

# Resistentieveredeling: Waar staan we nu?

Yuling Bai

Plant Breeding, Wageningen University & Research

# Afweermechanismen van de plant

## Plant defence strategies

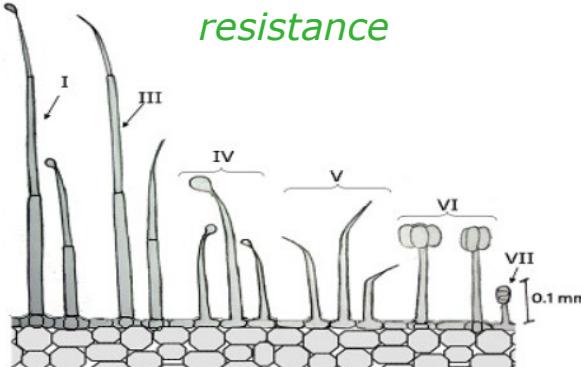
- Vermijden: Planten vermijden contact met insecten door componenten te gebruiken waar insecten niet tegen kunnen

*Avoidance: plant's capacity to avoid intimate contact with a potential attacker, usually by a particular morphology like smell, colour, etc*



[lotte.caarls@wur.nl](mailto:lotte.caarls@wur.nl)

De trichomen van de tomaat dragen bij aan resistentie voor witte vlieg  
*Tomato trichomes contribute to whitefly resistance*



EU project

 **Virtigation**  
Emerging viral diseases in tomatoes and cucurbits:  
Implementation of mitigation strategies for durable  
disease management

<https://www.virtigation.eu/partners/>

# Afweermechanismen van de plant

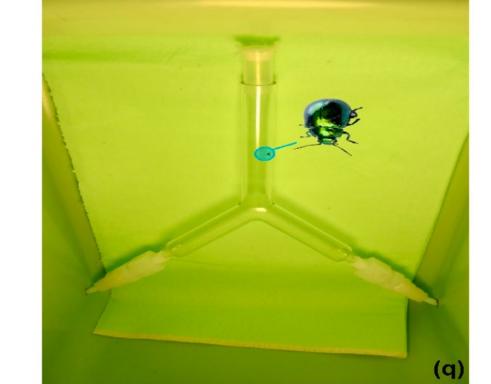
*Plant defence strategies*

## ■ Vermijden:

Avoidance:



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Insect wordt  
aangetrokken door  
de geur van de  
plant  
*attracted by the smell  
of the plant*

<https://www.mdpi.com/1999-4907/11/6/638/htm>

# Afweermechanismen van de plant

*Plant defence strategies*

- Vermijden: Planten vermijden contact met insecten door componenten te gebruiken waar insecten niet tegen kunnen

Avoidance: plant's capacity to reduce the chance of intimate contact with a potential attacker, usually by a particular morphology like *smell, colour, etc*

- Resistentie: De capaciteit van de plant om na contact de groei, ontwikkeling of reproductie van het insect te verminderen

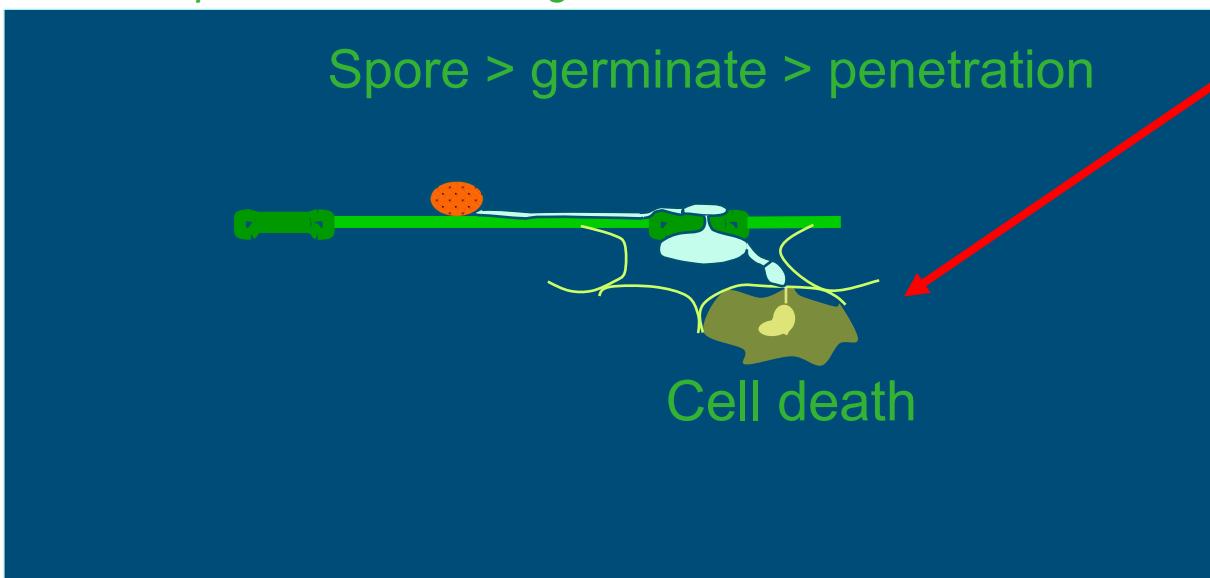
Resistance: plant's capacity to reduce the growth/ development/reproduction of the attacker after the intimate contact)

# Resistentie door dominate resistantie (R) genen

*Resistance via dominant resistance (R) genes*

Infectie process van de roestschimmel

*Infection process of rust fungi*

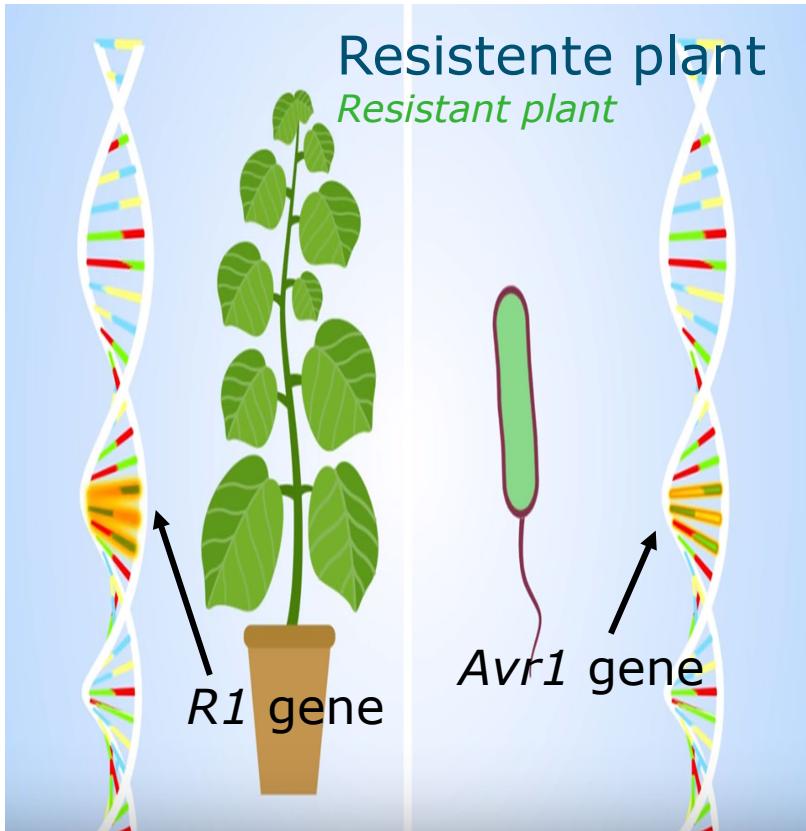


**Hypersensitive response (HR):**

Snelle gelokaliseerde celdood in de geïnfecteerde cel  
*Fast localized cell death in cells infected by pathogen.*

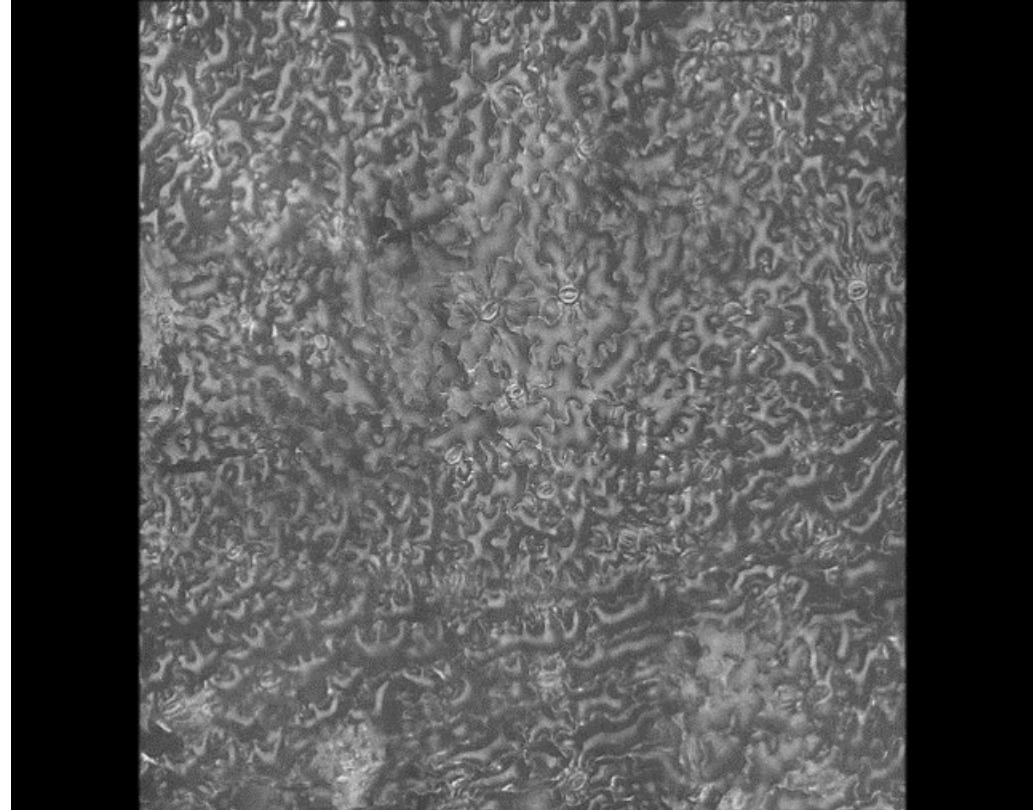
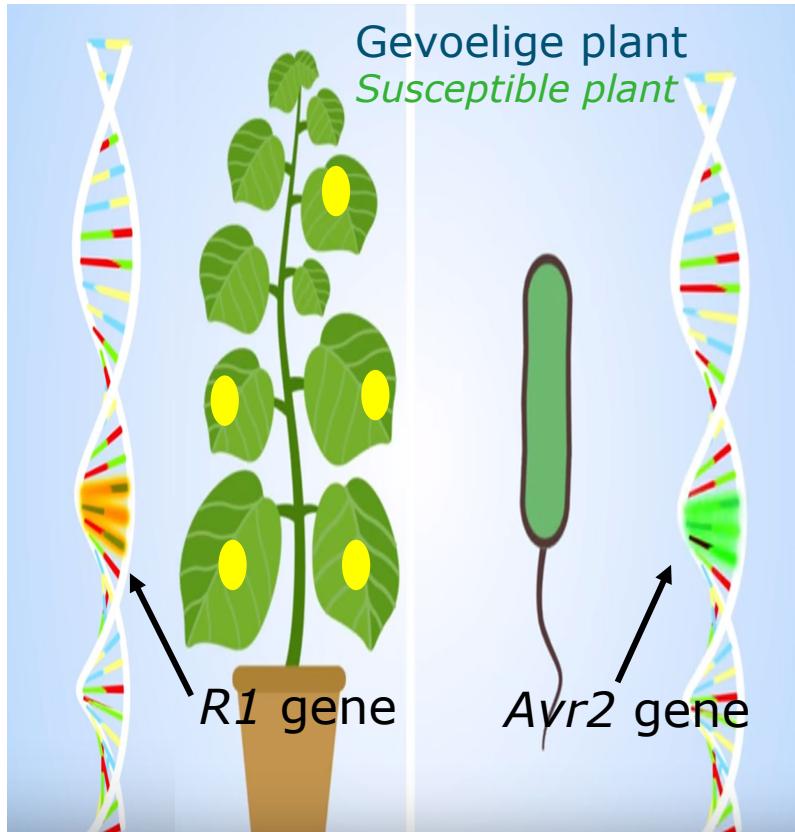
# R-gen: Gen om Gen

*R gene: the Gene-for-Gene Model*



# R-gen: Gen om Gen

*R gene: the Gene-for-Gene Model*



# Afweermechanismen van de plant

## *Plant defence strategies*

- Vermijden: Planten vermijden contact met insecten door componenten te gebruiken waar insecten niet tegen kunnen

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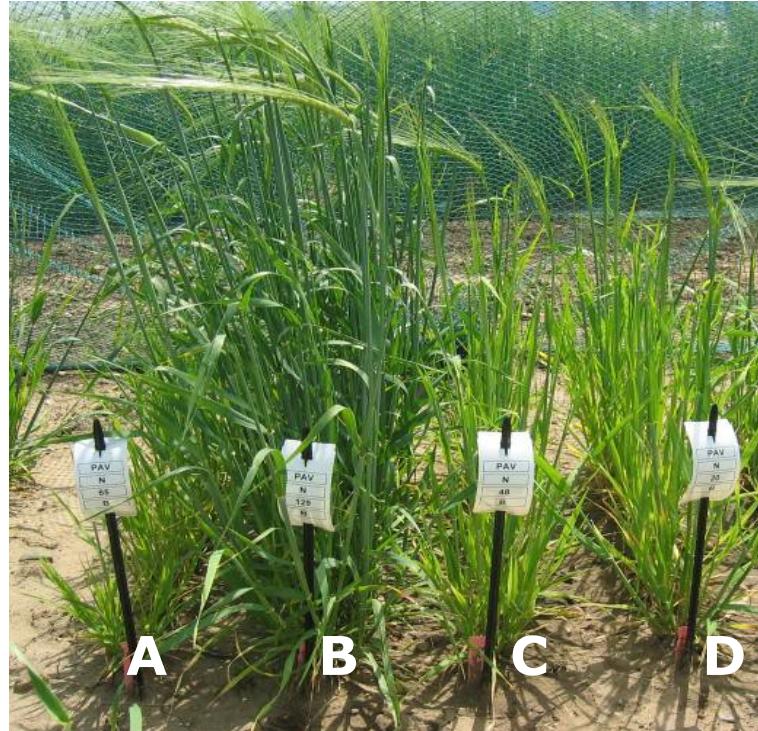
*Resistance: plant's capacity to reduce the growth/ development/reproduction of the attacker.*

- Tolerantie: de plant voorkomt symptoom ontwikkeling/ schade door het inperken van de infectie na contact

*Tolerance: plant's ability to restrict the symptoms or damage other than by restricting the amount of infection.*

# Afweermechanismen van de plant

*Plant defence strategies*



Vier gerst rassen geïnoduleerd met  
gerstevergelingsvirus (BYDV)

*Four barley cultivars were inoculated with barley yellow dwarf virus*

Verschil in symptomen na een paar weken

*After a few weeks, difference on plant growth was observed among the cultivars (see photo)*

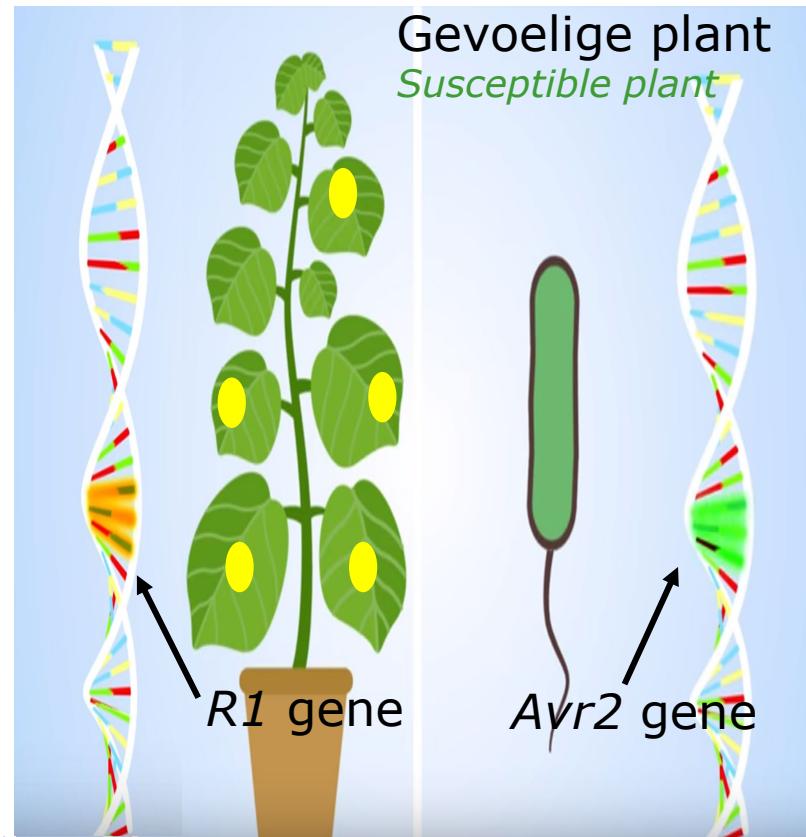
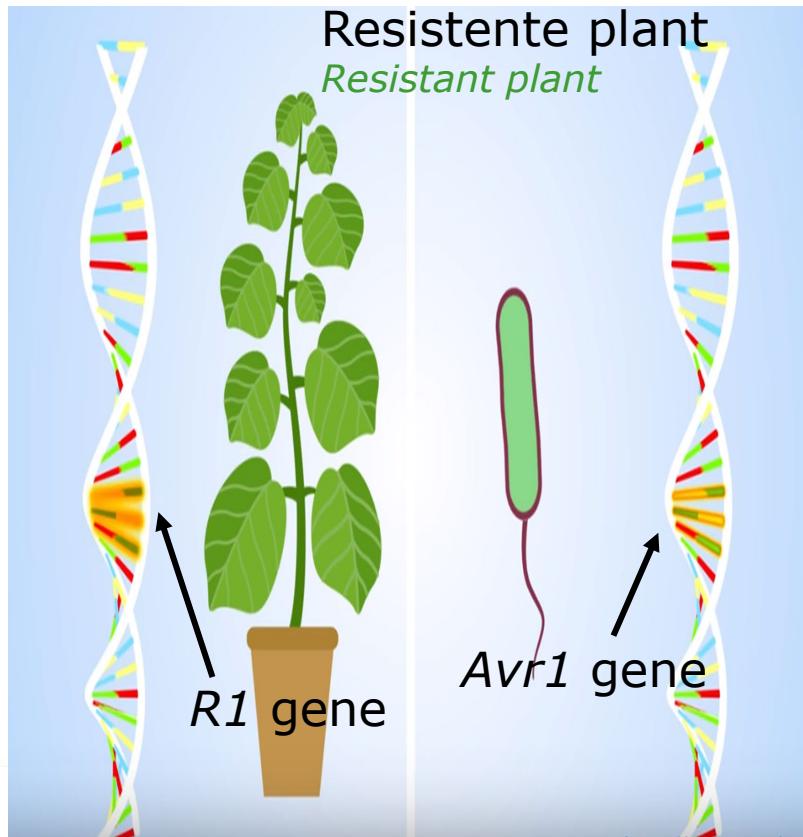
Is ras B resistent of tolerant? *Is cultivar B resistant or tolerant?*

**Virussen:**

- **Veredelen voor resistente of tolerante rassen?**
- **Kan je planten vaccineren?**

# R gen: ras-specifieke resistantie (Gen-om-Gen)

*R gene: race-specific resistance (the Gene-for-Gene Model)*



# De wapenwedloop tussen plant en pathogeen

*The arms race between plants and pathogens*

## Valse meeldauw in spinazie

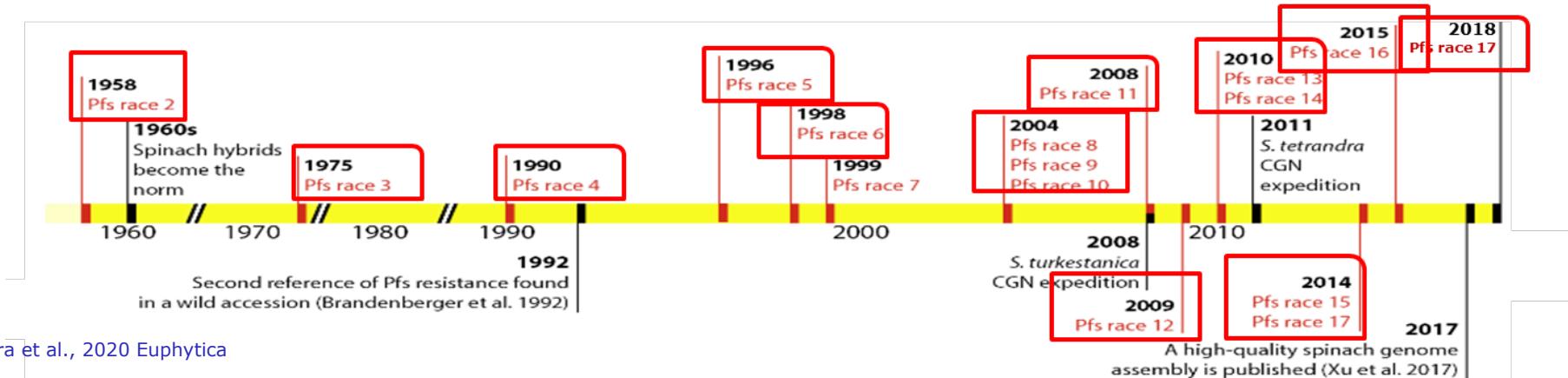
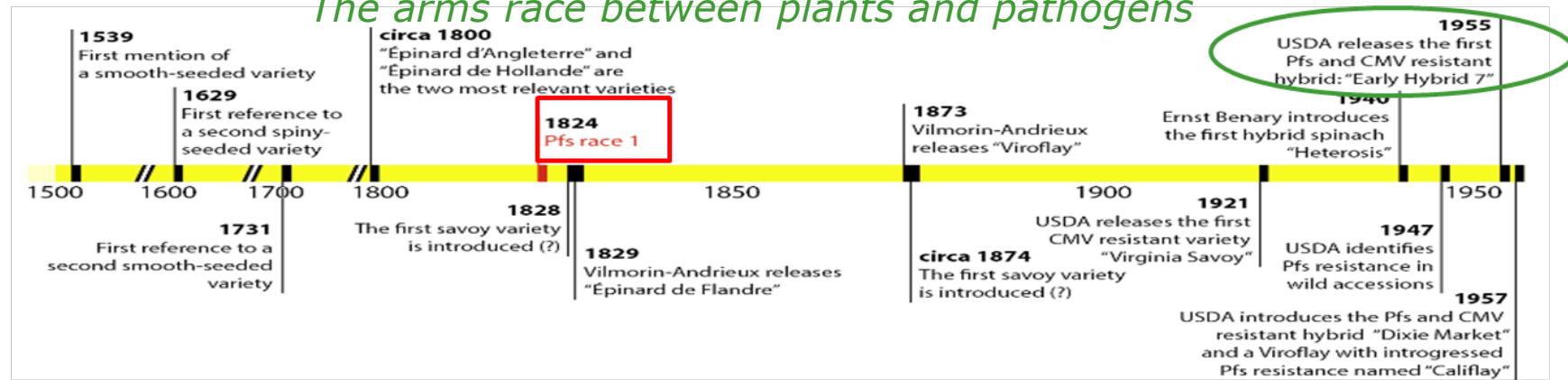
*Downy Mildew in Spinach*



Correll et al, 2011, European Journal of Plant Pathology

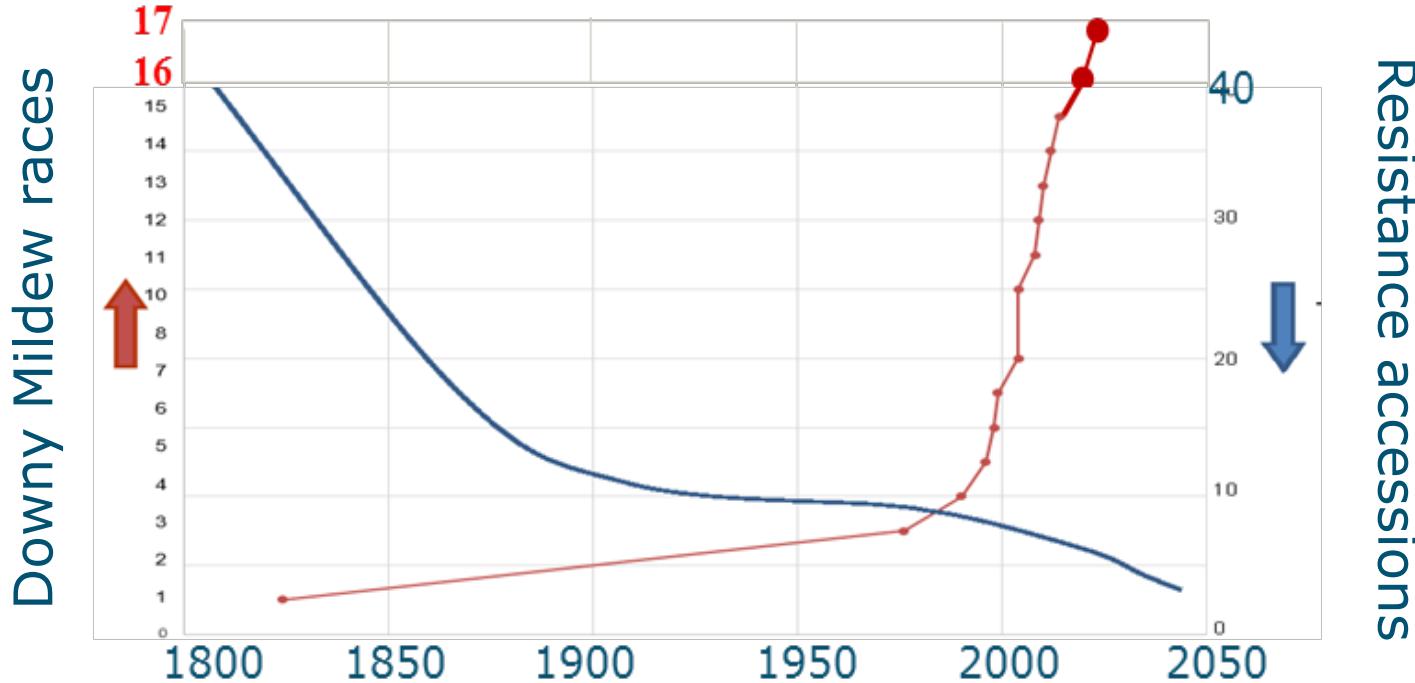
# De wapenwedloop tussen plant en pathogeen

*The arms race between plants and pathogens*



# De wapenwedloop tussen plant en pathogeen

*The arms race between plants and pathogens*



# Geen R genen voor resistentie tegen necrotrofen

*No R genes for diseases caused by necrotrophs*

- e.g. *Botrytis cinerea*
- Geen R-genen in wilde tomaten verwanten

*No R genes discovered so far in wild tomato relatives*



# Gevoeligheids genen

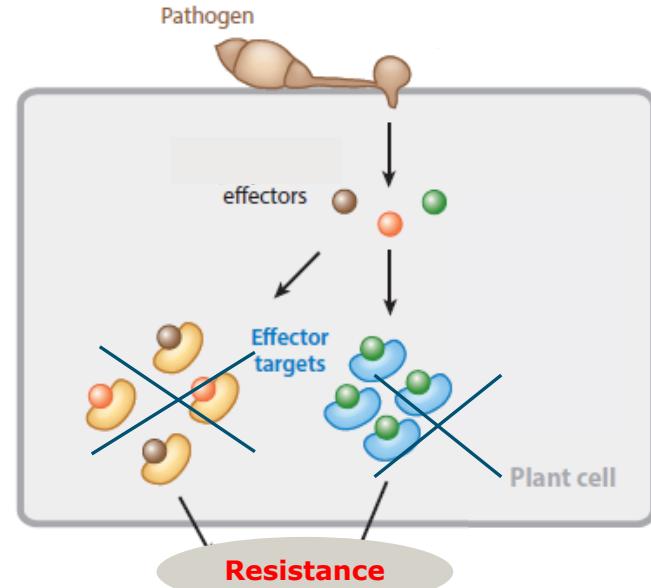
*Plant susceptibility (S) genes*

- **S-gen: een planten gen dat wordt gebruikt door een pathogen om ziekte te veroorzaken**

**S-gene:** A plant gene required/misused by pathogens to cause disease, e.g. genes coding effector targets

- **Genetisch, beschadigde/afwijkende S-genen resulteren in recessieve resistentie**

**Genetically, impaired S-genes lead to recessive resistance**



# Het S-gen concept in de afgelopen 10 jaar

*S-gene concept in the past 10 years*

- Zijn S-genen geconserveerd tussen planten soorten?

*Are S genes conserved across plant species?*

- Hoe verkrijg je mutanten die niet meer functioneel zijn?

*How to obtain loss-of-function mutants?*

- Natural mutations (*Mlo*, *Xa5* & *Xa13*, *eIF4E* & *eIF4G*)
- Mutagenesis
- RNAi and/or gene editing

- Hoe ga je om met pleiotropie (nadelige kenmerken)?

*How to deal with the pleiotropic phenotypes?*

# Zijn S-genen geconserveerd tussen planten soorten?

*Are plant S genes conserved across plant species?*

**S genen van Arabidopsis**

**Orthologues in gewassen**

**RNAi/CRISPR**

**PMR4**



**DND1**

**DMR1**

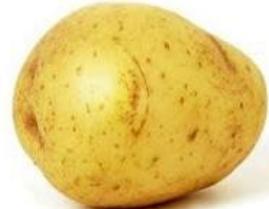
**DMR6**

**CESA3**

**SR4**

**Many others**

**Orthologues in gewassen**



Resistance to

**Powdery mildews**

**Late blight**

***Botrytis***

***Clavibacter***

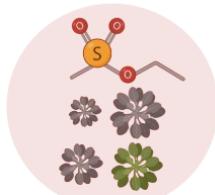
***Verticillium***

.....

# Hoe verkrijg je niet functionele mutanten?

*How to obtain loss-of-function mutants?*

Mutagenized populations



S gene orthologs



Natural mutant >>> Mutagenesis >>> Gene Editing (CRISPR)

Forward Genetics Screens



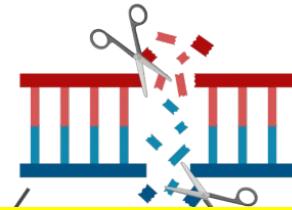
Reverse Genetics Screens



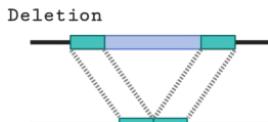
Effector  
S gene protein

Germplasm

Modification of S genes via precise genome editing



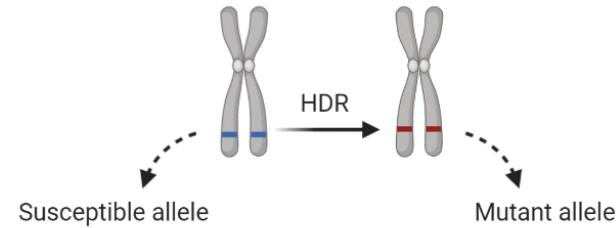
Induction of targeted indels



Introduction of SNPs via base editing

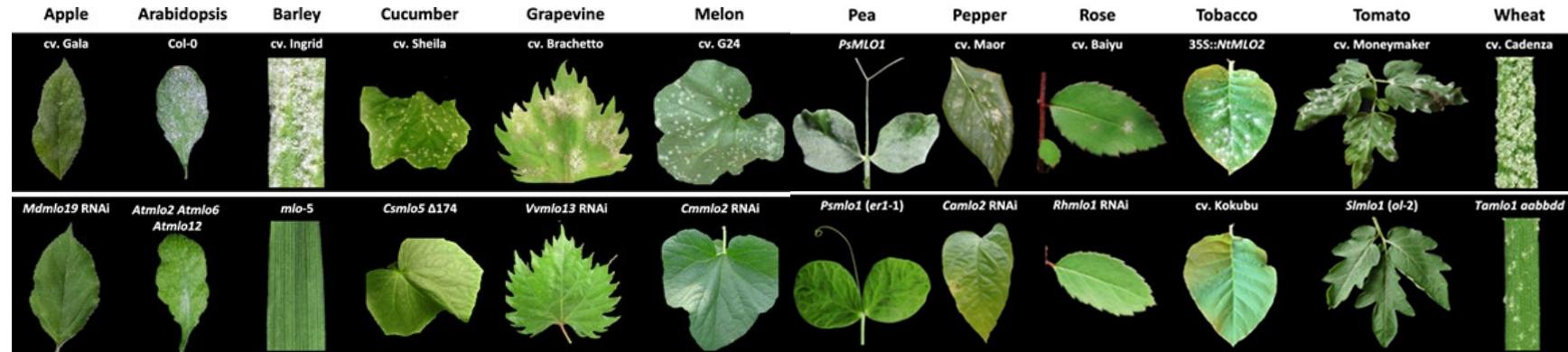
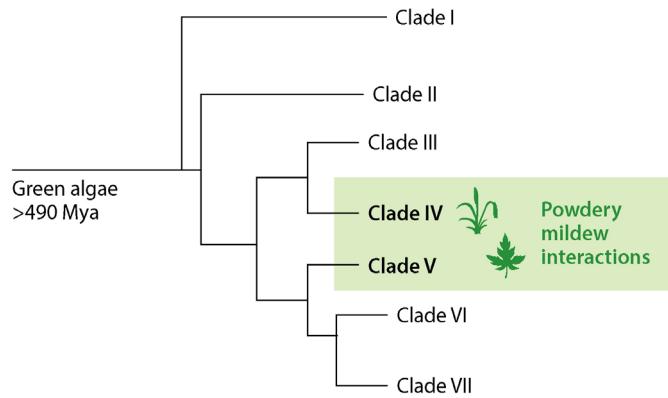


Allele replacement through HDR



# Het *Mlo*-gen: S-gen voor echte meeldauw

The *Mlo* gene: *S* gene for powdery mildews



## Brief Communication

# Mutation of *PUB17* in tomato leads to reduced susceptibility to necrotrophic fungi

Miguel Ramirez Gaona<sup>1</sup>, Ageeth van Tuinen<sup>1</sup>, Danny Schipper<sup>1</sup>, Akihito Kano<sup>2</sup>, Pieter J. Wolters<sup>1</sup>, Richard G. F. Visser<sup>1</sup>, Jan A. L. van Kan<sup>3</sup>, Anne-Marie A. Wolters<sup>1</sup> and Yuling Bai<sup>1,\*</sup> 

<sup>1</sup>Plant Breeding, Wageningen University & Research, Wageningen, The Netherlands

<sup>2</sup>Plant Breeding and Experiment Station, Takii & Company Limited, Konan, Japan

<sup>3</sup>Laboratory of Phytopathology, Wageningen University & Research, Wageningen, The Netherlands



Miguel Ramirez Gaona



Anne-Marie Wolters



Ageeth van Tuinen



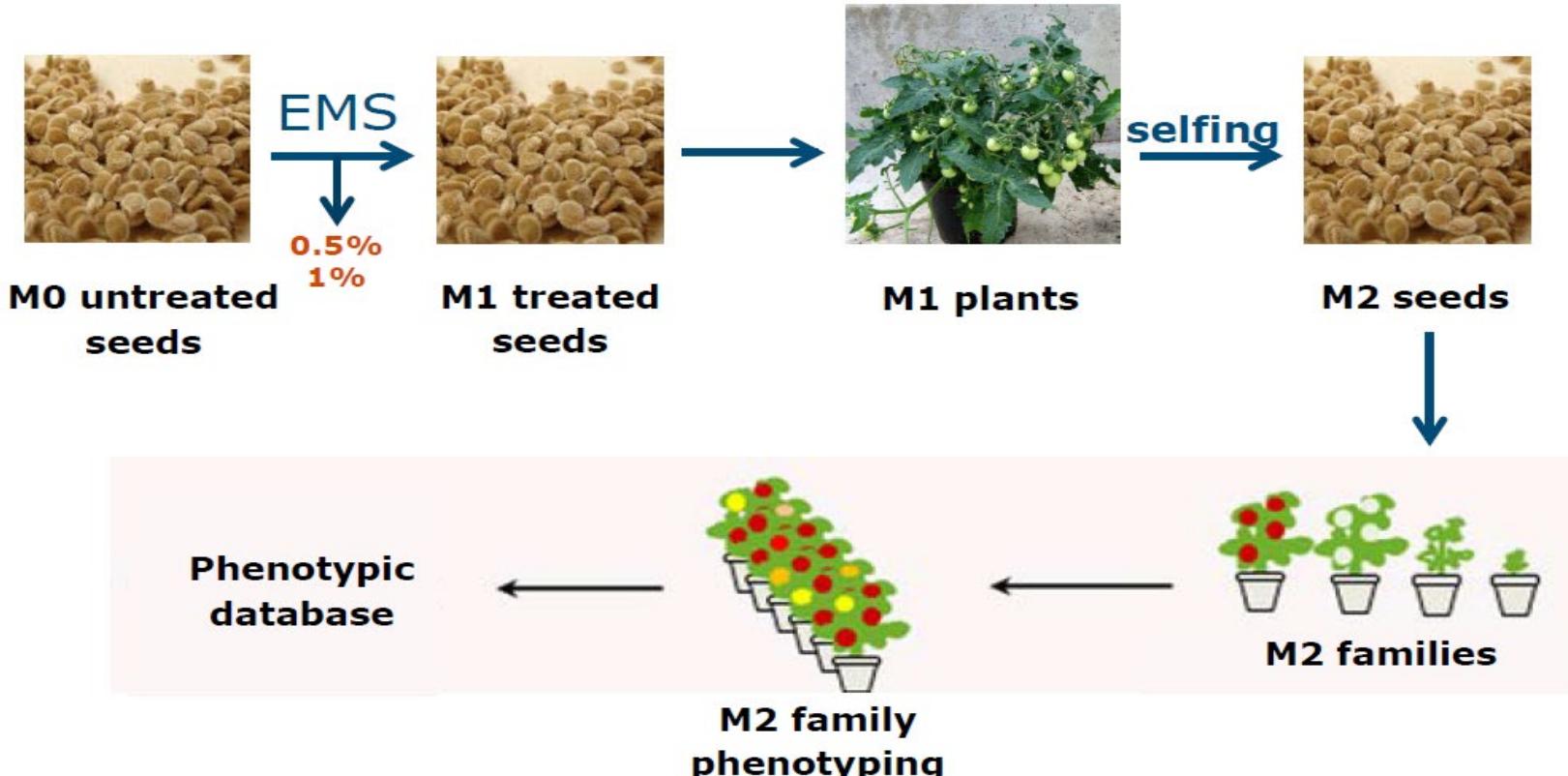
Jan van Kan



Danny Schipper

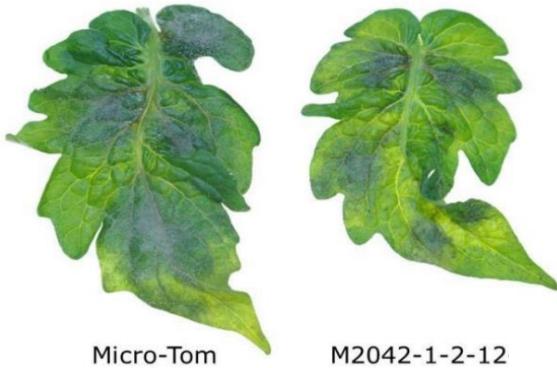
# Mutagenese om niet functionele mutanten te maken

*Mutagenesis to obtain loss-of-function mutants*

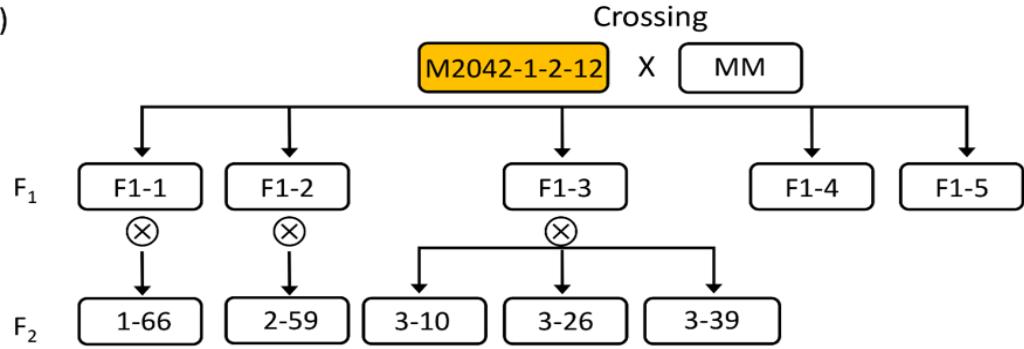


# BSA-sequentie analyse voor gen identificatie

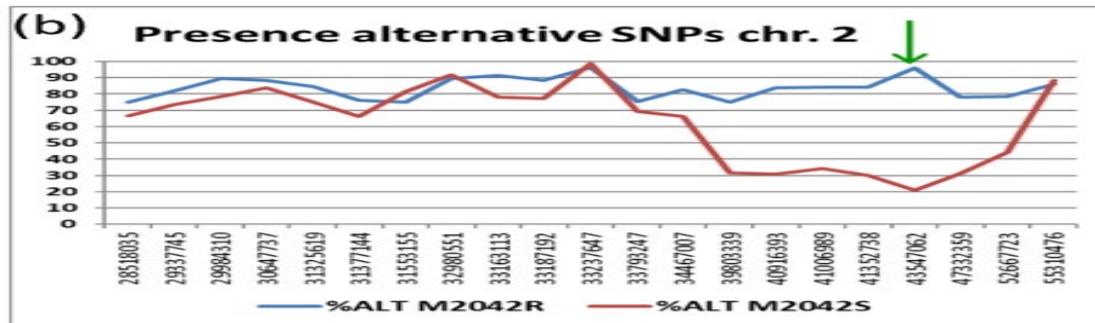
*BSA-sequence analysis to identify the causal gene*



(b)

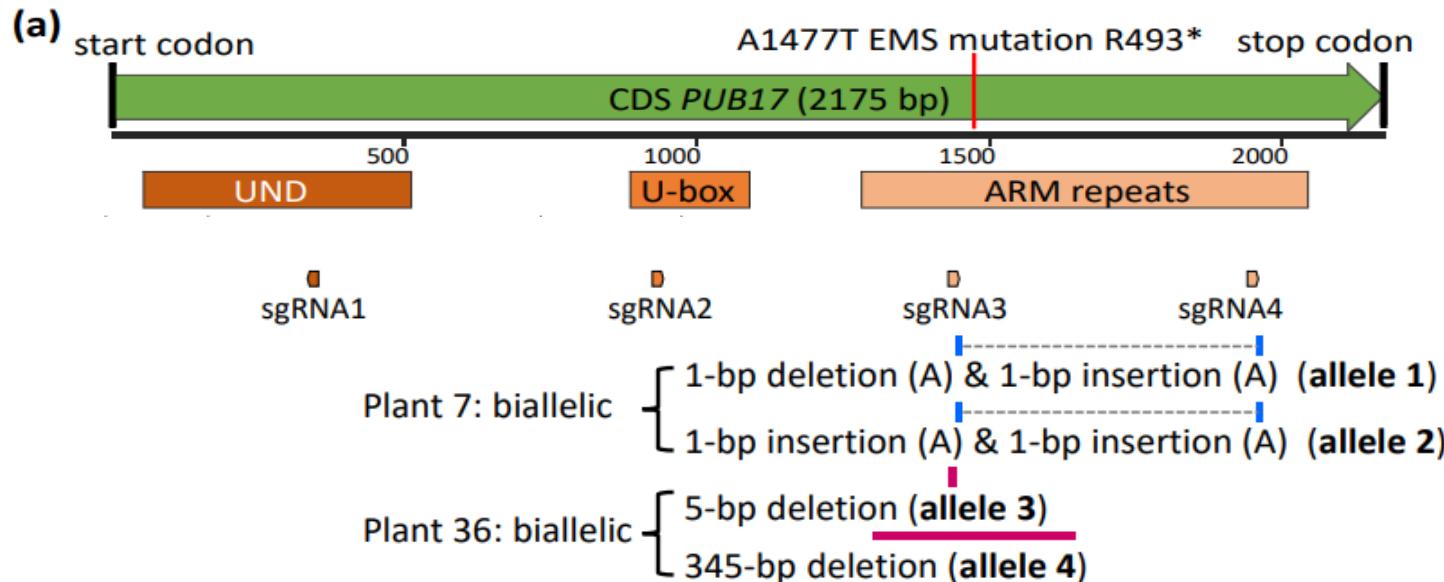


R and S pools used in BSA: bulked segregant analysis

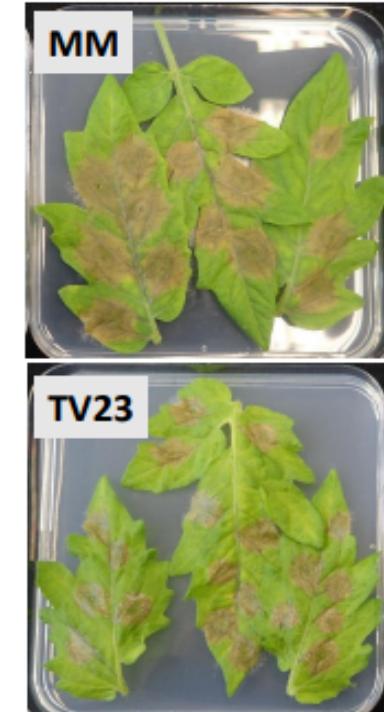
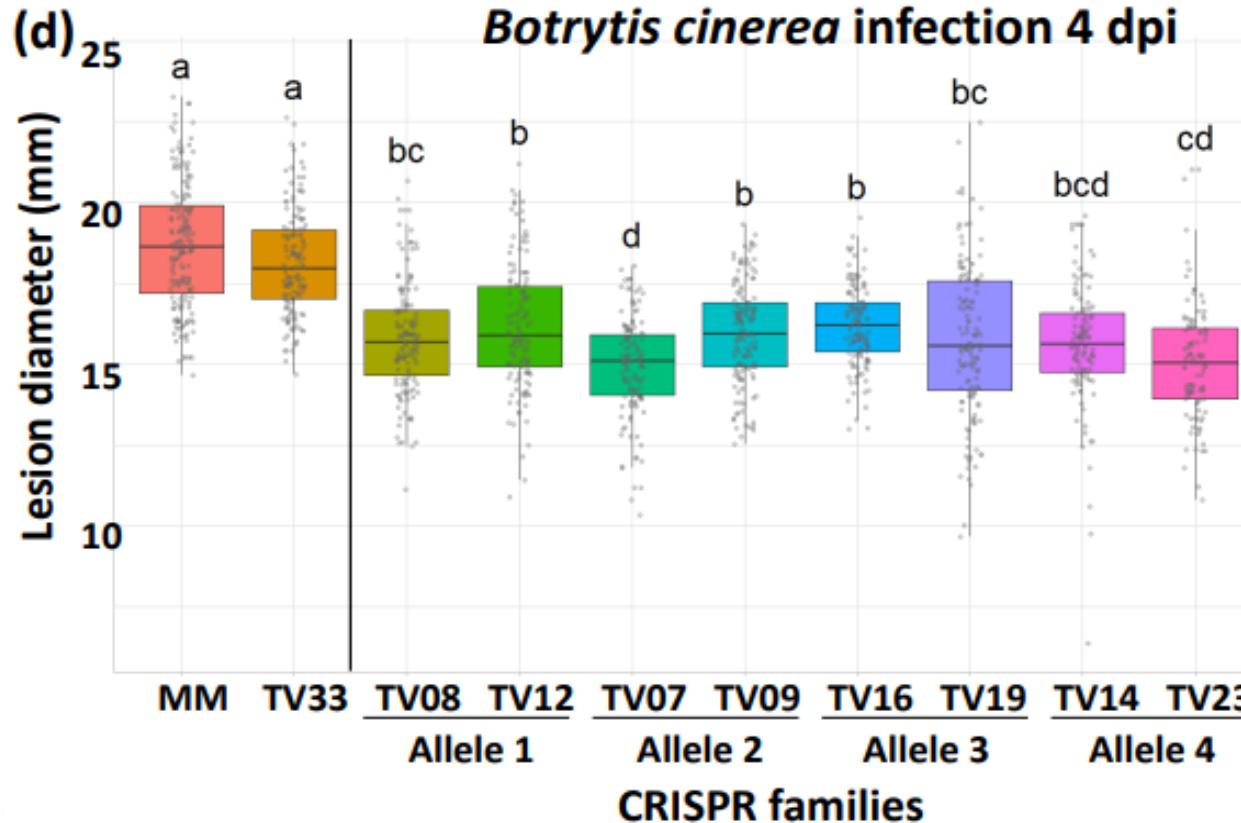


# Het causale gen/*The causal gene*: PUB17

- PUB17: a U-box E3 ubiquitin ligase; mutation R493\*.



# Het causale gen/*The causal gene*: PUB17



# Hoe voorkom je pleiotropie?

*How to deal pleotropic effect?*



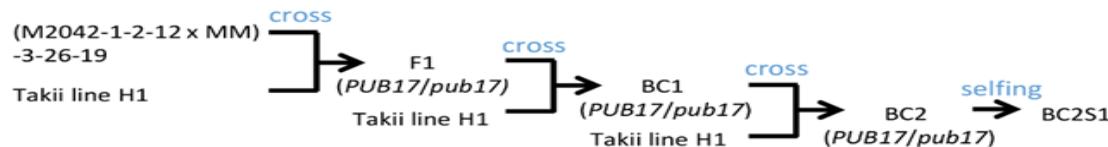
WT

*pub17*

# Pleiotropie is afhankelijk van de achtergrond

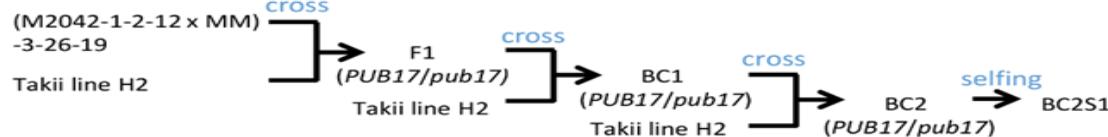
*Pleiotropic effect is background dependent*

(a) Line H1 backcross



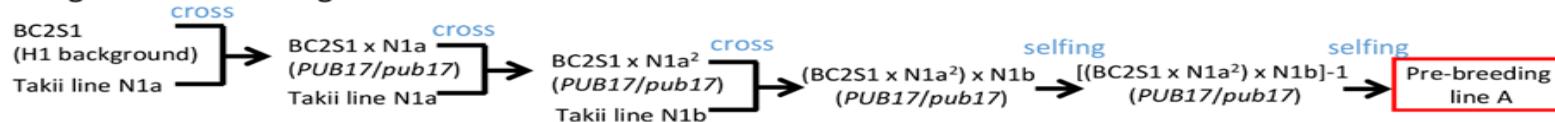
BC2S1 (*pub17/pub17*)  
no pleiotropic effect

Line H2 backcross

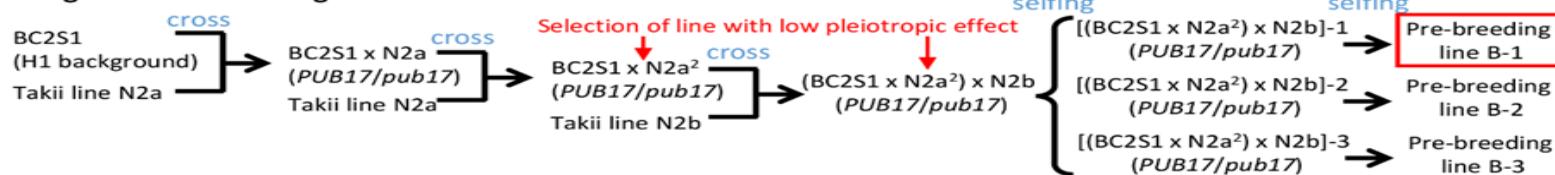


BC2S1 (*pub17/pub17*)  
pleiotropic effect; breeding stopped

(b) Pedigree of Pre-breeding line A

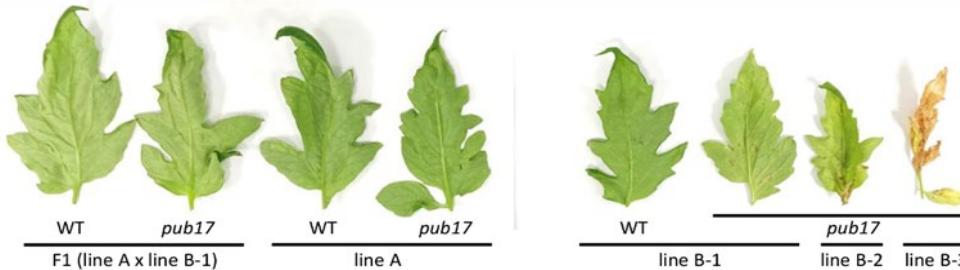


Pedigree of Pre-breeding line B

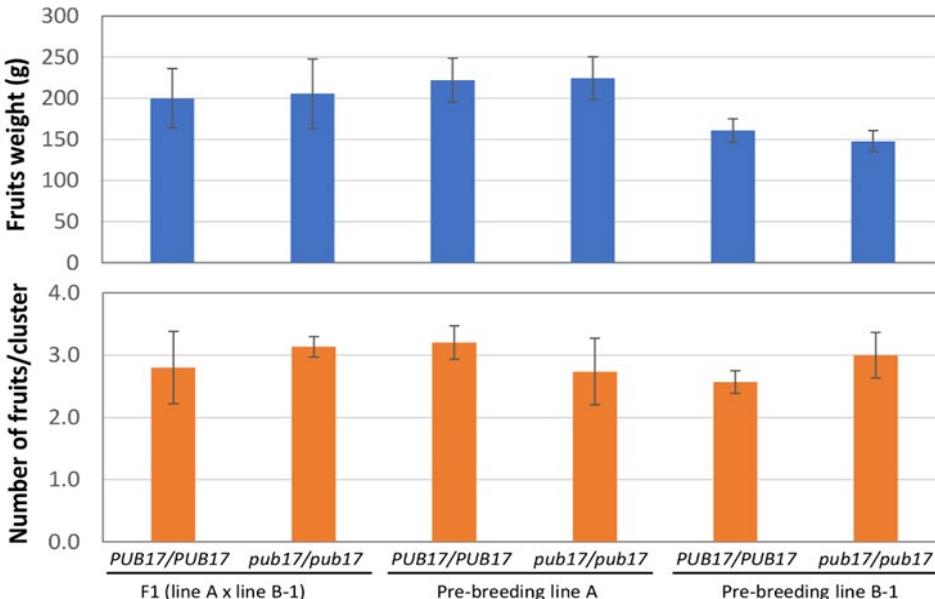


# *pub17* mutatie in tomaten cultivars!

(c)



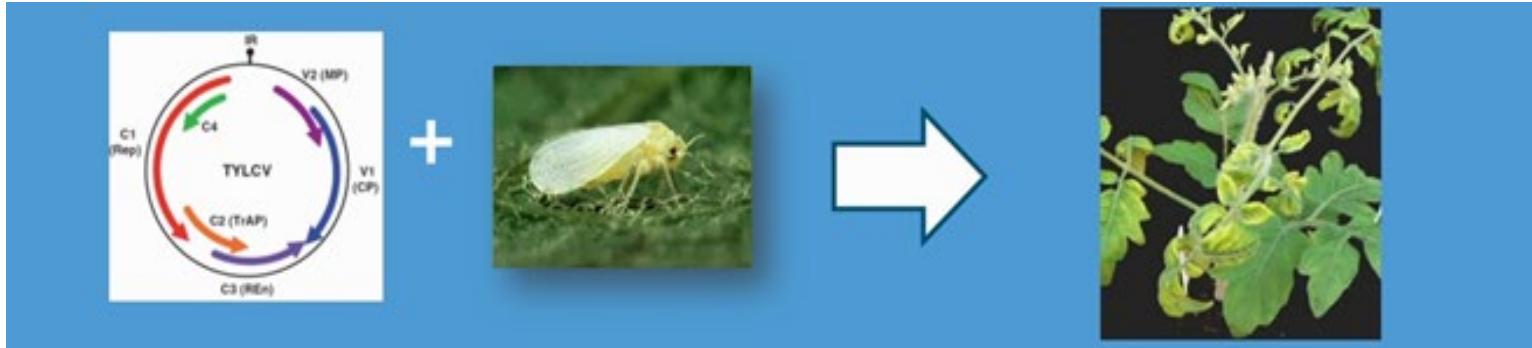
(d)



# S-gen voor virus resistantie

*S genes in resistance to viruses*

- >50% zijn recessieve resistantie genen
  - Virussen hebben weinig genen
  - Ze hebben hulp nodig van de plant



TYLCV: tomato yellow leaf curl virus, ~ 6 genen

# *PriL een S-gen voor geminivirussen*

PriL as an S-gene for geminiviruses

*DNA primase large subunit is an essential plant gene for geminiviruses, putatively priming viral ss-DNA replication*

Lampros Siskos<sup>1</sup>, Maria Antoniou<sup>1</sup>, Jose Riado<sup>2</sup>,  
Montserrat Enciso<sup>2</sup>, Carlos Garcia<sup>2</sup>, Daniele Liberti<sup>3</sup>,  
Danny Esselink<sup>1</sup>, Andrey G. Baranovskiy<sup>4</sup>, Tahir H. Tahirov<sup>4</sup>,  
Richard G. F. Visser<sup>1</sup>, Richard Kormelink<sup>5</sup>, Yuling Bai<sup>1</sup>  
and Henk J. Schouten<sup>1\*</sup>

# Het S-gen concept in de afgelopen 10 jaar

*S-gene concept in the past 10 years*

- S-genen zijn **plantengenen** die worden gebruikt door pathogenen/  
*plant genes that are exploited by pathogens*
  - Binnendringen/*to enter*, i.e. the *mlo* gene
  - Onderdrukken van afweer/*suppress plant defence responses*, *PUB17* gene
  - Verwerven van nutrienten/*acquire nutrients*, rice *Sweet* genes
  - Veroorzaken van symptomen/*cause symptoms*, pepper *Upa20* gene
  - ...
- S-genen zijn plantengenen die gebruikt kunnen in de  
resistentieveredeling/ *S genes are plant genes can be exploited for breeding resistance to biotic stresses*

# Het S-gen concept in de afgelopen 10 jaar

*S-gene concept in the past 10 years*

## ■ S-genen van planten:

- kunnen worden geïdentificeerd/ *can be identified*
- kunnen worden gemodificeerd/ *can be modified*
- zijn geconserveerd/ *conserved across plant species*
- veranderingen kunnen leiden tot pleiotropie/  
*may lead to pleiotropic effect if it is impaired*

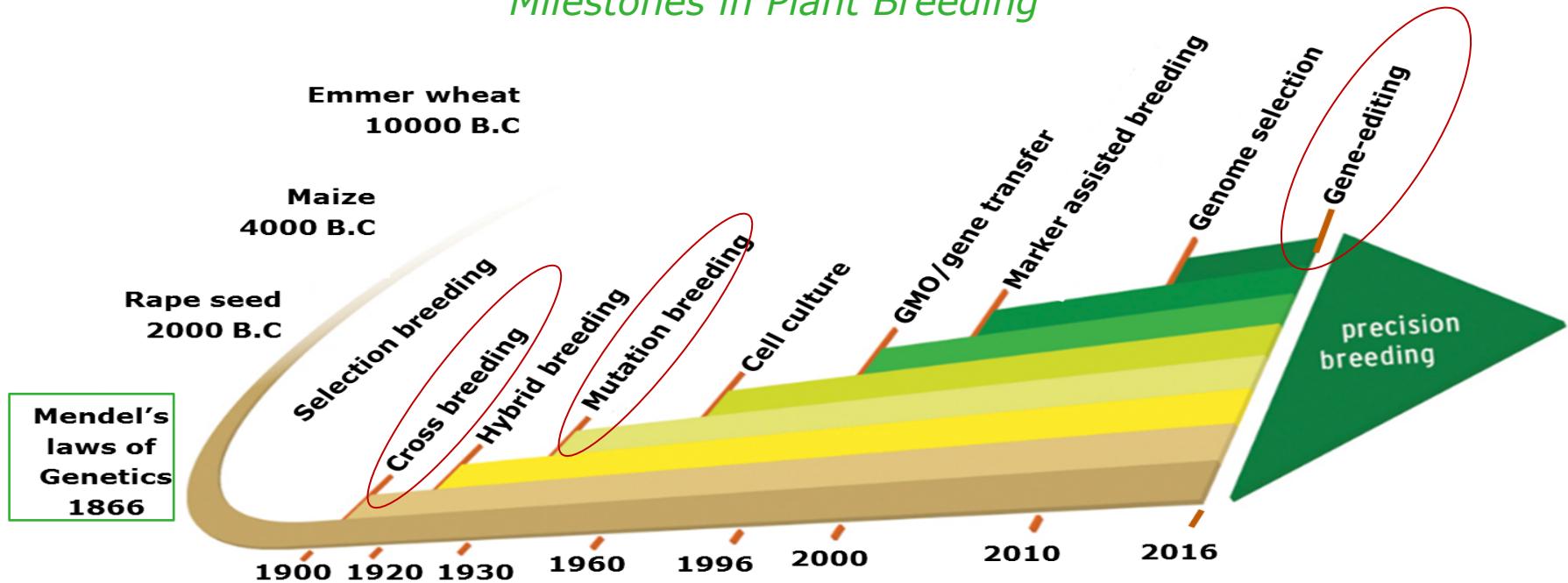
## ■ S-genen van planten in resistentieveredeling

*Plant S genes in resistance breeding*

- .....

# Mijlpalen in plantenveredeling

*Milestones in Plant Breeding*



Gene Editing belongs to New Genomic Techniques (NGT)

# Reactiveren van R-genen & modificeren van S-genen

## Repair R genes



Daniel Moninolopez



Jack Vossen



Henk Schouten



Anne-marie  
Wolters



Mireille van  
Damme



Eleni  
Koseoglou

## S genes



Arnaud Bovy



Jan Schaart



Paul Arens



Suzan Gabriels

# Afweermechanismen van de plant

## *Plant defence strategies*

- Resistentie voor biotische stress/ *Resistance to biotic stresses*
  - Monogene resistentie/ *Monogenic resistance*
    - R & S genen
  - Polygene resistentie/ *Polygenic resistance*
    - Kwantitatieve eigenschappen/ *Quantitative traits*, difficult to use in breeding
    - Nuttig voor genomics & digitale fenotyping/ *Helpful with genomics and digital phenotyping*
    - Genomische selectie/ *Genomic selection*
- Resistentie voor abiotische en gekoppelde stressfactoren *Resistance to abiotic and combined stresses*

# Respons van S-genen op biotische & abiotische stress

*Plant S genes in responses to biotic & abiotic stresses*

Plant Stress 13 (2024) 100541

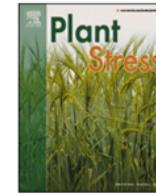


Contents lists available at ScienceDirect

Plant Stress

ELSEVIER

journal homepage: [www.sciencedirect.com/journal/plant-stress](http://www.sciencedirect.com/journal/plant-stress)



Knock-out of *SlDMR6-1* in tomato promotes a drought-avoidance strategy and increases tolerance to Late Blight

Alex Maioli<sup>a</sup>, Federica De Marchi<sup>a</sup>, Danila Valentino<sup>a</sup>, Silvia Gianoglio<sup>a,b</sup>,  
Davide Lucien Patono<sup>a</sup>, Fabio Miloro<sup>a</sup>, Yuling Bai<sup>c</sup>, Cinzia Comino<sup>a</sup>, Sergio Lanteri<sup>a</sup>,  
Claudio Lovisolo<sup>a</sup>, Alberto Acquadro<sup>a</sup>, Andrea Moglia<sup>a,\*</sup>

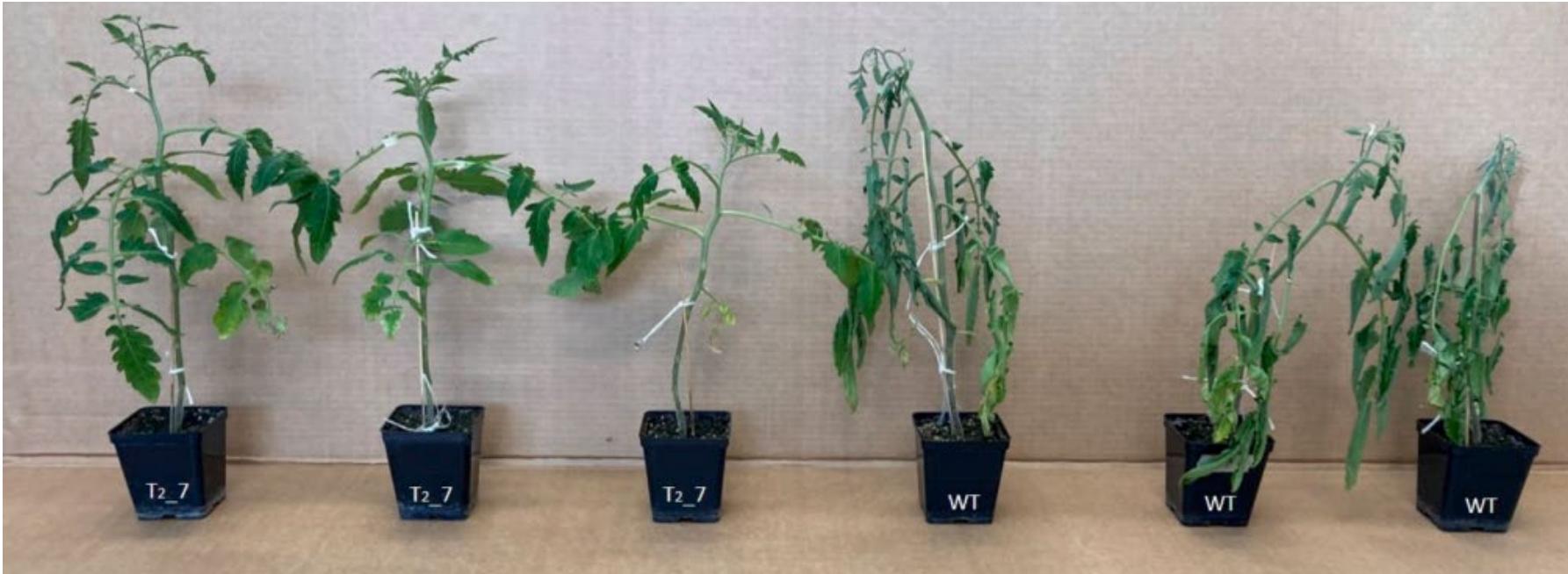
<sup>a</sup> DISAFA, Department of Agricultural, Forest and Food Sciences, University of Torino, 10095, Grugliasco, TO, Italy

<sup>b</sup> Instituto De Biología Molecular y Celular De Plantas (IBMCP) CSIC-UPV, Ingeniero Fausto Elio/n, 46022, Valencia, Spain

<sup>c</sup> Plant Breeding, Wageningen University & Research, 6708, PB, Wageningen, the Netherlands

# Respons van S-genen op biotische & **abiotische** stress

*Plant S genes in responses to biotic & **abiotic** stresses*



Drought stress analysis. T2\_7 & WT plants growing in a greenhouse  
after 7 days of withholding water

# Acknowledgements

## WUR:

- Jan van Kan  
(the *Pub17* gene)
- Richard Kormelink  
(the *Prl* gene)



## The *mlo* mutant

### Aachen University

- Ralph Panstruga

### University of Bari

- Stefano Pavan
- Claudio De Giovanni
- Luigi Ricciardi

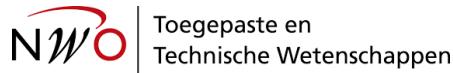
### Univ. of Foggia

- Concetta Lotti



2023 Dec.  
Including  
MSc thesis  
students

# Acknowledgements



Bayer CropScience



EAST-WEST SEED



KWS



TAKII EUROPE B.V.

