DEPLOYING STATE OF THE ART TECHNOLOGIES FOR LOSS OF SUSCEPTIBILITY AGAINST PATHOGENS ACROSS CROPS

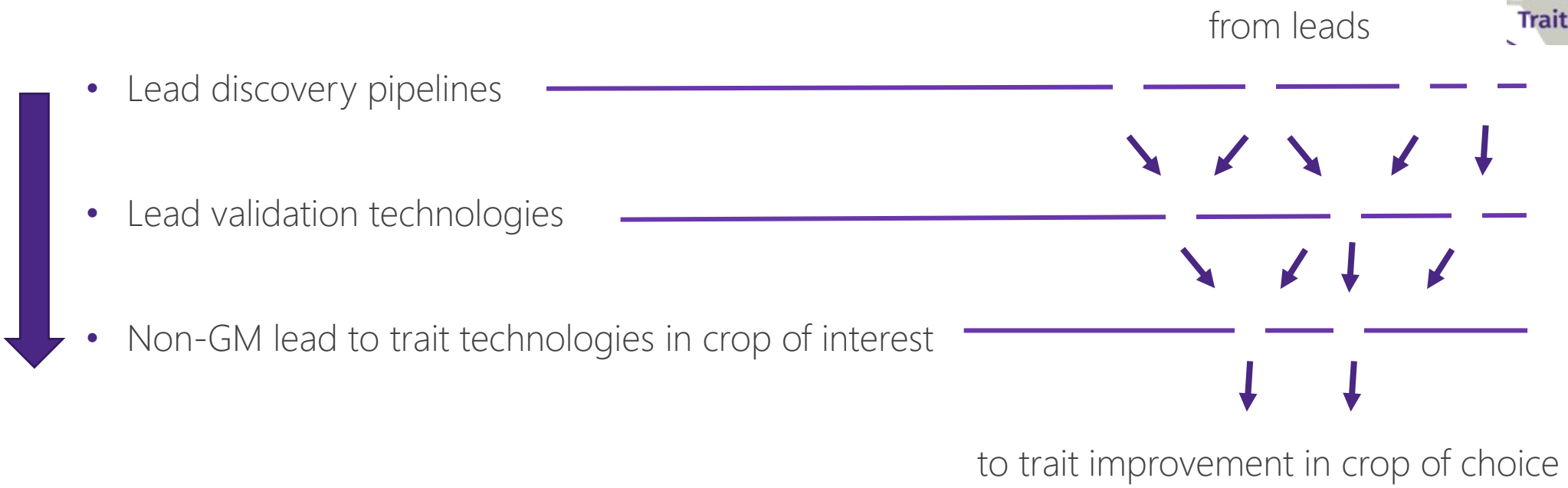
Marcel Prins / CIB April 13, 2021

START PRESENTATION

LEAD DISCOVERY, VALIDATION AND CROP IMPLEMENTATION

'A lead is nice, but how to bring the *right* lead into the *right* crop'

> The KeyGene trait discovery innovation platform combines:



LEAD DISCOVERY, VALIDATION AND CROP IMPLEMENTATION

Lead discovery pipelines

- KeySeeQ® KeyGene's transcriptomics-based LD platform
- Protein-protein interactomics/effectoromics (*in vitro* and *in silico*) platforms
- Leads from breeding in other crops; proprietary and public
  supplemented by specific trait expertise (in house and partnered)

LEAD DISCOVERY, VALIDATION AND CROP IMPLEMENTATION

Lead validation options

> Model/crop plant lead gene homolog over-expression, silencing or k.o.

> Lead gene k.o. by editing in crop of interest (**limited by crop regeneration capacity**)
  - Specific allele replacement by GE or k.o. plus transgenesis

> KeyPoint® breeding in 'any' crop

in practice: a combination of the above plus **proper phenotyping platforms**

LEAD DISCOVERY, VALIDATION AND CROP IMPLEMENTATION

Lead implementation in crop of choice

> Natural variation: depends greatly on available genetic diversity of the crop and breeding options

> Novel variation: Preferably **non-GM** independent from where you are in the world

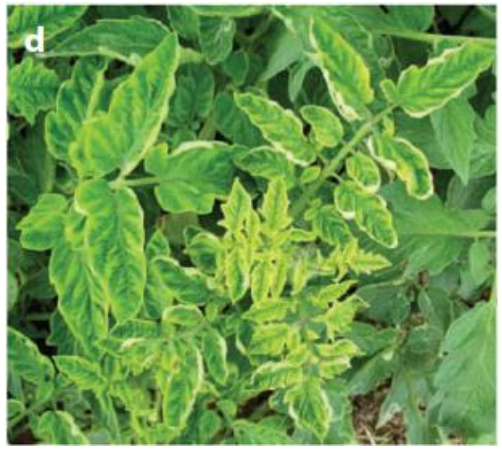
- Depends on the availability of mutant populations, type of mutants and mutation density

Loss of susceptibility proof-of-application cases:

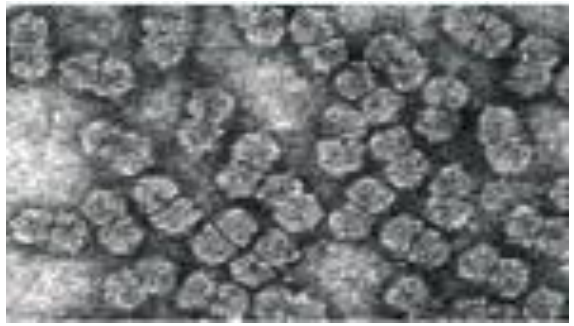
✓*Geminiviruses in pepper*

✓*Powdery mildew in wheat*

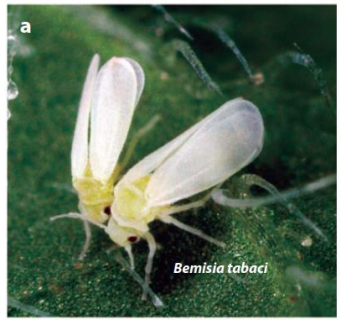
GEMINIVIRUSES

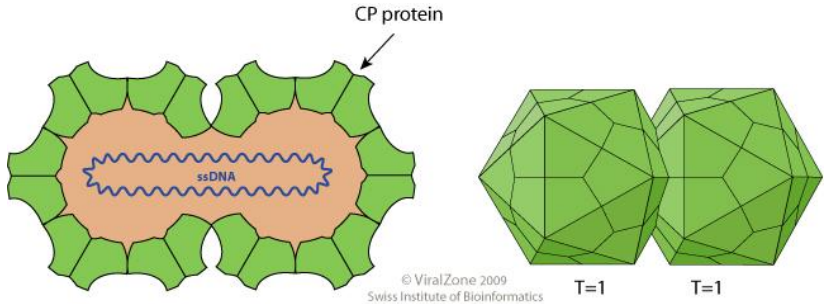


- Simple but highly damaging pathogens



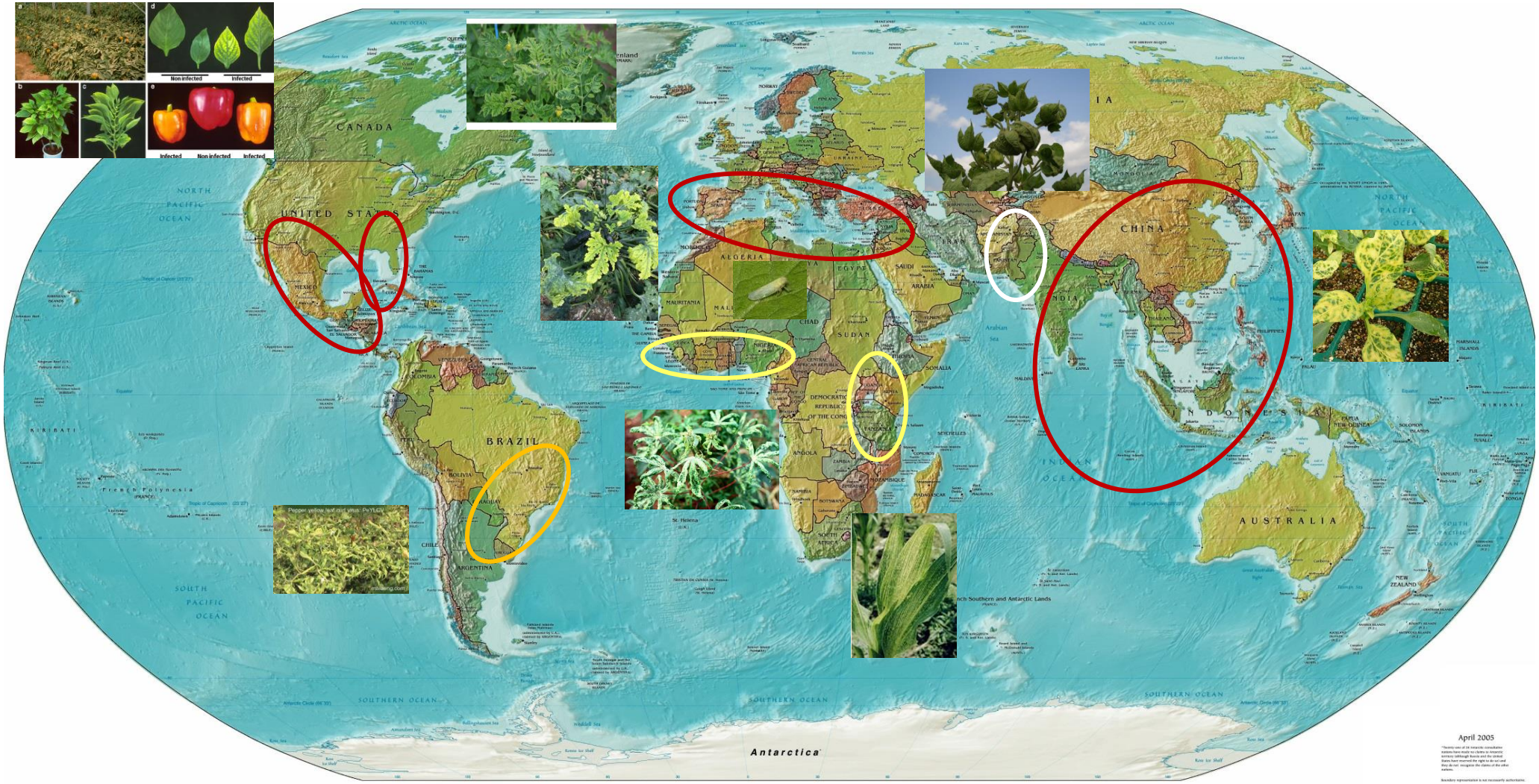
- One of the largest and most important families of plant viruses
- Transmitted by leafhopper, treehopper and whitefly
- Cause of intensive insecticide spraying (zero tolerance)





GEMINIVIRUSES

> Rapidly emerging problems in a wide range of crops throughout the world



LACK OF SUITABLE GEMINIVIRUS RESISTANCE IN PEPPER

> Suitable resistance sources are *not available* for breeding in commercial pepper varieties

> Several interesting *sources do exist* in other plants/crops (e.g. in tomato)

- R genes: some good, some already overcome by the virus, not easily transferred (non-GM) crop to crop
- S(usceptibility) genes: more rare, more durable, more ubiquitous, *transferrable??*

> Aim:

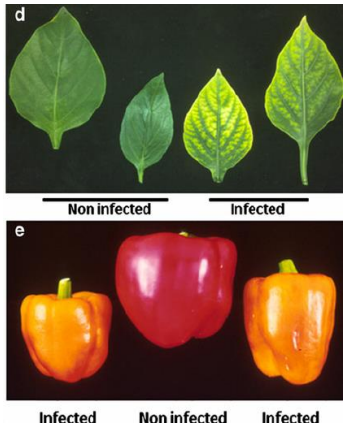
Generate mutated forms of S genes with decreased susceptibility to Geminiviruses

> How:

Discover S gene leads (by any means)

Transfer leads to crops starting with pepper

In a **non-GM** way by **KeyPoint®** breeding



GEMINIVIRUS RESISTANCE IN PEPPER

Literature lead: tomato S gene *ty-5* (aka *pelota*); LoS allele caused by a single aa change

Approach for pepper:

> Identification of the pepper Ty-5 S gene homolog: Delta Twin Pro (DTP)

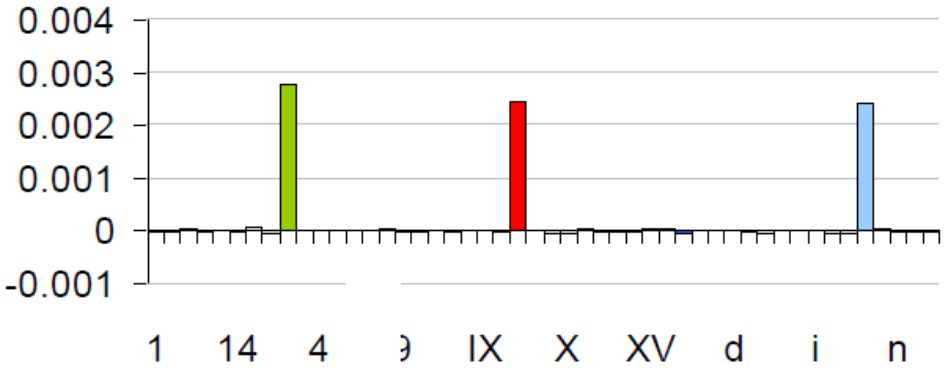
> KeyPoint® breeding using KeyGene pepper M2 populations

GEMINIVIRUS RESISTANCE IN PEPPER

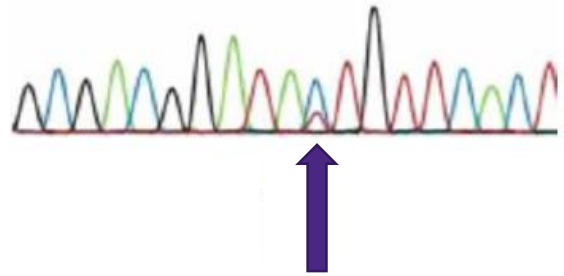
KeyPoint® breeding

- Existing M2 population: isolate DNA of individual plants/families
- Pool DNA for sequence based gene-specific mutant-identification (KeyPoint®)

detection of novel variants



verification of novel variant plants



- Unique pooling coordinates identify individual mutant plant families

GEMINIVIRUS RESISTANCE IN PEPPER

KeyPoint® breeding on KeyGene pepper population (4,000 M2 families)

> 21 mutant families discovered
  - 19 single amino acid changes
  - Two knock outs (stop codon and frame shift)

> Select homozygous offspring (LoS alleles are recessive!)

> Multiply homozygous M3/M4 seeds

> Distribute among experts of our partners for phenotyping

GEMINIVIRUS RESISTANCE IN PEPPER

Virus assays on KeyPoint® mutants in susceptibility gene DTP

- symptomless plants; (nearly) virus free


CONTROLS


MUTANTS

Courtesy dr Ryohei Arimoto, Takii

- k.o. versions of the gene loose susceptibility
- no effect of 19 individual single aminoacid changes
- Mechanical, agrobacterium, grafting and whitefly-mediated inoculation
- Monopartite and bipartite pepper-infecting geminiviruses
- Across the world

GEMINIVIRUS RESISTANCE IN PEPPER

> A range of test systems, viruses and locations; laboratory, greenhouse and field testing

GEMINIVIRUS RESISTANCE IN PEPPER

✓ Field testing 2020 confirms validity of new source of geminivirus resistance in pepper

Susceptible control DTP

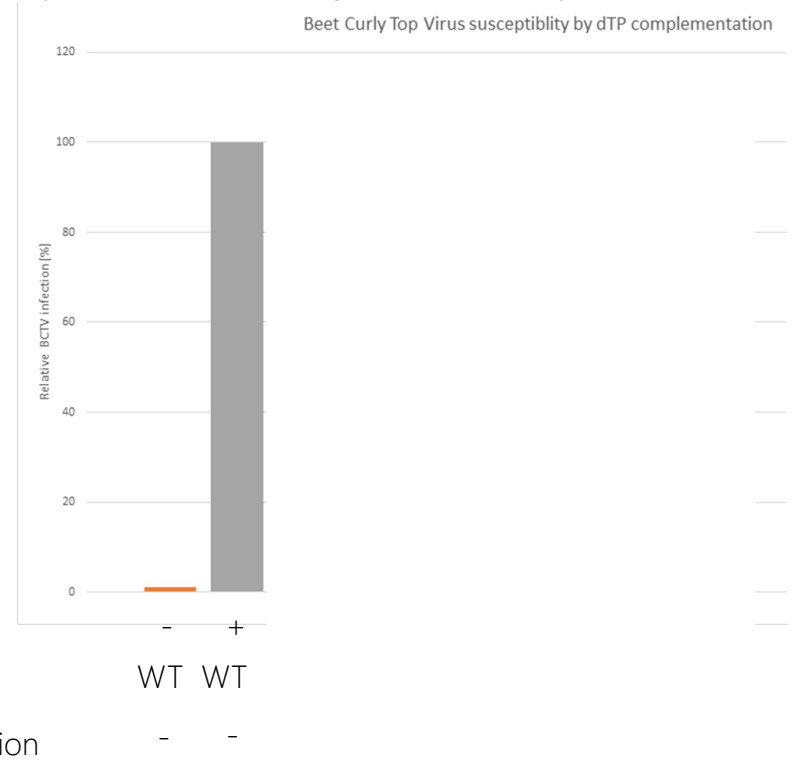
Courtesy Huay Chong Lim (Enza zaden)

Susceptible control DTP

Courtesy of Sushil Kumar (RijkZwaan)

GEMINIVIRUS RESISTANCE IN OTHER CROPS

- Arabidopsis DTP k.o. mutant is resistant to geminivirus (BCTV)
- Arabidopsis DTP complementation reverts to susceptible
- Complementation by DTP homologs from crops indicates a ‘universal’ role as S gene



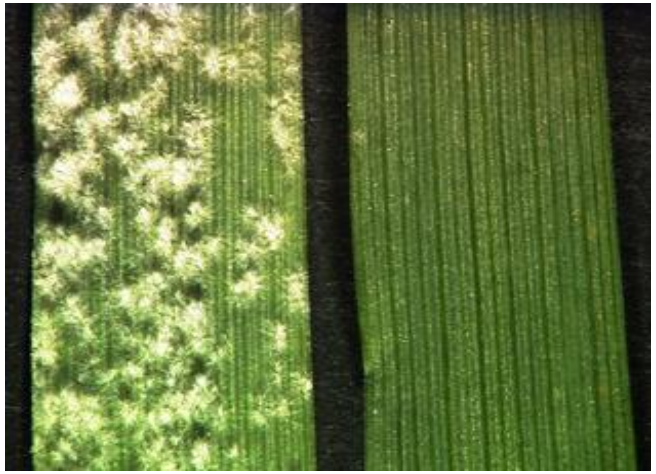
GEMINIVIRUS RESISTANCE IN OTHER CROPS

Ongoing:

- Introgress DTP alleles into elite lines for commercialization (@customer companies)
- Generate and test DTP mutants in other vegetable crops (@KG/customer companies)
- Collaborations on DTP in cassava and cotton
- Investigating opportunities in other geminivirus effected crops

CASE 2: POWDERY MILDEW RESISTANCE IN WHEAT

- MLO = Mildew resistance Locus O
  - Recessive resistance to powdery mildew (PM)
  - First identified in **Barley** (1942; X-ray mutant)
  - MAP based cloning of *Mlo/mlo* gene alleles from Barley
    by KeyGene & JIC (UK); 1997!



Büschges, et al., 1997

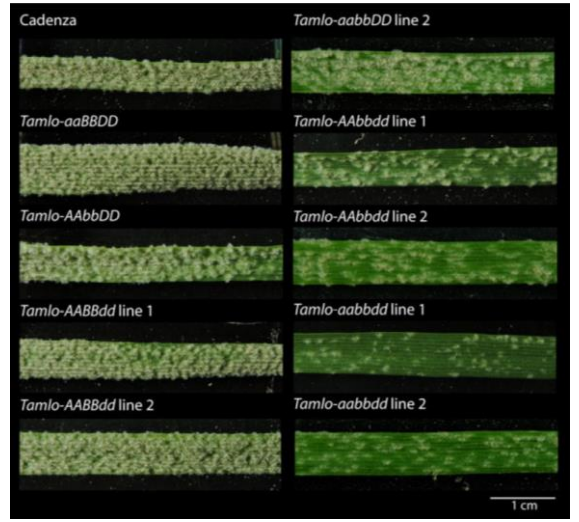
- 'Archetype' susceptibility factor
  - Functional gene => susceptible to PM
  - mutated/dysfunctional gene => resistance to PM
    homozygous mutant alleles required for resistance

WHEAT PM MUTATION BREEDING @ KEYGENE

- Bread wheat has a hexaploid (6n) genome
  - 3 homeologs TaMlo-A1, TaMlo-B1, TaMlo-D1 *(Elliot et al. 2002)*
  - All 3 homeologs proven functional in PM susceptibility

    e.g. by complementation of Arabidopsis *mlo2/6/12*

- KeyPoint® Wheat M2 population screens
- 110 putative mutants of interest (TaMlo-A1 = 58; TaMlo-B1 = 14; TaMlo-D1 = 38)



Acevedo-Garcia et al., 2016

- Crossings towards triple mutant for phenotyping
  - STOP and splice variants for TaMLO-A1 and B1 -> double mutant still susceptible (milder)

WHEAT PM MUTATION BREEDING @ KEYGENE

- (only) Triple homozygous TaMLO k.o. mutants are symptomless/resistant to powdery mildew infection

Proof of concept for NON-GM PM RESISTANT WHEAT

- Trait innovation and KeyPoint® LoS Breeding extended to a polyploid crop

LOSS OF SUSCEPTIBILITY BREEDING TEAM

Acknowledgements for truly collaborative accomplishments

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