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## Resistance to aphids in melon: R-cluster and QTLs

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# Resistance to aphids in melon: R-cluster and QTLs

**“Plant-Insect-Symbiont interaction meeting”**

2<sup>nd</sup> annual meeting of Japan-France PISI-net

Le Rheu

4-6<sup>th</sup> November 2019

GAFL – CBGP – Pathologie Végétale

EPGV/CNRGV/Gentyane/Genotul

BAPOA

# Vat Resistance

# Vat Resistance

First described in 1979 (Pitrat & Lecoq)

A remarkable phenotype : Vat confers resistance  
to *Aphis gossypii*

To viruses only, and only when, they are inoculated by *Aphis gossypii*

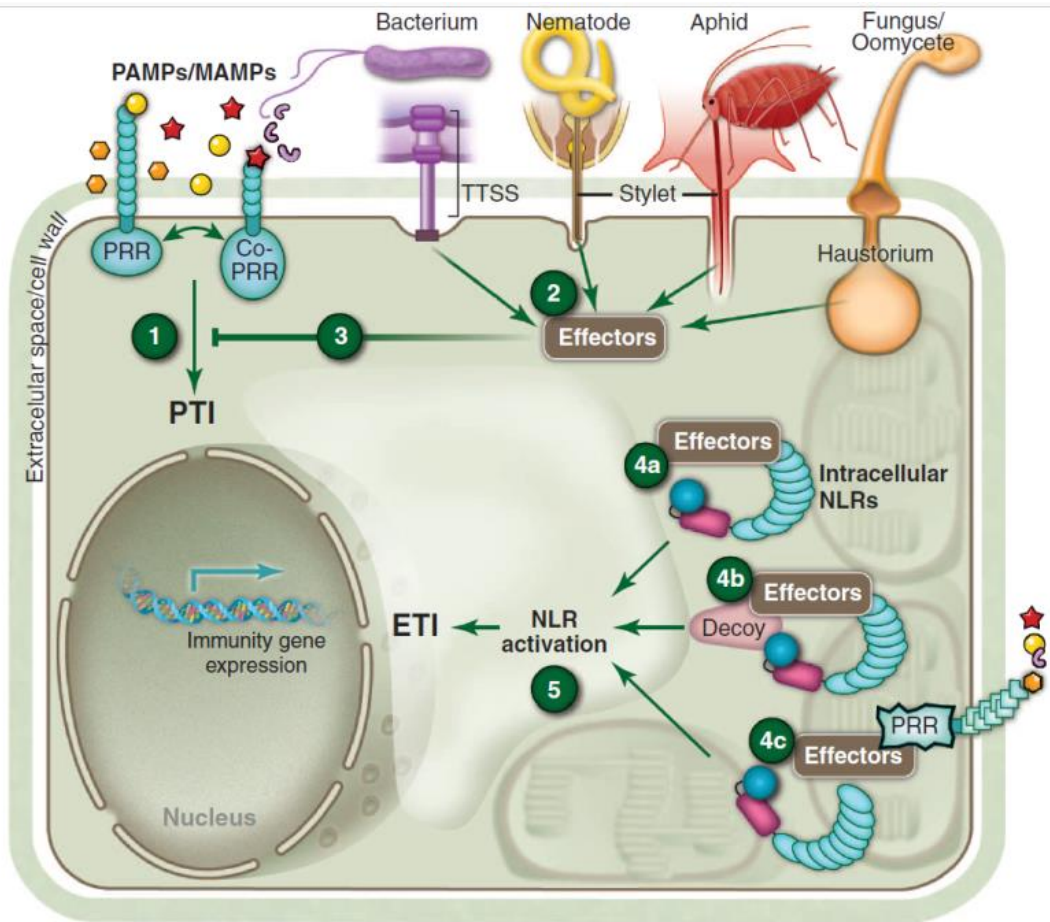
In 1987 a first Charentais line was proposed to growers

In 2000 hybrids with the Vat resistance produced by private companies are highly successful  
80% of the melon produced in South-East of France

In 2008 fine mapping and chromosome walking studies ( Dogimont et al 2008, 2014)

**Vat belongs to CC-NBS-LRR resistance gene family**

# Vat resistance belongs to NB-LRR R gene family

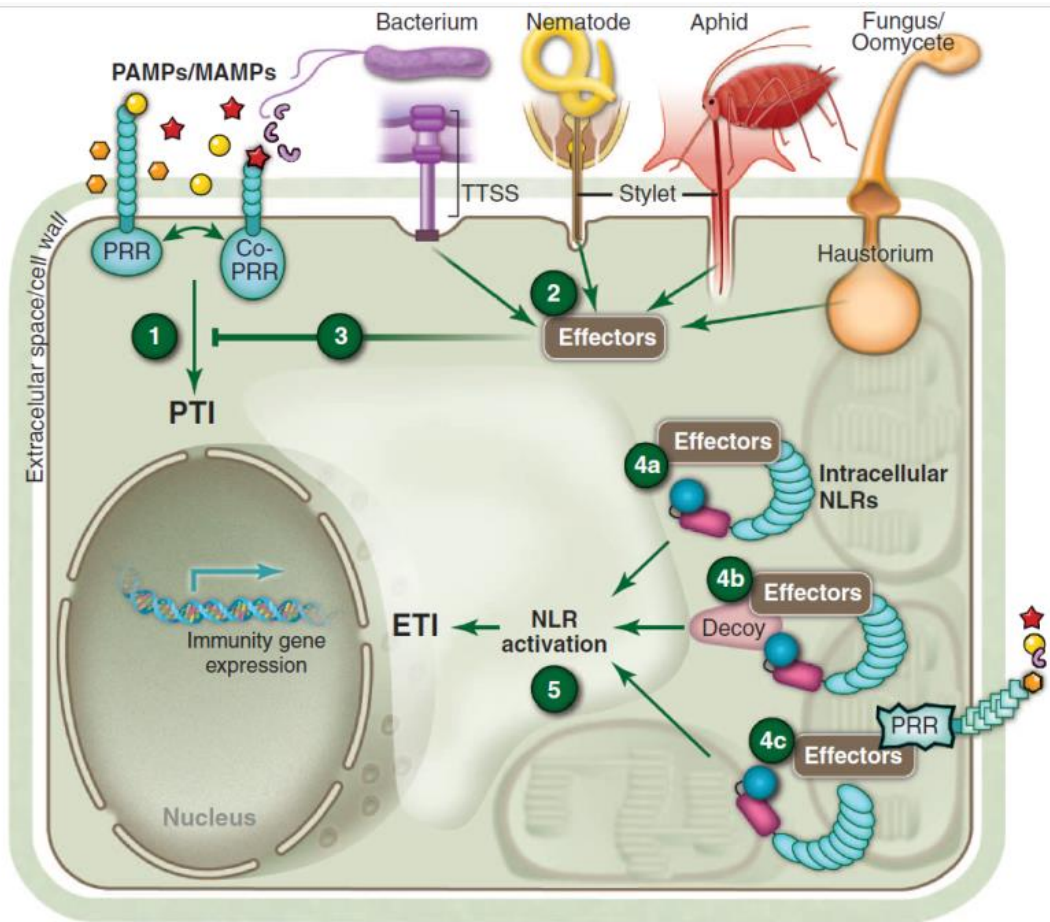


Each pathogen express an array of effectors inhibiting PTI

R protein is activated by the product of a specific pathogen isolate effector gene.

R genes are present in multigene clusters,  
True alleles exist across genetic backgrounds.

# Vat resistance belongs to NB-LRR R gene family



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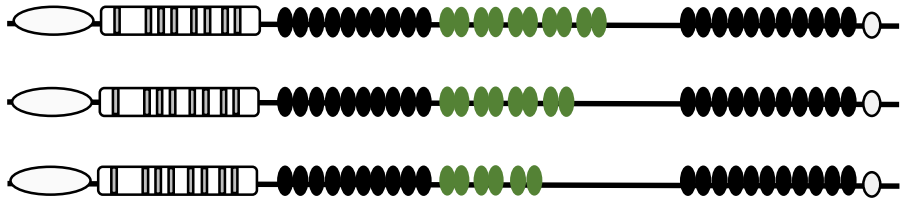
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## The first Vat genes characterized

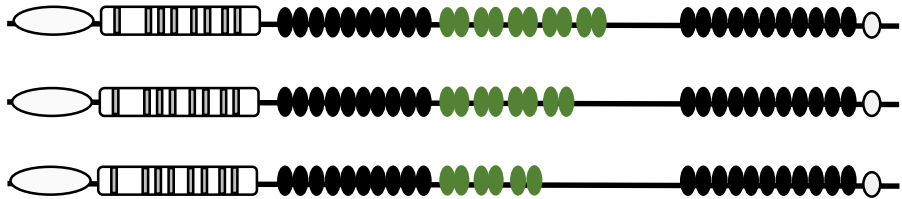
CC - NB - LRR1 LRR2 LRR3 - Nterm



# Vat resistance belongs to NB-LRR R gene family

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CC - NB - LRR1 LRR2 LRR3 - Nterm



### **PHENOTYPES that have been validated with transgenic plants**

Resistance to podwery mildew race 3-5

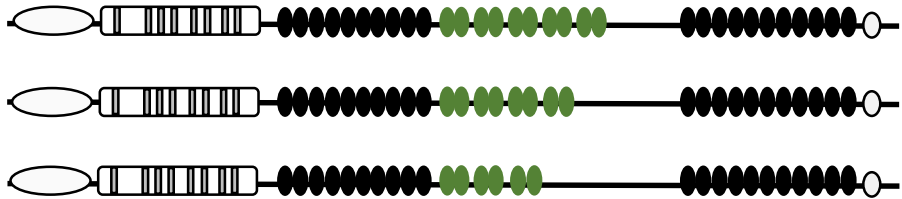
Resistance to aphid clone NM1 and to viruses when transmitted by NM1



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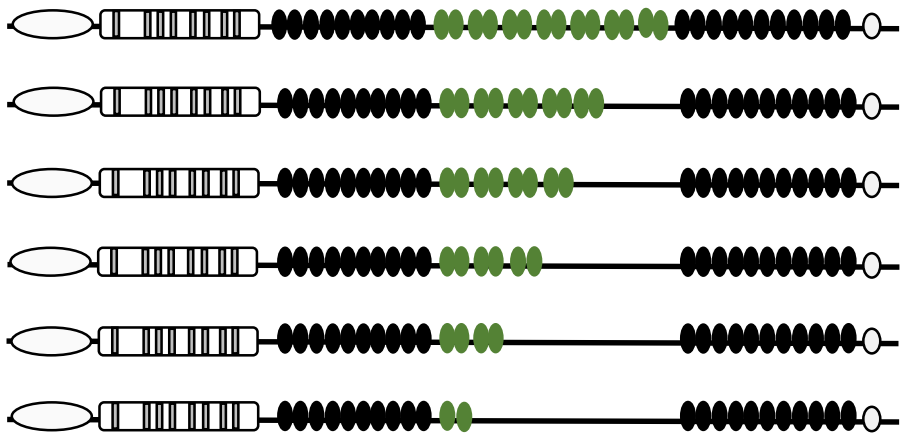
Resistance to aphid clone NM1 and to viruses when transmitted by NM1

Long range PCR to characterize Vat homologs in the melon diversity

# Vat resistance belongs to NB-LRR R gene family

## 35 Vat homologs from 20 melon lines

CC - NB - LRR1 LRR2 LRR3 - Nterm



1300 to 2900 pb

### **PHENOTYPES that have been validated with transgenic plants**

Resistance to podwery mildew race 3-5

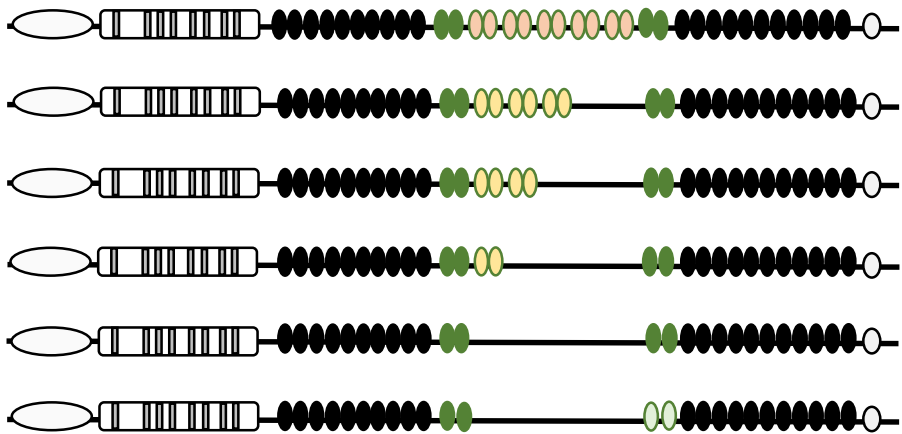
Resistance to aphid clone NM1 and to viruses when transmitted by NM1

and SNPs ...

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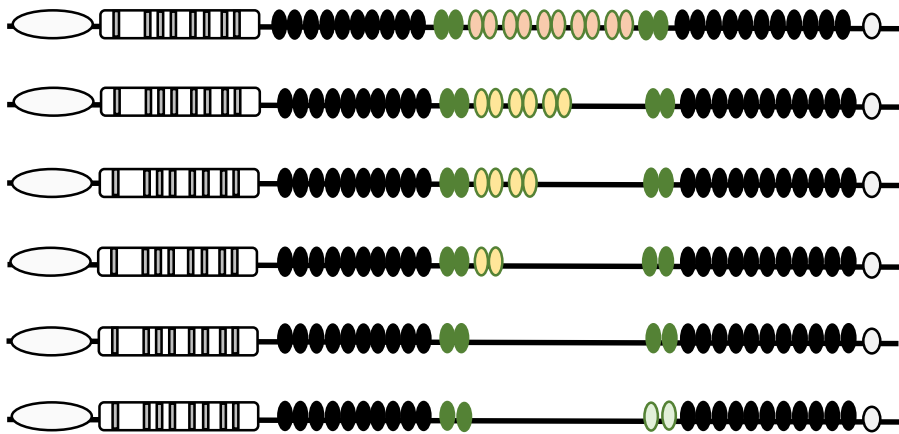
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Probability to have non synonymous SNP when the codon is different,

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## 35 Vat homologs from 20 melon lines

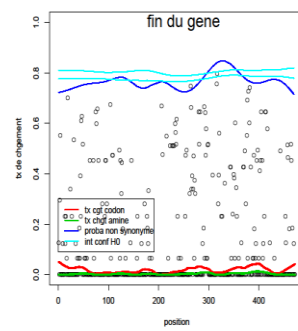
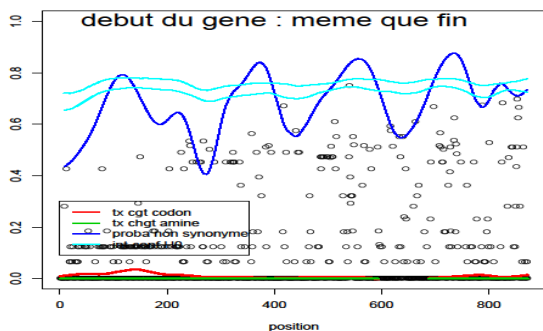
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**PHENOTYPES that have been validated with transgenic plants**

Resistance to podwery mildew race 3-5

Resistance to aphid clone NM1 and to viruses when transmitted by NM1



Probability to have non synonymous SNP when the codon is different,

=> Non synonymous SNPs are more often observed at the end of the NB pattern ans in the LRR1 and LRR2 patterns

Vat homologs are expected  
in the same genomic area

Our sequencing objectives : get a robust assembly of the Vat cluster

ADN with very long fragments

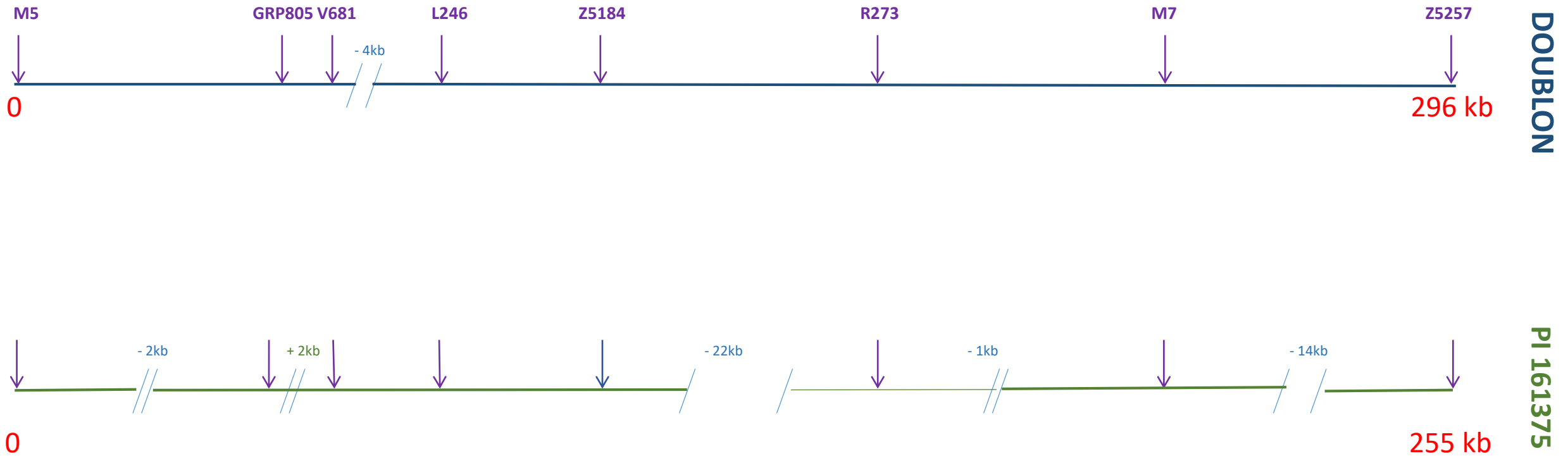
enough reads that overlap each homolog => sup 3 kb

reads that overlap at least two or three homologs => sup 50 kb

=> Nanopore + Illumina or PacBio sequencing + Optical map

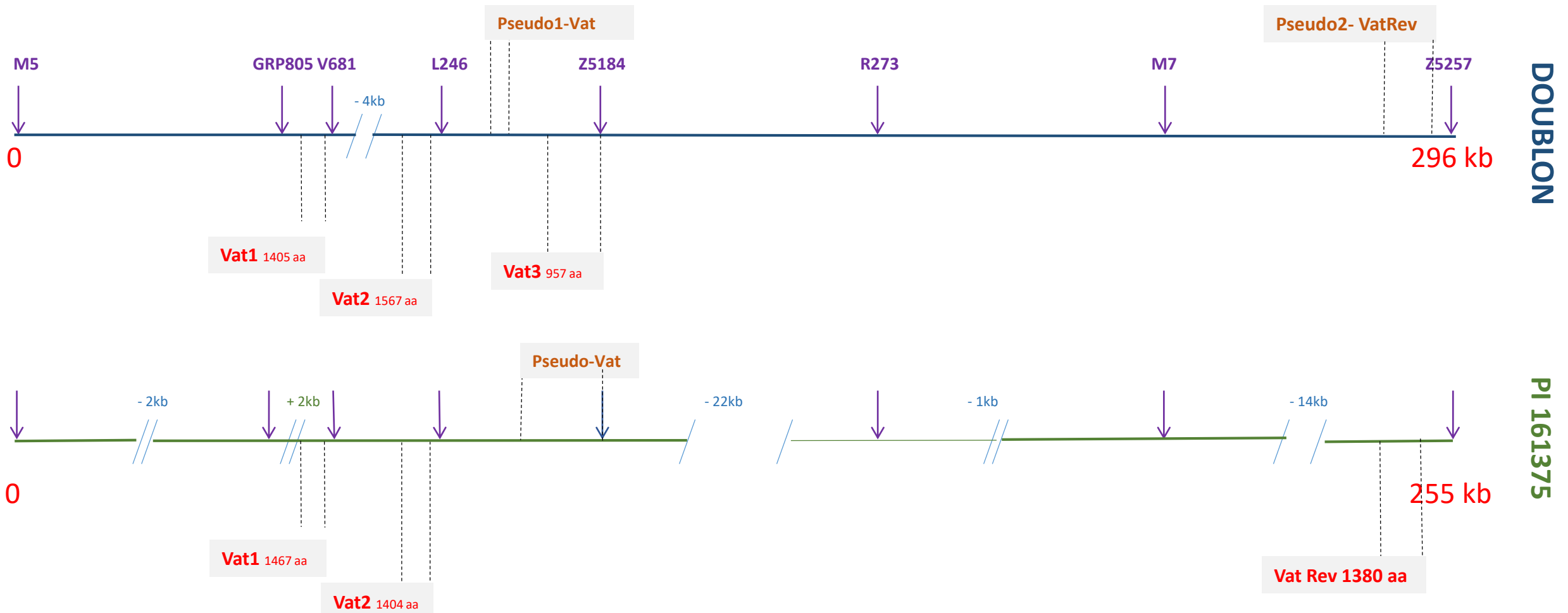
# Vat resistance genes belong to a cluster

- Genetic markers



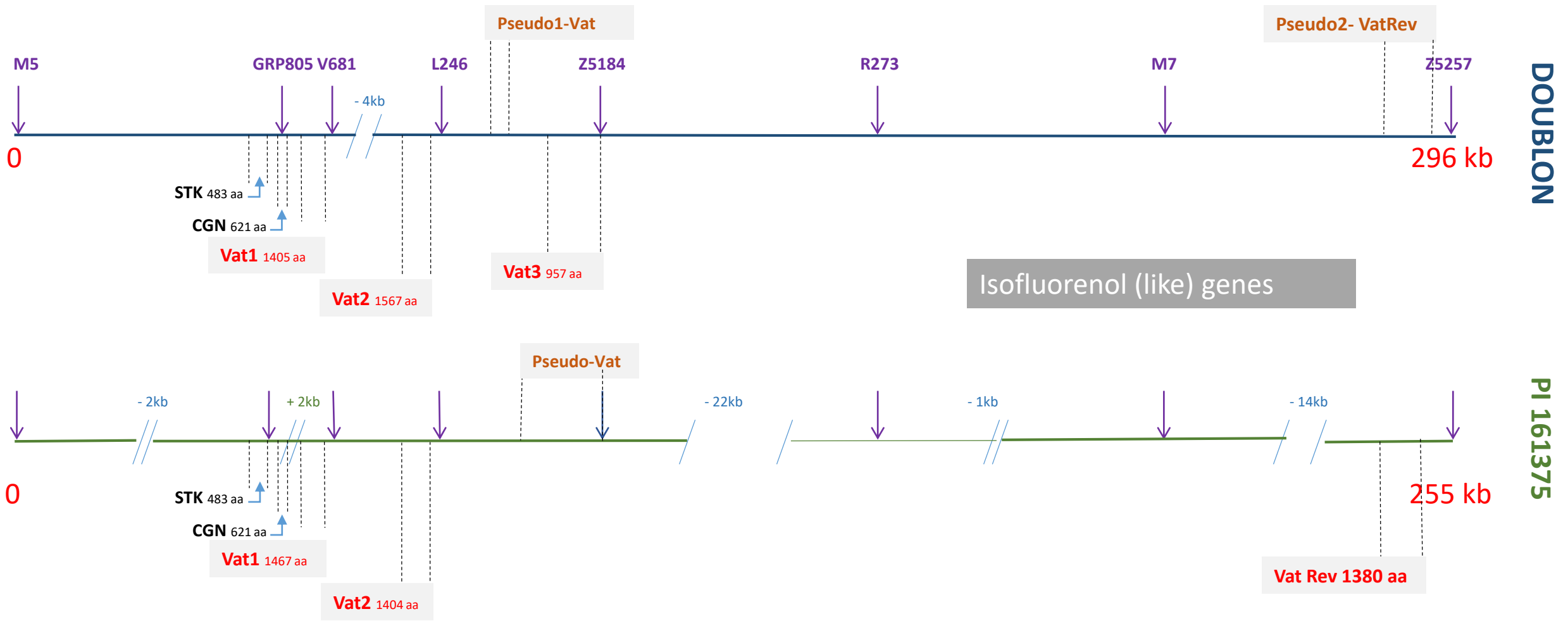
# La résistance Vat : famille des NB-LRR

- Vat1, Vat2, Vat3 genes that have an ORF up to exon 4
- PseudoVat sequences have early STOP codons or large insertion or large deletion
- Genetic markers



# La résistance Vat : famille des NB-LRR

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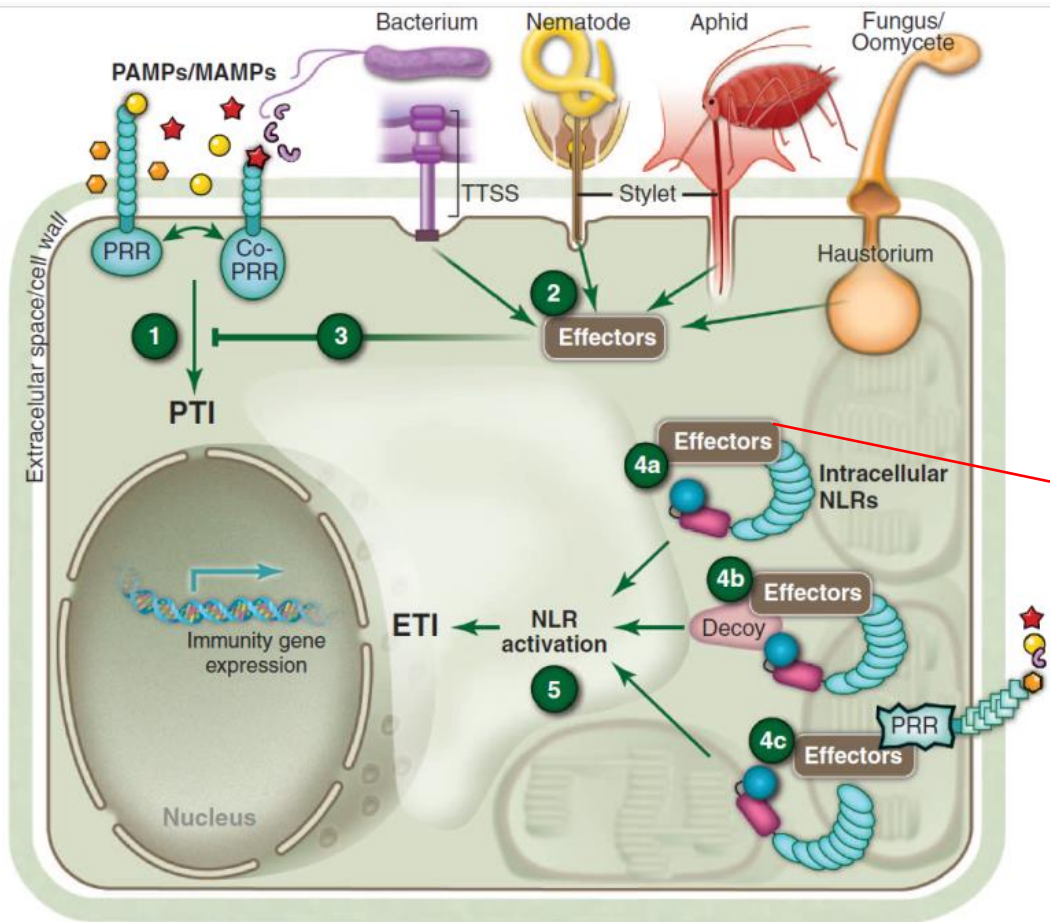
# La résistance Vat : famille des NB-LRR

In summary, what we know from 4 *C. melo* lines....

- Vat Homologs are clustering in 200-300 kb
- Some sequences (genes STK, CGN, 50 S ?) surrounding this cluster are highly conserved
- 2 – 5 Vat ORFs per cluster
- 1 – 2 Pseudo Vat sequences per cluster

Unbalance crossing-over are probably involved in building this cluster  
aside insertion of transposons

# Vat resistance belongs to NB-LRR R gene family



Each pathogen isolate express an array of effectors.

VAT protein is activated by the product of a specific *Aphis gossypii* effector gene.

R genes are present in multigene clusters, True alleles exist across genetic backgrounds.

# Vat resistance belongs to NB-LRR R gene family

## A specific *Aphis gossypii* effector gene

- Syntax
  - Avirulent allele product in aphid triggers resistance in Vat plants
  - (a)Virulent does not trigger resistance in Vat plants
- The hypothesis
  - (a)Virulent allele is recessive
  - This recessive allele is express or not
  - The peptide is excreted in saliva

# Vat resistance belongs to NB-LRR R gene family

## A specific *Aphis gossypii* effector gene

- Tools
  - Head Transcriptomes from one (a)Virulent clone and two Avirulent clones
  - Illumina sequencing
  - BUSCO = 94-96%
- In silico analysis
  - (a)Virulent allele is recessive
  - This recessive allele is express or not = CDS present or not
  - => 700 candidates
  - The peptide is excreted in saliva
  - => 50 candidates

# Vat resistance belongs to NB-LRR R gene family

## A specific *Aphis gossypii* effector gene

- de novo sequencing by Sanger of the candidates in the three clones
  - => None candidate fitted the hypothesis
- Transcriptome quality ?
  - => We are developing full genomes with Nanopore + Illumina + Optical map

# Vat resistance belongs to NB-LRR R gene family

To summarize where we are today

Vat plants/ *A. gossypii* interaction set up using a complex genetic framework in melon

Which level(s) of polymorphism does play a key role in phenotype ?

Vat plants/ *A. gossypii* interaction set up using an unknown genetic system in *A. gossypii*

Which effector does trigger the Vat resistance ?

**We are building high quality genomes -melon and aphids- to address both questions**



# Vat Resistance

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To viruses only and only when they are inoculated by *Aphis gossypii*

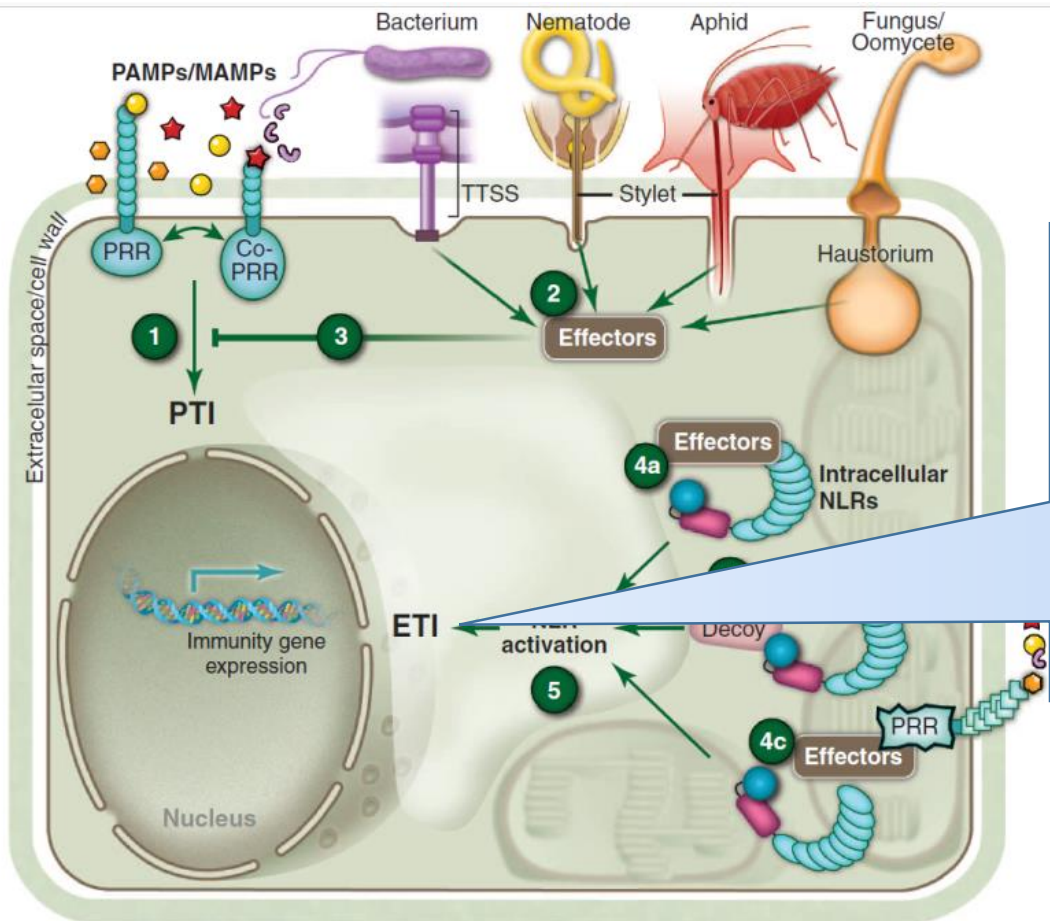
In 1987 a first Charentais line was proposed to growers

In 2000 hybrids with the Vat resistance produced by private companies are highly successful  
80% of the melon produced in South-East of France

**Since 2010 Vat resistance is jeopardized in South East of France**



# Erosion of Vat effect : which mechanism ?



Plant resistance to colonization by aphids

Resistance to virus when they are inoculated by *A. gossypii*

=

Indicating that ETI occurs independantly of aphid genetic background

# Vat resistance belongs to NB-LRR R gene family

## A specific *Aphis gossypii* effector gene

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  - Product of the Avirulent allele in aphid triggers ETI in Vat plants
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# Vat resistance belongs to NB-LRR R gene family

## A specific *Aphis gossypii* effector gene

- Syntaxe
  - Product of the Avirulent allele in aphid triggers ETI in Vat plants => **resistance to virus**
  - Product of the (a)Virulent does not trigger ETI in Vat plants => **no resistance to virus**
- The hypothesis
  - (a)Virulent allele is recessive
  - This recessive allele is express or not
  - The peptide is excreted in saliva

# Erosion of Vat effect : which mechanism ?

Clone triggering  
resistance to viruses

Clone not triggering  
resistance to viruses

High colonisation



*Melon Vat*

Low colonisation



# Erosion of Vat effect : which mechanism ?

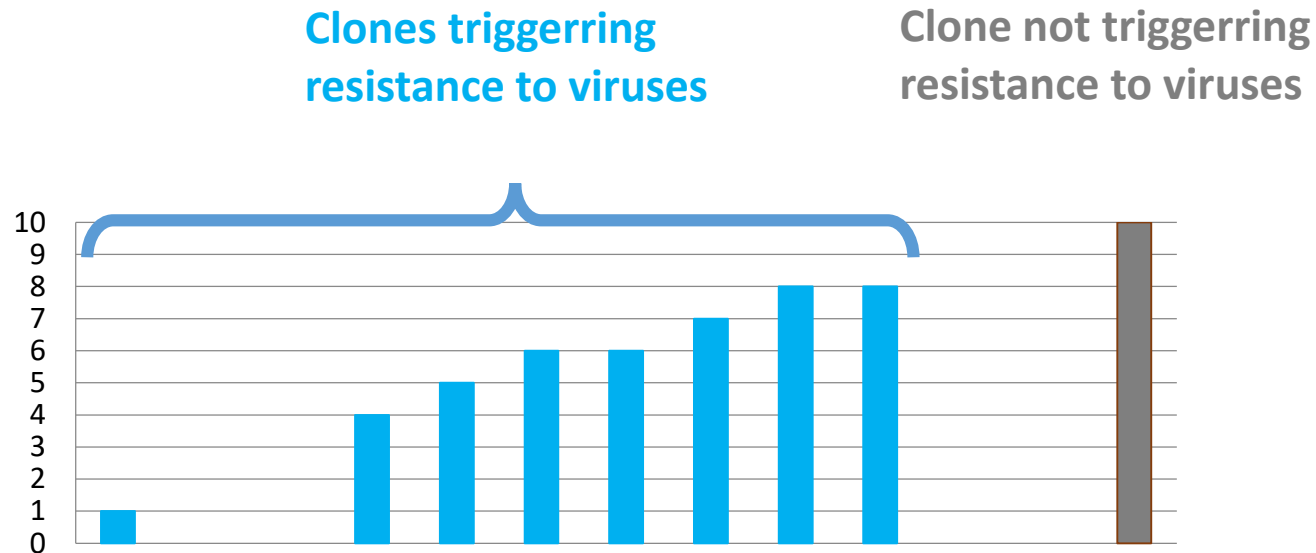
Some Avirulents clones are adapted to ETI

High colonisation



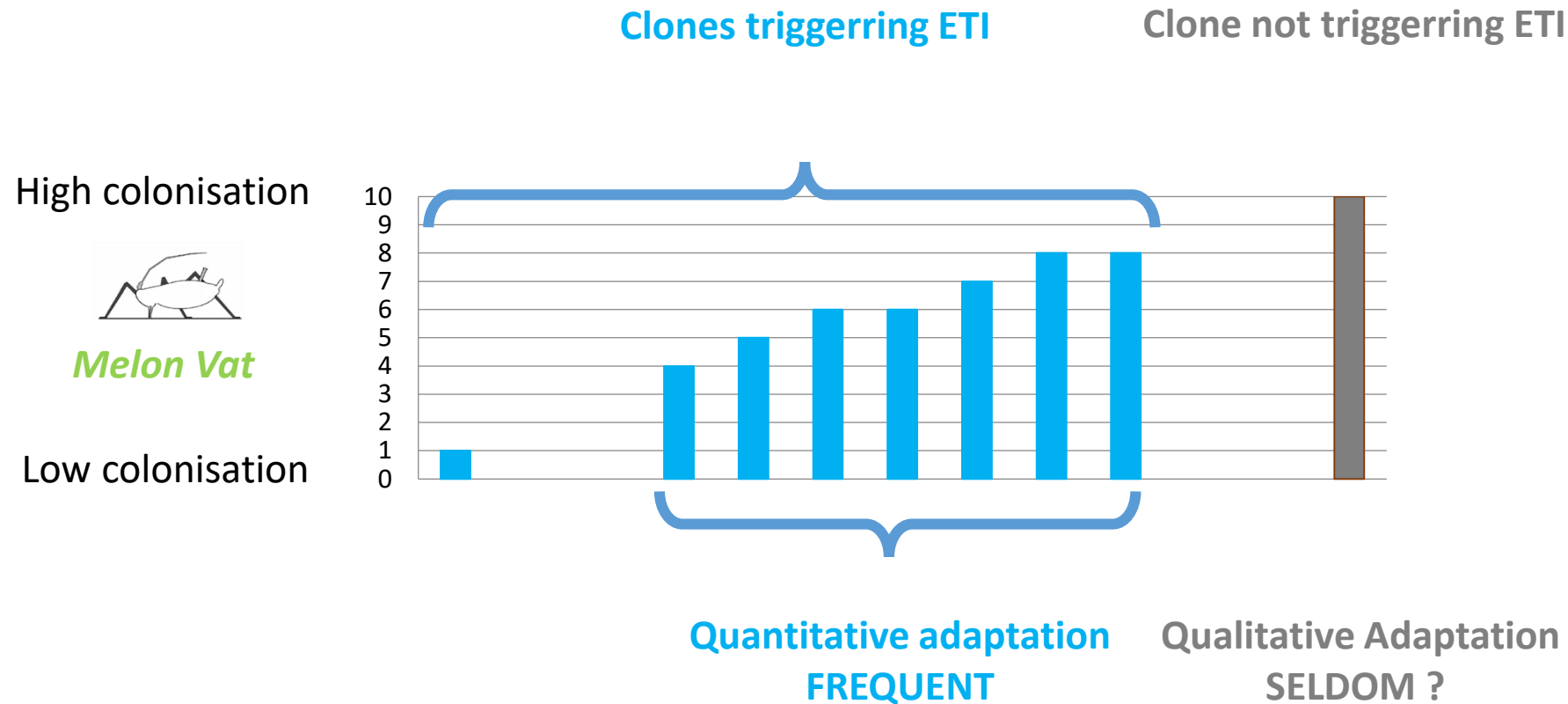
*Melon Vat*

Low colonisation



# Erosion of Vat effect : which mechanism ?

Some Avirulents clones are adapted to ETI



# Erosion of Vat effect : which mechanism ?

Southeastern France

Vat largely used by growers since 2000

> 3000 spring migrants aphids early collected in plots  
in melon fields since 2004

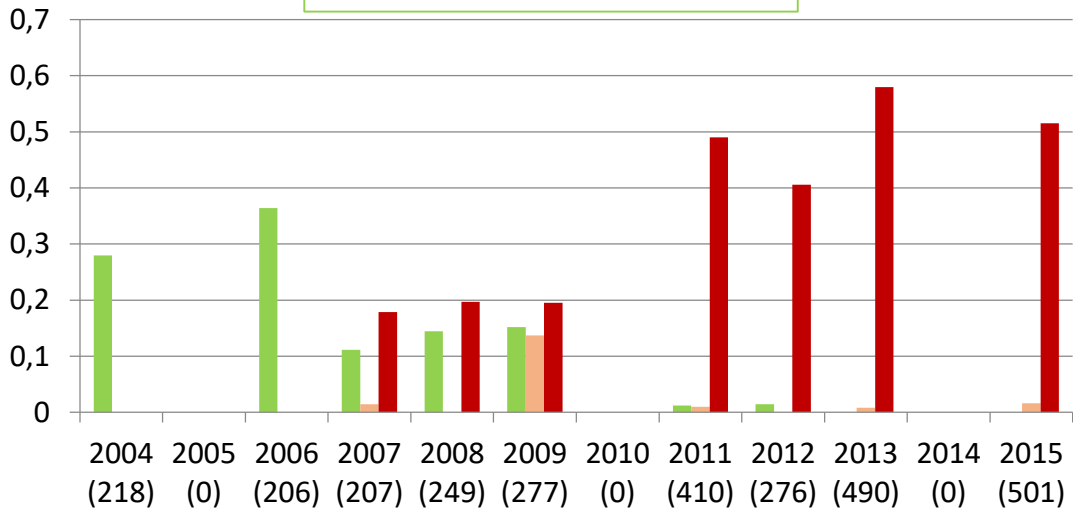
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3 most frequent clones





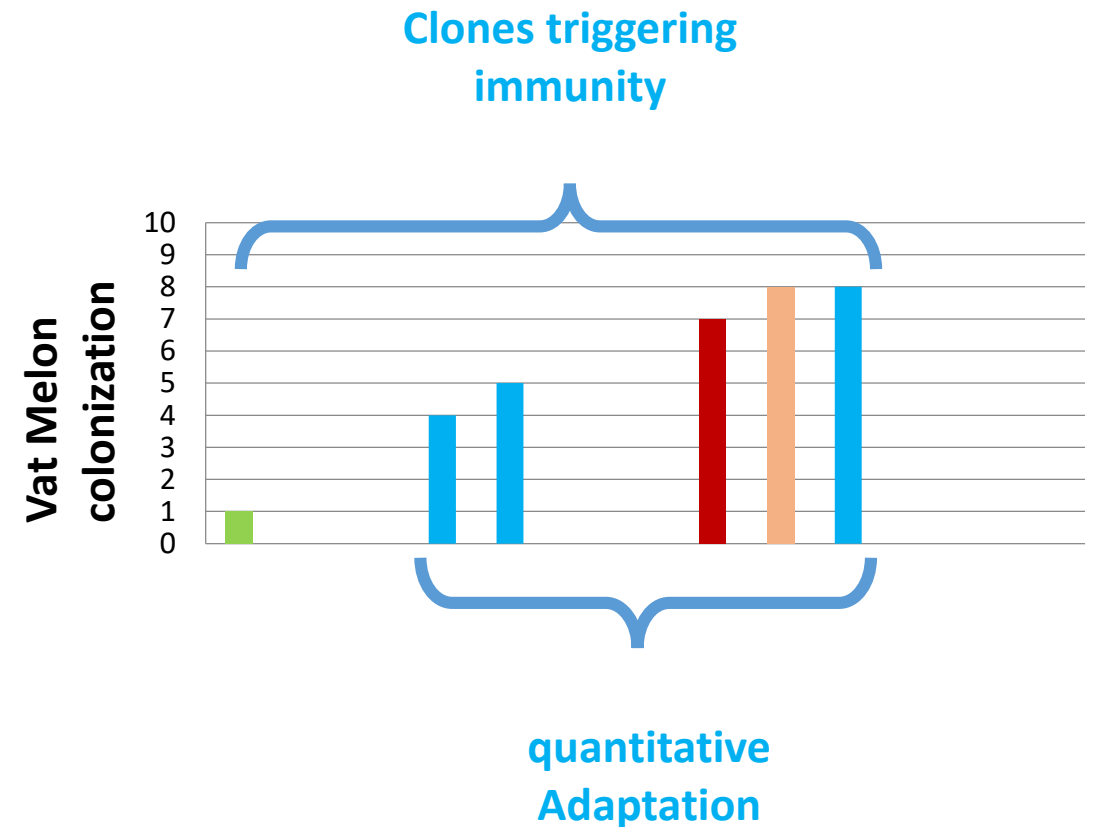
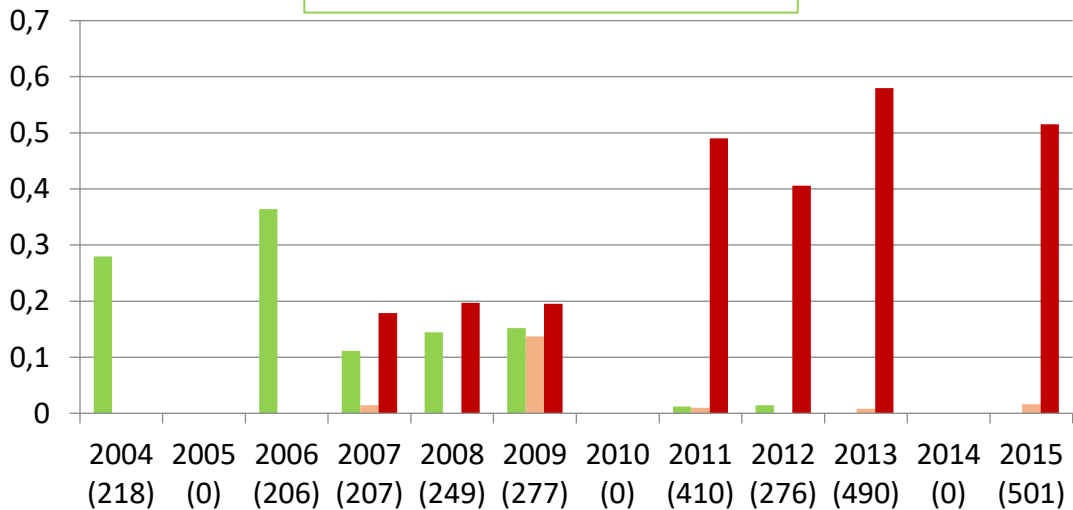
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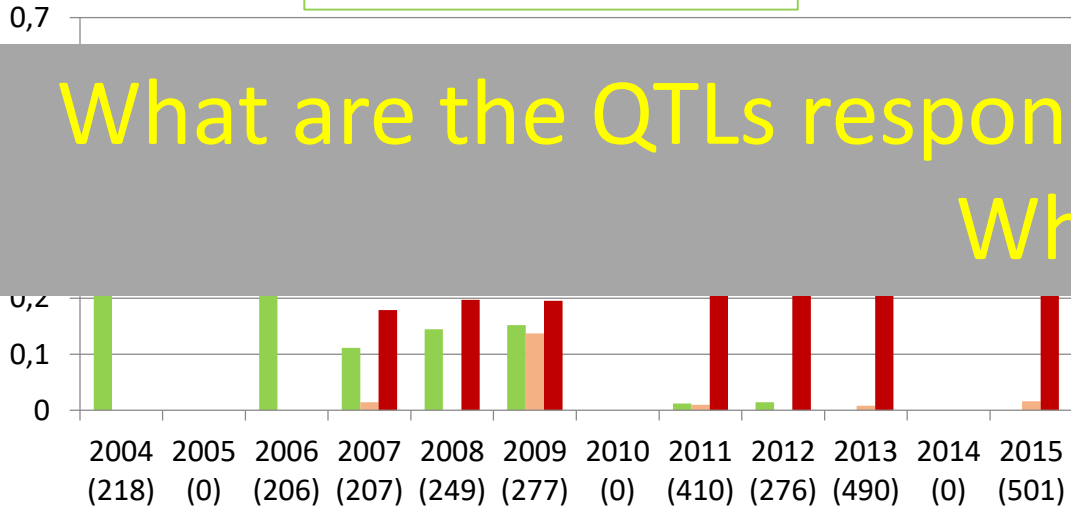
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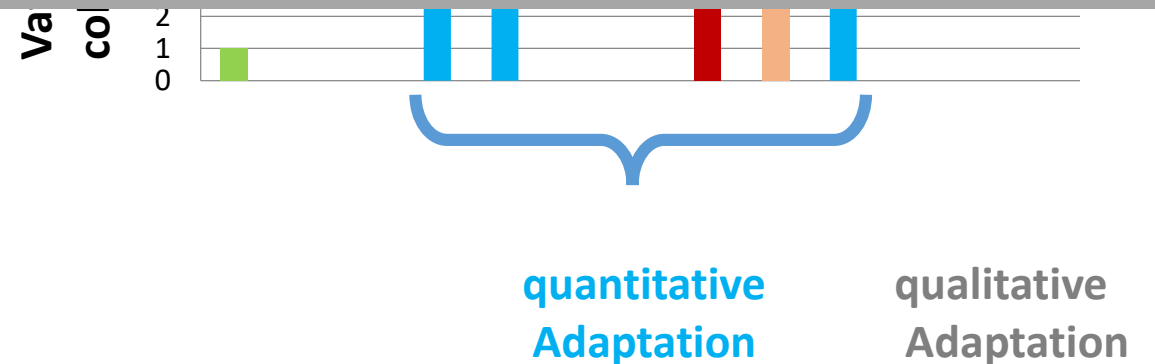
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Clones triggering immunity

What are the QTLs responsible of the quantitative adaptation ?  
Which strategy ?



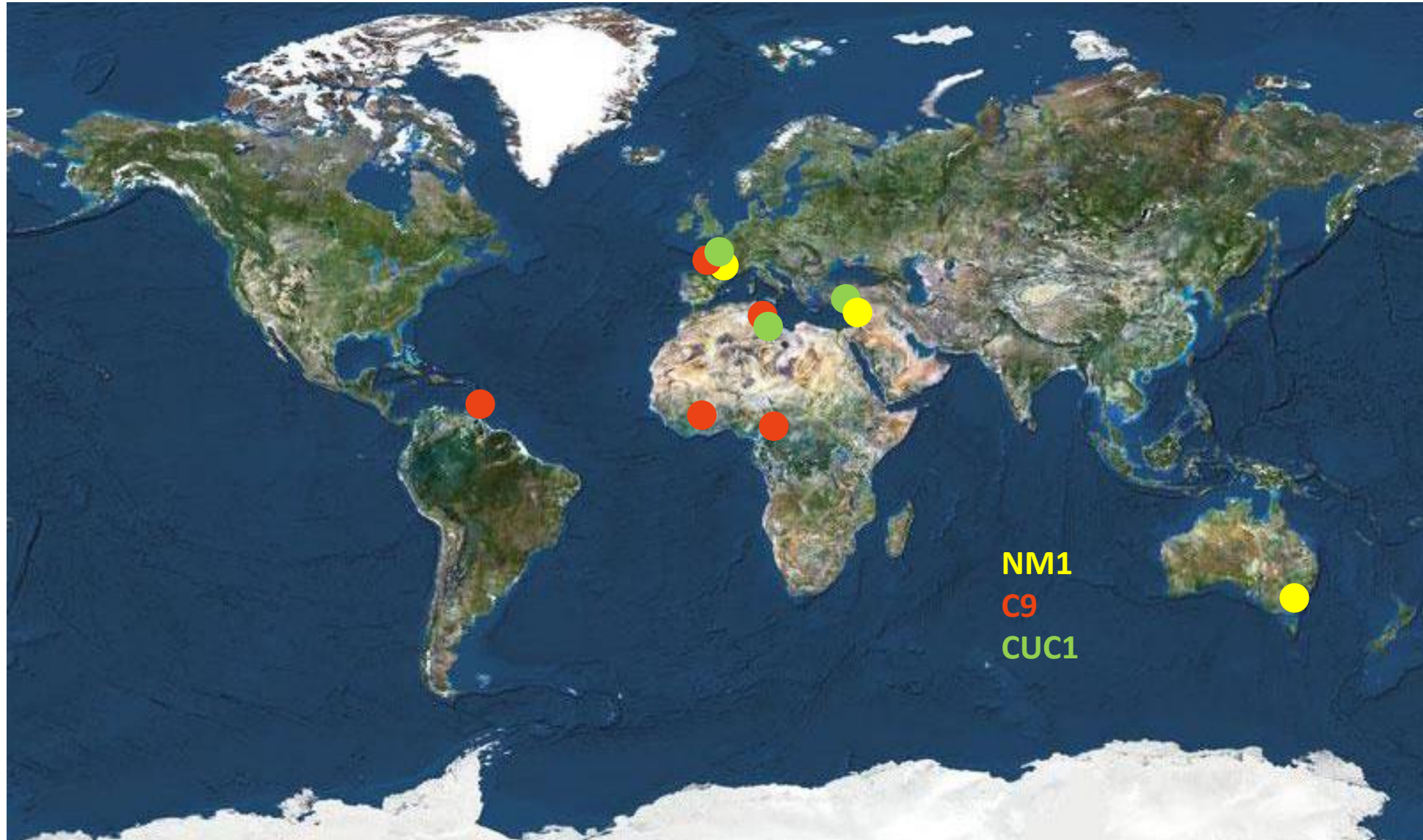
# non-Vat Resistance : a genetic background for Vat durability ?

Resistance to *Aphis gossypii* sensu stricto :

Observed in a melon line with a clone not triggering resistance to virus

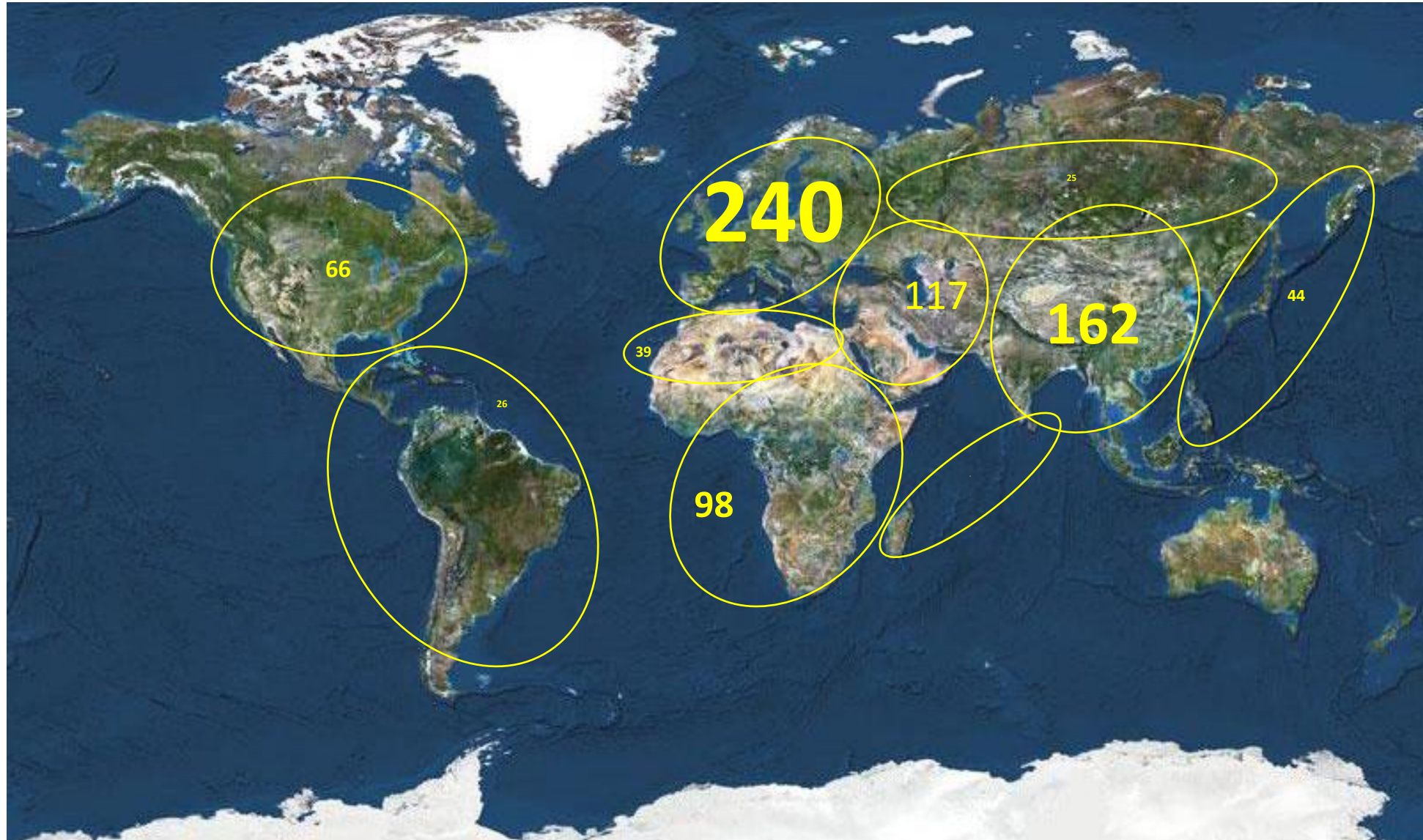
# non-Vat Resistance : un genetic background for Vat durability ?

3 cosmopolitan clones



# non-Vat Resistance : a genetic background for Vat durability ?

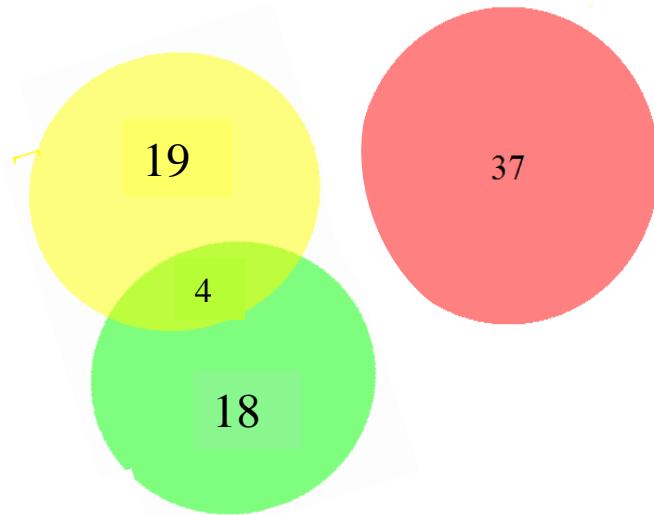
837 melon lines infested by at least one of these 3 clones



# non-Vat Resistance : un genetic background for Vat durability ?

## Number of resistant melon lines

Strong resistance

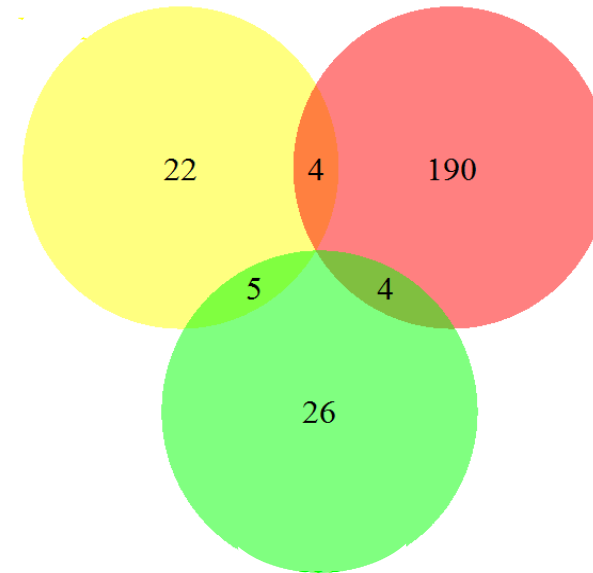


R NM1 5 %

R C9 8 %

R CUC1 6 %

Strong, partial or heterogenous resistance



R NM1 7 %

R C9 40 %

R CUC1 10 %

# non-Vat Resistance : a genetic background for Vat durability ?

One melon accession / one clone => partial resistance to aphids & no resistance to virus

What are the QTLs responsible of the quantitative resistance ?





# non-Vat Resistance : a genetic background for Vat durability ?

One melon accession/ one clone => partial resistance to aphids no resistance to virus

Production of 500 F2 ( from a cross R XS ) with private companies

DNA production for the 500 F2, Seeds production of 500 F3

Phenotypage of the F3 => bulk of DNA for the most resistant and the most susceptible F2 according to the F3 phenotype

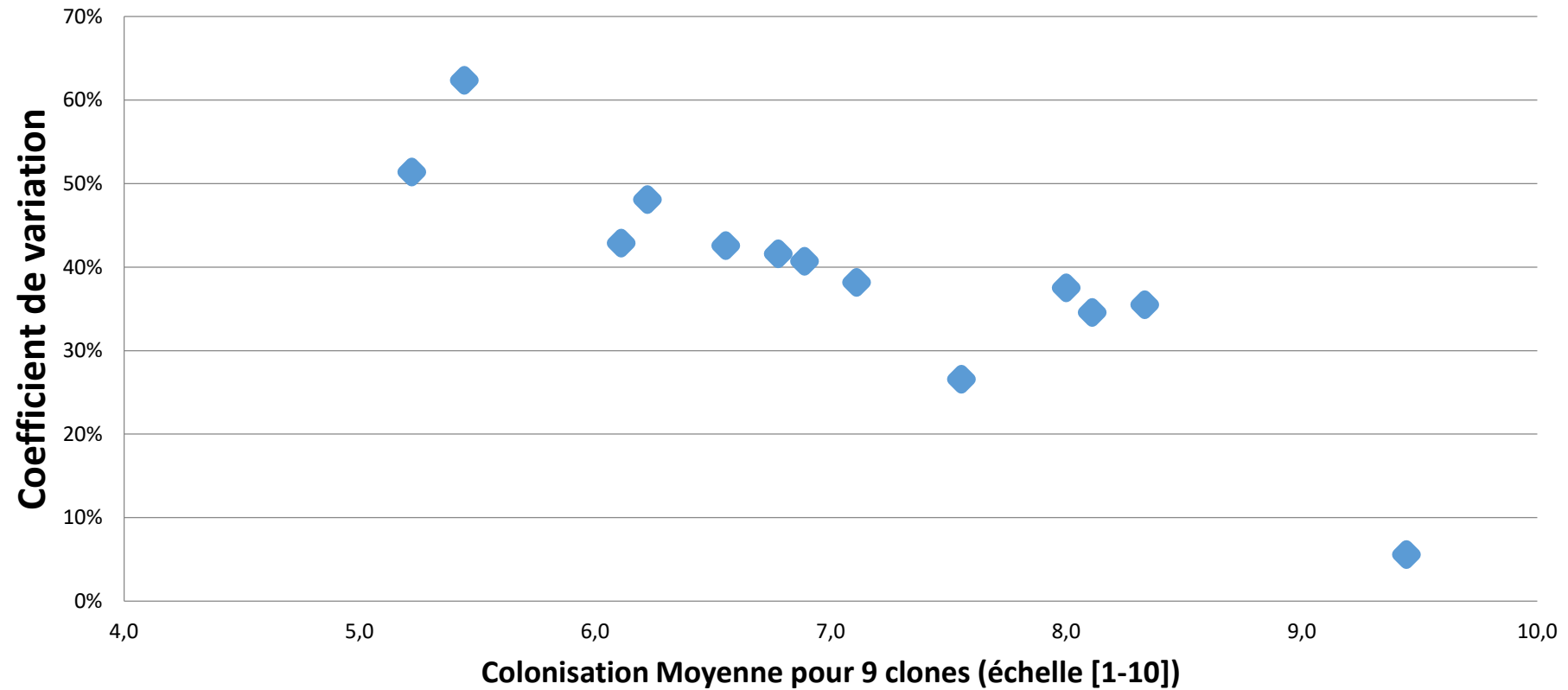
Illumina sequencing of each bulks

QTL seq analyses on the basis of a high quality genome for each R and S parents

# La résistance non-Vat : un background pour la durabilité de Vat

Certaines accessions expriment une résistance partielle plus stable

## 13 accessions



# La résistance non-Vat : un background pour la durabilité de Vat

Quelle(s) accession(s), Quel(s) clone(s) ?

Choisir un clone qui ne déclenche pas la résistance aux virus....

Génotypage d'une descendance F2/Phénotypage des F3, puis une stratégie QTLseq (CASDAR)

Genome Wide Analysis Study ? Analyse d'un grand nombre d'accessions (matrice SNP + phénotypage ) (H2020 ?)

Quel trait mesuré ?

Colonisation ?

Trait spécifique de durabilité (SMACH)

# La résistance Vat : famille des NB-LRR

## *Aphis gossypii* clones express an array of effectors

- Les hypothèses
    - Peptides dans la salive, spécifiques des races d'hôtes d'*Aphis gossypii*,
    - Orthologues existant chez les autres espèces,
  - Recherche de candidats
    - In silico => 52 candidats
    - Amplification de 10 cDNA chez 17 clones de différentes races d'hôte
  - Un 'mega-CDS' ancêtre pour tous les clones Cucurbitacées
    - 3 gènes sous sélection positive
- => Impliqués dans la spécialisation sur Cucurbitacées ?

