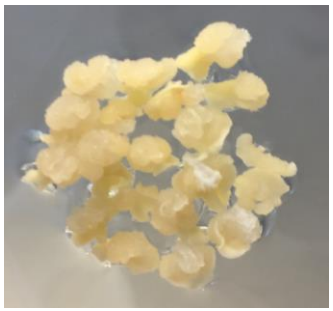
Bombardment advantages

- Universal DNA delivery system
- Transformation of plant species and genotypes recalcitrant to Agrobacterium-based transformation
- Mutation possible without integration of DNA

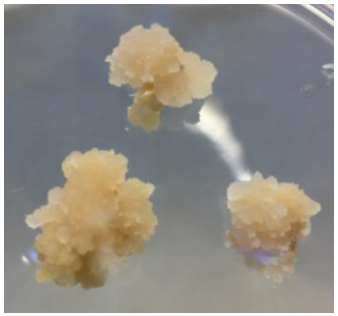
Results with bombardment of cultivar Rika, using two different CRISPR/Cas9 constructs



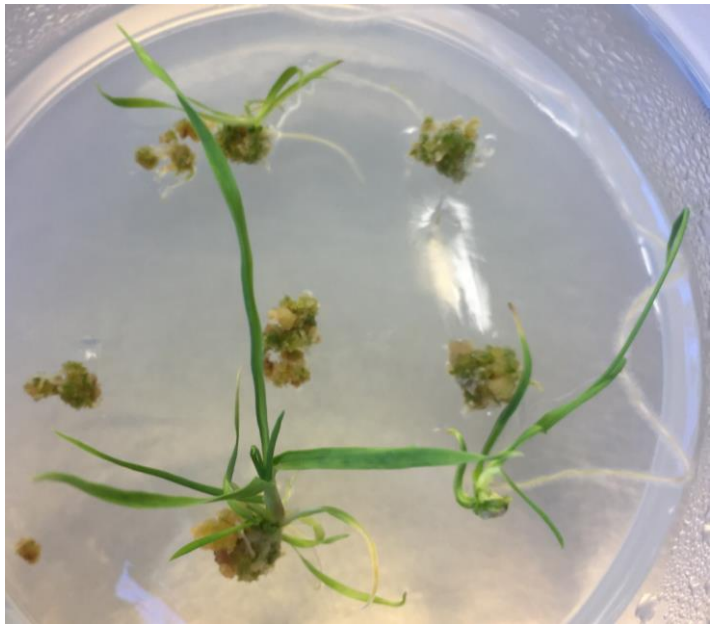
Embryos on callus induction media



One week later, ready for bombardement



One month after bombardment



Calli with leaves growing on shoot induction media

- Two plants with stable integration of CRISPR/Cas9 constructs, but no mutations obtained in T0 nor in its progeny
- Ongoing efforts to increase the bombardment efficiency

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Thanks for your attention!

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