

# The Quest for Durable Disease Resistance, a Case Study - Turnip mosaic virus Resistance in Brassicas

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# Outline

- I You shall have no other ~~gods~~ pathogens before me -  
Background
- II ~~Love~~ Know thine enemy - Pathogen diversity
- III Thou shalt not kill - Plant resistance to TuMV
- IV ~~Do not~~ work on Sundays - Identity of the resistance genes
- V Thou shalt not worship graven images -  
Arabidopsis can confuse!
- VI You shall not commit adultery - Be careful  
what you cross your plants to!
- VII What next?



# I. The importance of the *Potyviridae* and *Turnip mosaic virus* (TuMV)

## *Potyviridae*

- Largest group of plant viruses
- Contains some of the most important and damaging plant viruses
- *Turnip mosaic virus* (TuMV) transmitted by many (89) aphid species non-persistently
- It has a very wide host range and infects many different crops, including all brassicas, lettuce, peas, radish, watercress, horseradish, ornamental plants, many weeds and rhubarb

# I. The importance of *Turnip mosaic virus* (TuMV)

## *Turnip mosaic virus*

- Causes severe symptoms including internal symptoms in *B. oleracea* cabbage
- It also infects and causes severe symptoms in *B. rapa*
- TuMV infects oilseed rapa and swede (*B. napus*) and severely reduces seed yield in the former

# II. Know thine enemy - diversity of TuMV

- In order to identify broad-spectrum resistance to Turnip mosaic virus it is important to know about variation in the virus
- We studied:
  - biological
  - serological
  - geographical and
  - genetic

diversity of TuMV

# Biological and geographical diversity

## Pathotypes of TuMV

Virus pathotype	Differential plant line			
	Rape S6	Rape R4	Swede 165	Swede S1
1	+	0	0	+
2	R	R	0	R
3	+	+ <sub>N</sub>	0	+
4	+	+	+	+
5	+	+	0	+
6	+	+ <sub>N</sub>	0	R
7	+	0	0	R
8	+	+ <sub>N</sub>	R <sub>N</sub>	R
9	+	R <sub>N</sub>	0	R
10	+	+	0	0
11	R	+	0	R
12	+	+	+ <sub>N</sub>	+

Jenner & Walsh, (1996). Plant  
Pathology 45, 848-856.

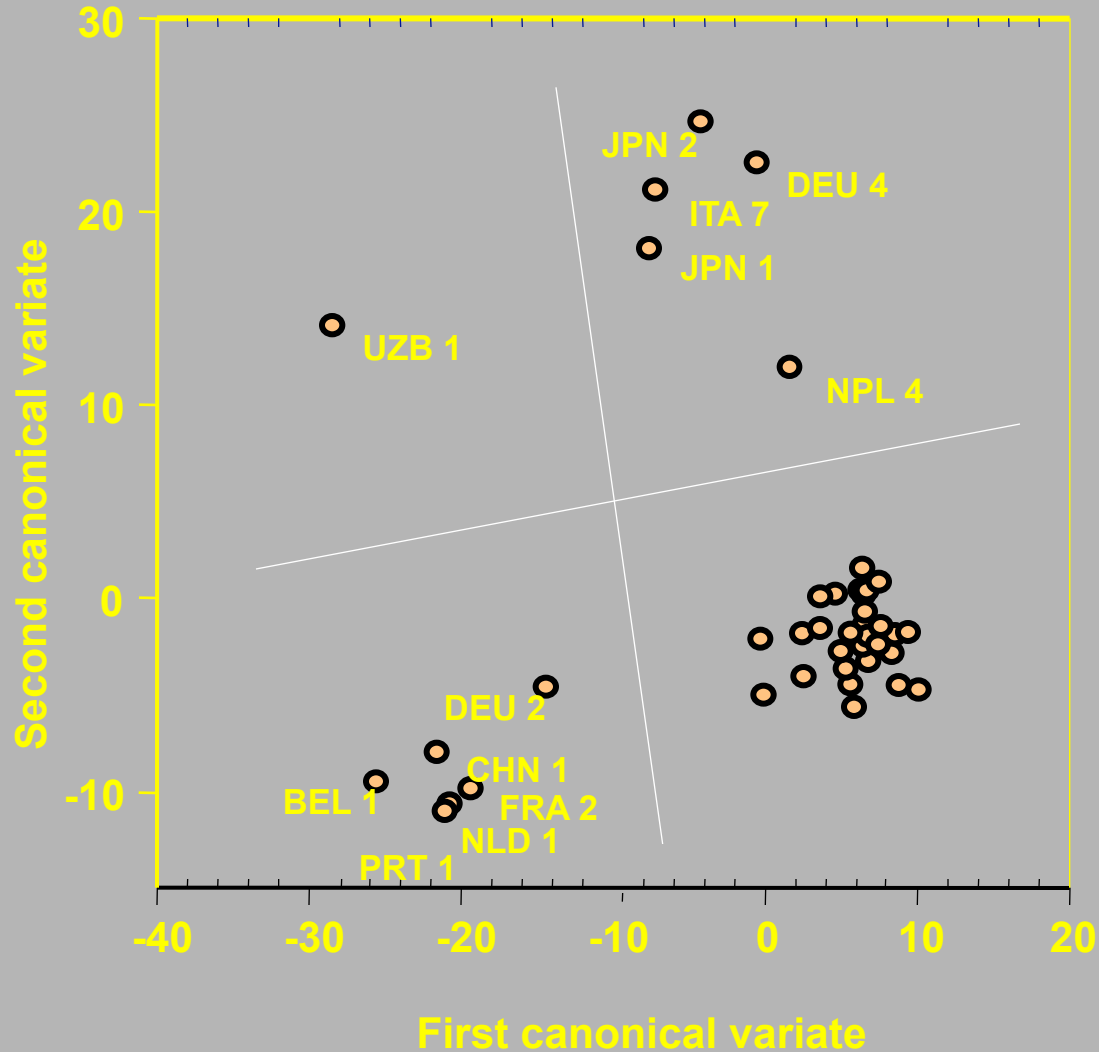
# Biological and geographical diversity

## Pathotypes of TuMV

Virus pathotype	Differential plant line			
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5	+	+	0	+
6	+	+ <sub>N</sub>	0	R
7	+	0	0	R
8	+	+ <sub>N</sub>	R <sub>N</sub>	R
9	+	R <sub>N</sub>	0	R
10	+	+	0	0
11	R	+	0	R
12	+	+	+ <sub>N</sub>	+

Jenner & Walsh, (1996). Plant Pathology 45, 848-856.

# Serological and geographical diversity

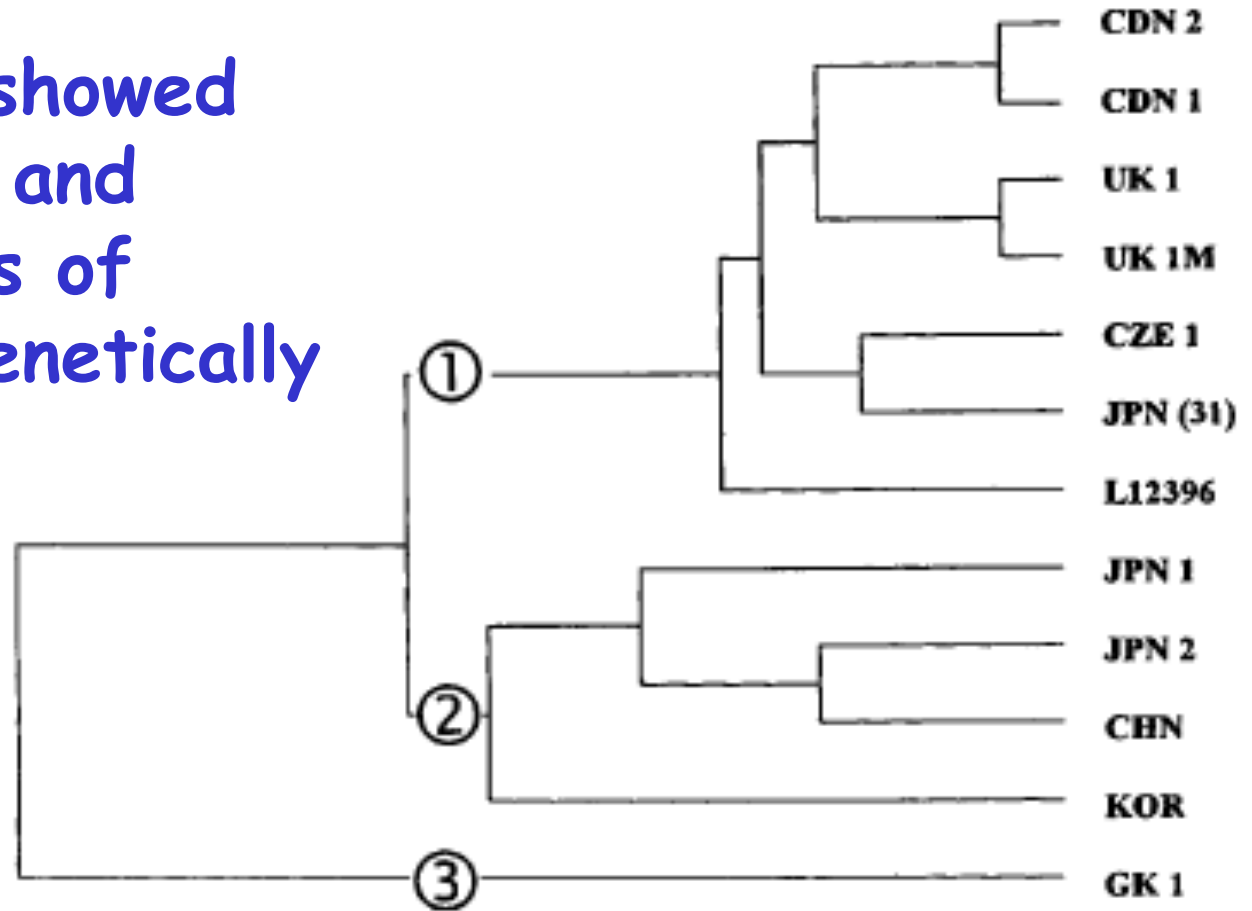


Jenner, Keane, Jones & Walsh, (1999).  
Plant Pathology 48, 101-108.



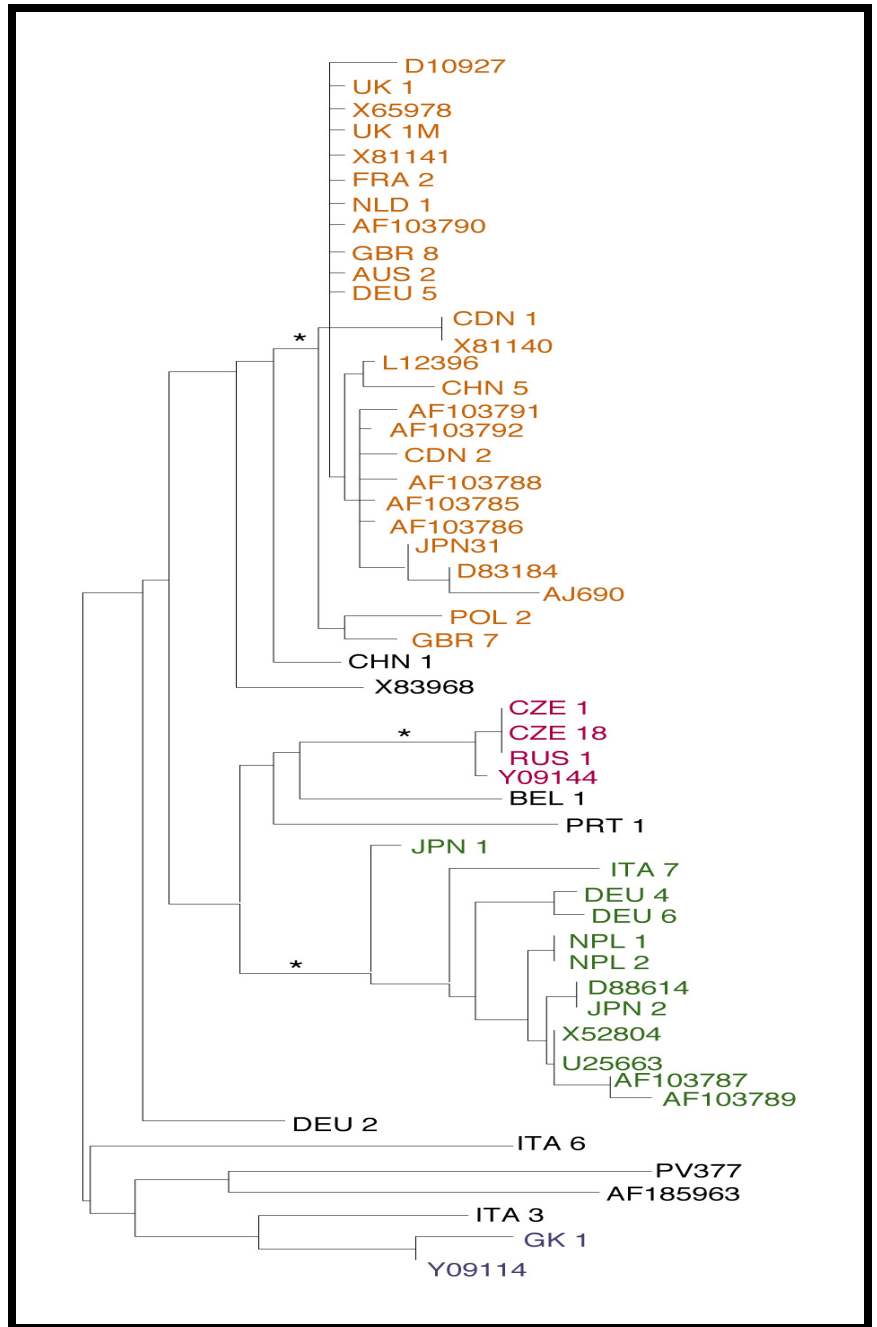
# Genetic variation

In 1997, we showed that brassica and radish isolates of TuMV were genetically distinct



# Genetic variation

Further sequencing and whole genome sequencing confirmed these differences

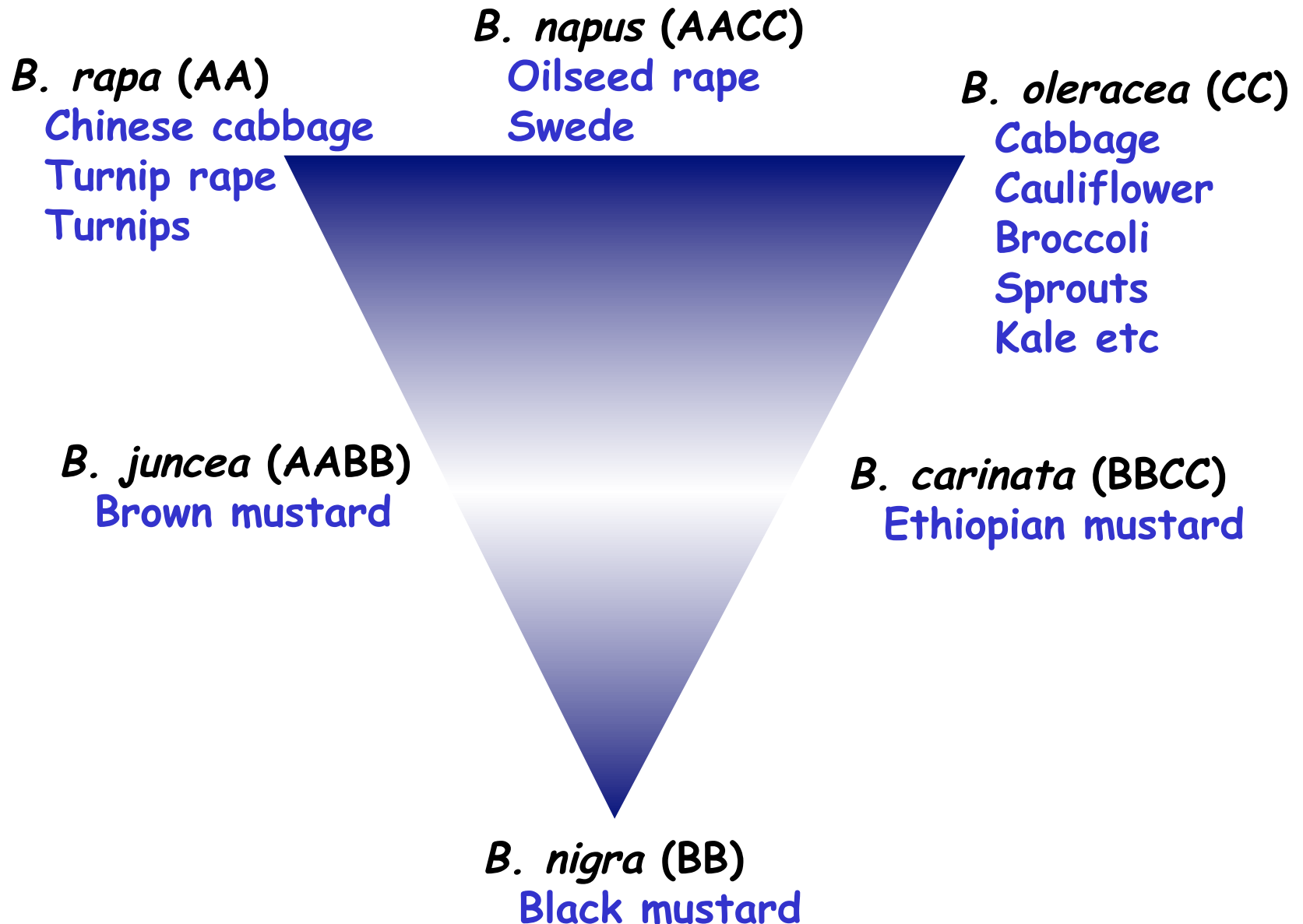


Tomimura, Gibbs, Jenner, Walsh & Ohshima (2003). *Molecular Ecology* 12, 2099 - 2111.

# III. Thou shalt not kill - Plant resistance to TuMV

- Having studied the variation in the pathogen - TuMV, we were then able to go about looking for resistance to TuMV in brassicas
- We looked for resistance in:
  - The C genome of *Brassica oleracea*
  - The A genome of *Brassica rapa*
  - The A+C genome of *Brassica napus*

# Sources of resistance: Triangle of U

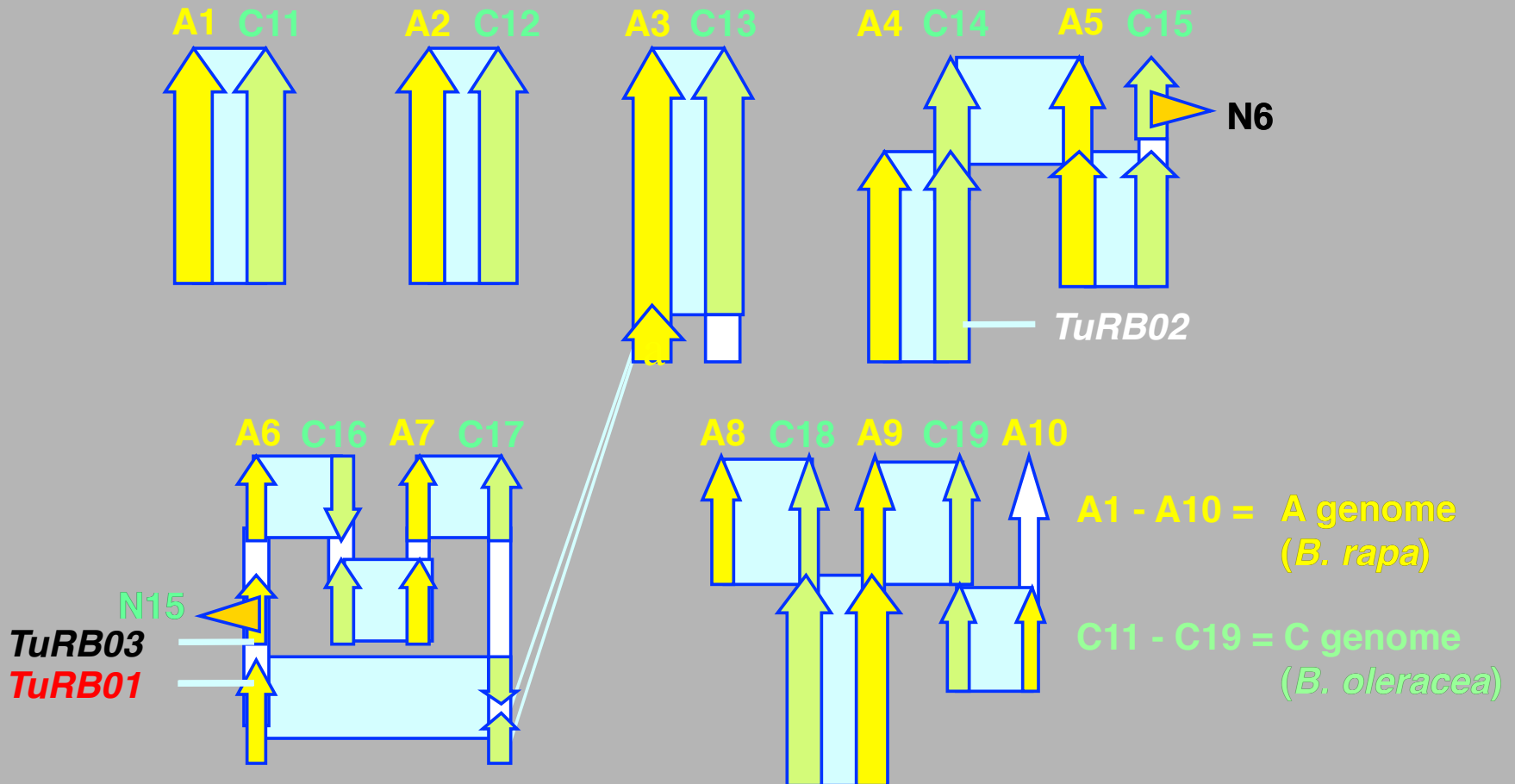


# Sources of resistance to TuMV in *Brassica*

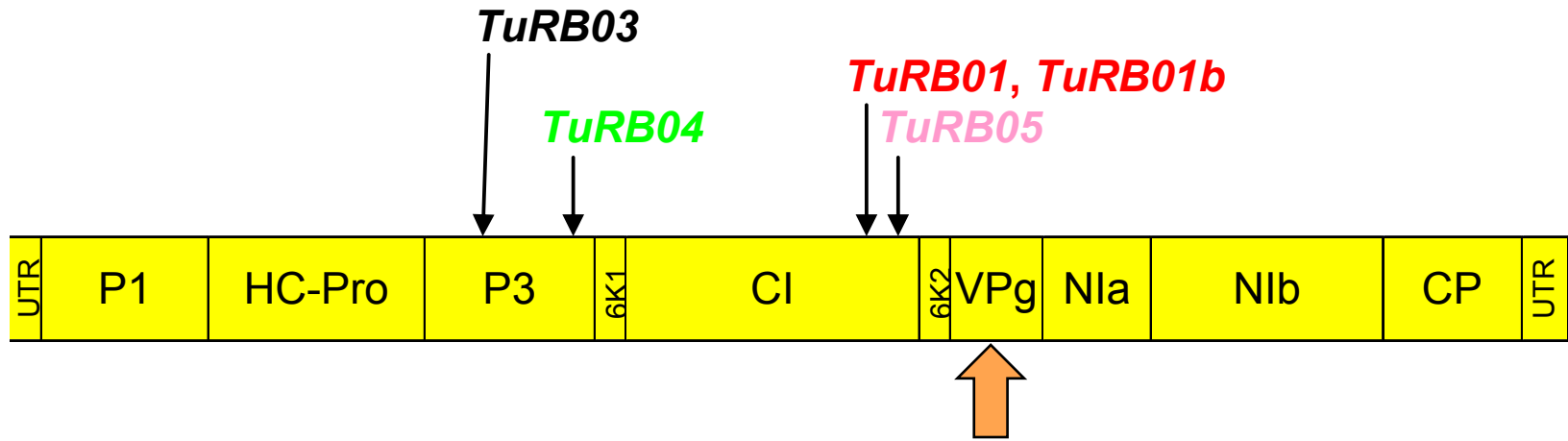
Plant line	Virus isolate (pathotype)			
	UK 1 (1)	CZE 1 (3)	CHN 5 (3)	CDN 1 (4)
N-o-1 ( <i>TuRBO1</i> )	0	+ <sub>N</sub>	+ <sub>N</sub>	+
No. 2	0	+	+	+
No. 22 ( <i>TuRBO3</i> )	+ <sub>N</sub>	+ <sub>N</sub>	+ <sub>N</sub>	0
Ap 1	+	+	R/+	+
M 1	0/R	R/+	+	+
G 1	0	R/+ <sub>N</sub>	+ <sub>N</sub>	+
165	0	0	0	+
V 1	0	0/+	0/R	+
0-2'-5	0	0	R	0/R
No. 1	0	0	R	R
RLR22	R	R	R	R
BP-4	R/0	R/0	R	R/0

# 2. Past research - mapping TuMV resistance genes

TuMV resistance genes mapped in the *Brassica* genome



# Past research - pathogenicity / avirulence factors



# IV. ~~Do not~~ work on Sundays - Identity of plant resistance genes

Most recently  
focussed on  
*Brassica rapa* line  
RLR22 with  
broad-spectrum  
TuMV resistance

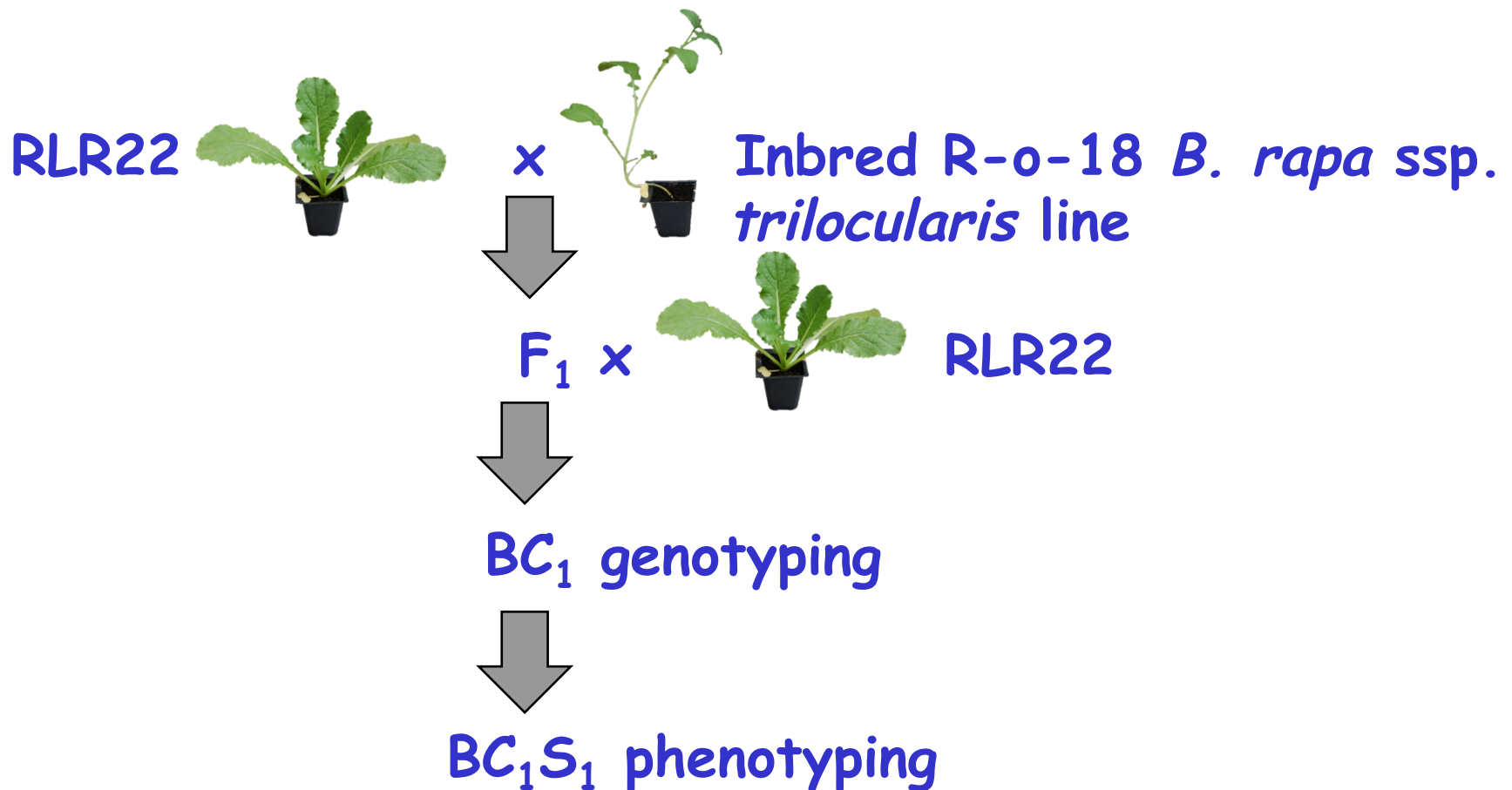


# Parental phenotypes

	TuMV isolate (pathotype)							
	UK 1 (1)	CZE 1 (3)	CHN 5 (3)	CDN 1 (4)	JPN 1 (7)	DEU 7 (8)	GK 1 (9)	UK 4 (12)
RLR22	R	R	R	R	O	R	R	R
R-o-18	+	+	+	+	+	+	+	+

Walsh, J.A. et al. (2002). *European Journal of Plant Pathology* **108**: 15-20.

# Resistance gene mapping strategy



Segregation ratios in F<sub>1</sub>, BC<sub>1</sub> and BC<sub>1</sub>S<sub>1</sub> generations suggested the resistance was controlled by a recessive gene and a dominant gene.

# Two gene model

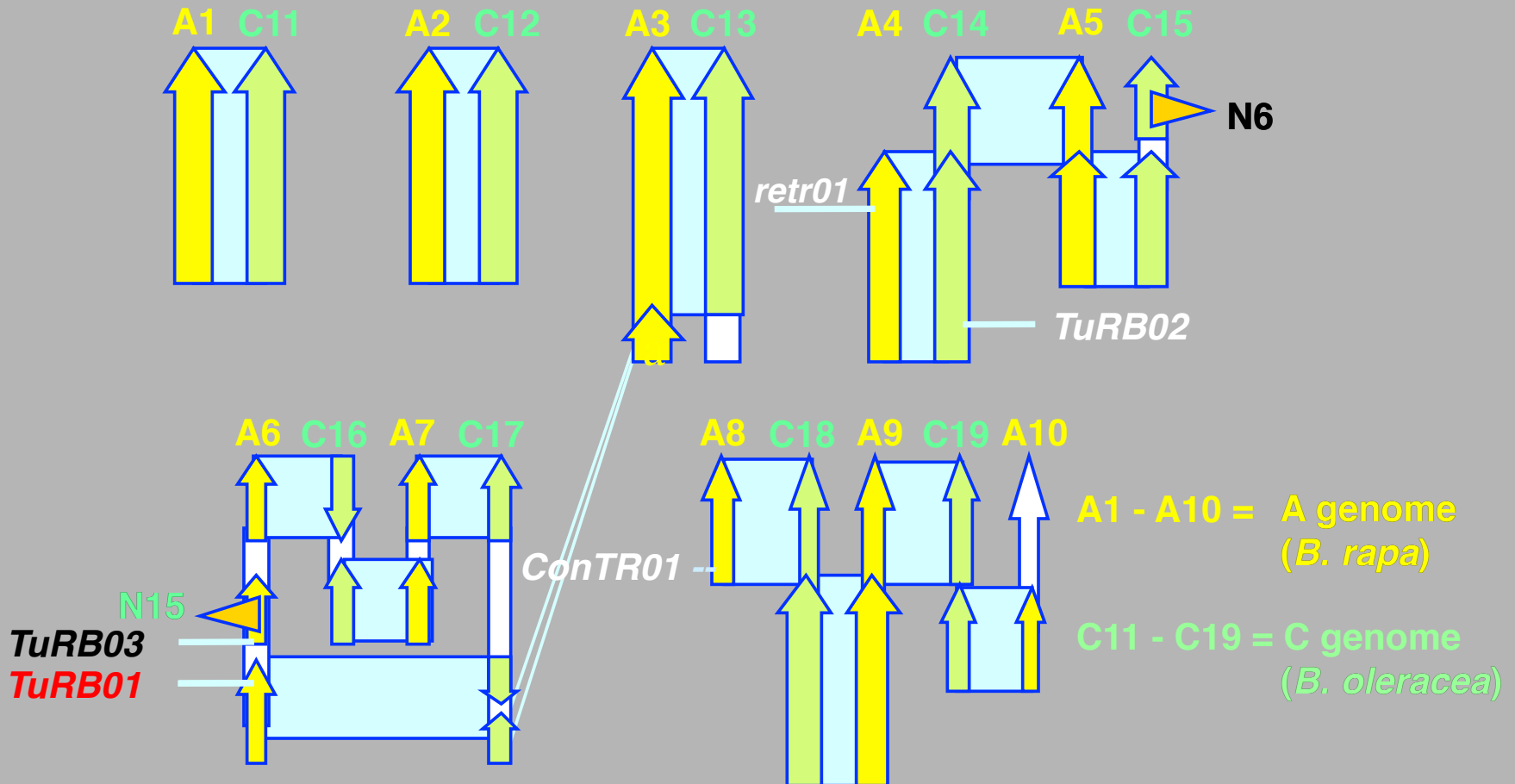
Recessive resistance gene:

recessive TuMV resistance 01 *retr01*

Dominant resistance gene:

Conditional TuMV resistance 01 *ConTR01*

# Mapping the TuMV resistance genes



# Identity of the resistance genes indicated from the R-o-18 cross

Recessive resistances have been discovered to some potyviruses

- More than half the resistance genes associated with potyviruses are recessive - compared to only 20% against other plant viruses (Provvidenti and Hampton, 1992)
- Recessive resistance was believed to be based on a passive mechanism - Resistant plants are lacking in a specific host factor that is required for the virus to complete its life-cycle (Fraser, 1986)
- Many of these recessive resistances have been found to be based on *eIF4E* or *eIF(iso)4E*

Potyvirus uses the eukaryotic translation initiation complex to translate their positive sense RNA genome into protein

Potyvirus use the eukaryotic translation initiation complex to translate their plus strand RNA into protein

Mutations in the plant *eIF4E* gene result in the potyviral VPg protein being unable to bind with the *eIF4E* protein

# Identity of the resistance genes indicated from the R-o-18 cross

- Probes for members of the *eIF4E* and *eIF(iso)4E* gene families were used to locate these genes in the A genome.
- Probes for one of the copies of *eIF4E* and one of the copies of *eIF(iso)4E* were closely linked to the *ConTR01* locus.
- A probe for one of the other copies of *eIF(iso)4E* was closely linked to the *retr01* locus.



# V. Thou shalt not worship graven images - *Arabidopsis* can confuse

2002 Mutation and transposon knockout of *Arabidopsis* *eIF(iso)4E* demonstrated the requirement of this gene for TuMV susceptibility

*Arabidopsis* transposon knock-out line *Col-0::dSpm*  
*eIF(iso)4E* was found to be resistant to TuMV (Duprat  
*et al.*, 2002) SO .....

# V. Thou shalt not worship graven images - *Arabidopsis* can confuse

- We found that *Brassica rapa* has 3 copies of *eIF4E* one of which is a pseudogene
- We also found that *Brassica rapa* has 3 copies of *eIF(iso)4E*
- Copies of two *eIF4E* and two *eIF(iso)4E* genes were identified in a BAC library of the susceptible plant line R-o-18 and a fosmid library of the resistant plant line RLR22 and cloned
- The genes were transformed in to the *Arabidopsis* mutant line *Col-0::dSpm eIF(iso)4E* which is resistant to TuMV

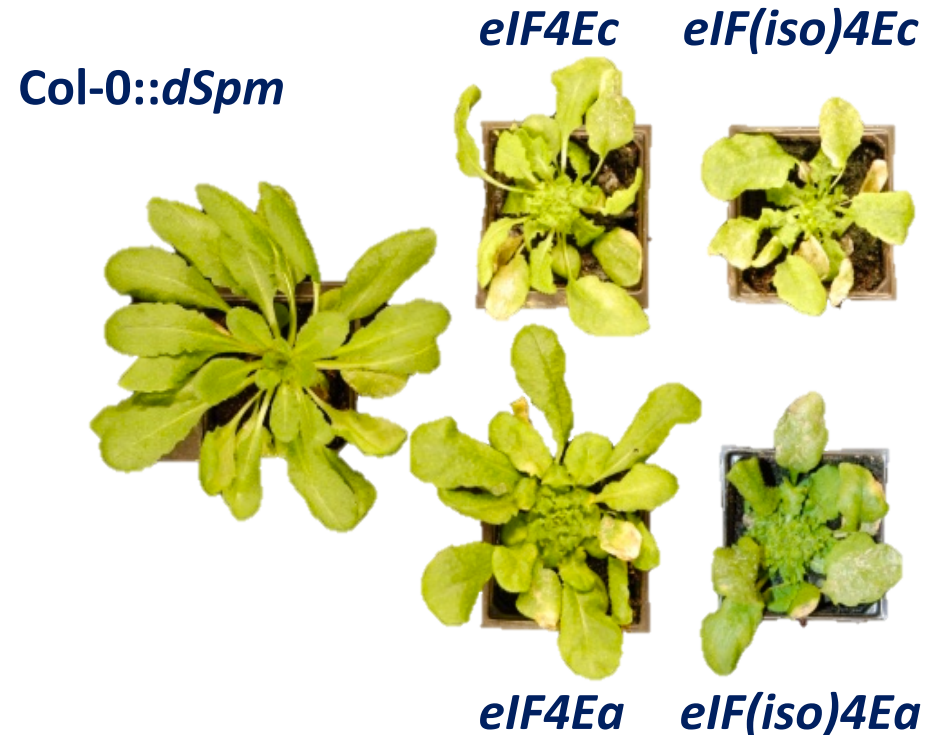
# V. Thou shalt not worship graven images - *Arabidopsis* can confuse

- This showed that TuMV can use multiple copies of both genes from R-o-18 when ectopically expressed in *Arabidopsis*



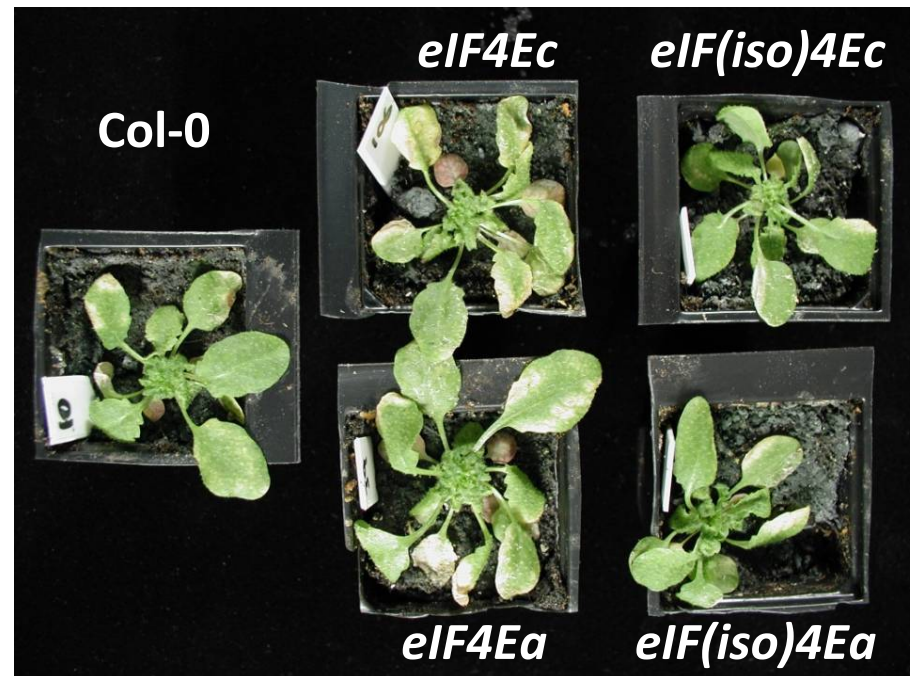
# V. Thou shalt not worship graven images - *Arabidopsis* can confuse

- This also showed that TuMV can use multiple copies of both genes from RLR22 when ectopically expressed in *Arabidopsis*



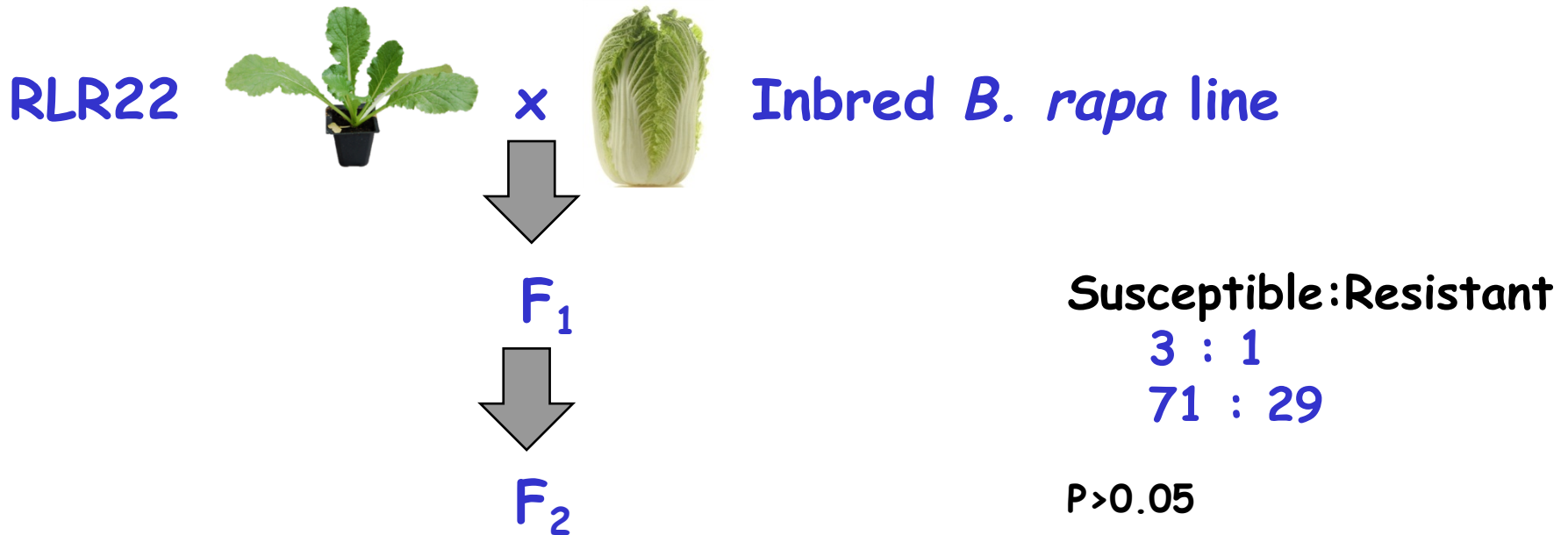
# V. Thou shalt not worship graven images - *Arabidopsis* can confuse

- Control transformations in Col-0



Jenner *et al.* (2010). *Mol. Plant-Microbe Interactions* 23: 498-1505.

# VI. You shall not commit adultery - Be careful what you cross your plants to

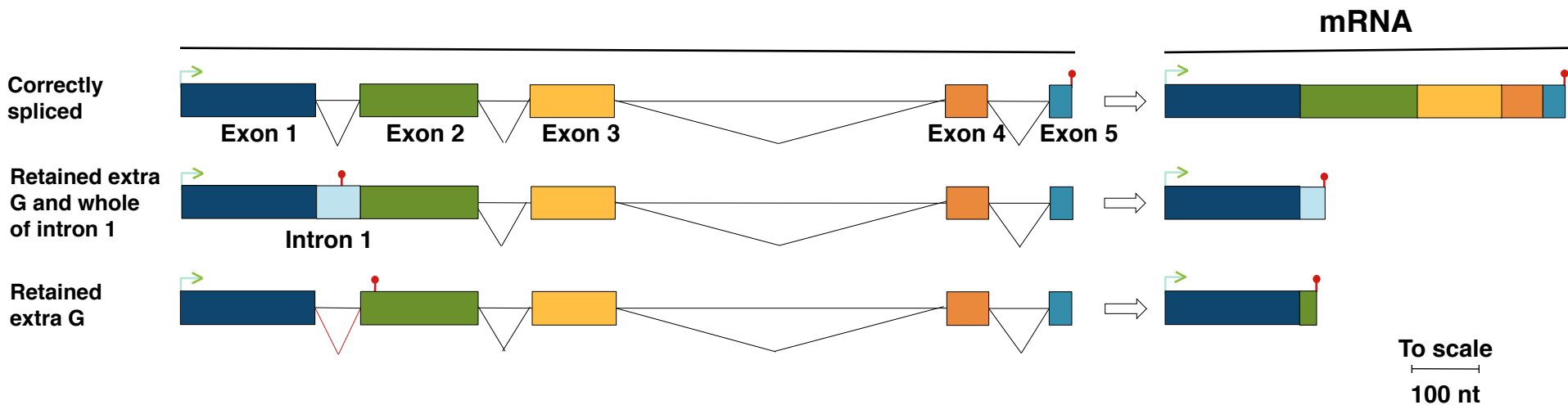


Plants with genotype	Phenotype	
	Resistant R	Susceptible +
<i>RR</i>	0	25
<i>Rr</i>	0	46
<i>rr (retr01)</i>	29	0

# Identity of the resistance gene indicated from the *B. rapa* cross

- gDNA sequencing of candidate *eIF(iso)4E.a* revealed an extra G at the exon 1 - intron 1 splice site in RLR22 compared to susceptible lines
- gDNA sequencing of two other lines with broad-spectrum resistance revealed they also possessed the extra G

# The resistance mechanism - mis-splicing of *eIF(iso)4E.a*



Expression studies revealed the mis-splicing of *eIF(iso)4E.a*

⇒ *retr01* was identified as *eIF(iso)4E.a*

Nellist *et al.* (2014). *The Plant Journal* **77**: 261-268.



# What next?

## Alternative sources of broad-spectrum resistance to TuMV

Plant Line	Broad-spectrum Resistance	Indel at position 201 bp	Phenotype	Mis-spliced?
RR	✓	No	0	No
CP	✓	No	0	No
BR	✓	No	0	No
BP	✓	Yes	R	Yes
RLR22	✓	Yes	R	Yes
JB	✓	Yes	R / +	Yes / No
TD	<i>x (TuRBO1)</i>	No	+	No
R-o-18	<i>x</i>	No	+	No

# Acknowledgements:

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Bill Briggs The Syngenta logo, consisting of the word "syngenta" in a lowercase, sans-serif font, with a green leaf icon above the letter 'a'.

Rifei Sun, Xiaowu Wang, Shujiang Zhang, Wei Qian



**Thank you for your attention**