



# Resistance Breeding – cornerstone for meeting future challenges

Frank Ordon

# Introduction



**ask the experts**

# PLANET UNDER PRESSURE

## Too Many People on Earth?

World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100

21 June 2017, New York

The current world population of 7.6 billion is expected to reach 8.6 billion in 2030, 9.8 billion in 2050 and 11.2 billion in 2100, according to a new United Nations report being launched today. With roughly 83 million

Matt Annis (Author)

[https://books.google.de/books/about/Planet\\_Under\\_Pressure.html?id=0wK7oQEACAAJ&redir\\_esc=y](https://books.google.de/books/about/Planet_Under_Pressure.html?id=0wK7oQEACAAJ&redir_esc=y)

ipcc  
INTERGOVERNMENTAL PANEL ON climate change

## Climate Change 2022

### Mitigation of Climate Change

Working Group III contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change

<https://www.wcrp-climate.org/news/wcrp-news/1879-ipcc-wg3-ar6>

WFP  
World Food Programme

## Food security implications of the Ukraine conflict

SAVING LIVES  
CHANGING LIVES

The Russian invasion of Ukraine has major implications for food security across the world as well as the region, given both countries' major roles in global food markets and Russia's prominence in global energy trade.

The conflict comes at a time of unprecedented humanitarian needs, as a ring of fire circles the world with climate shocks, conflict, COVID-19 and rising costs driving millions closer to starvation.

A total 44 million people in 38 countries are in need of humanitarian assistance and overall global needs for humanitarian assistance are higher than ever.

As funding levels off due to donor nations' treasuries being stretched, and in the face of rising food costs, WFP has already had to reduce rations for refugees and other vulnerable populations across East Africa and the Middle East. This includes Yemen, where 16.2 million people are food insecure and there are pockets of famine-like conditions.

The conflict in Ukraine has plunged global food and energy markets into turmoil, raising high food prices even further. These increases, once passed onto domestic markets, will limit people's access to food. They will also ultimately increase operational costs for WFP, as raising its response at a time when people need it most.

March 2022

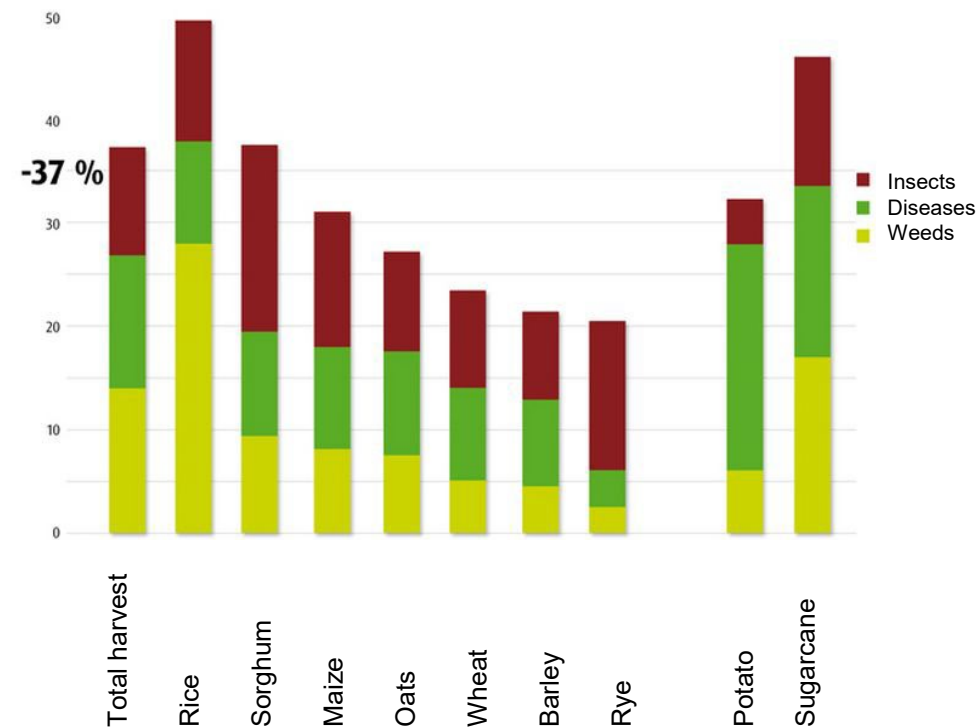
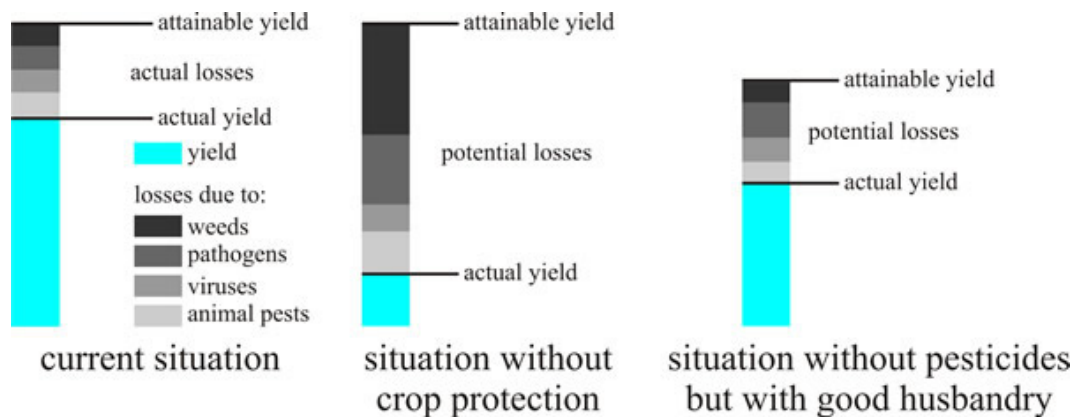
<https://www.wfp.org/publications/food-security-implications-ukraine-conflict>

[www.julius-kuehn.de](http://www.julius-kuehn.de)





# Yield losses



**Fig. 2.** Typical crop losses and yield levels estimated with and without various protection regimes. The value of crop protection practices (shown at left as 'current situation') can be calculated as the percentage of potential losses prevented by all the crop protection measures that are employed (compare with centre panel). In contrast, the impact of pesticide use on crop productivity (right hand panel) takes into account consequential changes in the agricultural system (for example, **use of alternative varieties of the crop**, modified crop rotation, reduced fertiliser use), which are provoked by the abandonment of pesticides and which are often accompanied by reduced attainable yield. Redrawn after Oerke, 2006.

Moore et al. 2021

<http://www.transgen.de/pflanzenforschung/pflanzengesundheit/>

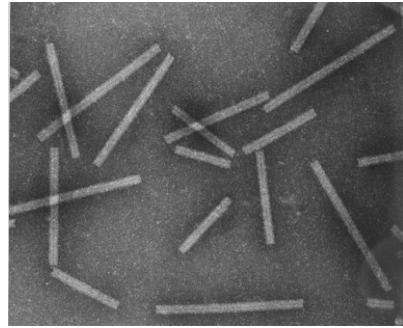
# Pests and diseases in the background of climate change



Insects



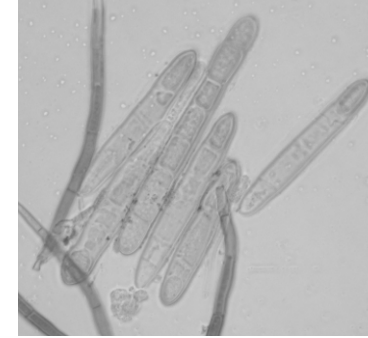
Viruses



Bacteria



Fungi



+2°C = 213 Mio t loss  
in wheat, maize  
and rice due to  
insects

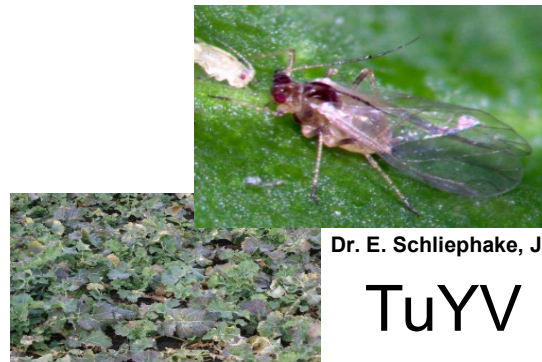
Deutsch, C.A.; Tewksbury, J.J.; Tighelaar, M.; Battisti, D.S.; Merrill, S.C.; Huey, R.B.; Naylor, R. Increase in crop losses to insectpests in a warming climate. *Science* 2018, 361, 916–919.

Chmielewski, 2007: Rise in average temperature about 3-6°C facilitates moving north of insects of about 1000 km

Fungal pathogens adapted to higher temperature like stem rust in wheat or *Cercospora beticola* will gain importance



Commons.wikimedia.org/wiki/File: Suikerbiet

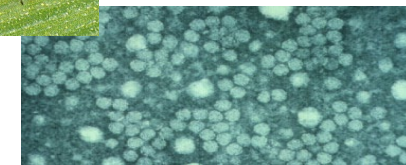


Dr. E. Schliephake, JKI

TuYV



BYDV



Dr. A. Habekuss, JKI



# Breeding progress in wheat

scientific reports

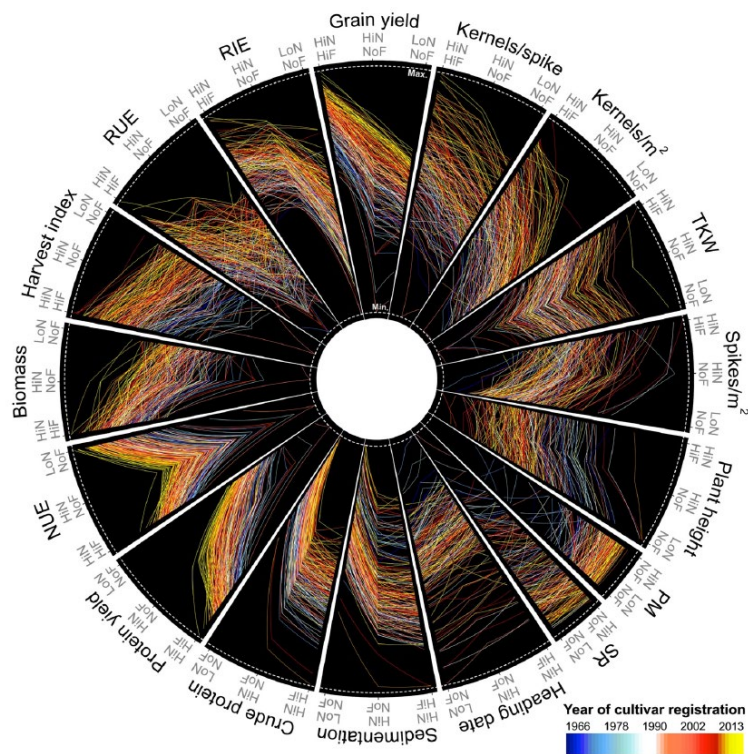


Article | Published: 17 June 2019

## Breeding improves wheat productivity under contrasting agrochemical input levels

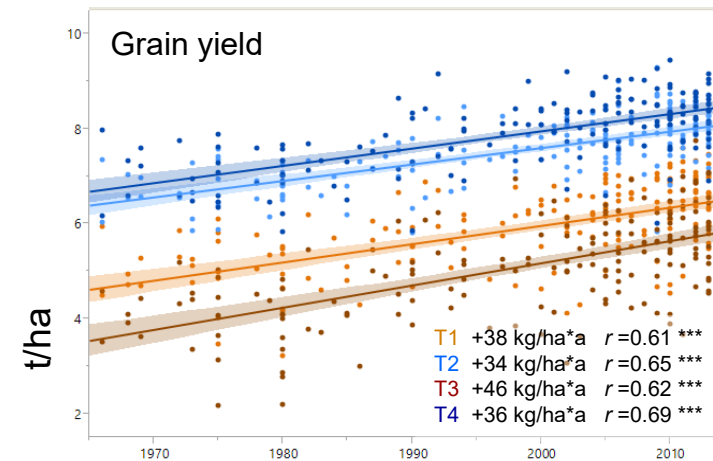
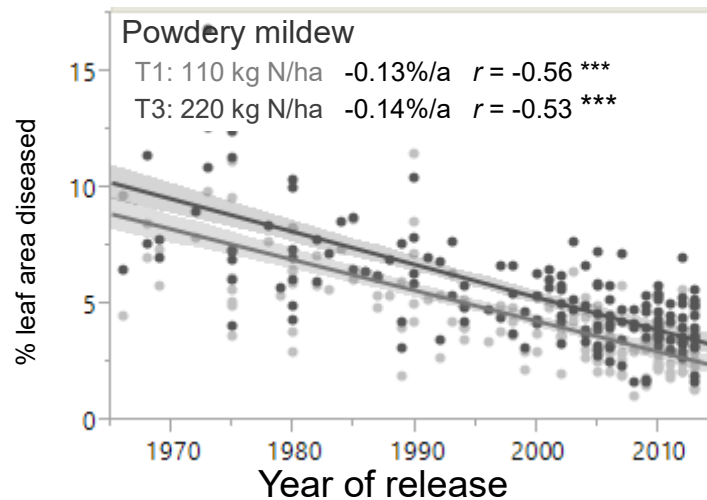
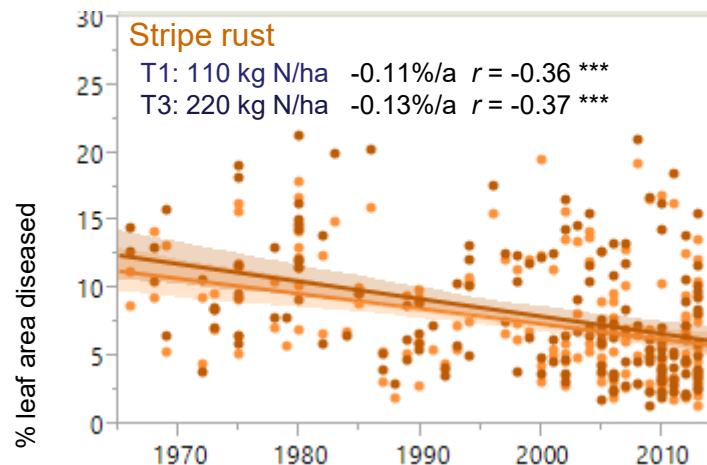
Kai P. Voss-Fels, Andreas Stahl, Benjamin Wittkop, Carolin Lichthardt, Sabrina Nagler, Till Rose, Tsu-Wei Chen, Holger Zetzsche, Sylvia Seddig, Mirza Majid Baig, Agim Ballvora, Matthias Frisch, Elizabeth Ross, Ben J. Hayes, Matthew J. Hayden, Frank Ordon, Jens Leon, Henning Kage, Wolfgang Friedt, Hartmut Stützel & Rod J. Snowdon

Nature Plants (2019)

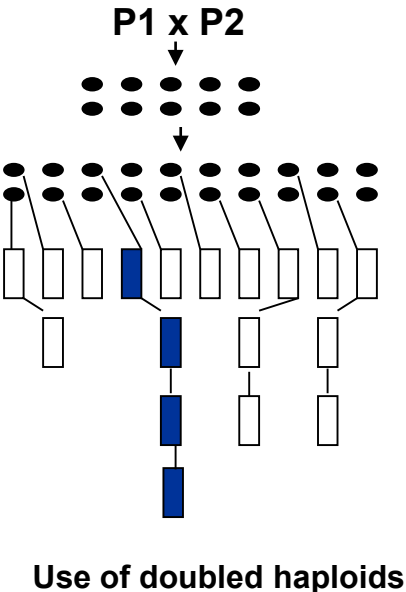
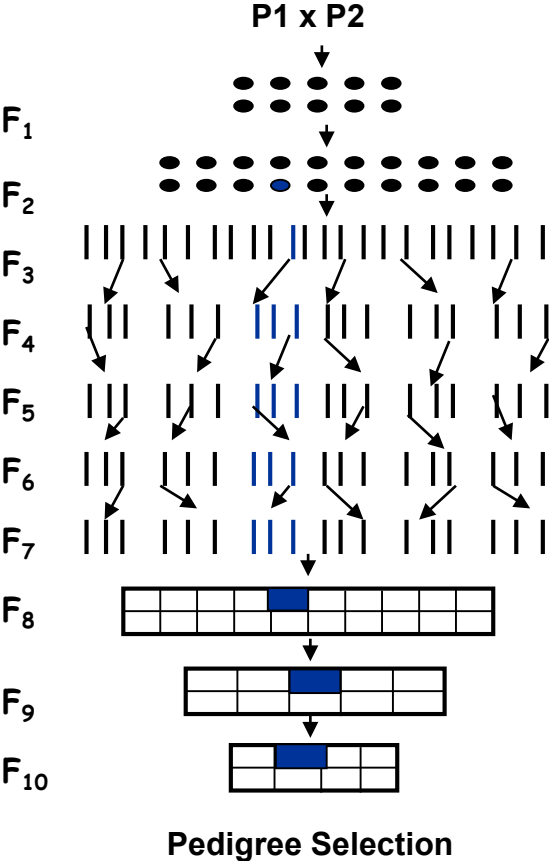


## Breeding progress for pathogen resistance is a second major driver for yield increase in German winter wheat at contrasting N levels

Holger Zetzsche<sup>1,2</sup>, Wolfgang Friedt<sup>2</sup> & Frank Ordon<sup>2</sup>



# Breeding progress in wheat

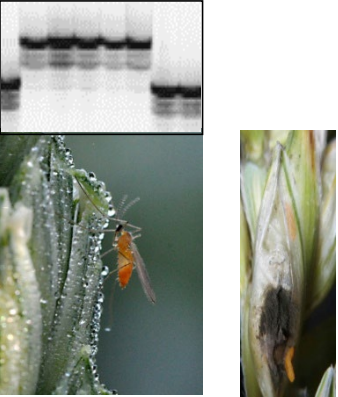
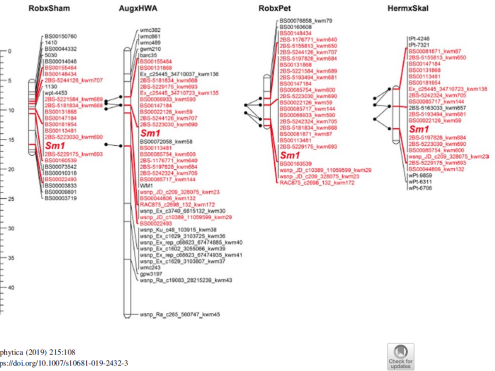


## Marker based selection

Theor Appl Genet (2016) 129:1507–1517  
 DOI 10.1007/s00270-016-2720-4  
 ORIGINAL ARTICLE

### A saturated SNP linkage map for the orange wheat blossom midge resistance gene *Sm1*

Muhammad T. Kassab<sup>1,2</sup>, Sabrina Haas<sup>3</sup>, Edgar Schliephake<sup>3</sup>, Clare Lewis<sup>4</sup>, Frank M. You<sup>1</sup>, Curtis J. Pozniak<sup>5</sup>, Ilona Krámer<sup>6</sup>, Dragan Perovic<sup>7</sup>, Andrew G. Sharpe<sup>8</sup>, Pierre R. Robert<sup>9</sup>, Michael Koehler<sup>10</sup>, Ian R. Wise<sup>11</sup>, Paul Frenwick<sup>12</sup>, Simon Berry<sup>13</sup>, James Simmonds<sup>14</sup>, Delphine Bourcade<sup>15</sup>, Patrice Seneffart<sup>16</sup>, Laure Duchalais<sup>16</sup>, Olivier Robert<sup>17</sup>, Jutta Förster<sup>17</sup>, Julian B. Thomas<sup>18</sup>, Wolfgang Friedl<sup>19</sup>, Frank Ordon<sup>1</sup>, Christof Laus<sup>4</sup>, Curt A. McCartney<sup>20</sup>



### Mapping of quantitative trait loci (QTL) for resistance against *Zymoseptoria tritici* in the winter spelt wheat accession HTR1410 (*Triticum aestivum* subsp. *spelta*)

Franziska Karlstedt<sup>1</sup>, Doris Kophinke<sup>2</sup>, Dragan Perovic<sup>3</sup>, Andreas Jacobi<sup>4</sup>, Klaus Pöhlen<sup>5</sup>, Frank Ordon<sup>1</sup>

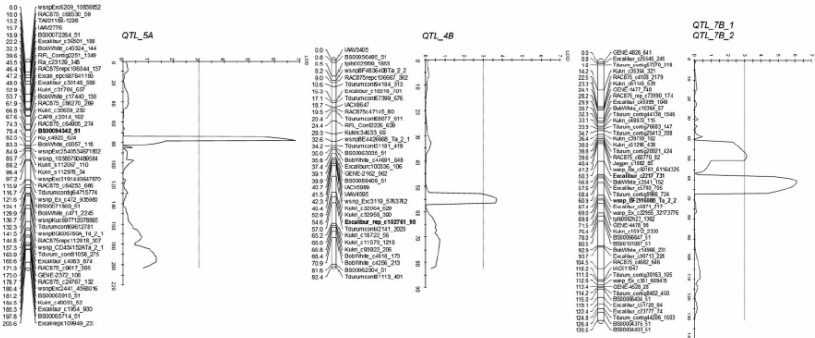
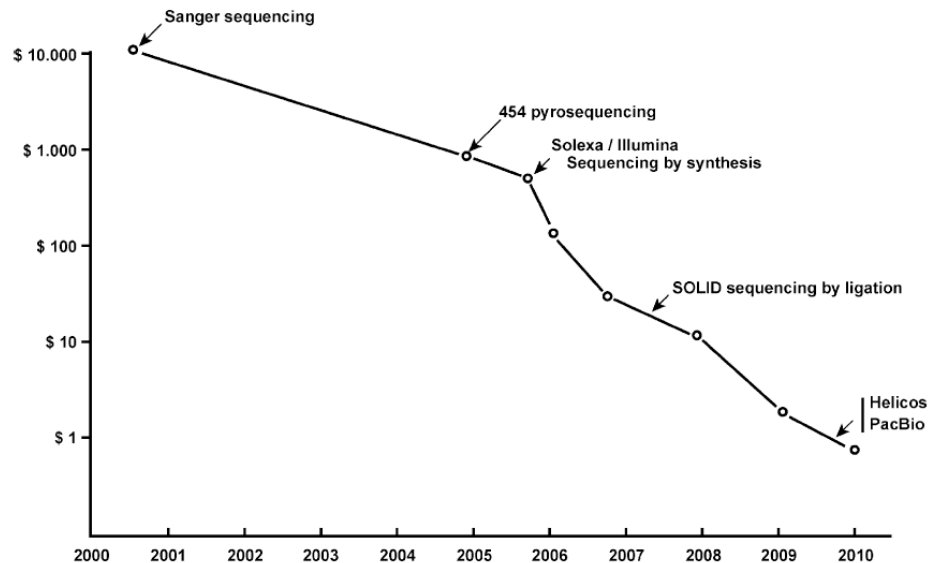


Fig. 3 Results of QTL analyses for *Zymoseptoria tritici* resistance in the DH population HTR1410 × susceptible parental lines

# Plant breeder's toolbox



Marker type	RFLPs	Genomic SSRs	AFLPs	EST SNPs/SSRs	DArTs	BOPAs/OPAs	iSelect		Genotyping by sequencing
Throughput	single marker application	single marker application	few marker application	single marker application	6K	1,5K	9K	50K	
Multiplexing	no mutiplexing	few markers multiplexing	low multiplexing	few markers multiplexing	platform/ simultaneous analysis	platform/ simultaneous analysis	platform/ simultaneous analysis	platform/ simultaneous analysis	simultaneous multiplexing NGS/GBS
Amount of D N A	Large amount	low amount	low amount	low amount	low amount	low amount	low amount	low amount	low amount
Quality of D N A	very good	average	average	average	very good	very good	very good	very good	very good



An estimate of the evolution of sequencing costs over the last 10 years. Costs are given for sequencing a megabase using a logarithmic scale.

M. Delseny et al. (2010) *Plant Sci.* 179: 407-422



## ARTICLE

OPEN

doi:10.1038/nature22043

### A chromosome conformation capture ordered sequence of the barley genome



Article

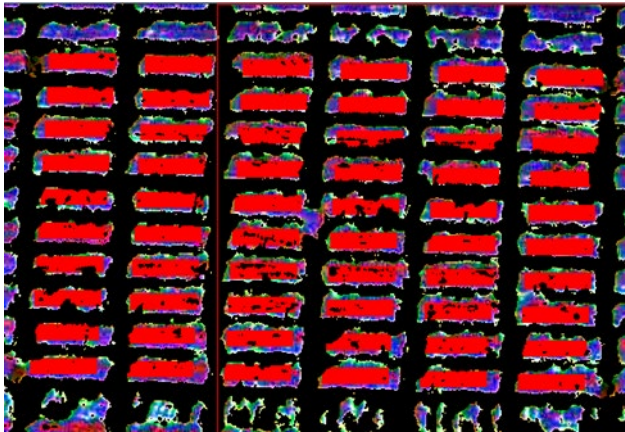
### Multiple wheat genomes reveal global variation in modern breeding



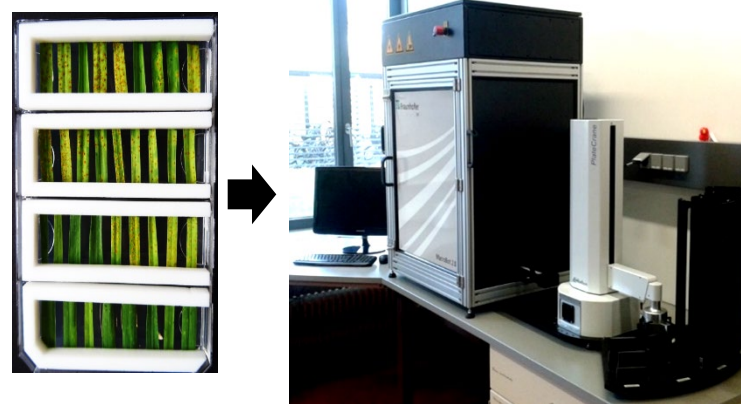
# Systematic use of plant genetic resources

## Genome wide association studies GWAS

### Phenotyping

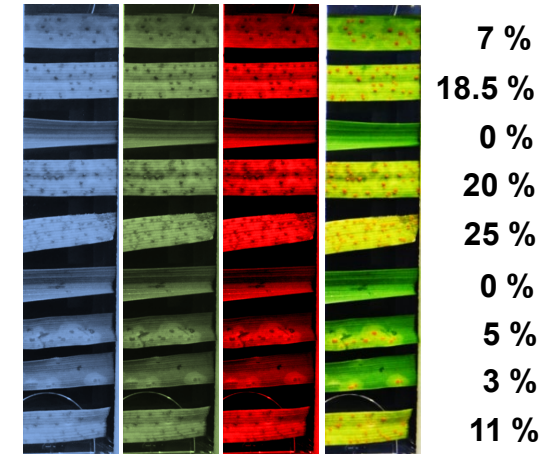


Tattaris M, Reynolds MP, Chapman SC, 2016. A direct comparison of remote sensing approaches for high-throughput phenotyping in plant breeding. *Front. Plant Sci.* 7: 1131.



High throughput phenotyping

A. Serfling und U. Beukert

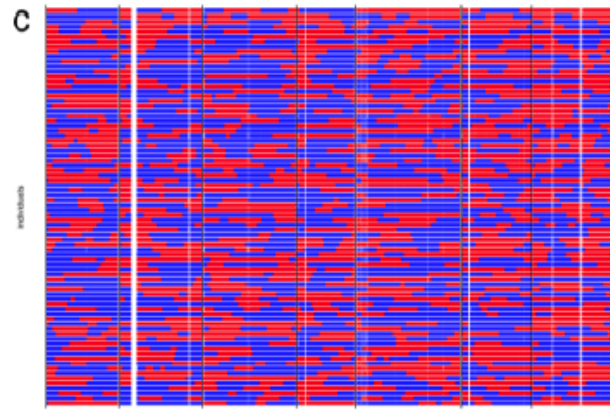


Automatic detection of leaf area infected

### Genotyping



<https://www.illumina.com/systems/sequencing-platforms/hiseq-2500.html>



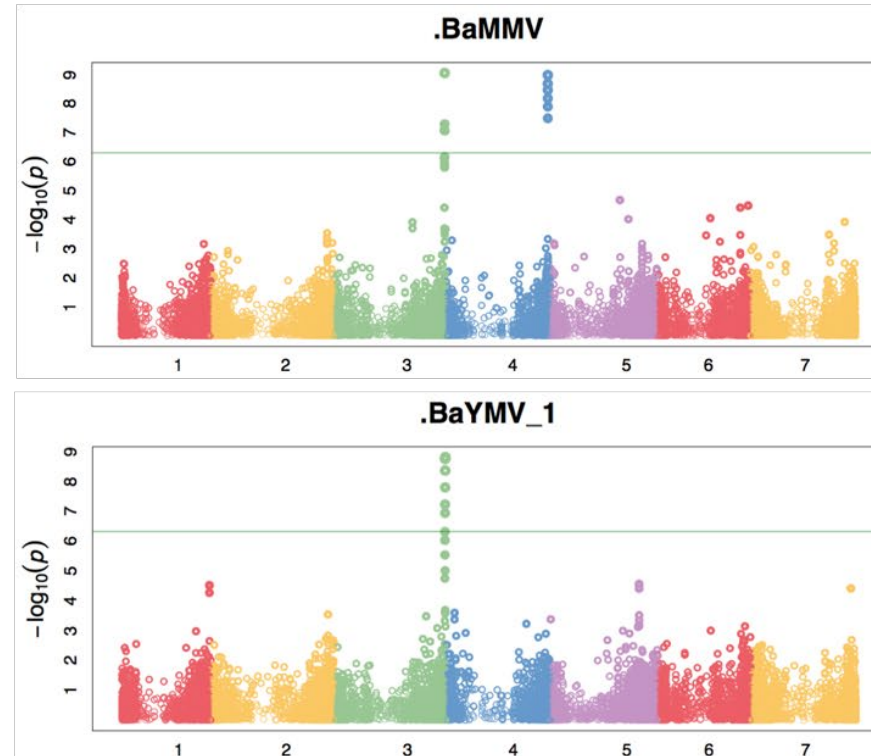
N. Stein



<https://www.illumina.com/products/by-type/microarray-kits/infinium-iselect-custom-genotyping.html>

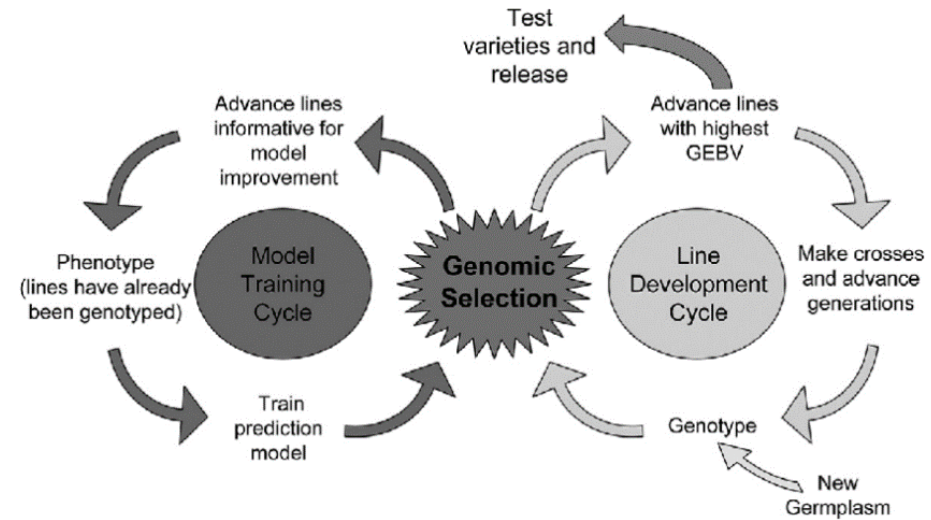
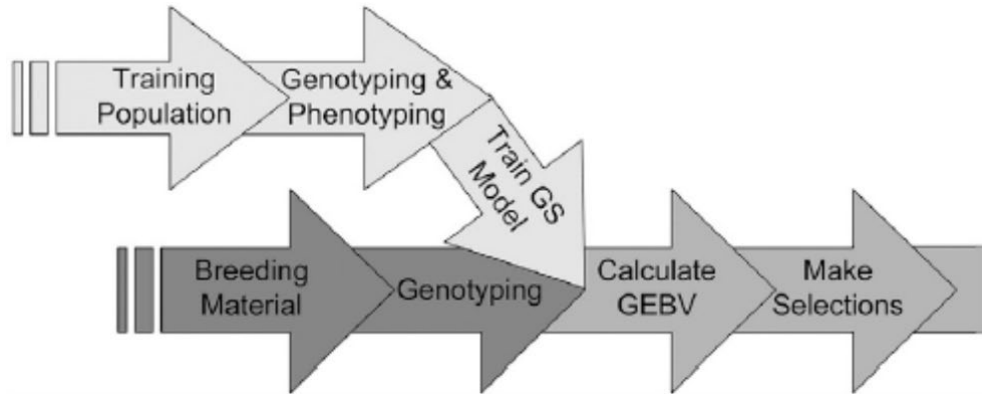
# Systematic use of genetic resources

## Genomwide association studies (GWAS)



Millner et al. 2018: Nature Genetics

# Genomic Selection



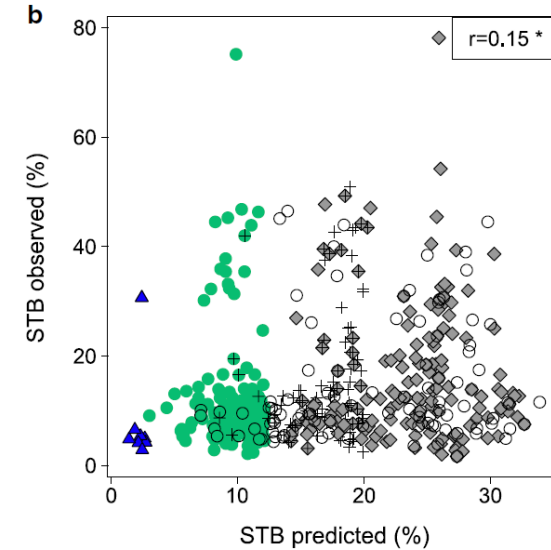
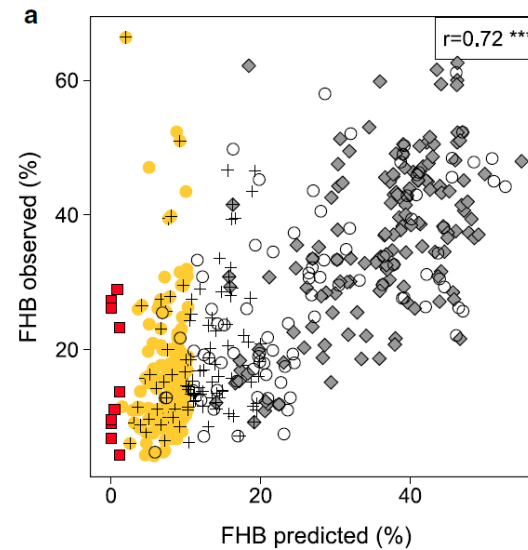
Heffner et al. 2009

Theoretical and Applied Genetics (2019) 132:2425–2437  
<https://doi.org/10.1007/s00122-019-03364-7>

ORIGINAL ARTICLE

## An experimental approach for estimating the genomic selection advantage for Fusarium head blight and Septoria tritici blotch in winter wheat

Cathérine Pauline Herter<sup>1</sup> · Erhard Ebmeyer<sup>2</sup> · Sonja Kollers<sup>2</sup> · Viktor Korzun<sup>2</sup> · Thomas Miedaner<sup>1</sup>

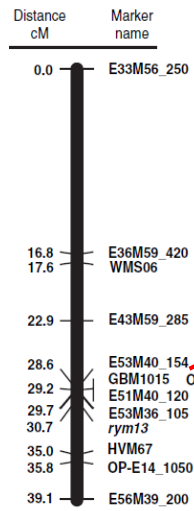




# Isolation von resistance genes: map based cloning

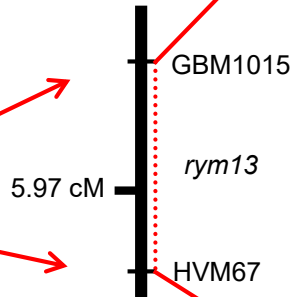


1. Mapping of the gene of interest on low to medium resolution

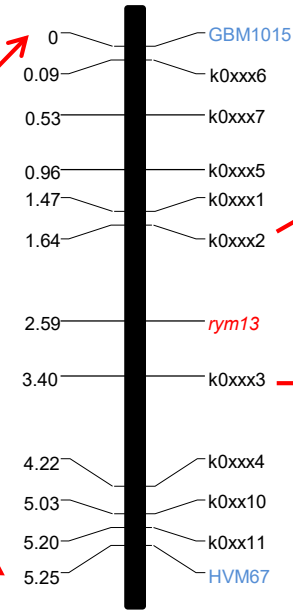


Humbroich *et al.* 2010

2. Construction of a high resolution mapping population (5191 F<sub>2</sub>-plants)



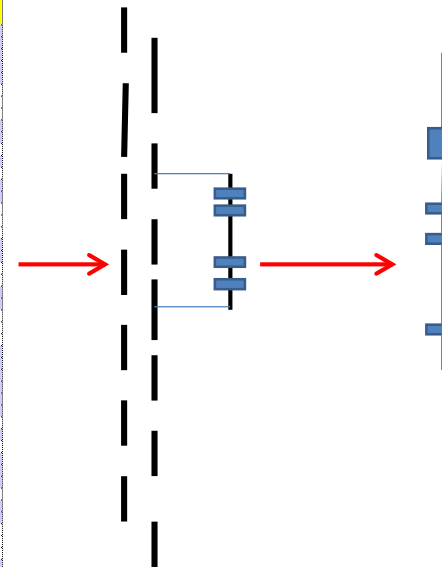
3. Phenotyping of segmental RILs (F<sub>4</sub>) and marker saturation using available high density maps



4. Further marker saturation by using sequence information from rice, sorghum brachypodium and NGS data from barley (GenomeZipper)

	brachypodium	rice	sorghum
	Bradi1gms1	Oz03gms1	Sb01gms1
	Bradi1gms2	Oz03gms2	Sb01gms2
	Bradi1gms3	Oz03gms3	Sb01gms3
	Bradi1gms4	Oz03gms4	Sb01gms4
	Bradi1gms5	Oz03gms5	Sb01gms5
	Bradi1gms6	Oz03gms6	Sb01gms6
	Bradi1gms7	Oz03gms7	Sb01gms7
	Bradi1gms8	Oz03gms8	Sb01gms8
	Bradi1gms9	Oz03gms9	Sb01gms9
	Bradi1gms10	Oz03gms10	Sb01gms10
	Bradi1gms11	Oz03gms11	Sb01gms11
	Bradi1gms12	Oz03gms12	Sb01gms12
	Bradi1gms13	Oz03gms13	Sb01gms13
	Bradi1gms14	Oz03gms14	Sb01gms14
	Bradi1gms15	Oz03gms15	Sb01gms15
	Bradi1gms16	Oz03gms16	Sb01gms16
	Bradi1gms17	Oz03gms17	Sb01gms17
	Bradi1gms18	Oz03gms18	Sb01gms18
	Bradi1gms19	Oz03gms19	Sb01gms19
	Bradi1gms20	Oz03gms20	Sb01gms20
	Bradi1gms21	Oz03gms21	Sb01gms21
	Bradi1gms22	Oz03gms22	Sb01gms22
	Bradi1gms23	Oz03gms23	Sb01gms23
	Bradi1gms24	Oz03gms24	Sb01gms24
	Bradi1gms25	Oz03gms25	Sb01gms25
	Bradi1gms26	Oz03gms26	Sb01gms26
	Bradi1gms27	Oz03gms27	Sb01gms27
	Bradi1gms28	Oz03gms28	Sb01gms28
	Bradi1gms29	Oz03gms29	Sb01gms29
	Bradi1gms30	Oz03gms30	Sb01gms30
	Bradi1gms31	Oz03gms31	Sb01gms31
	Bradi1gms32	Oz03gms32	Sb01gms32
	Bradi1gms33	Oz03gms33	Sb01gms33
	Bradi1gms34	Oz03gms34	Sb01gms34
	Bradi1gms35	Oz03gms35	Sb01gms35
	Bradi1gms36	Oz03gms36	Sb01gms36
	Bradi1gms37	Oz03gms37	Sb01gms37

5. Identification of a BAC contig and candidate genes based on the physical map of barley or re-sequencing of the target interval



6. Identification of *rym13* by transformation, TILLING, RNAi

Strategy for marker development and isolation of virus resistance genes in barley using genomic tools exemplified for *rym13* being effective against BaMMV/BaYMV (Lehmann *et al.* unpublished)

# Isolation von resistance genes: *rym4* und *rym11*



The Plant Journal (2005) 42, 912–922

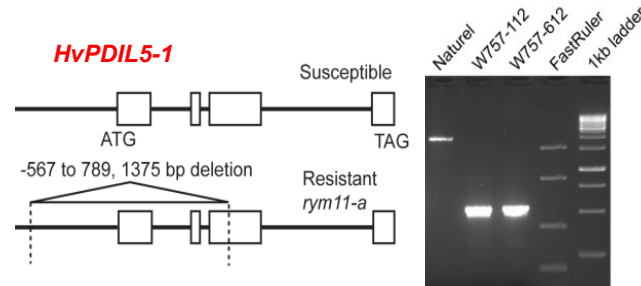
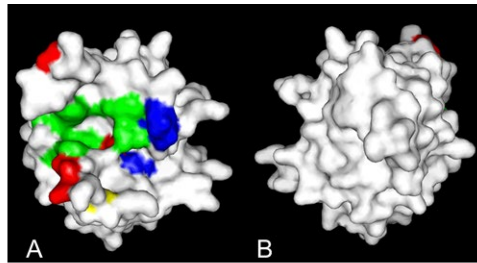
doi: 10.1111/j.1365-3113X.2005.02424.x

The eukaryotic translation initiation factor 4E confers multiallelic recessive *Bymovirus* resistance in *Hordeum vulgare* (L.)

Nils Stein<sup>1,4</sup>, Dragan Perovic<sup>1,7</sup>, Jochen Kumlehn<sup>1</sup>, Bettina Pellio<sup>2</sup>, Silke Stracke<sup>1</sup>, Stefan Streng<sup>1</sup>, Frank Ordon<sup>2,3</sup> and Andreas Graner<sup>1,7</sup>

## PROTEIN DISULFIDE ISOMERASE LIKE 5-1 is a susceptibility factor to plant viruses

Ping Yang<sup>a</sup>, Thomas Lüpken<sup>b</sup>, Antje Habekuss<sup>b</sup>, Goetz Hensel<sup>c</sup>, Burkhard Steuernagel<sup>d,1</sup>, Benjamin Kilian<sup>a</sup>, Ruvini Ariyadasa<sup>a</sup>, Axel Himmelbach<sup>a</sup>, Jochen Kumlehn<sup>c</sup>, Uwe Scholz<sup>d</sup>, Frank Ordon<sup>b,2</sup>, and Nils Stein<sup>a,2,3</sup>



frontiers in PLANT SCIENCE

REVIEW ARTICLE published: 25 December 2012 doi: 10.3389/fpls.2012.00276

Cellular chaperones and folding enzymes are vital contributors to membrane bound replication and movement complexes during plant RNA virus infection

Jeanmarie Verchot\*

Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, USA

### ER Quality Control Machinery

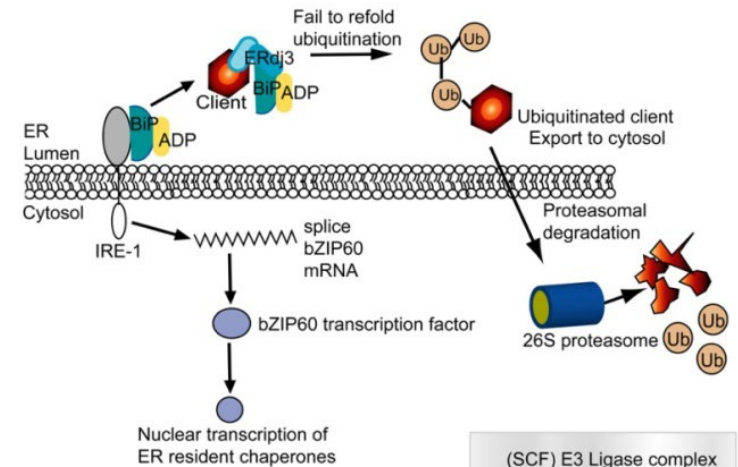
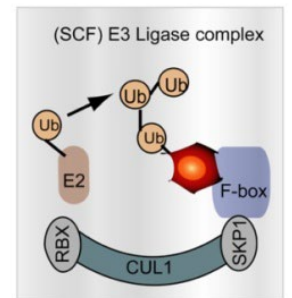
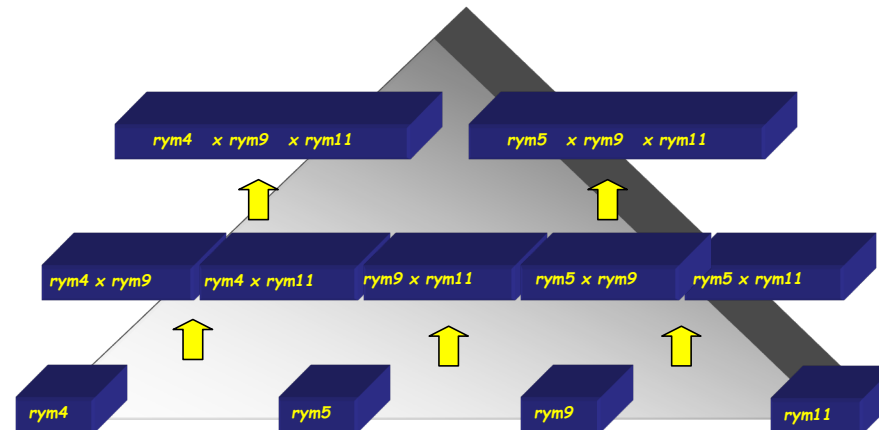
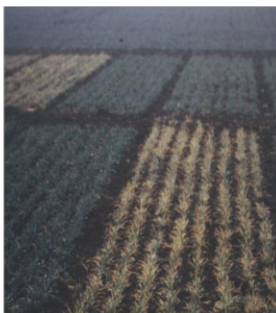
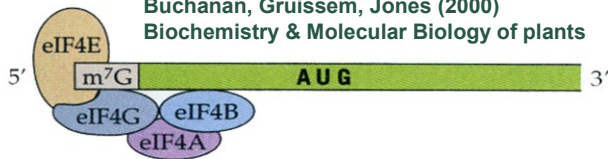


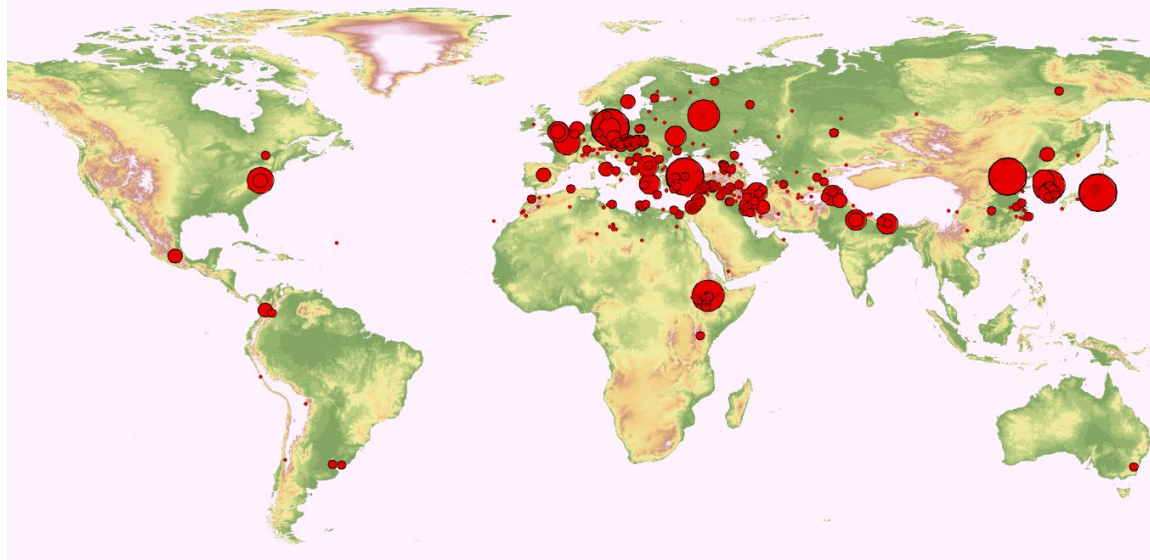
figure taken from:  
Buchanan, Grussem, Jones (2000)  
Biochemistry & Molecular Biology of plants



# Allele mining: *rym11*

*PROTEIN DISULFIDE ISOMERASE LIKE 5-1* is a susceptibility factor to plant viruses

Ping Yang<sup>a</sup>, Thomas Lüpken<sup>b</sup>, Antje Habekuss<sup>b</sup>, Goetz Hensel<sup>c</sup>, Burkhard Steuernagel<sup>d,1</sup>, Benjamin Kilian<sup>a</sup>, Ruvini Ariyadasa<sup>a</sup>, Axel Himmelbach<sup>a</sup>, Jochen Kumlehn<sup>c</sup>, Uwe Scholz<sup>d</sup>, Frank Ordon<sup>b,2</sup>, and Nils Stein<sup>a,2,3</sup>



365 Wild barley (*H. spontaneum*)

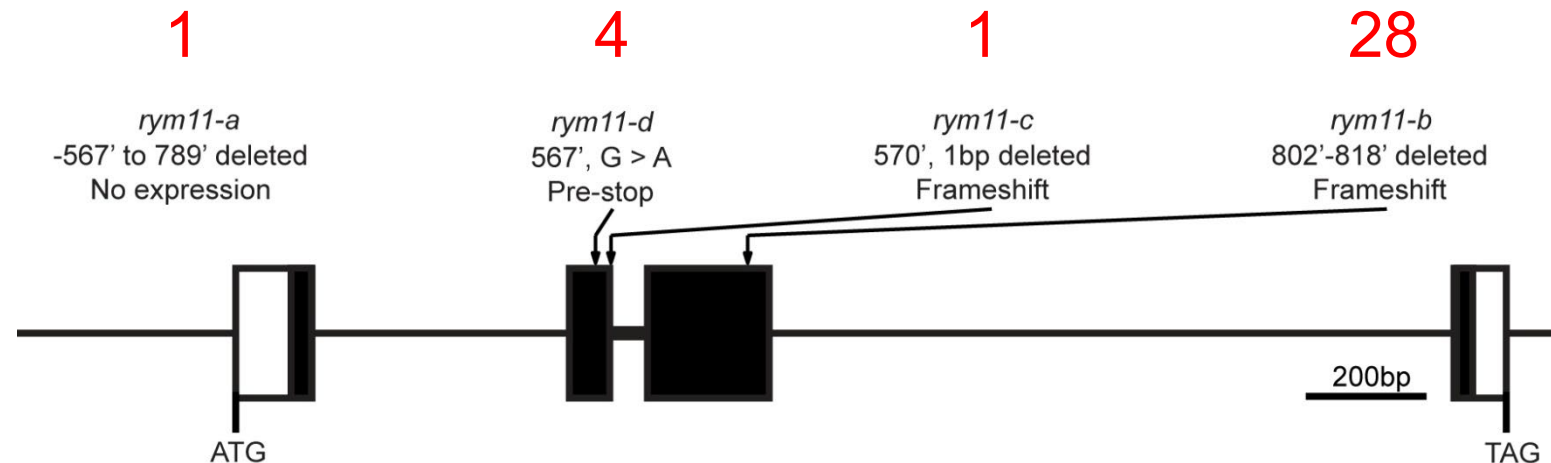
847 Landrace (*H. vulgare*)

559 Cultivar (*H. vulgare*)

5 *H. agriocrithon*

Total = 1.816 accessions

Number of accessions carrying different alleles

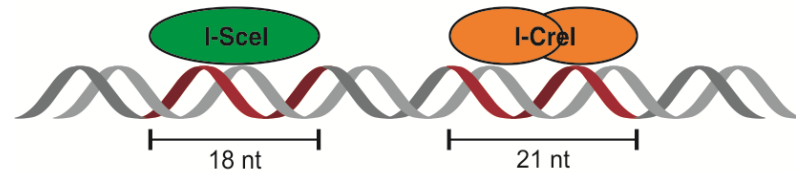




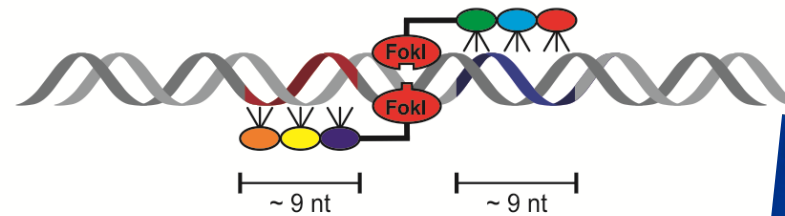
# Plant breeder's toolbox - Genome editing



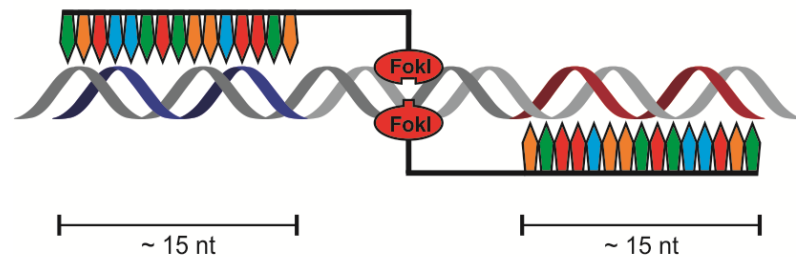
## Meganucleases



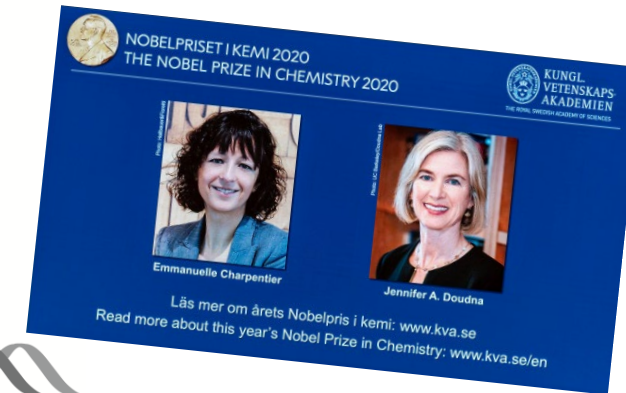
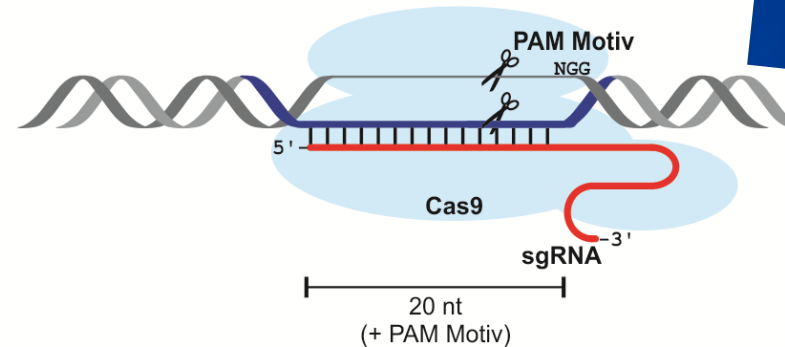
## ZFNs



## TALENs



## CRISPR/Cas System

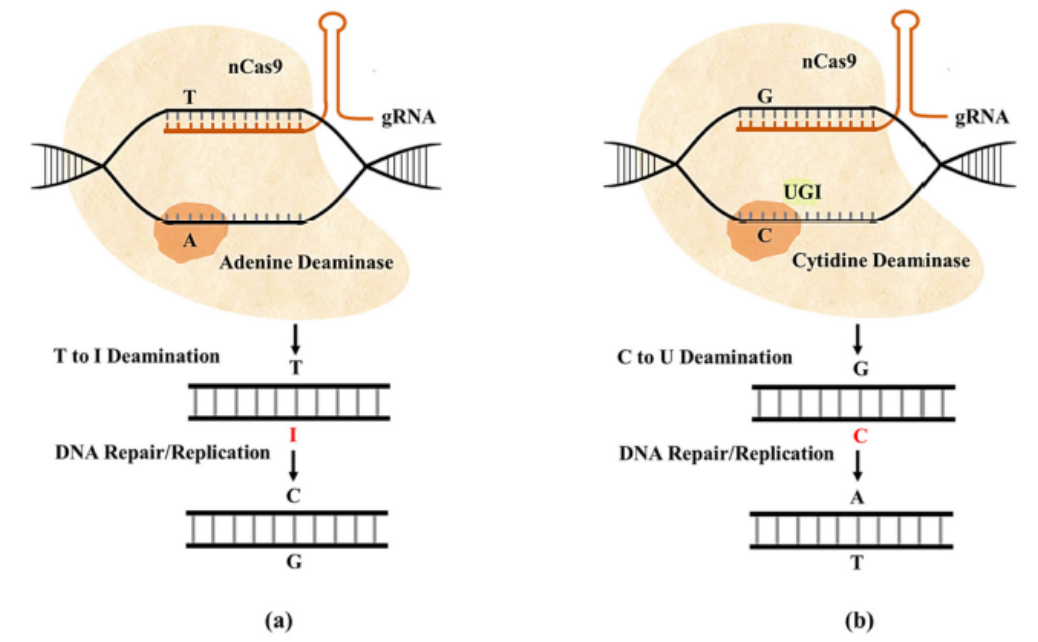
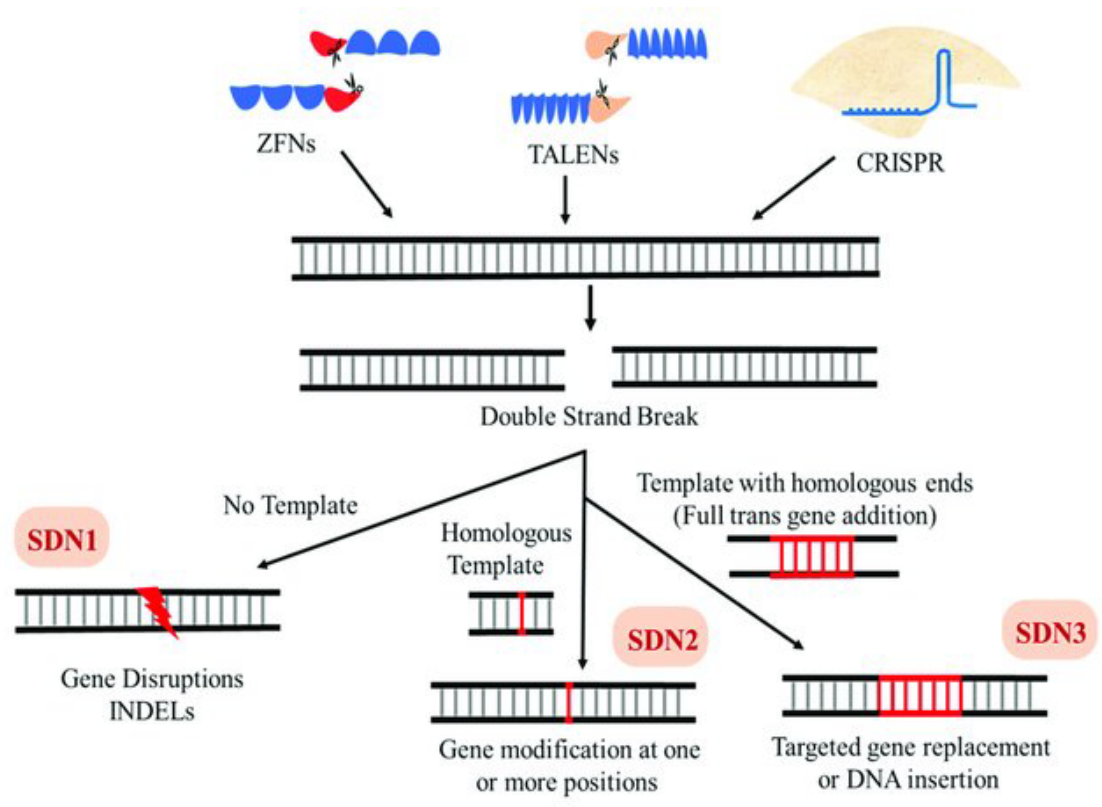


<https://www.forschung-und-lehre.de/karriere/charpentier-und-doudna-erhalten-chemie-nobelpreis-3168>

- ZFNs Zinc-Finger Nucleases
- TALENs Transcription Activator-Like Effector Nucleases
- CRISPR Clustered Regularly Interspaced Short Palindromic Repeats
- Cas CRISPR-associated, RNA-guided endonuclease

Puchta and Fauser (2014) The Plant Journal

# Plant breeder's toolbox - Genome editing



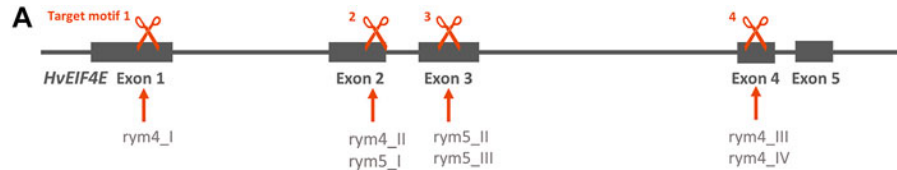
**Figure 6.** Schematic diagram of base editing with nickase Cas9 (nCas9). (a) ABE system uses nCas9 and adenine deaminase to catalyze transformation of adenine into guanine. ABE deaminates adenine to inosine (I), thus converting T-A to T-I. Repair machinery recognizes I as G and repair T-I as C-G; (b) CBE system utilizes nCas9 and cytidine deaminase to catalyze transformation of cytosine to uridine. Uracil glycosylase inhibitor (UGI) prevents U:G mismatch from being repaired back to C:G, and U is ultimately transformed into T.

# Genome editing – *rym4*



## Targeted Knockout of Eukaryotic Translation Initiation Factor 4E Confers Bymovirus Resistance in Winter Barley

OPEN ACCESS  
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**B**

Target motif no.	Target motif incl. PAM	Mutation efficiency in protoplasts (%)	Primary transgenic plants (T <sub>0</sub> )	Primary mutant plants (T <sub>0</sub> = M <sub>1</sub> )
1	ACAACCCGCAGGGCAAGTCCCGG	17.1	4	4 (100 %)
2	ATTTGTGCCAATGGCGGTAAATGG	0	27	0
3	CGTCAGCGTGCCTAAGAACCAGG	3.7	50	0
4	ATGCTAAGAGGTCCGACAAAGG	0	-	-

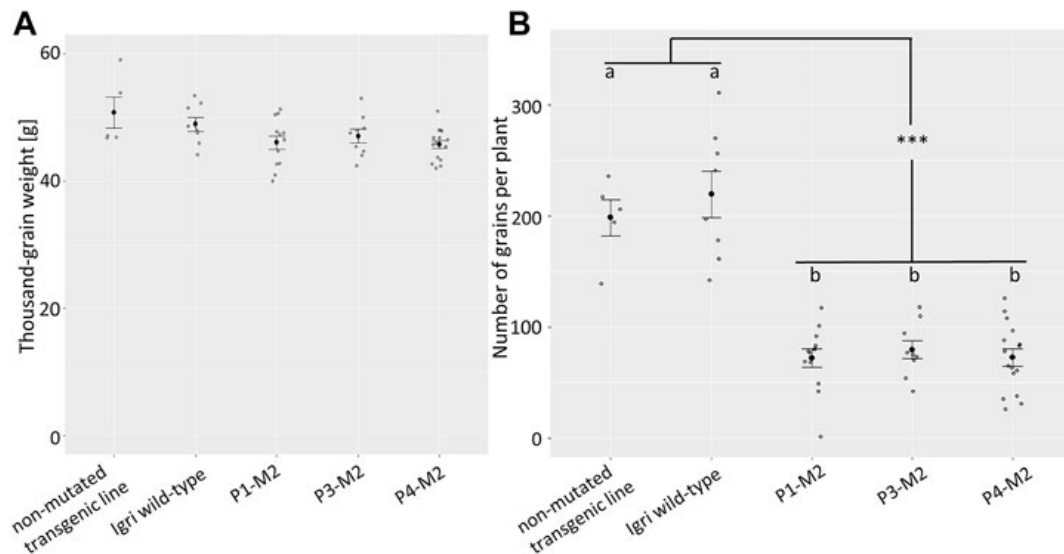
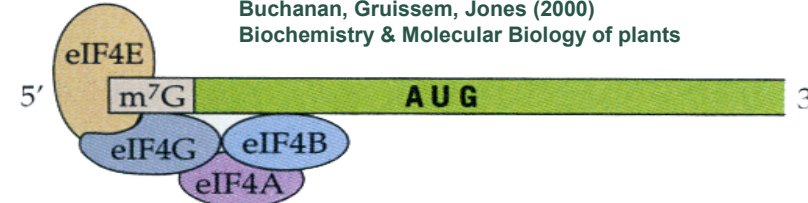


figure taken from:  
Buchanan, Grisse, Jones (2000)  
Biochemistry & Molecular Biology of plants



**C**

Primary mutants	Mutation	No. of M <sub>2</sub> plants tested	BaMMV detection by ELISA
P1	+A	13	0/13
P3	+T	9	0/9
P4	+T	16	0/16
lgri wt	none	8	7/8



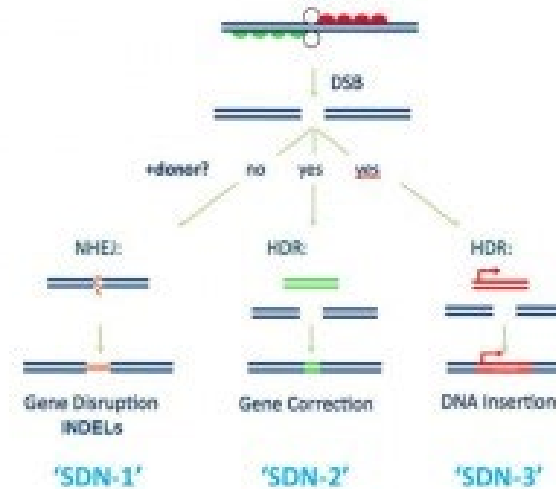
# Ruling of the Court of Justice of the EU, 25th July, 2018



Picture: Court of Justice of the European Union

- All organisms generated by mutagenesis are GMO.
- Organisms generated by targeted mutagenesis underly the strict regulations and are not exempted from any obligations.

## Double-strand break repair



<https://prri.net/scientific-topics/new-breeding-techniques/genome-editing/site-directed-nuclease-sdn-genome-editing>

# Summary and future prospects



Improvements in plant breeder's toolbox, e.g. the availability of high throughput marker technologies, genome sequences etc., and the possibilities of genome editing, have transferred plant breeding to a new era.

Knowledge on resistance gene sequences will facilitate efficient allele mining as well as the targeted editing of respective alleles, e.g. by CRISPR/Cas, resulting in a much faster combination of resistance with superior agronomic traits.



**Modern breeding for resistance will be the cornerstone for an environmental friendly and productive plant production in the background of climate change and the loss of insecticides and fungicides.**



**Thank you for your attention**