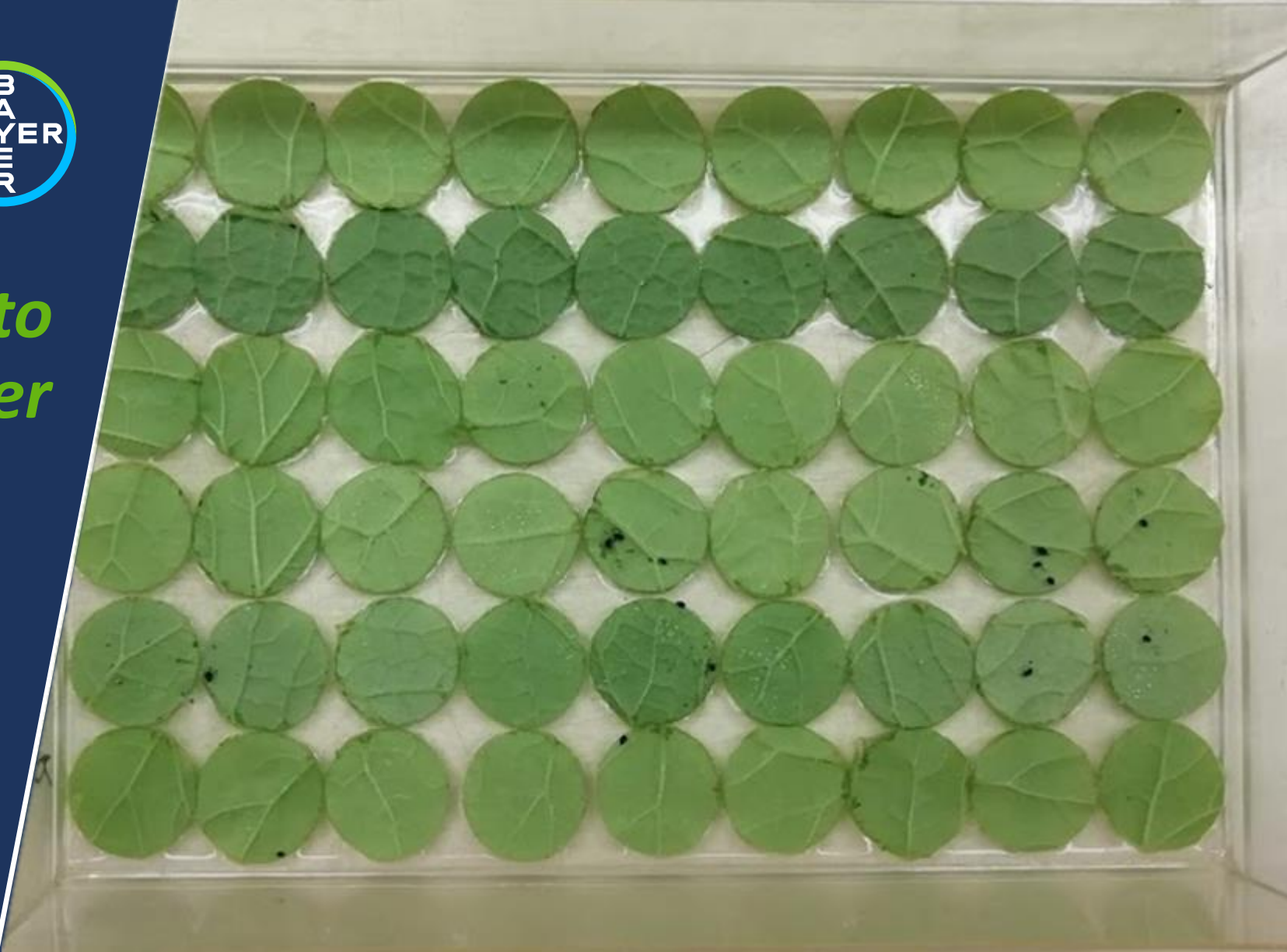


Cucurbit attractivity to Aphis gossypii is under polygenic control

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Strip plant-aphid interactions to provide to farmers crops less exposed to disease

Aphid is a major threat for cucurbit crops, due to herbivory and virus it transmit.

Resistance to *A. gossypii* and aphid transmitted viruses are currently studied in diverse plant species

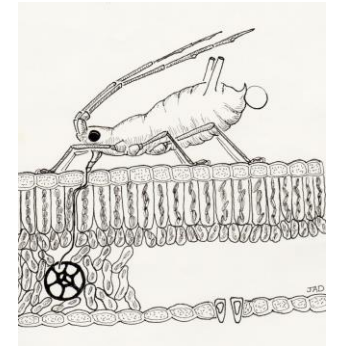
→ The Vat gene has been successfully introduced in melon cultivated varieties

The discovery of novel genetic lever of resistance could help with current resistances.

Breed for unattractive varieties to aphids could

- Delay herbivory and virus damages to adult plant stage
- Reduce aphid impact on yields
- Limit aphid selective pressure and prevent overcoming clone selection

Illustration: vineyard and rose bush



In addition, could give additional information for epidemiology open-field trial pooling several varieties.

The plant attractivity to aphid: a new index to quantify plant-aphid interaction



Historically:

Acceptation and colonization tests.

- Aphid restricted to one plant
- Entire plant



Dissect mechanisms of plant first virus infection:

The plant attractivity test.

- Aphid choose between 4 varieties
- Miniature test
- Non-destructive

The protocol conception: test a maximum of plant in a minimum of time and space

→ Inspired from powdery mildew testing protocol

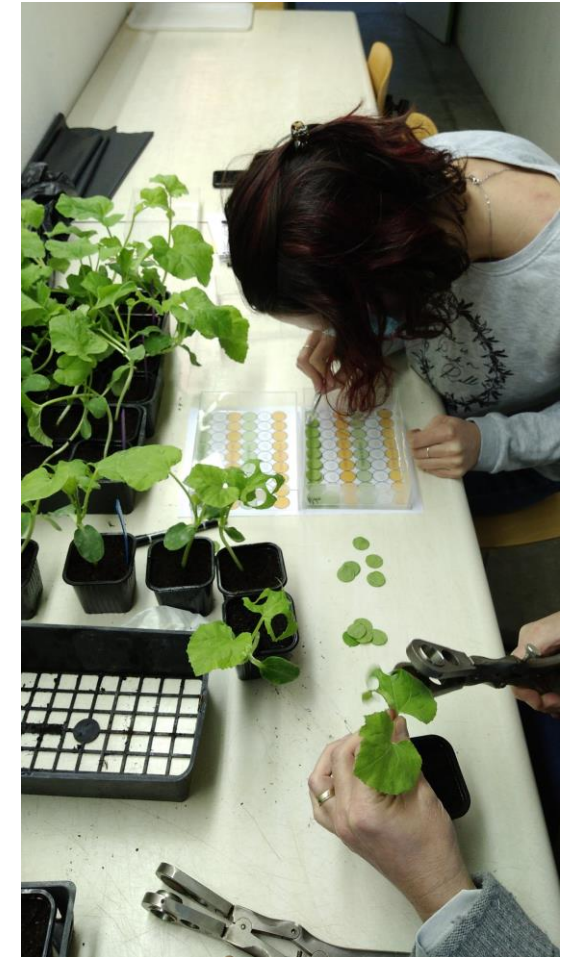
Box preparation:

- Agar media (4g/L of agar and 10g/L of mannitol)
- Random pairing of melon varieties within boxes (9 punches each)
- One susceptible melon control, Vedrantaïs (18 punches)
- One resistant melon control, Virgos (18 punches)
- 18 synchronized and apterous aphids

Aphids moved freely within the box, no edge effects.

Phenotyping 24h after the deposit :

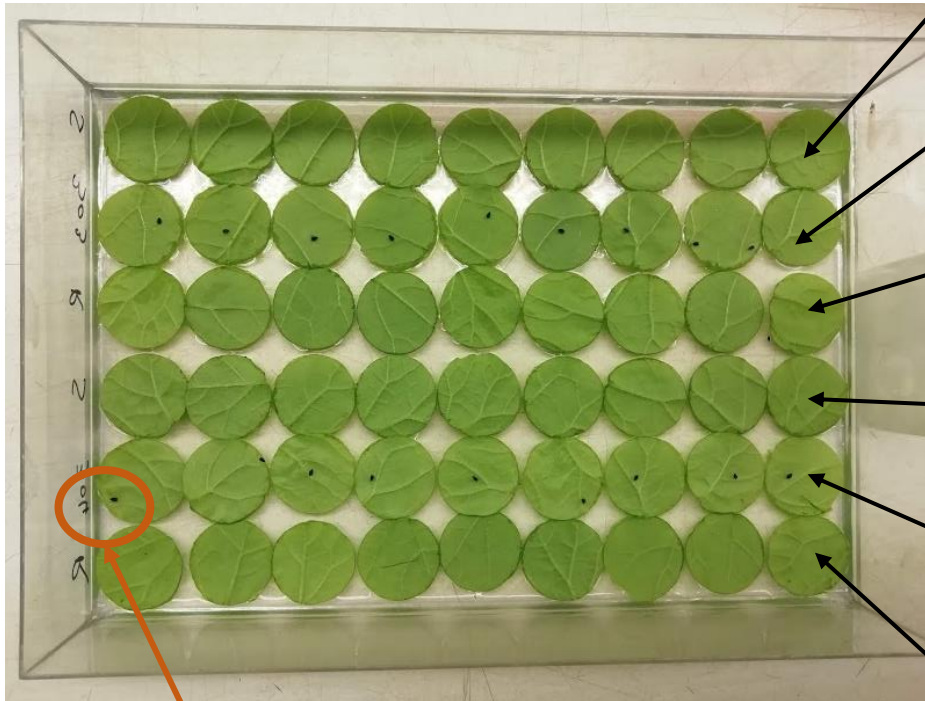
- Count of feeding aphids on each leave punch
- Idem for egg-laying



Preparation of the box

The protocol conception: An exemple of notation

Deposit



1 aphid per leaf variety to test
= **18 aphids** per box

Susceptible

1st variety

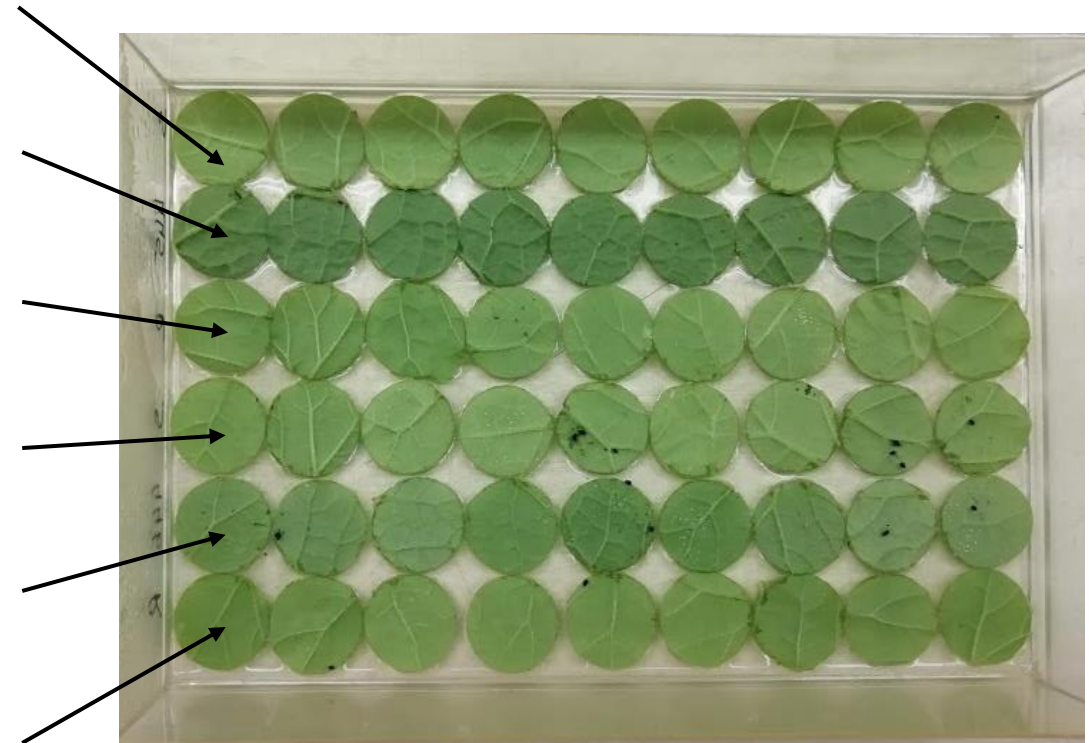
Resistant

Susceptible

2nd variety

Resistant

24h later



Susceptible = 10 aphids

Resistant = 2 aphids

1st variety = 1 aphid

2nd variety = 5 aphids

The attractivity, a statistical index: Simulation and distribution deviation

Two hypotheses:

- Aphids acted independently,
- Aphid's behaviours inside the box are statistically similar

$$P_g(x = n_g) = (p_{gi})^{n_g}$$

$$p_{gi} = \frac{a_{ig}}{\sum a_{i,b}}$$

5000 simulations

Theory: Aphid will feed on the most attractive Genotype

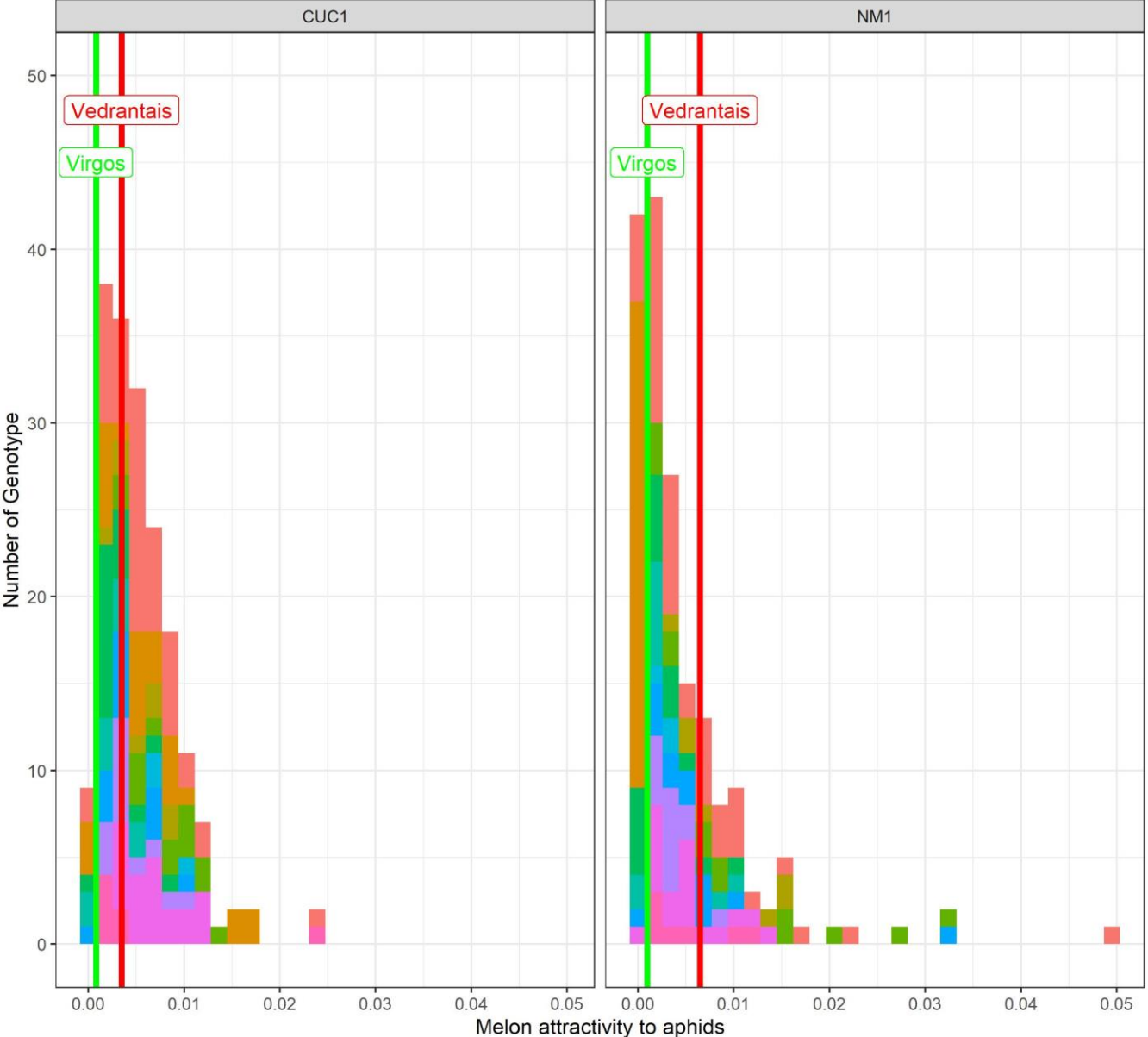
- More or less aphid than controls? (similar in each box)
- More or less aphids than the other variety

Attractivity ranged from 0 to 1.

- 0 = non-attractive variety for aphids
- 1 = plant super attractive for aphids

Attractivity distribution

Comparison of melon attractivity to two aphid clones: CUC1 and NM1



- Controls are well estimated
- Phenotypic variation observed
- More variance for NM1
... but only one repetition

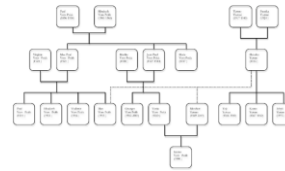
Application: map attractiveness QTLs by GWAS

Genome Wide Association Studies (GWAS) is a statistical method associating a phenotype variation to a single genetic variation.

1	-1	-1	1	-1	-1	-1	0	-1	0	1	-1	0
-1	-1	1	1	1	1	1	1	1	1	1	-1	1
1	-1	-1	1	-1	1	1	1	0	1	-1	1	-1
1	1	1	1	1	1	1	1	1	1	1	1	1
1	-1	-1	1	0	-1	-1	-1	-1	0	1	-1	0
1	-1	-1	-1	-1	-1	-1	0	0	1	0	1	-1
1	-1	-1	0	1	-1	-1	0	-1	1	1	-1	1

Phenotypic variation, Y , attractiveness:

- 2 aphid clones, CUC1 and NM1 (Vat for proof of concept)
- Respectively 3 and 1 repetition



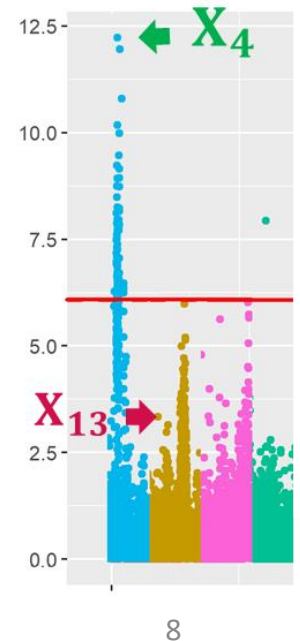
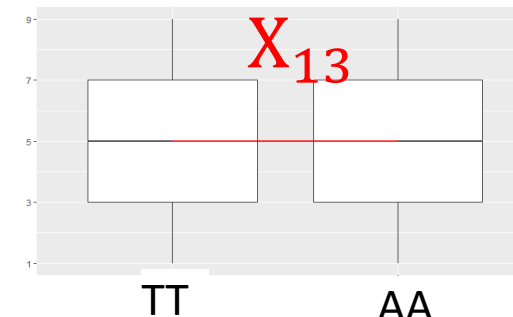
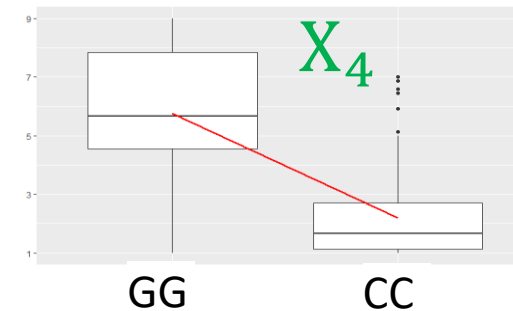
$$Y = \mu + X_M \beta + X_W v + Z_G g + e, \quad g \rightarrow N(0, \sigma_K^2 I)$$

Genetic variation, Y , Single Nucleotide Polymorphism (SNP) :

- 150 melon varieties from a collection of diversity
- 45k SNP (short-reads sequencing) distributed on 12 chr

Phenotypic variation, X_M , attractiveness:

- 2 aphid clones, CUC1 and NM1 (Vat for proof of concept)
- Respectively 3 and 1 repetition



Multiloci GWAS map several QTL with small effects in melon for CUC1 and NM1



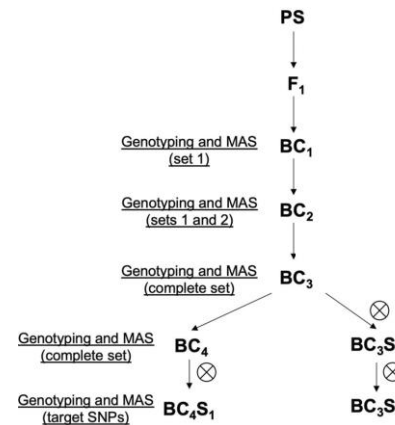
→ Heritability is improved with repetitions

→ Not enough statistical power: more repetition or local score from Fariello *et al.* 2017

How can we use GWAS results of melon attractivity to aphids?

1. QTL mapping by segregant population to enable marker assisted selection
 → pyramid genetic levers in cultivated varieties to improve the resistance durability

MEL_1779 x Zo_ME_0673_ct

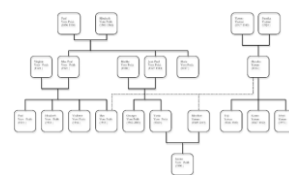


2. Use attractivity as a correcting variable in the context of aphid transmitted virus studies



1	-1	-1	-1	1	-1	-1	-1	0	-1	0	1	-1	0
-1	-1	1	1	1	1	1	1	1	1	1	1	-1	1
1	-1	-1	1	1	-1	1	1	1	0	1	-1	1	-1
1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	-1	-1	-1	0	-1	-1	-1	-1	0	1	-1	0	0
1	-1	-1	-1	1	-1	-1	-1	0	0	1	0	-1	-1
1	-1	-1	0	1	-1	-1	-1	0	0	-1	1	-1	1

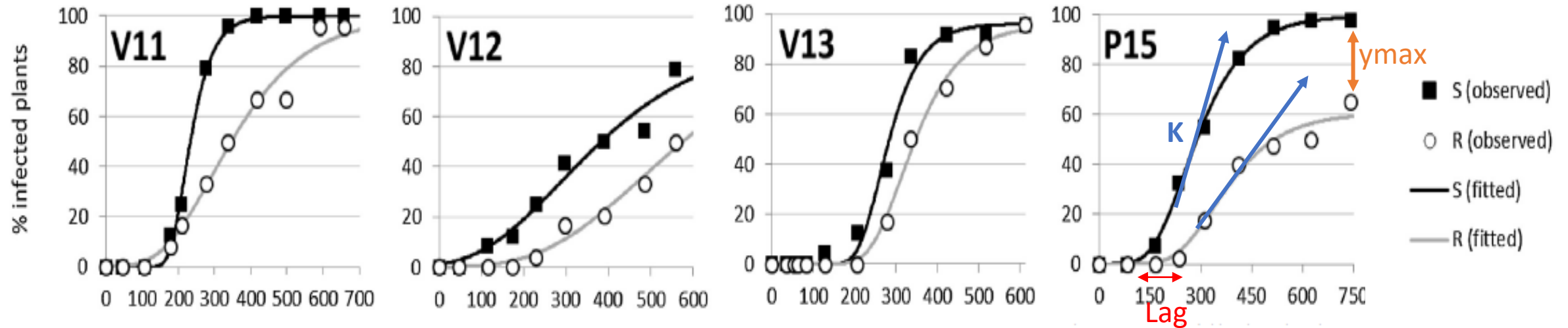
X_4 X_{13}



$$Y = \mu + X_M \beta + X_W v + Z_G g + X_A a + e, \quad g \rightarrow N(0, \sigma_K^2 I)$$

Aphid attractivity and virus resistance the example of Vat

CABYV disease progress in melon crop, adapted from *Schœny et al., 2017*



Gompertz curves are estimated from three parameters:

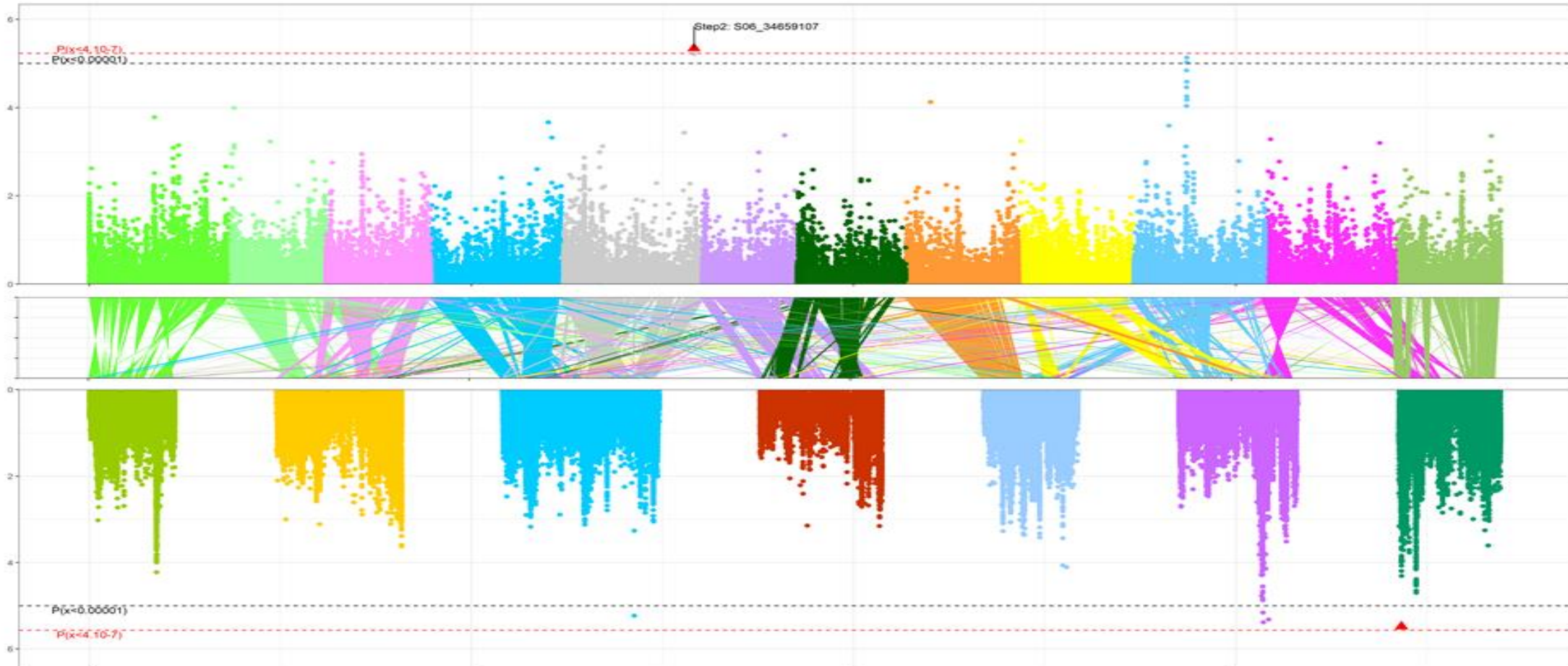
- Lag = Infection delay
- K = Infection speed
- Y_{max} = Maximum of infection

In V12 and P15, the difference of infection delay result in different maximum of infection.

→ Less attractive plants could be less impacted by virus disease

→ Vat seems to be a component of attractivity (infection delay) so it should be mapped with this phenotype

Potential synteny between melon and cucumber for attractivity to CUC1



→ Not enough statistical power: more repetition or local score from Fariello et al. 2017

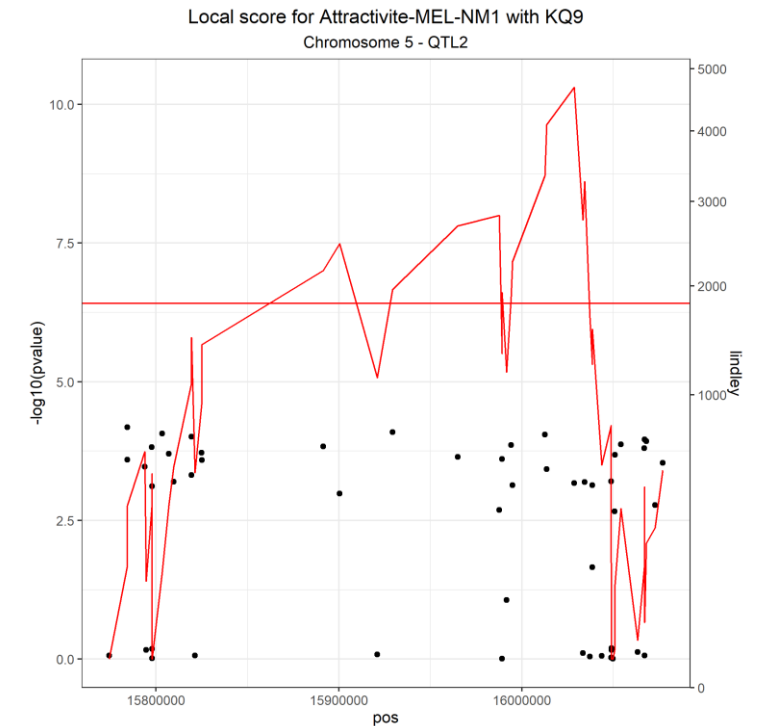
What is next?

Improve phenotyping protocol:

- Is the mix of volatile within the box a limit to our protocol?
- For statistical purpose, we hypothesized that aphid acted independently
→ Does a gregarious instinct exist in aphids?
- Test with other aphid clones/species to represent open field diversity

Improve mapping approach:

- Increase the number of repetition to improve the experimental heritability and favor QTL mapping
- Re-sequence some melon lines to improve the SNP matrix quality
- Once we have a higher SNP density than before, test local score approach



Take home message:

- **Attractivity is a promising trait resulting from choice test**
- **Its statistical power is highly dependent to repetition number**
- **Several QTL successfully map**
- **Including one close to Vat for NM1**
- **Maybe syntenic QTLs within cucurbits**
- **Many different ways to improve and test our approach**

Test our theory in open field condition:

- Find more attractive cucurbit species
- Compare attractivity QTLs with virus infection delay QTLs in open field condition

INRAE



Thank you for your attention!

Do you have any question?

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