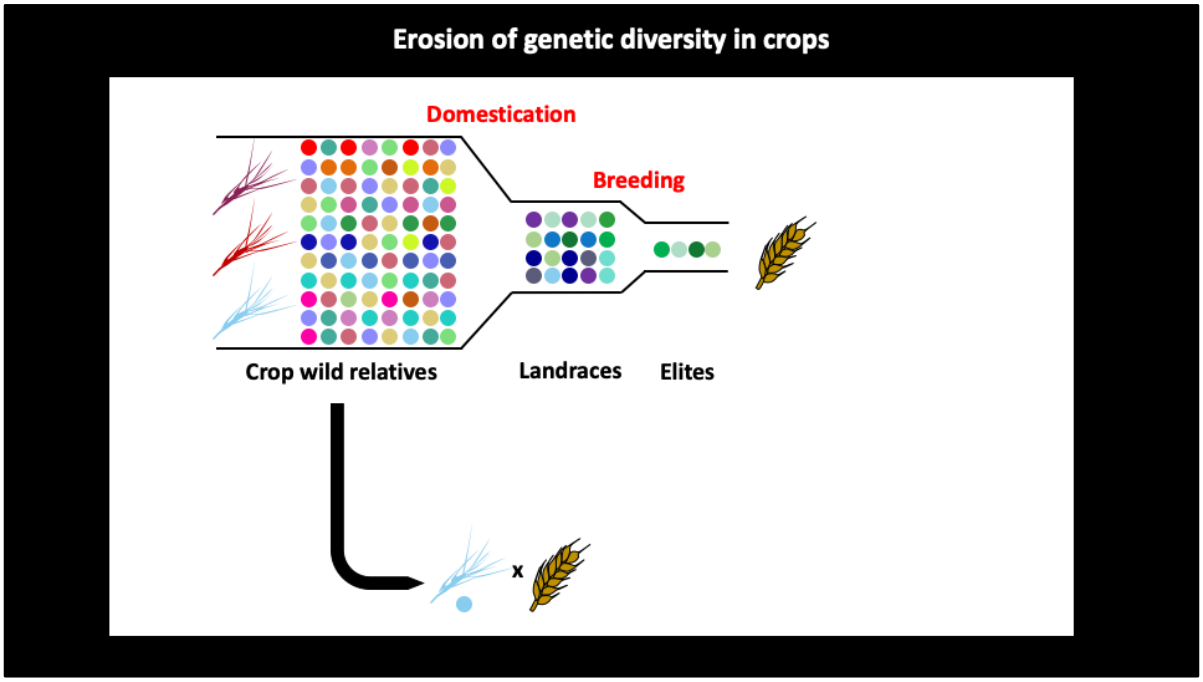
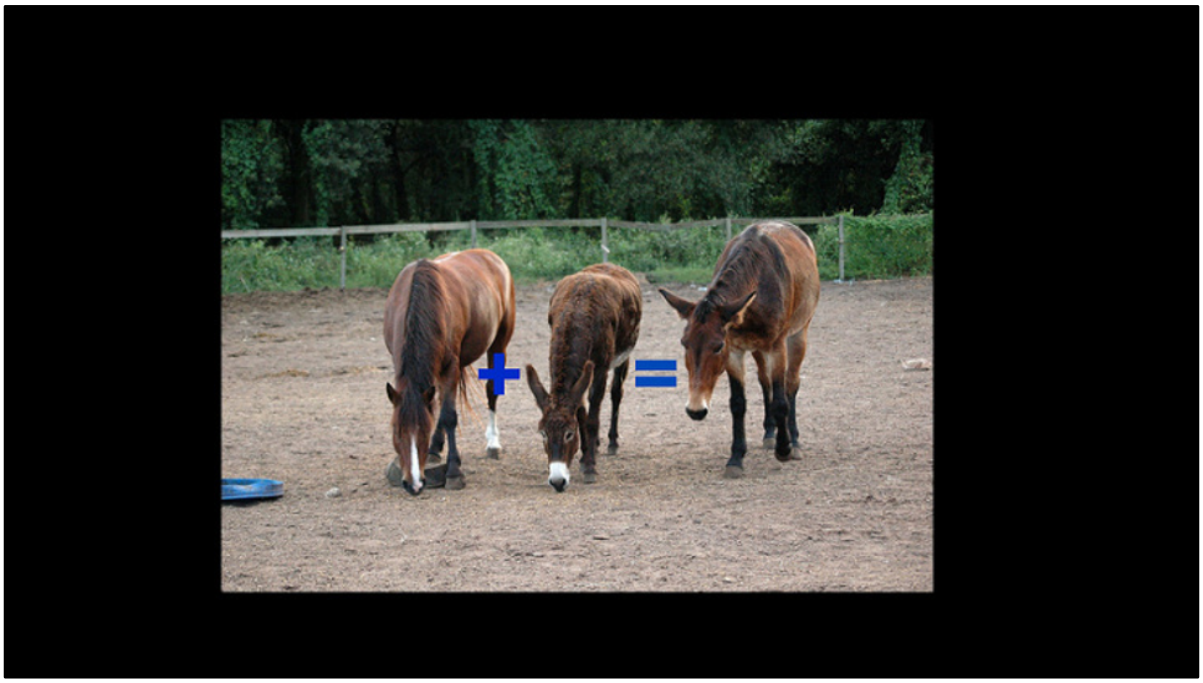
Disruptions to the supply of food have throughout history been associated with famine and civil unrest.
In 2010, unusual weather patterns led to reduced wheat harvests around the world. As a result, the price of bread soared, particularly in many wheat importing countries. The ensuing Arab Spring toppled governments across North Africa and the Middle East.

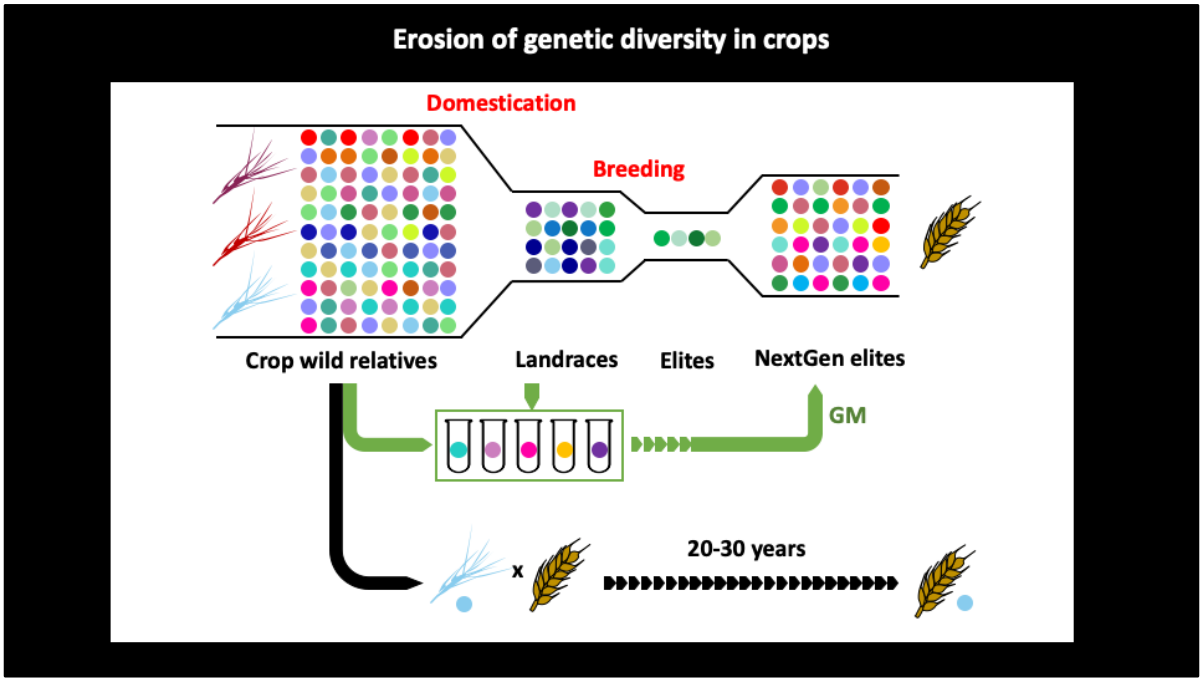
Disease, like the weather, can hit suddenly and unexpectedly.
A case in hand concerns the emergence of wheat stem rust in Bangladesh in 2016, likely the result of importation of contaminated grain from South America.
In a bid to try to stem the spread of the disease, farmer's fields across the country were burnt.
The potential for spread of the disease into the wheat belts of Punjab and China, a region producing 20% of the world's wheat and home to 200 million mostly poor people who depend on wheat, is a concern for food security.



Why are wheat plants so susceptible to disease?
Wheat has gone through genetic bottle necks, e.g. domestication and intensive breeding, which have reduced the diversity for disease resistance genes (represented by ‘smarties’).
This diversity has been further compartmentalized by the modern practice of monoculture (not shown in figure).
Therefore, when a disease emerges, it can spread very rapidly.
For more than 100 years farmers and breeders have tapped into the genetic diversity in the wild relatives of wheat by performing interspecific crosses (bottom part of figure)

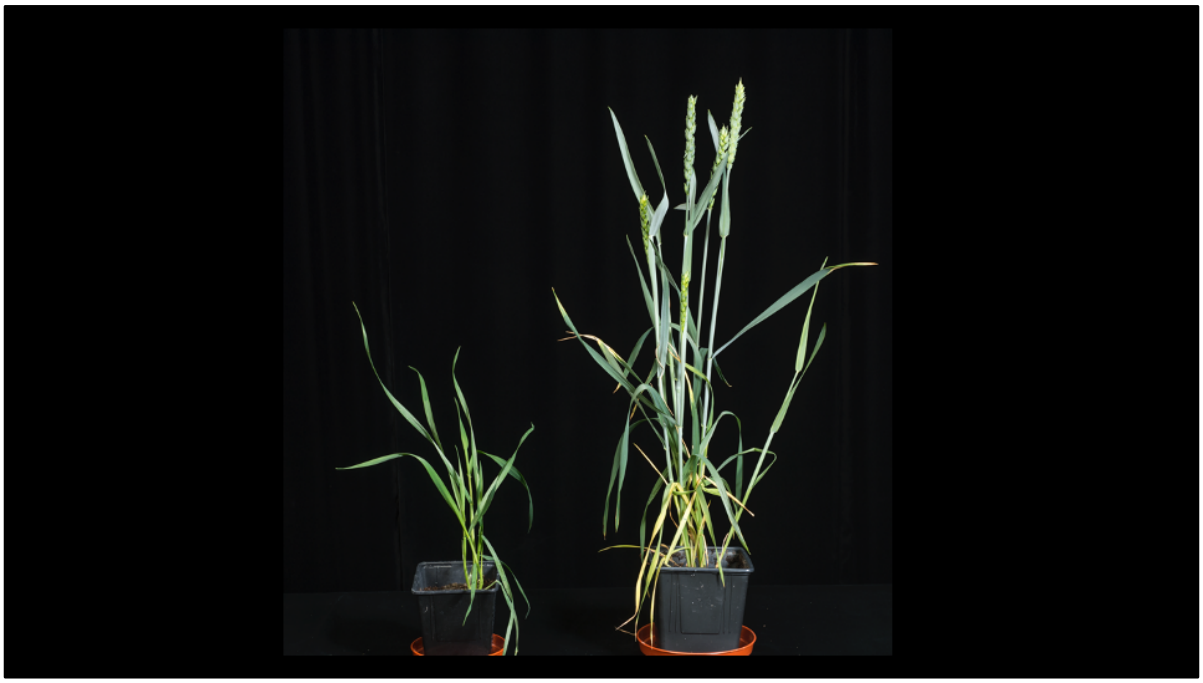


However, this is akin to crossing a racehorse with a donkey.



It takes many years of breeding to combine the best of both worlds and produce a wheat line which is commercially viable (*i.e.* disease resistance from the wild relative combined with bread making properties and high yield of the elite domesticated cultivar).
The cloning of disease resistance genes and introduction by transformation into elite cultivars can speed up the delivery of a novel resistance gene into wheat. Moreover, it allows the simultaneous stacking of multiple genes for disease resistance.
Such stacks would, from first principle, provide more durable disease resistance. Moreover, GM stacks would not suffer from 'linkage drag' from the wild relative, i.e. the co-introduction of deleterious alleles of linked genes.

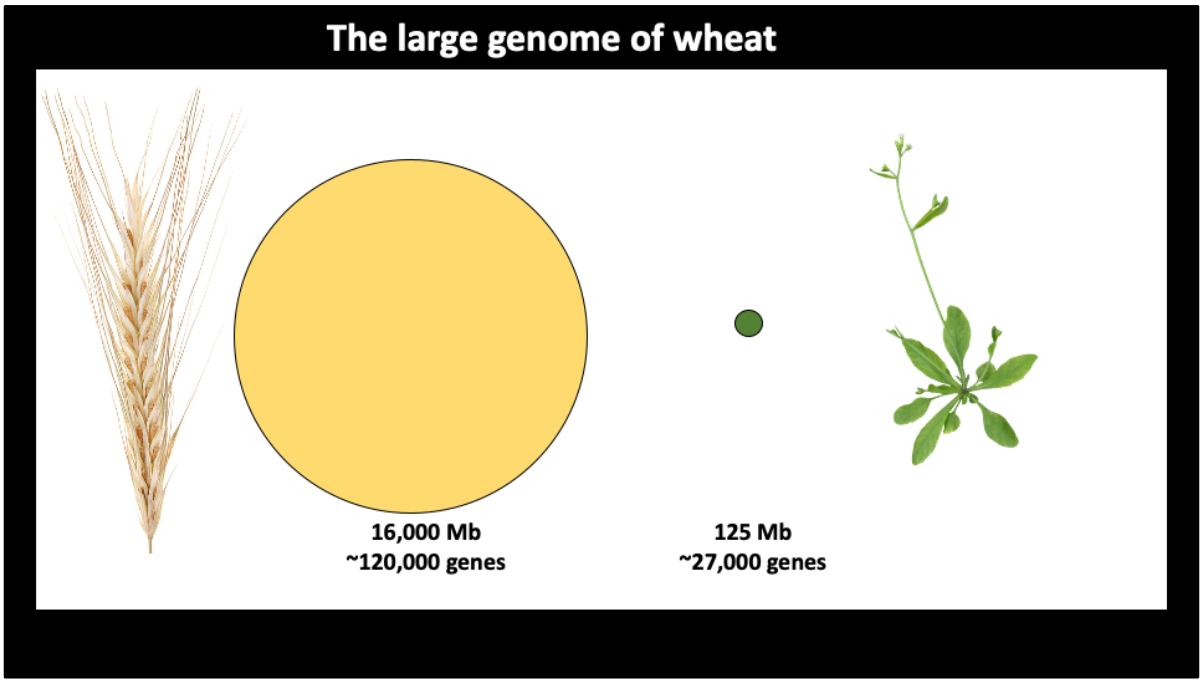
See e.g.
Wulff & Moscou (2014) Strategies for transferring resistance into wheat: from wide crosses to GM cassettes. Frontiers in Plant Sciences 5:692. doi: 10.3389/fpls.2014.00692.



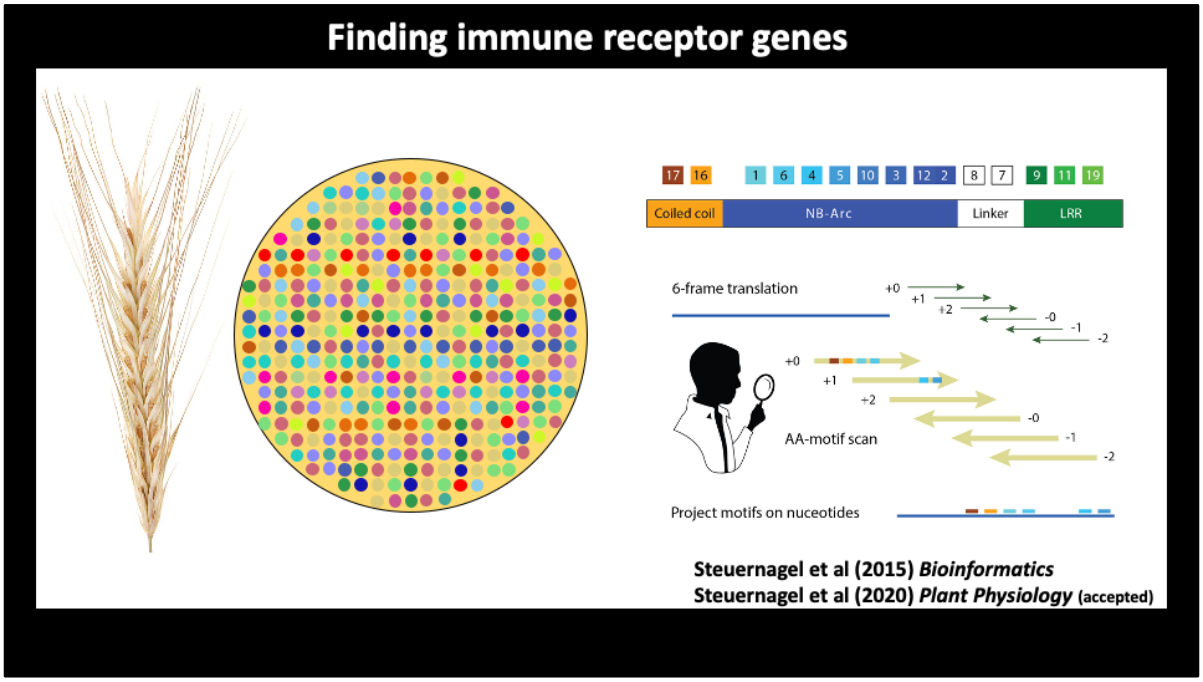
One of the limitations of working with wheat is the slow generation time, typically 4 to 5 months.
This can be improved with ‘speed breeding’, whereby generation times of 8 weeks can be achieved.
The left-hand plant was grown in a glass house with natural light supplemented with sodium vapour lamps. It is 5.5 weeks old. The right-hand plant was grown under speed breeding conditions and is also 5.5 weeks.

The rapid generation times are achieved by growing the plants under LEDs (light emitting diodes) enriched in the blue and red light spectra optimized for photosynthesis (indicated by white bars under spectral graphs).
Moreover, the daylength is increased to 22 hours.

Watson, Ghosh et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nature Plants 4:23-29.
Ghosh, Watson et al (2018) Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. Nature Protocols 13:2944-2963.



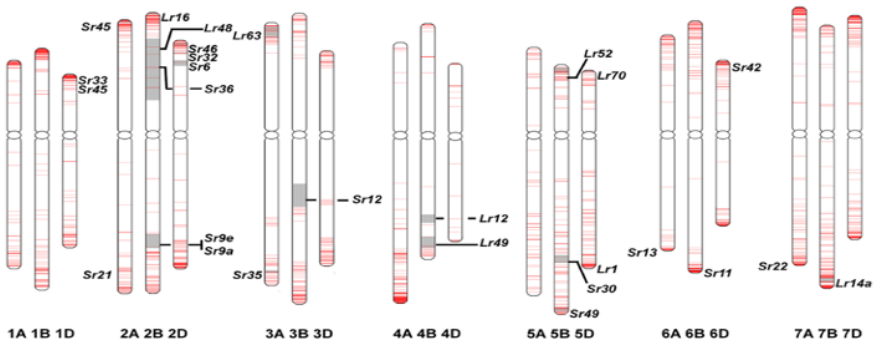
Another challenge of working with wheat is imposed by its huge genome, some 128 times bigger than *Arabidopsis thaliana*, the plant 'lab rat'.



Most resistance genes which have been defined by genetics and subsequently cloned have been found to encode nucleotide-binding and leucine rich repeat (NLR) proteins. The bioinformatics tools NLR-Parser and NLR-Annotator detect small motifs (numbered boxes) typical of NLRs to identify, *ab initio*, NLR loci in DNA sequences.

Steuernagel et al (2020) The NLR-Annotator tool enables annotation of the intracellular immune receptor repertoire. Plant Physiology doi: 10.1104/pp.19.01273.
Steuernagel, Jupe et al (2015) NLR-parser: rapid annotation of plant NLR complements. Bioinformatics 31:1665-7.

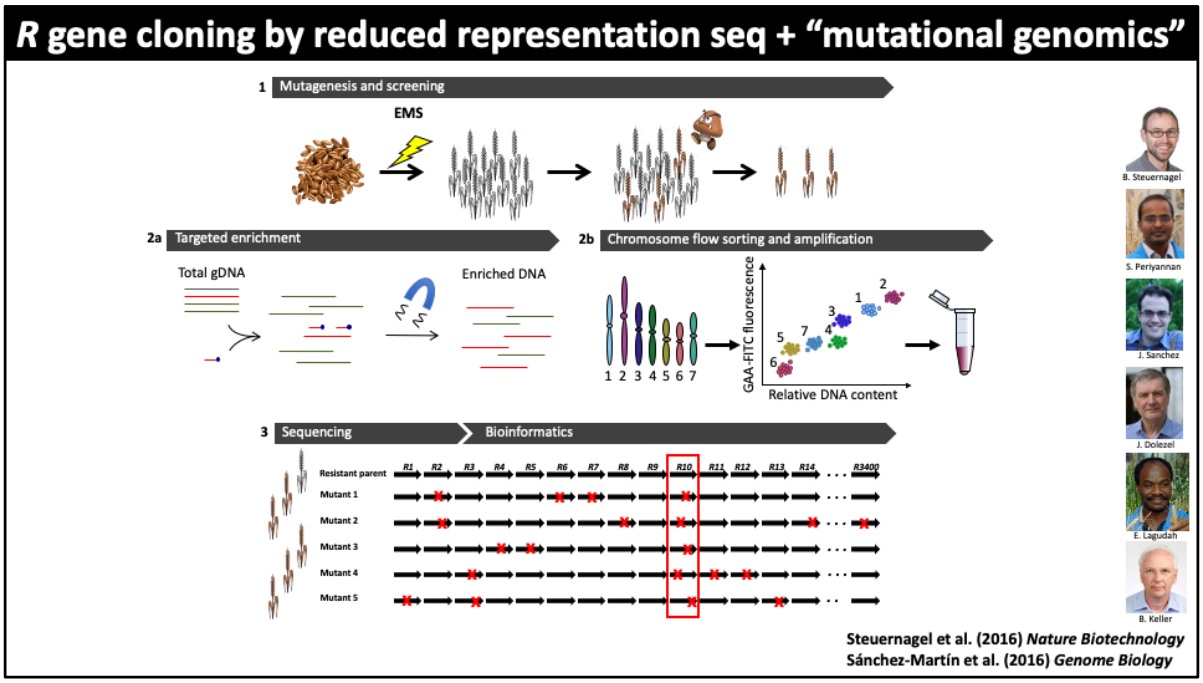
Finding immune receptor genes



Steuernagel et al (2020)

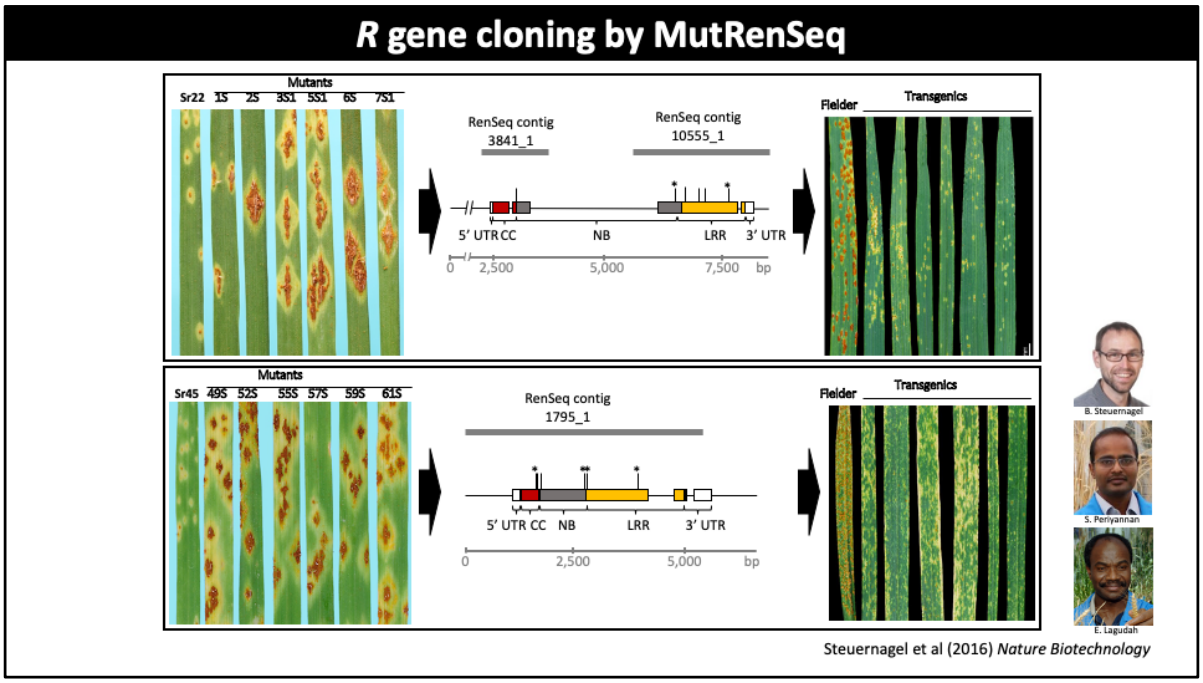
With NLR-Annotator, 3400 NLR loci can be detected in the wheat reference genome. By positioning genetically mapped resistance genes (illustrated here for some leaf rust and stem rust resistance genes) the number of NLR candidate genes inside a mapping interval can be determined.

Steuernagel et al (2020) The NLR-Annotator tool enables annotation of the intracellular immune receptor repertoire. Plant Physiology doi: 10.1104/pp.19.01273.



Top panel: Once a resistance gene has been genetically isolated (i.e. bred into a background susceptible to the pathogen isolate of interest), it can be cloned by "mutational genomics". A seed stock containing the genetically isolated resistance gene is mutated, typically with a chemical mutagen such as EMS, which introduces mostly single nucleotide variants (SNVs). The mutant population is screened to identify susceptible mutants. The phenotypes of these mutants are verified in the next generation.
Middle panel: To avoid having to sequence the entire 16 Gb wheat genome, genome complexity reduction is applied. Resistance gene enrichment sequencing (RenSeq) captures the NLR-encoding portion of the genome to achieve a ~1000-fold enrichment, while chromosome flow sorting achieves up to 21-fold enrichment in hexaploid wheat or 7-fold enrichment in diploid relatives. RenSeq imposes a bias for NLRs. Chromosome flow sorting is sequence-unbiased but requires knowledge of which chromosome the gene locates to.
Bottom panel: The sequences from the wildtype parent are compared to the mutants to search for a gene which is mutated in all of the mutants. The probability of this happening by chance alone for 5 mutants is very small, allowing the identification of causative mutations in a single gene.

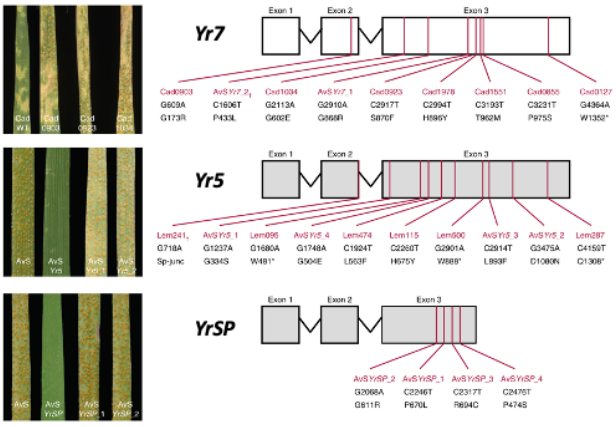
- Steuernagel, Periyannan et al (2016) Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nature Biotechnology 34:652-5.
- Sánchez-Martín, Steuernagel et al (2016) Rapid gene isolation in barley and wheat by mutant chromosome sequencing. Genome Biology 17(1):221.



Examples of two wheat stem rust resistance genes, *Sr22* and *Sr45*, cloned by MutRenSeq.

Steuernagel, Periyannan et al (2016) Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nature Biotechnology 34:652-5.

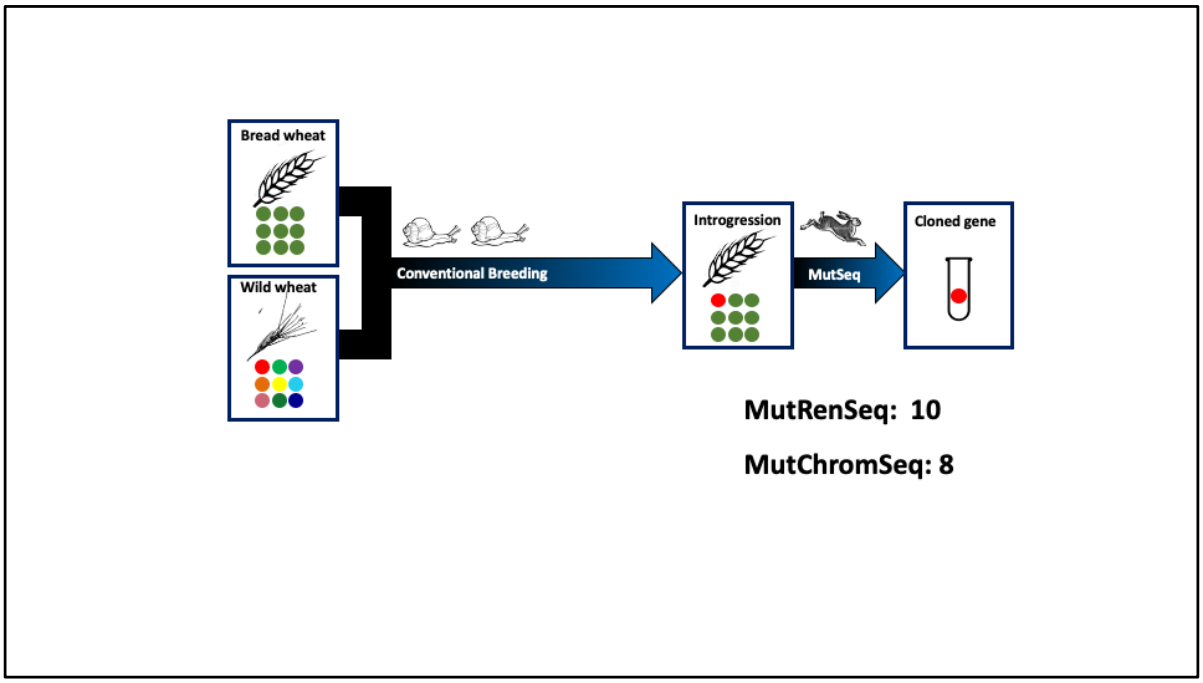
R gene cloning by MutRenSeq



Marchal et al (2018) ***Nature Plants***

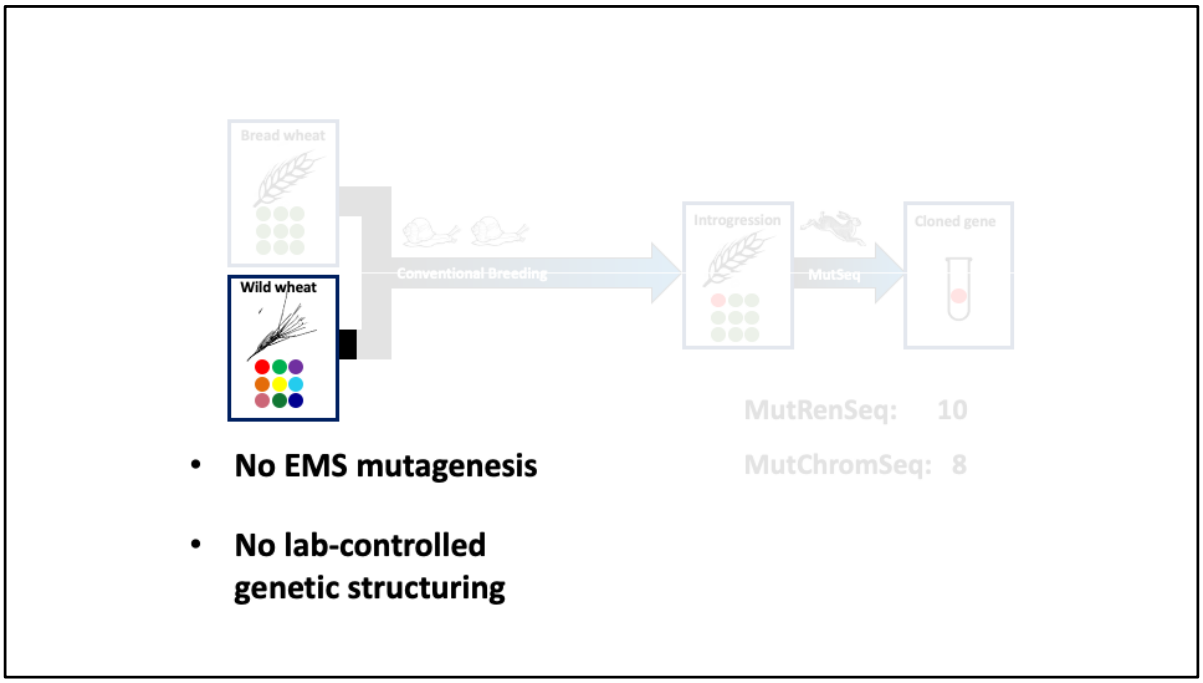
Example of three stripe rust resistance genes, *Yr7*, *Yr5* and *YrSp*, cloned by MutRenSeq.

Marchal, Zhang et al (2018) BED-domain-containing immune receptors confer diverse resistance spectra to yellow rust. Nature Plants 4:662-668.



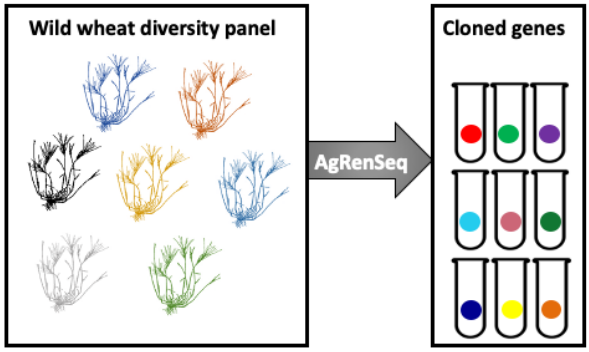
The MutRenSeq and MutChromSeq enabling technologies allow cloning genes fast once they have been genetically isolated.
However, the number of crosses and generations required to genetically isolate a gene makes this a slow process for any new gene identified in a wild relative.
Could the process work directly in a wild relative? This would allow tapping into the extraordinary wealth of genetic diversity outside of the wheat gene pool.

We genetically isolated a stem rust resistance gene in the wild diploid wheat relative *Aegilops sharonensis*, bulked up large amounts of seed, performed EMS mutagenesis, and grew up a mutant population. However, the plants were infertile. Compared to hexaploid bread wheat, which tolerates a higher load of EMS due to genetic redundancy, getting the EMS concentration just right in a diploid is more tricky.

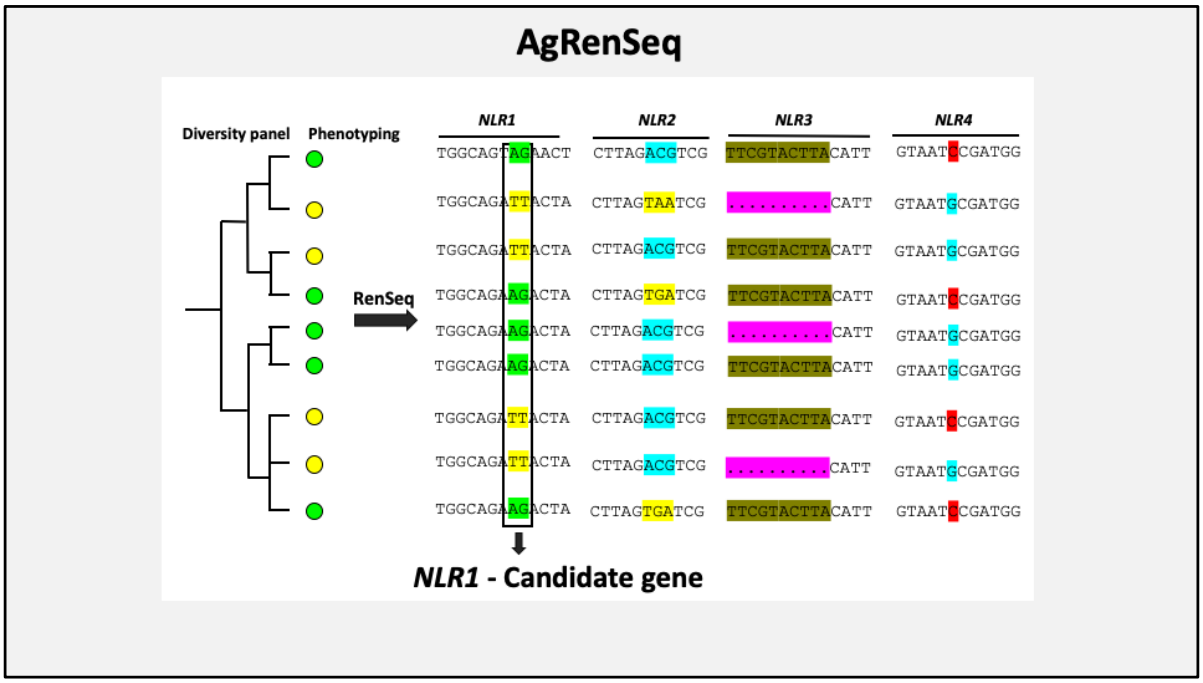


We therefore decided to try to develop a gene cloning method which would not require EMS mutagenesis or lab-controlled genetic structuring.

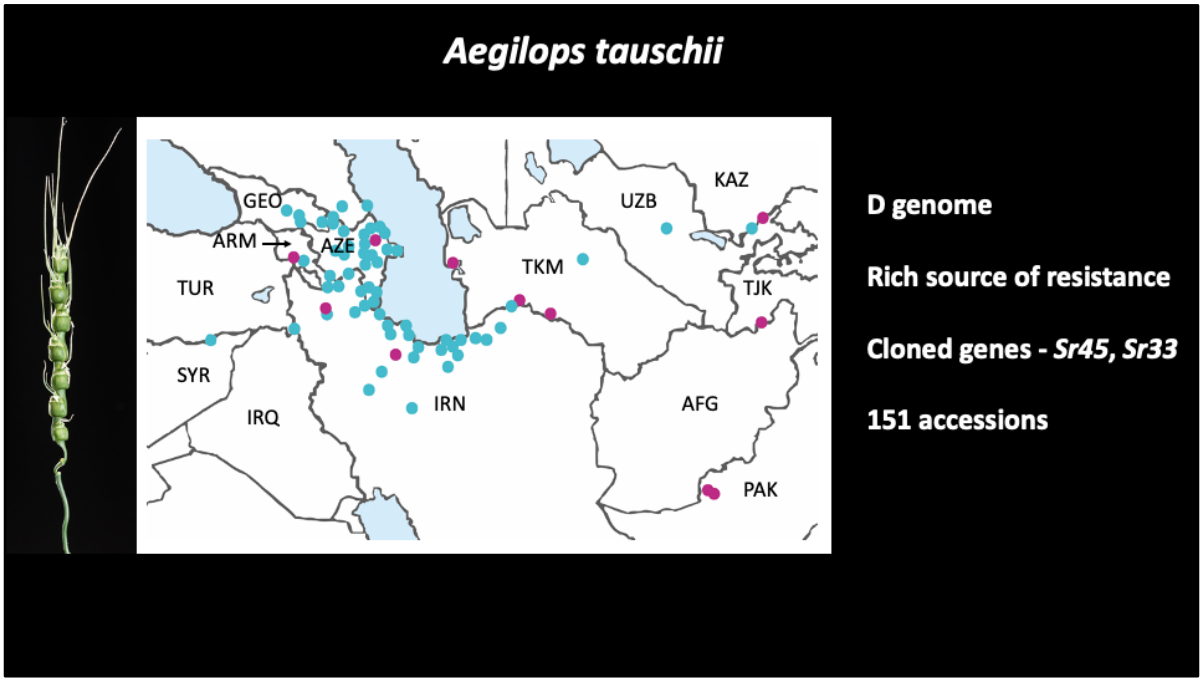
Association genetics + RenSeq = AgRenSeq



We decided to take advantage of nature's own genetic structure, the historical recombination and mutations which have accumulated in natural populations over thousands of years. We would couple this genetic structure by association genetics to RenSeq to identify resistance gene candidates. This concept, dubbed "AgRenSeq" would in principle, allow cloning multiple resistance genes from a diversity panel.

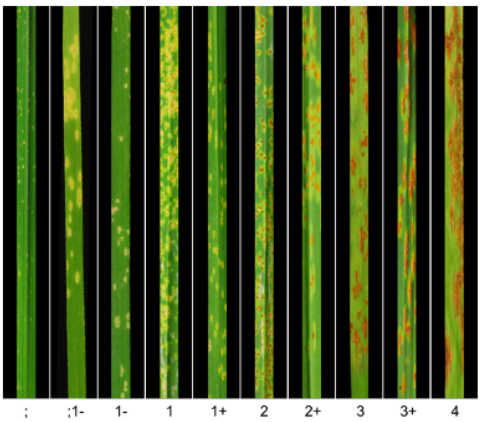


The proposed technology would require a genetically diverse panel which is scored for resistance (green circles) and susceptibility (yellow circles).
The NLR repertoires from each accession in the panel are sequenced.
The NLRs are lined up, and sequence polymorphisms which correlate with resistance are identified.
In the hypothetical example an AG dinucleotide polymorphism correlates perfectly with resistance, identifying *NLR1* as a candidate resistance gene.



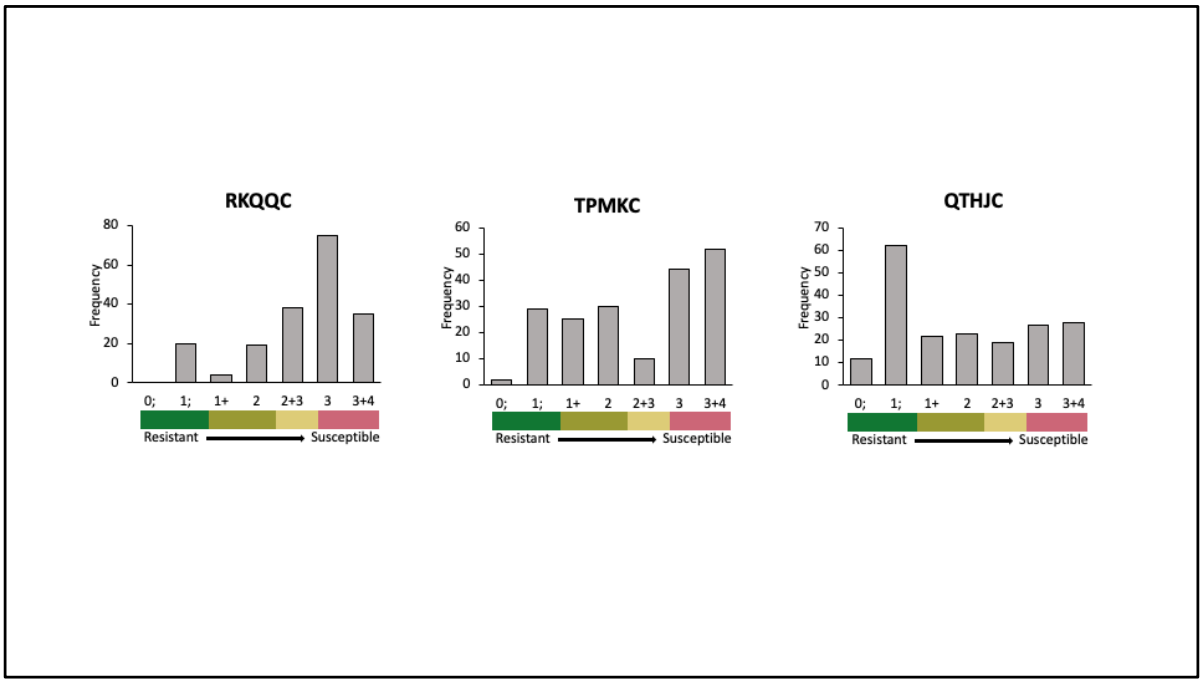
To reduce the concept to practice, we turned our attention to *Aegilops tauschii*, the D genome progenitor of hexaploid bread wheat.
This species has historically been a rich source of resistance to major diseases of wheat over the years.
Importantly, two *Aegilops tauschii* resistance genes, *Sr33* and *Sr45*, had already been cloned (after being transferred from *Aegilops tauschii* into wheat) providing perfect positive controls.
We configured a panel of 151 genetically diverse accessions from across the distributional range of the species.

Stem rust phenotyping

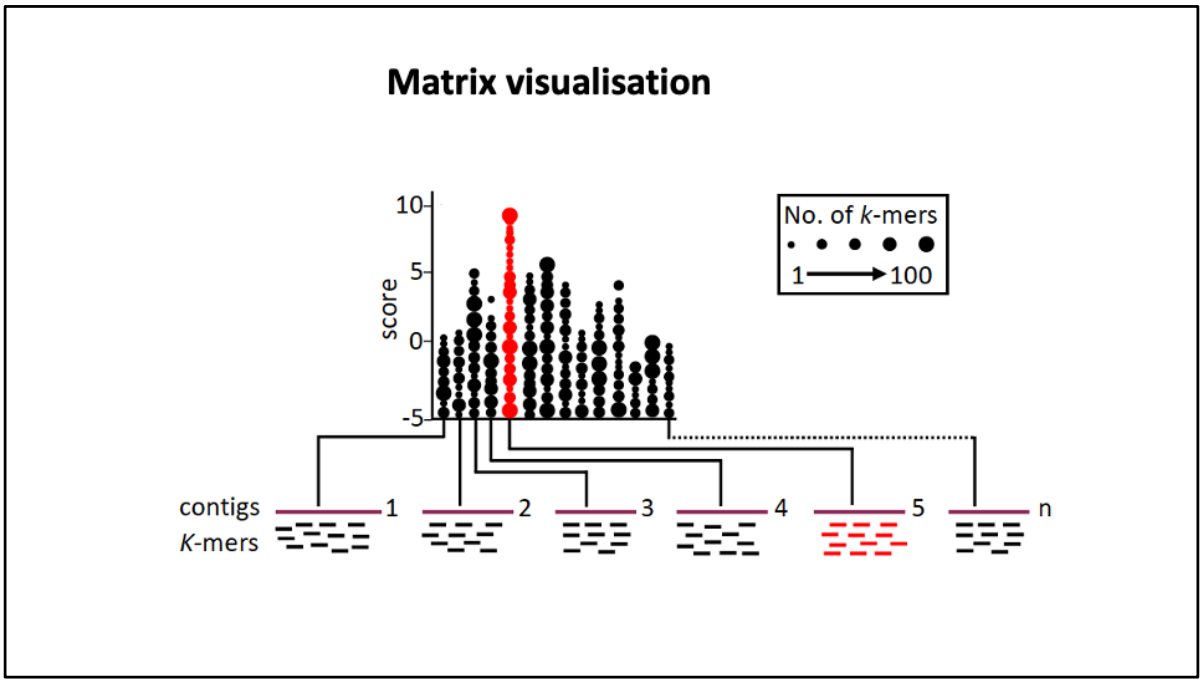


- RKQQC
- TPMKC
- QTHJC

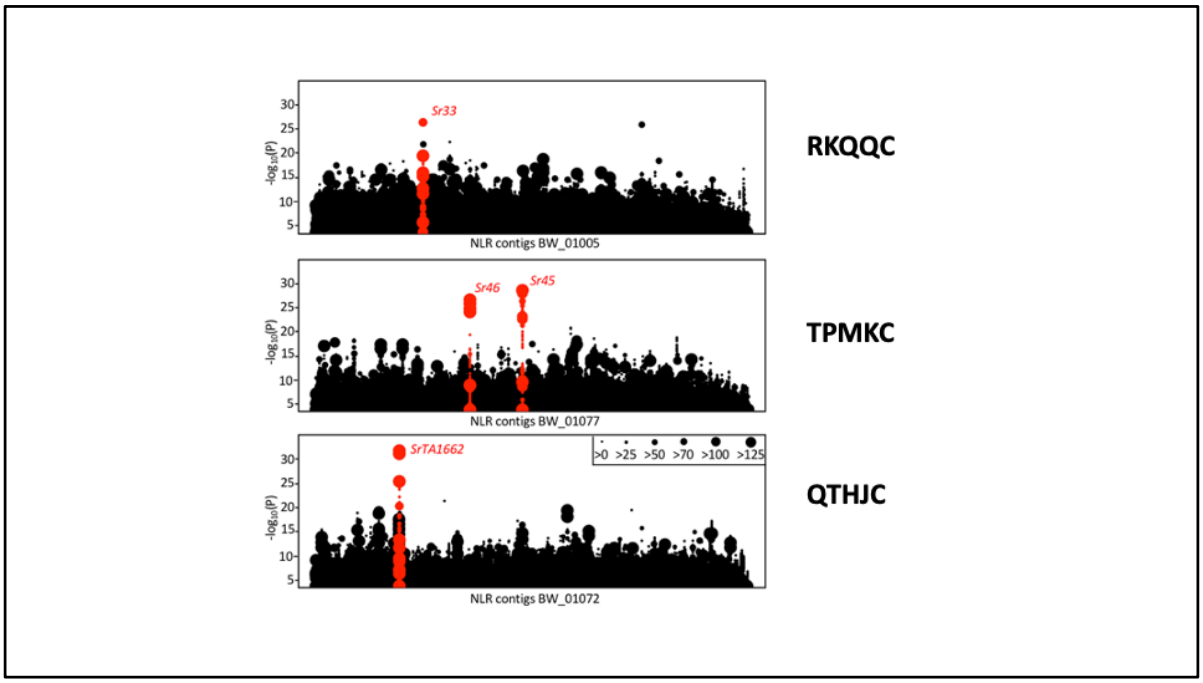
The panel was phenotyped with three races of *Puccinia graminis* f. sp. *tritici*, the causal agent of wheat stem rust.
The phenotypes ranged from complete immunity (indicated by a ";") to complete susceptibility (indicated by a "4").



The distribution of resistance across the panel for the three races.



To visualize the correlation between disease resistance in the panel and genotype, we developed a dot-column plot in which the resistance phenotype is indicated by a score on the y-axis and the NLR genotypes on the x-axis.
The RenSeq reads (NLR complement) from a resistant accession have been lined up along the x-axis.
Thus, each integer on the x-axis represents a RenSeq contig.
Each contig is represented by short sequences (*k*-mers) of 51 nucleotides.
The *k*-mers across the whole panel are correlated with resistance and given a value and this value is placed on the y-axis.
The higher the *k*-mers (dots) are placed on the y-axis, the better the correlation with resistance.
The dot size is proportional to the number of *k*-mers at a given position.
In the hypothetical example, one dot-column highlighted in red, is head and shoulders above the rest – this would be our candidate resistance gene.



What does it look like with real data?

Top panel:

Race RKQQC is diagnostic for *Sr33*. That is to say, the race is avirulent on accessions carrying *Sr33*, but overcomes resistance in most of the rest of the panel.

We observe a discrete peak for RKQQC which revealed a contig with perfect sequence correlation to *Sr33.*

Middle panel:

The plot for TPMKC reveals two peaks, one for the second positive control, *Sr45*, and another, new peak. The gene under this new peak was found by BLAST to locate to a region where the gene *Sr46* had been genetically mapped. Our colleague, Evans Lagudah in the CSIRO, Australia, had at the time recently cloned the gene, but not yet published it. Evans compared the two sequences, and low and behold, they were 100% identical.

Bottom panel:

With the QTHJC phenotypes we identified another novel peak. The gene under this peak localized by BLAST to a region where the gene *SrTA1662* had been genetically located, thus providing a high-confidence candidate for this gene.

Resistance gene cloning from a wild crop relative by sequence capture and association genetics


S. Arora

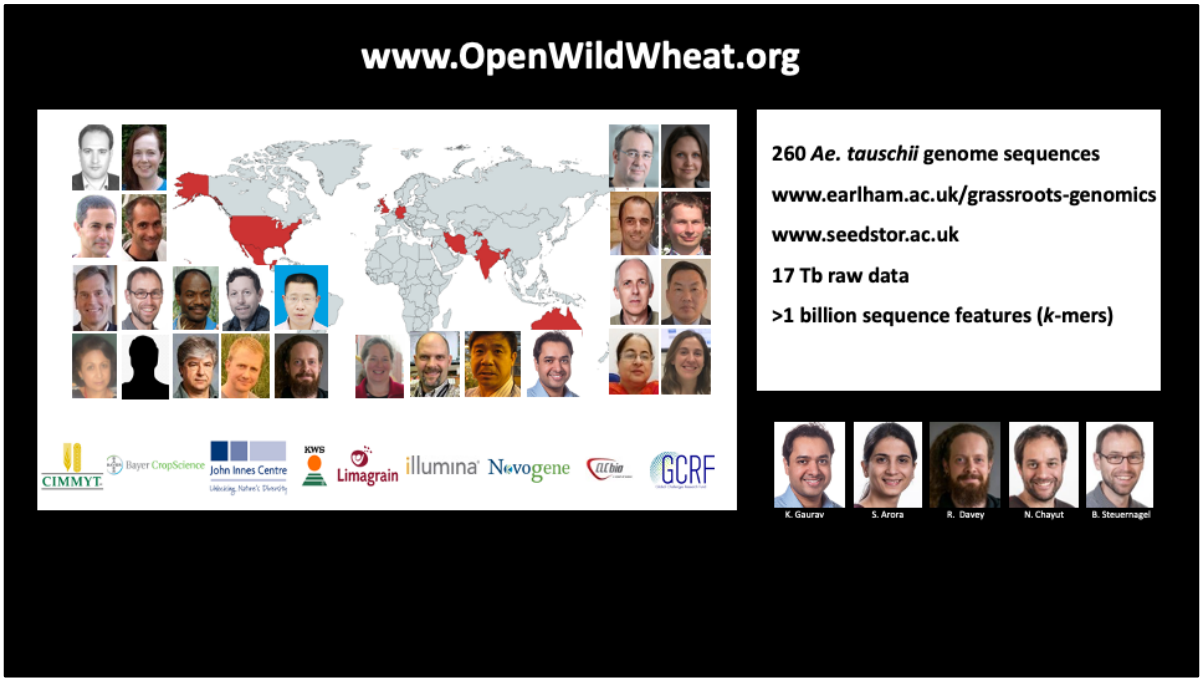

B. Steuernagel


K. Gaurav

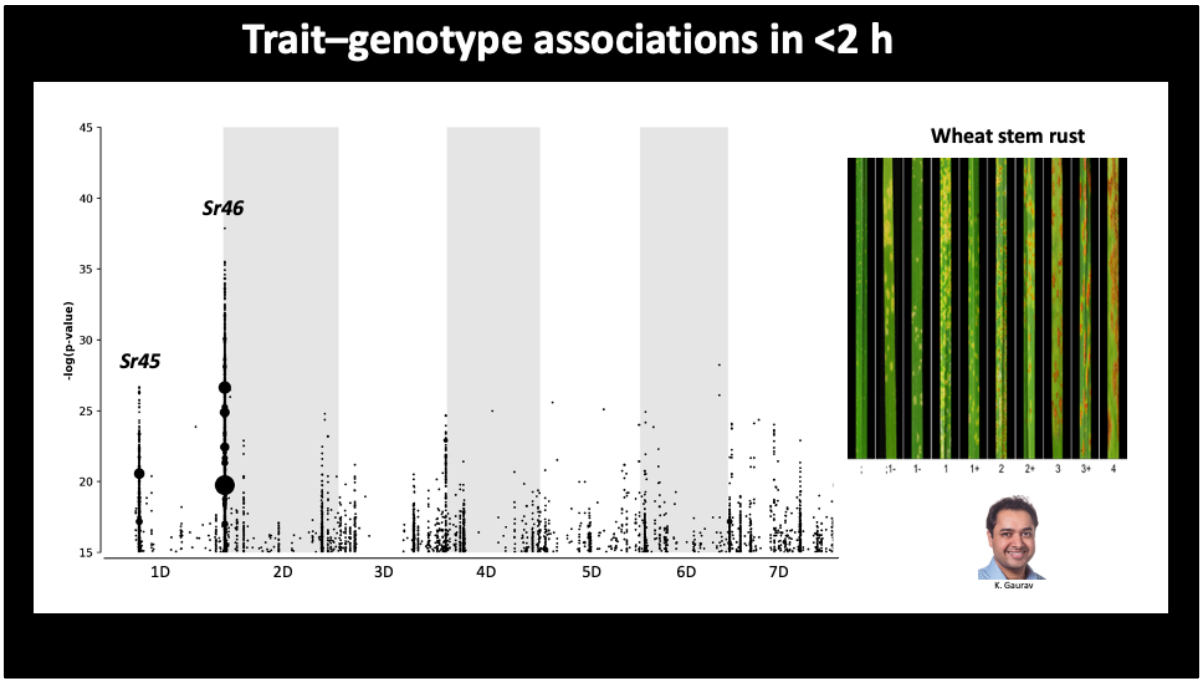
Sanu Arora, Burkhard Steuernagel, Kumar Gaurav, Sutha Chandramohan, Yunming Long, Oadi Matny, Ryan Johnson, Jacob Enk, Sambasivam Periyannan, Narinder Singh, M. Asyraf Md Hatta, Naveenkumar Athiyannan, Jitender Cheema, Guotai Yu, Ngonidzashe Kangara, Sreya Ghosh, Les J. Szabo, Jesse Poland, Harbans Bariana, Jonathan D. G. Jones, Alison R. Bentley, Mick Ayliffe, Eric Olson, Steven S. Xu, Brian J. Steffenson, Evans Lagudah & Brande B. H. Wulff ✉

Nature Biotechnology **37**, 139–143 (2019)

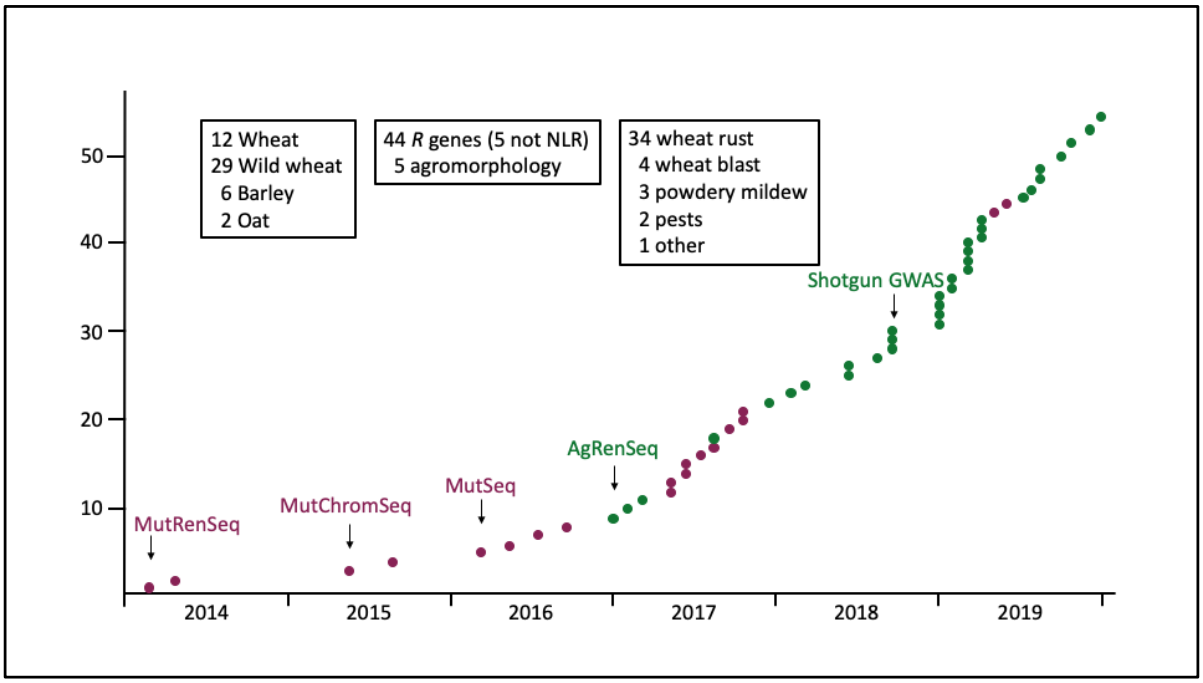
"AgRenSeq" publication.



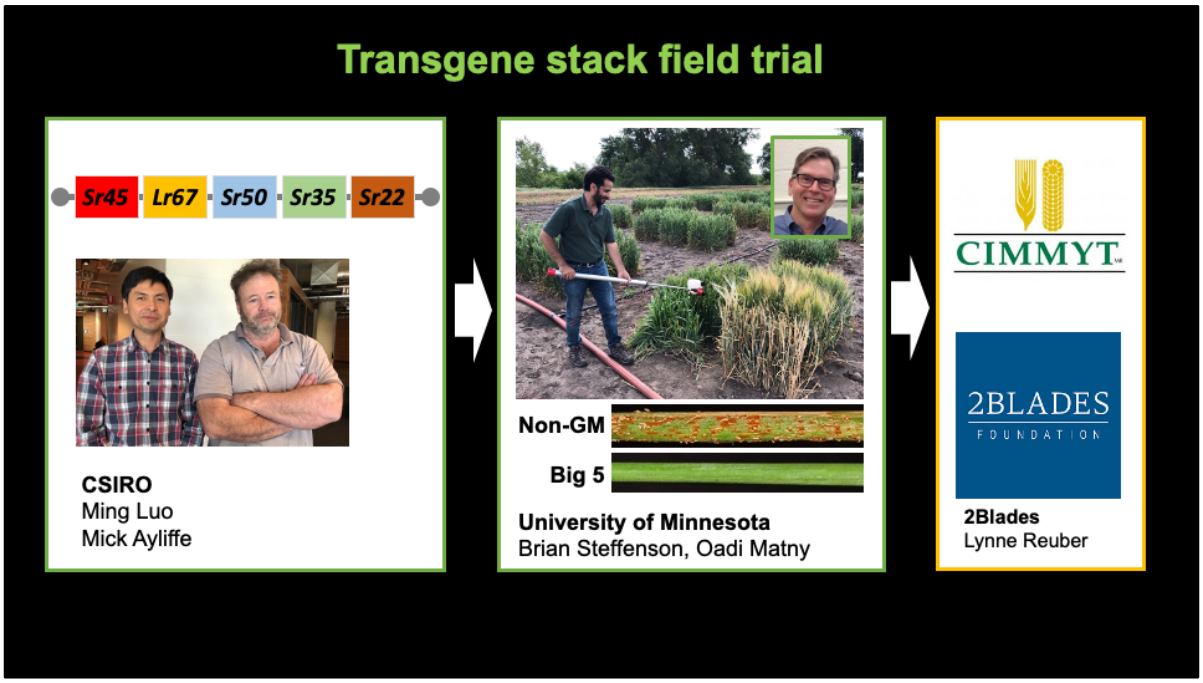
The AgRenSeq technology allows direct cloning of resistance genes in plant diversity panels without having to perform crosses or mutagenesis in the lab. A drawback, however, is that the resistance gene enrichment sequencing introduces a huge bias in that it assumes that the target gene of interest is an NLR. Yet, not all resistance genes are NLRs. To overcome this bias we decided to perform whole genome shotgun sequencing of every accession in the panel. To facilitate this we set up the Open Wild Wheat Consortium. Under the aegis of the OWWC we have to date sequenced 260 *Aegilops tauschii* genomes.



We have scaled up the AgRenSeq pipeline and made it more efficient to work on whole genome shotgun data. This has allowed re-identification of positive controls, such as *Sr45* and *Sr46*.



Using mutational genomics and association genetics, we have to date identified ~50 genes or high confidence candidate genes in domesticated cereals and their wild relatives, mostly for disease resistance.



The 2Blades Foundation has led an international 2Blades Wheat Rust Consortium to engineer resistance to wheat rusts. As part of this effort, Mick Ayliffe and Ming Luo (CSIRO, Canberra, Australia), have engineered a multi-*R* gene construct containing five Sr genes and transformed it into wheat. Brian Steffenson and Oadi Matny (University of Minnesota, USA) have field-trialled the stack, known as the "Big 5", and found that it confers complete immunity.

See: http://2blades.org/2019/03/12/wheat-lines-from-2blades-csiro-and-umn-exhibitexceptional-stem-rust-resistance-in-the-field/

The "Big 5" is a proof-of-concept and was engineered with conventional GM technology, so retains non-native DNA, e.g. the antibiotic selectable marker. However, all the *Sr* genes in the stack are from wheat or have been bred into wheat from wild or domesticated relatives, so it would be possible to generate a cis-genic version (i.e. with only same-species genes) of the "Big 5", which may be subject to less regulation.

2Blades has allied with CIMMYT to introduce resistance to wheat rusts into CIMMYT wheat lines, with the goal of distributing the lines to countries that have biosafety regulations in place to utilize these cultivars.

See: http://2blades.org/2016/12/07/2blades-cimmyt-alliance/

Wheat–the cereal abandoned by GM

Genetic modification of wheat for disease resistance could help stabilize food production

By **Brande B. H. Wulff**[1]
and **Kanwarpal S. Dhugga**[2]

SCIENCE 3 AUGUST 2018 • VOL 361 ISSUE 6401

Unlike other major broadacre crops, such as maize, soy and oilseed rape, which have been endowed with GM traits (e.g. for herbicide and insect resistance), no GM wheat has been approved. It would appear that low profit margins and public pressure from those that oppose GM crops have marginalized wheat to become an “orphan GM crop”. Currently, >20% of projected wheat fields are lost every year to disease. Important opportunities for GM disease resistant wheat are clearly being missed.

Wulff and Dhugga (2018). Wheat-the cereal abandoned by GM. Science 361:451-452.

Wheat engineered with multi-resistance gene stacks is likely the best long term solution to sustainably and efficiently manage disease resistance in wheat. With appropriately selected genes in the stack, this can create more durable resistance than has been possible hitherto. A major help in enabling this would be if the EU amends its 2001/18/EC legislation to facilitate cis-genesis, rather than burden the technology with GM regulation as at the moment. In the interim, we propose creating an international public-corporate partnership using mutational genomics and GWAS to clone the majority of wheat disease resistance genes against the major diseases of wheat to develop a ”Wheat Resistance Gene Atlas”. This, in combination with pathogen surveillance, speed breeding and marker assisted selection, would allow breeders to more rapidly and judiciously breed wheat varieties with improved and more durable disease resistance.

Amber Hafeez
Attilio Pascucci
Guotai Yu
Jiaqian Liu
Kumar Gaurav
Ngoni Kangara
Sanu Arora
Sreya Ghosh
Tom O'Hara

Alumni:

Asyraf Hatta
Burkhard Steuernagel
Clemence Marchal
Deepika Narang
Guozhong Sun
Lucy McCann
Macarena Forner
Suzi Pearce
Yajuan Yue

John Innes Centre
Unlocking Nature's Diversity

2Blades

norwich research park

CerealPath

PCGIN

SCIENCE FOR AFRICA

UPM

Monsanto's Beachell-Borlaug International Scholars Program

BBSRC
bioscience for the future

Newton Fund

Your logo here
£10,000 per month

THE SAINSBURY LABORATORY