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”
2nd annual meeting of Japan-France PISI-net
Le Rheu
4-6th November 2019

GAFL – CBGP – Pathologie Végétale
EPGV/CNRGV/Gentyane/Genotul
BAPOA
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.

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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.

The first Vat genes characterized belong to the NB-LRR R gene family.
Vat resistance belongs to NB-LRR R gene family

The first Vat genes characterized

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
The first Vat genes characterized belong to NB-LRR R gene family.

**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

**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**

**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

1300 to 2900 pb

and SNPs ...
Vat resistance belongs to NB-LRR R gene family

35 Vat homologs from 20 melon lines

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

Probability to have non synonymous SNP when the codon is different,
Vat resistance belongs to NB-LRR R gene family

35 Vat homologs from 20 melon lines

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

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 LLR1 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

- 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 Vat resistance genes belong to a cluster
La résistance Vat : famille des NB-LRR

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Vat1, Vat2, Vat3 genes that have an ORF up to exon 4

Pseudo1-Vat sequences have early STOP codons or large insertion or large deletion

Genetic markers

Vat1 1405 aa
Vat2 1404 aa
Vat3 957 aa

Isofluorenol (like) genes

STK 483 aa
CGN 621 aa

Vat Rev 1380 aa
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 expresses 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.

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
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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
To summarize where we are today

Vat resistance belongs to NB-LRR R gene family

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
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

*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 independently of aphid genetic background
A specific *Aphis gossypii* effector gene

- **Syntax**
  - Product of the Avirulent allele in aphid triggers ETI in Vat plants
  - Product of the (a)Virulent does not trigger ETI 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

- 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

Low colonisation

Melon Vat
Erosion of Vat effect: which mechanism?

Some Avirulents clones are adapted to ETI.

Clones triggering resistance to viruses

Clone not triggering resistance to viruses

High colonisation

Low colonisation

Melon Vat
Erosion of Vat effect: which mechanism?

Some Avirulents clones are adapted to ETI

- Clones triggering ETI
- Clone not triggering ETI

**Melon Vat**

- High colonisation
- Low colonisation

**Quantitative adaptation**
- FREQUENT

**Qualitative Adaptation**
- SELDOM?
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
Erosion of Vat effect: which mechanism?

Southeastern France
Vat largely used by growers since 2000

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3 most frequent clones
Southeastern France
Vat largely used by growers since 2000

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

3 most frequent clones

Clones triggering immunity

quantitative Adaptation
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

3 most frequent clones

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

Clones triggering immunity

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

3 most frequent clones

Quantitative Adaptation
Qualitative Adaptation
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 heterogeneous 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?
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
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)
Aphis gossypii clones express an array of effectors

- Hypotheses
  - Peptides in saliva, specific to host races of Aphis gossypii,
  - Orthologues existing in other species,

- Candidate search
  - In silico => 52 candidates
  - Amplification of 10 cDNA in 17 clones of different host races

- 'Mega-CDS' ancestor for all Cucurbitaceae clones
  - 3 genes under positive selection

=> Involved in specialization on Cucurbitaceae?