

Genetic, molecular and physiological characterization of the *HaOr7* resistance gene to *Orobanche cumana* in sunflower.



Objectives of my work

The only *HaOr* gene
already mapped

Major resistance genes: *Or* genes

HaOr5 → race E
HaOr7 → race F

What is the role of the *HaOr7* gene for the resistance to race F?

Map-based cloning
of the gene

Functional
characterization

Physiological
characterization

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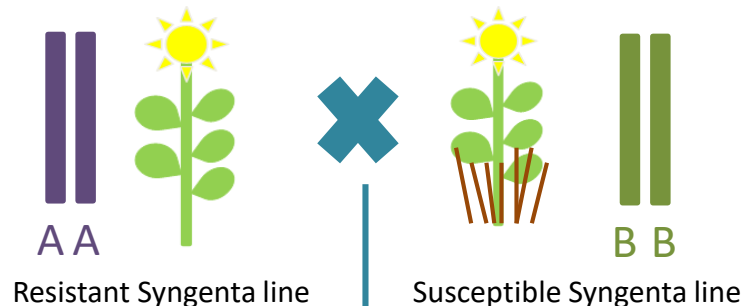
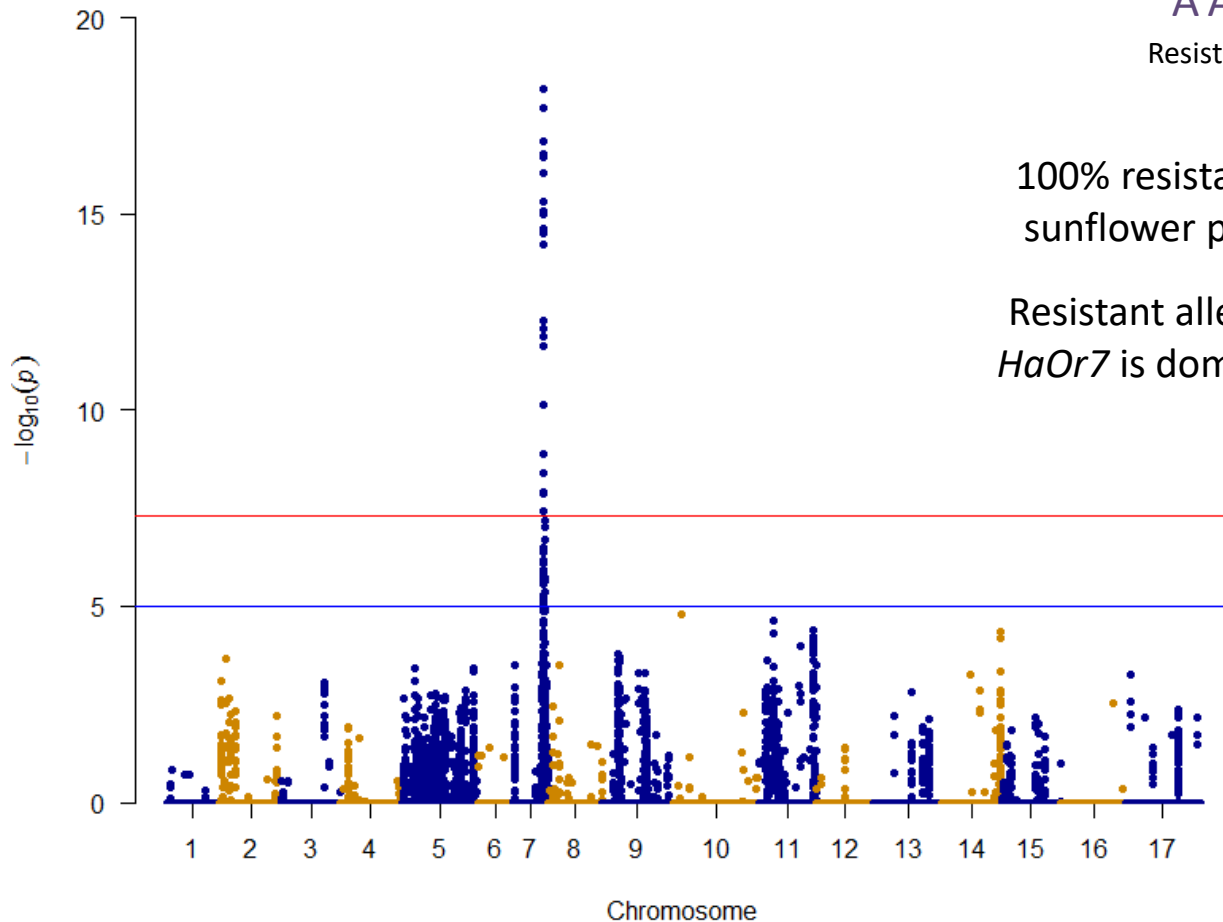
Mapping

Map-based cloning of the gene

GWAS on 55 lines :

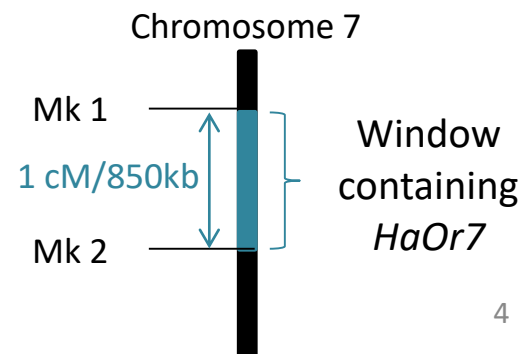
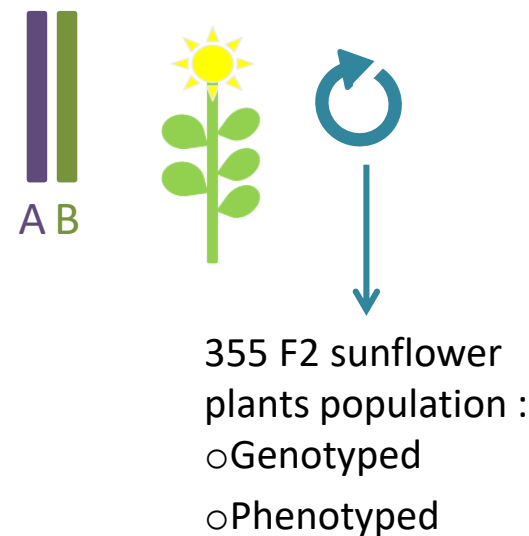
- 21 *HaOr7*- susceptible lines
 - 34 *HaOr7*+ resistant lines
- 600k SNPs AXIOM chip

With the same origin !



100% resistant F1 sunflower plants

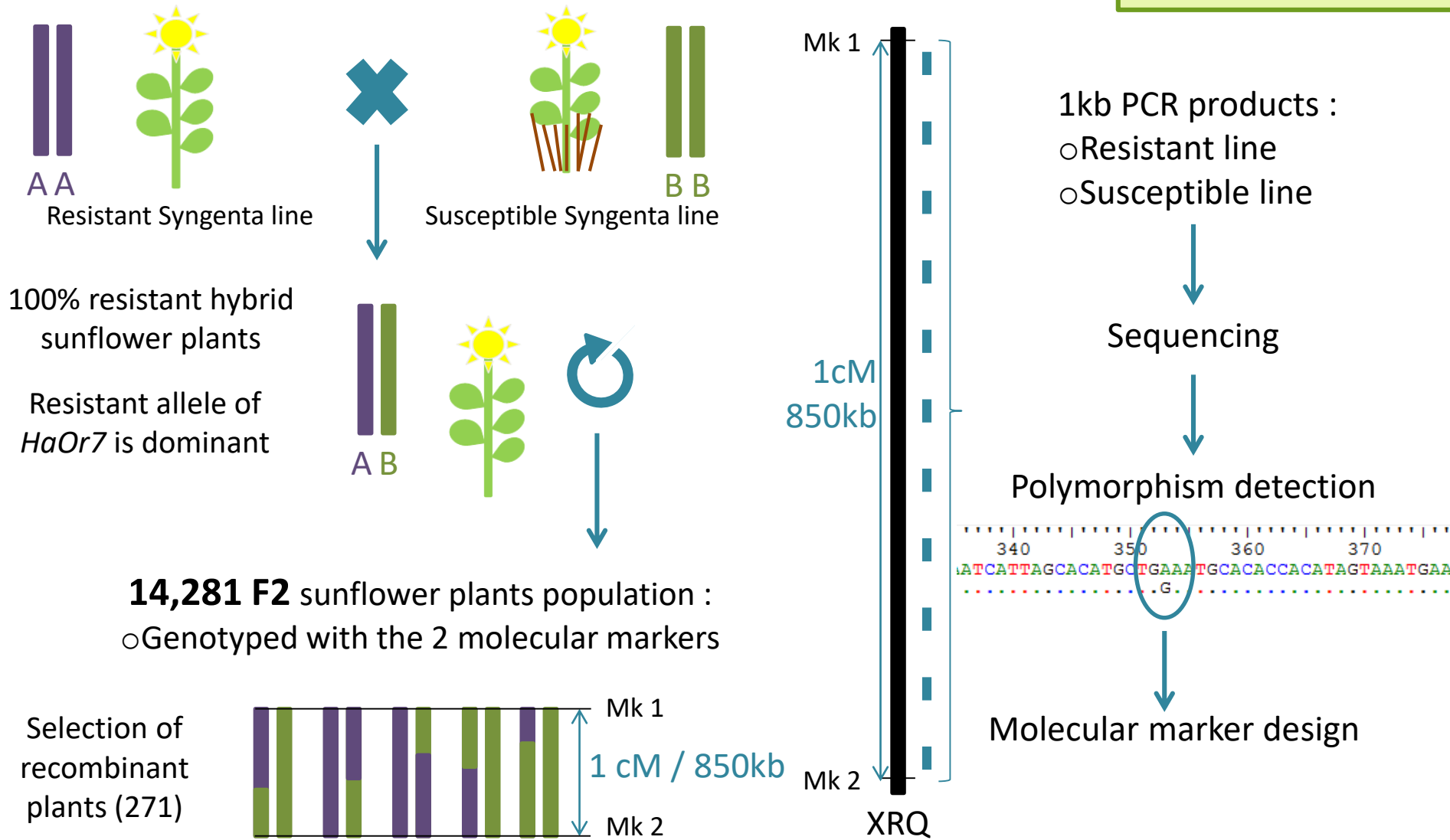
Resistant allele of *HaOr7* is dominant



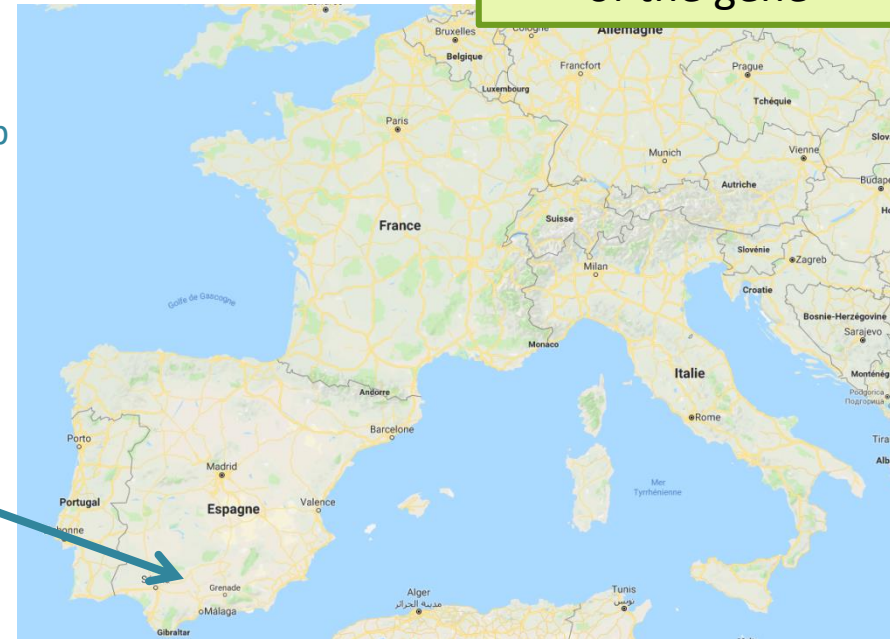
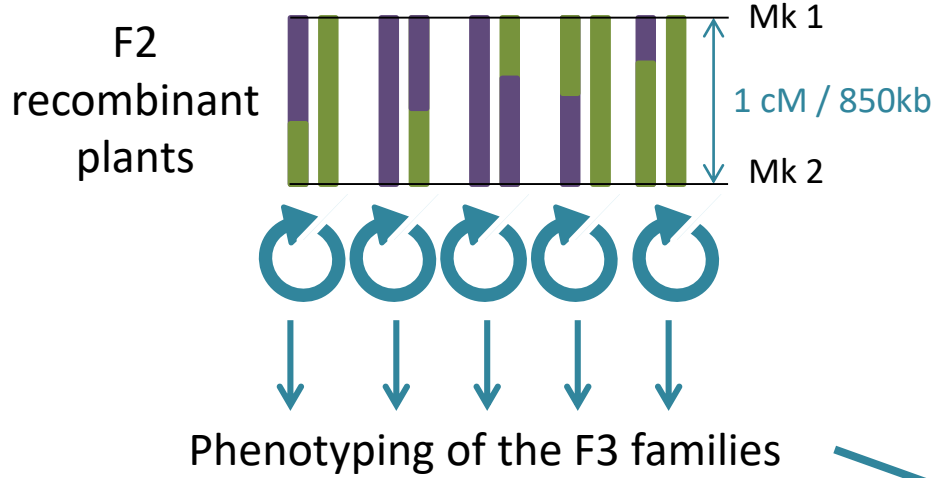
We located *HaOr7* in a 1cM/850kb window on chromosome 7

Fine mapping

Map-based cloning of the gene



We found 271 recombinant plants in the 1cM window, genotyped with 91 molecular markers



In a field in South of Spain (Ecija) 2016

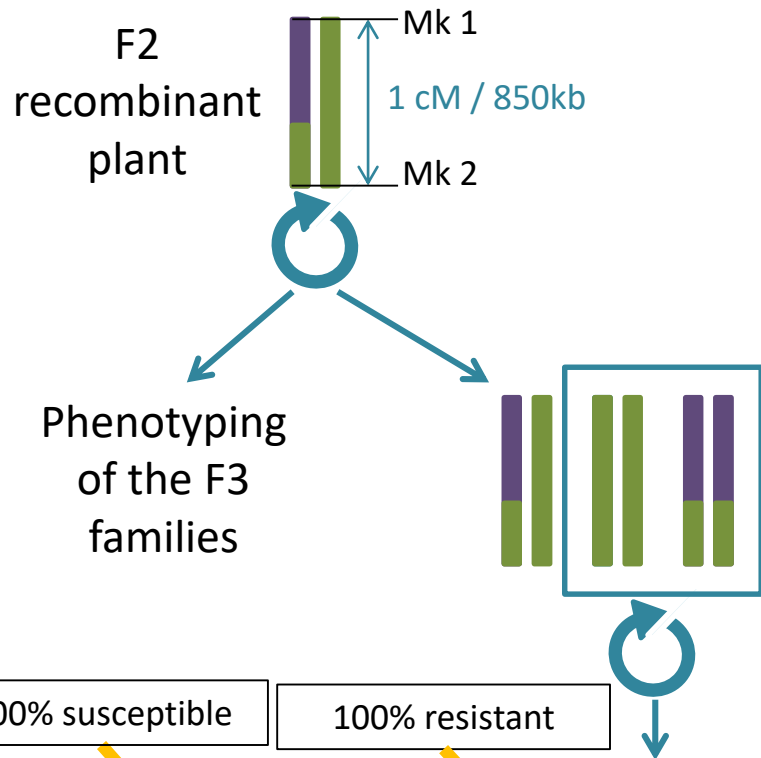
100% susceptible

100% resistant

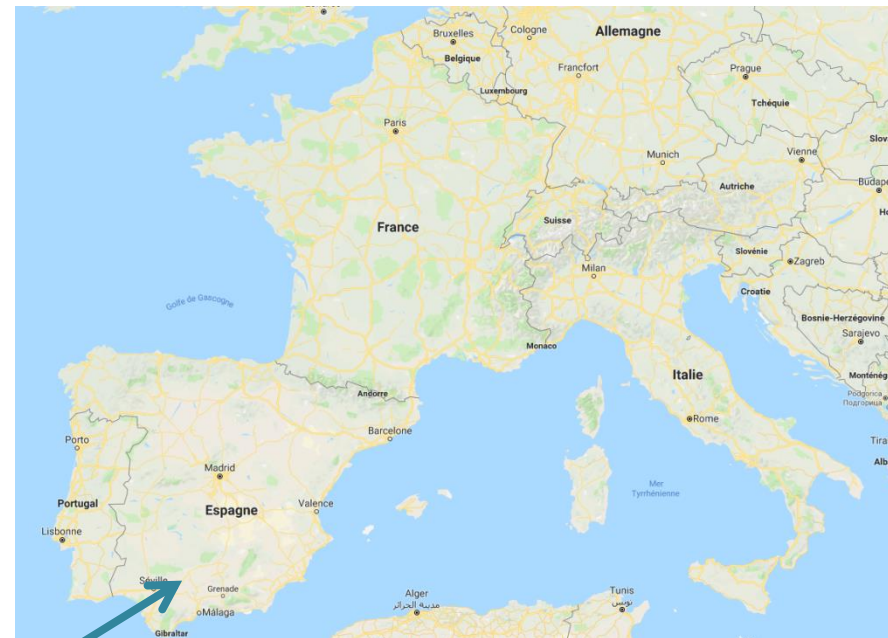


Expected ratio :
 $\frac{3}{4}$ resistant
 $\frac{1}{4}$ susceptible

We phenotyped almost all the 271 F3 families



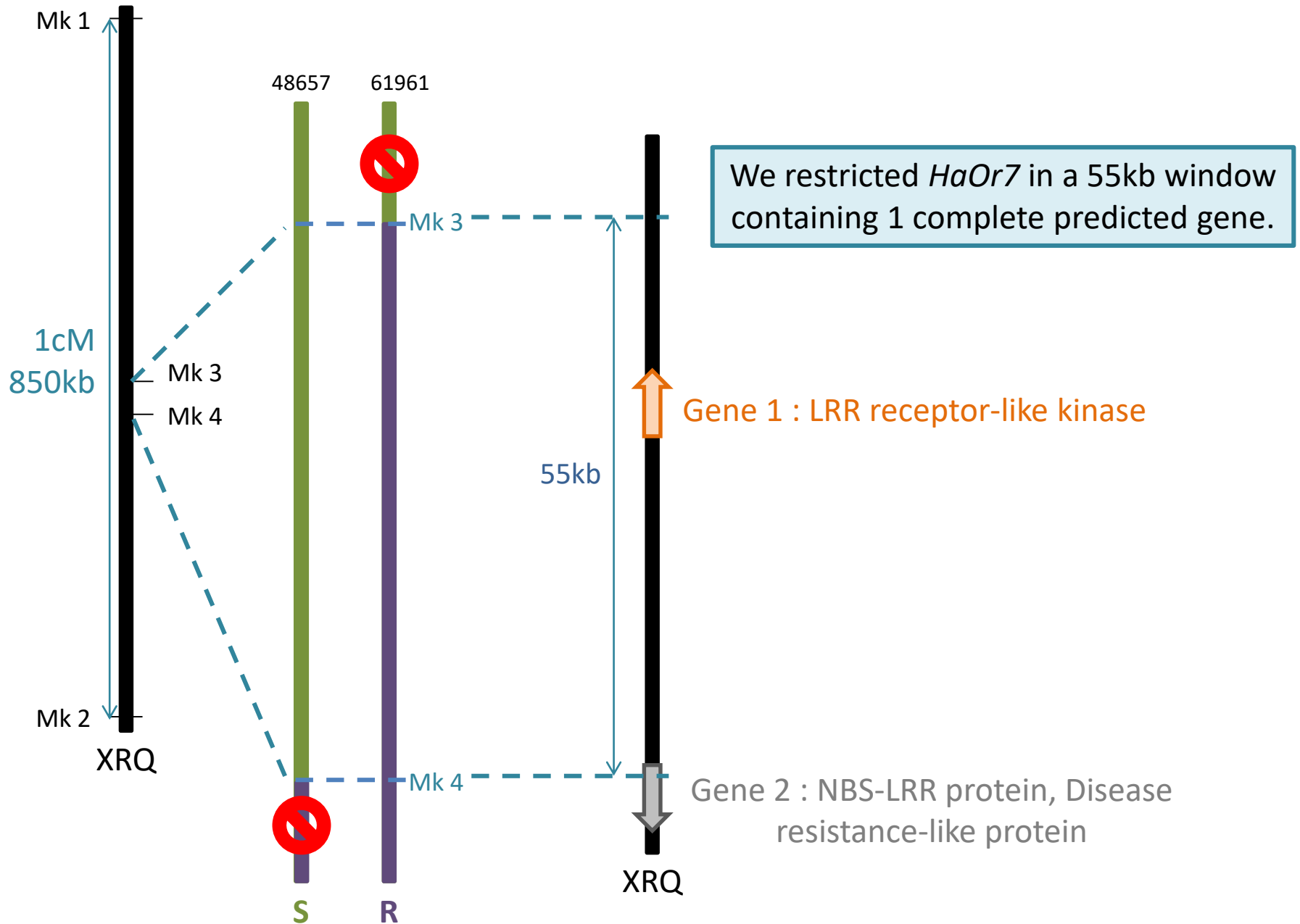
Genotyping of some F3 individuals :
selection of the fixed ones



In the same field in Ecija (2017)



We confirmed the F3 phenotype
with the fixed F4 phenotypes



Genomic data

Map-based cloning
of the gene

CNRGV created BAC clone libraries :

- Susceptible line
- Resistant line



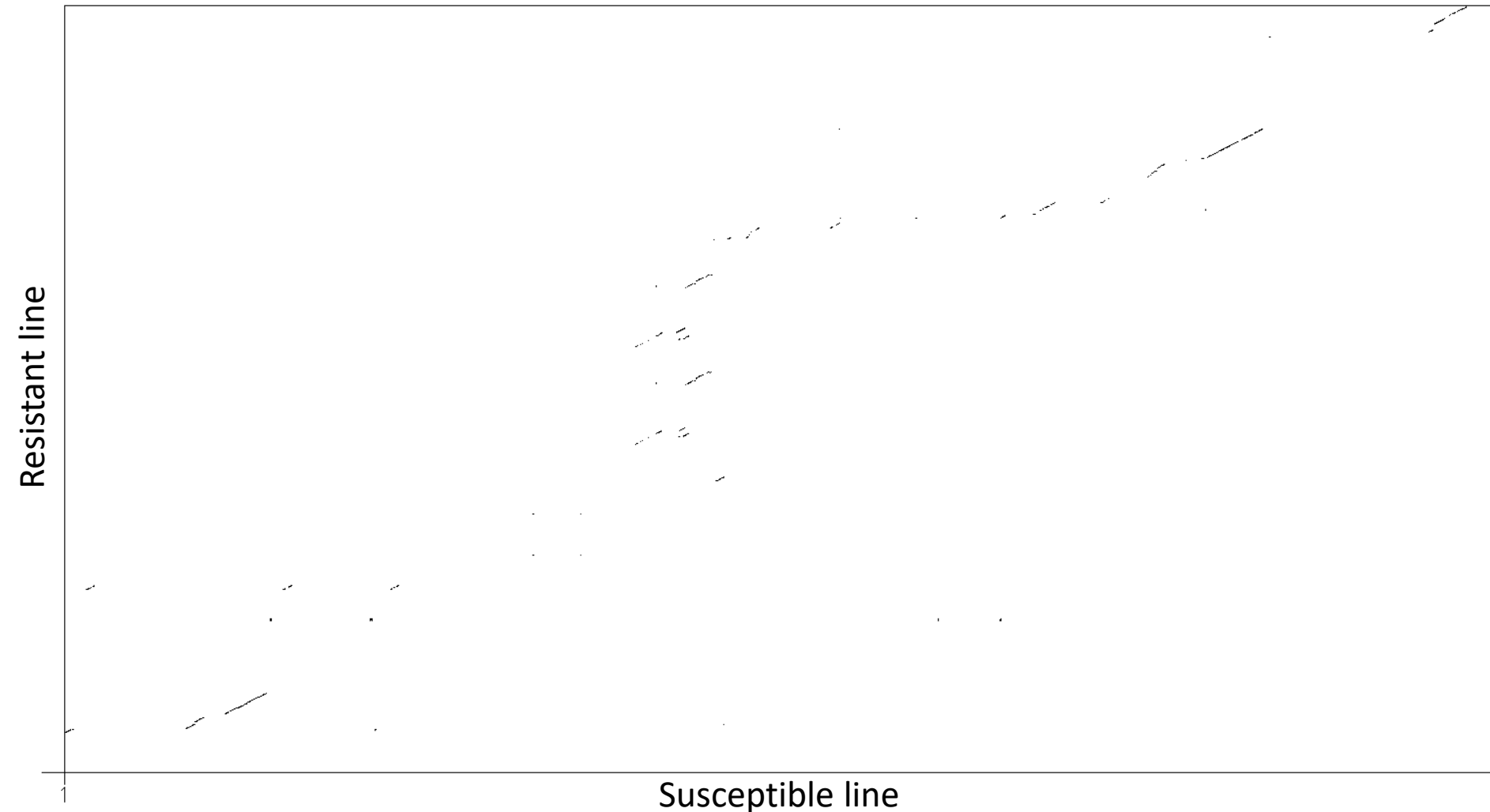
Screened with
primers designed
in the region

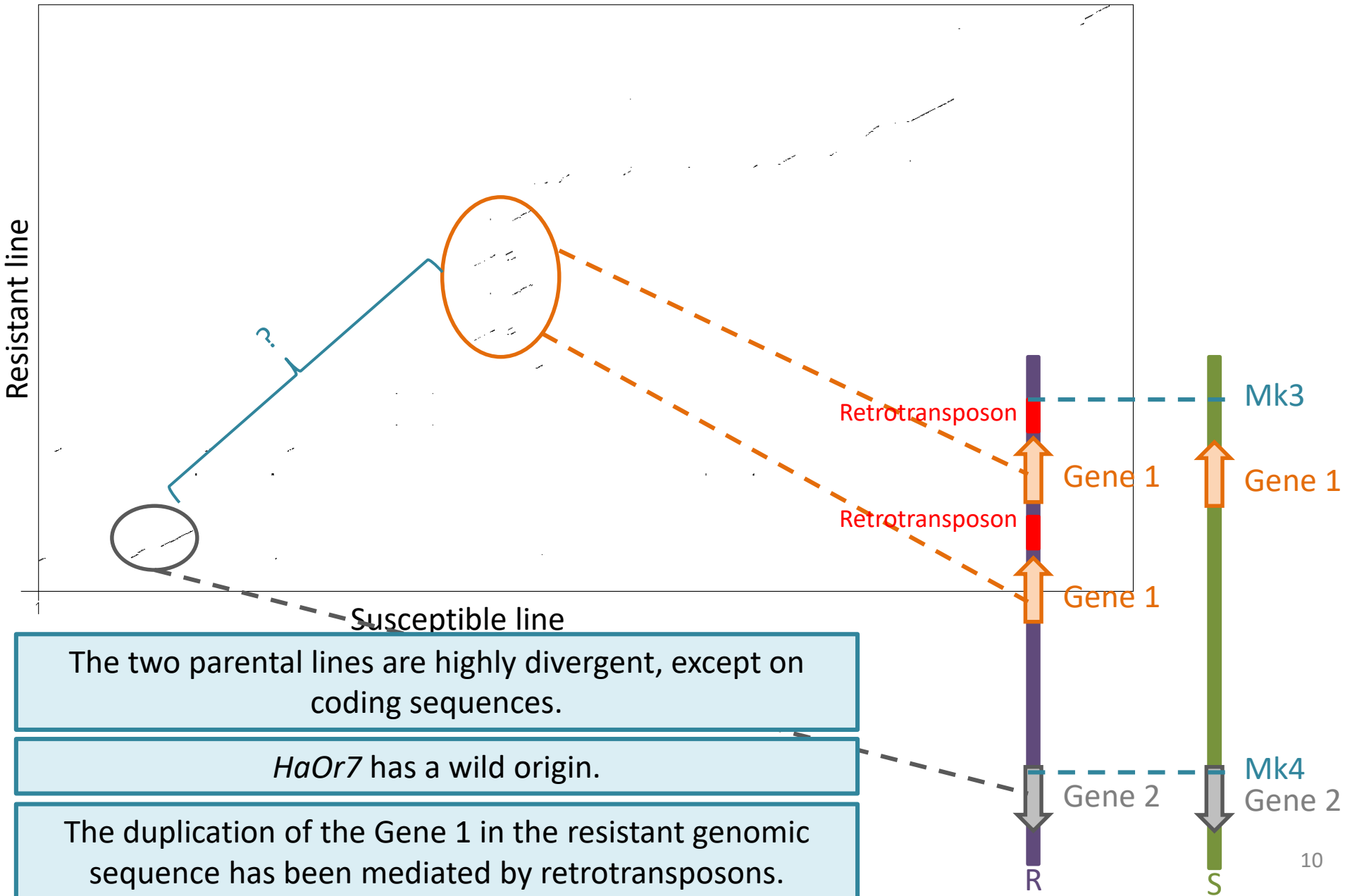


We obtained BAC
clones for the 2
parental lines



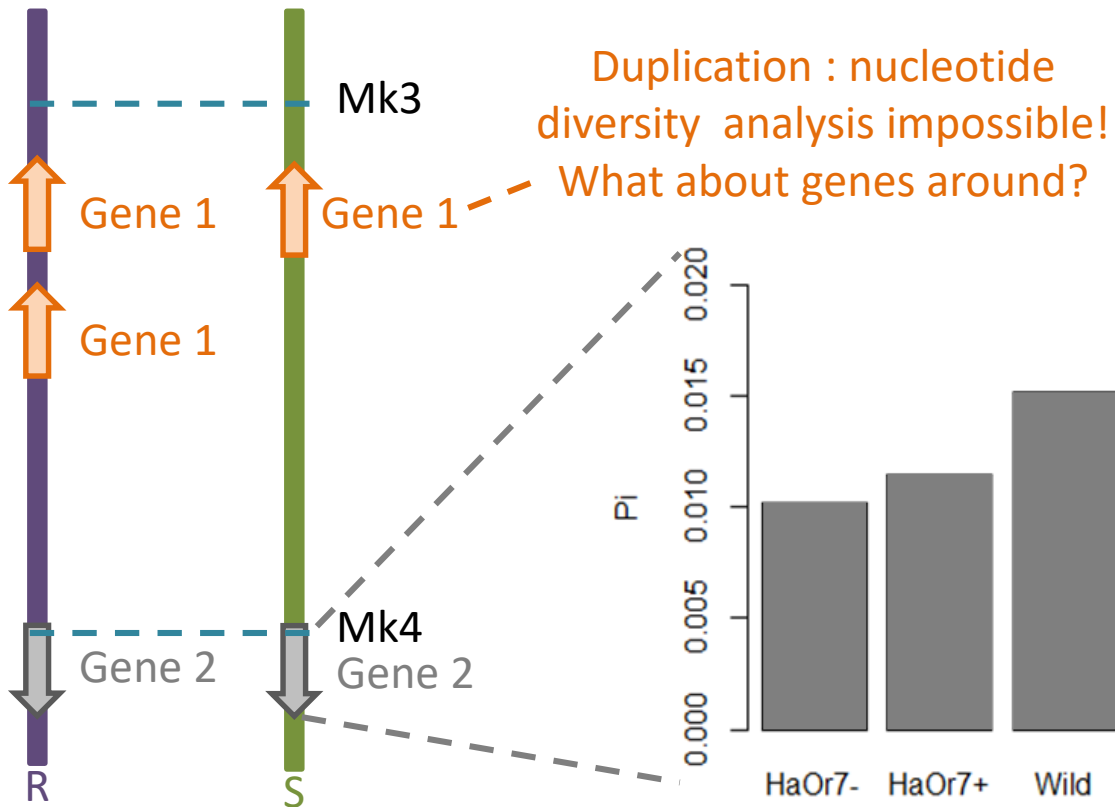
Sequenced





170 sunflower accessions :

- *HaOr7+* resistant cultivated lines
- *HaOr7-* cultivated lines (resistant and susceptible)
- Wild *H. annuus* and wild relatives



Sequencing 1kb PCR products on all individuals of the panel : number of alleles (π)

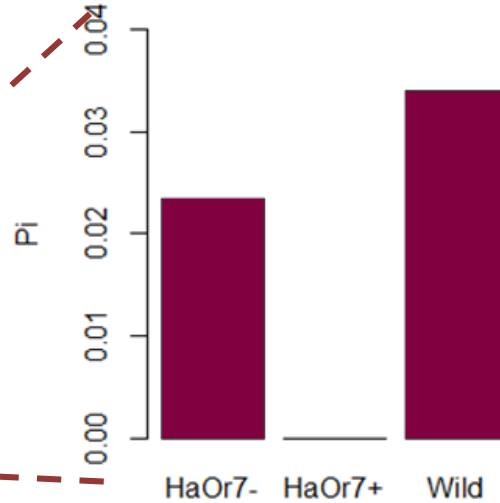
Several alleles on *HaOr7+* individuals :
No hitchhiking.

Candidate genes and hitchhiking

Map-based cloning of the gene

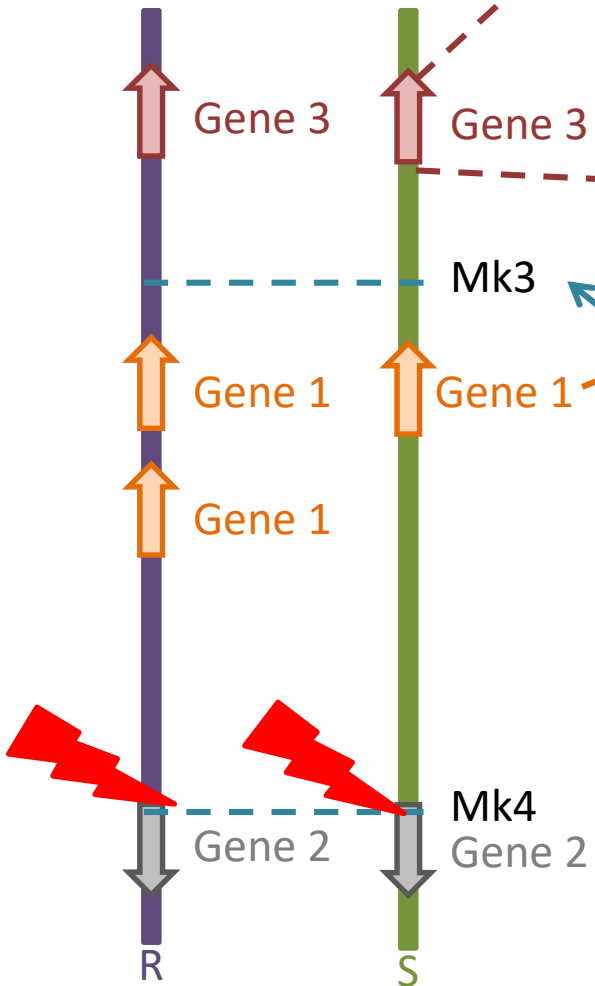
170 sunflower accessions :

- *HaOr7+* resistant cultivated lines
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Only one allele on *HaOr7+* individuals : Hitchhiking

Sequencing 1kb PCR products on all individuals of the panel : number of alleles (π)



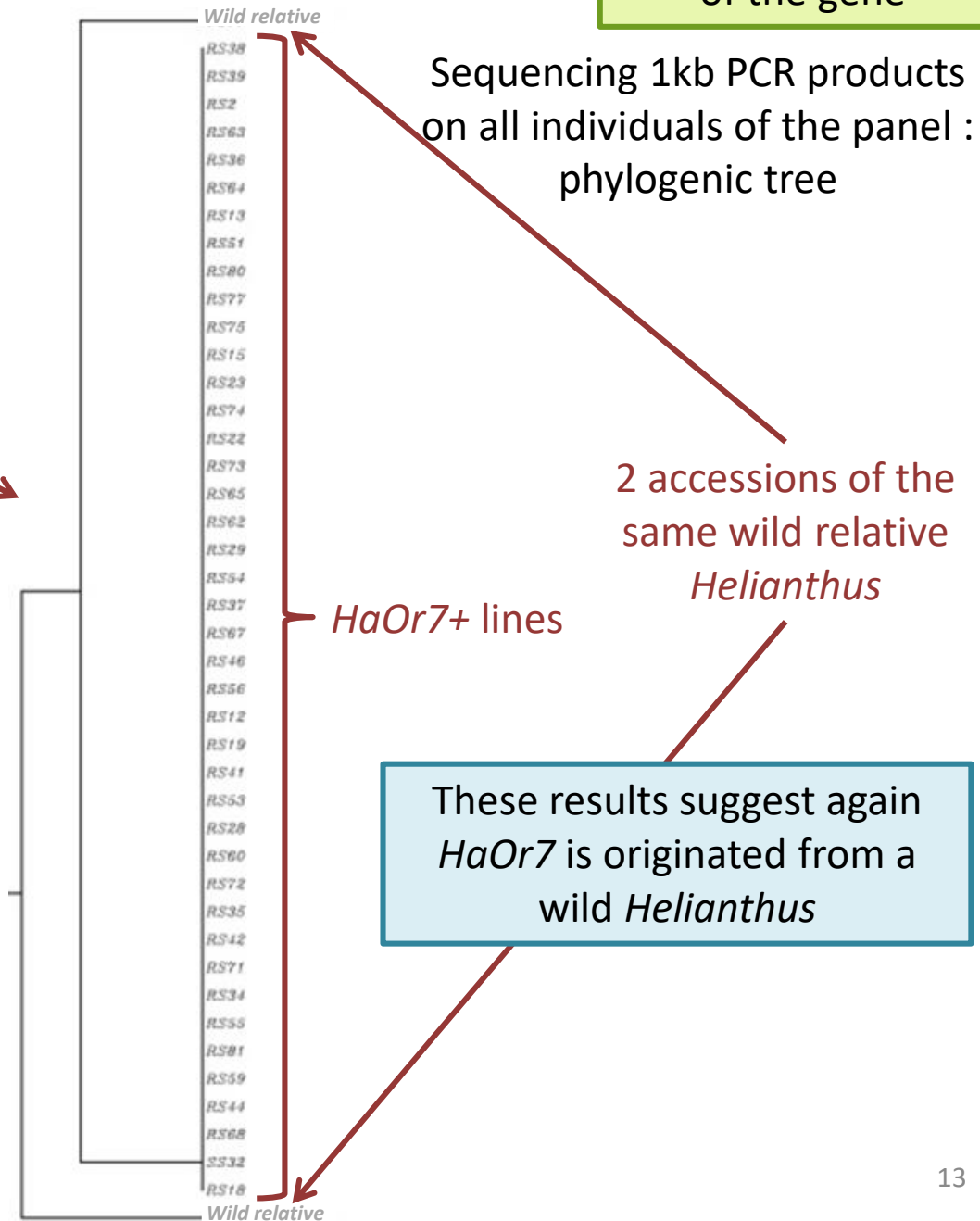
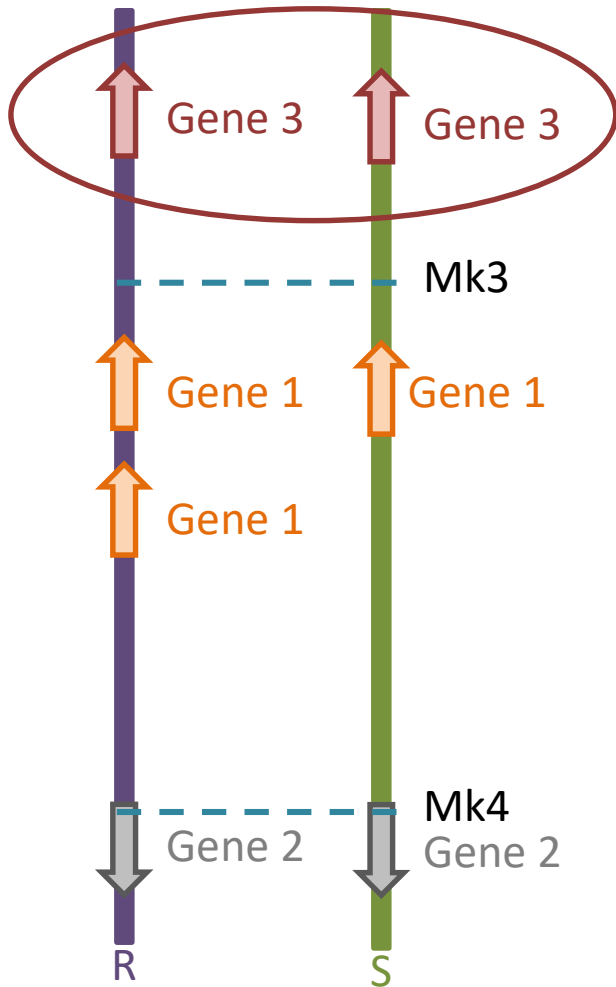
Duplication : nucleotide diversity analysis impossible!

1 recombinant plant in the fine mapping population (14,281 individuals)

25 recombinant plants in the fine mapping population (14,281 individuals)

170 sunflower accessions :

- *HaOr7+* resistant cultivated lines
- *HaOr7-* cultivated lines (resistant and susceptible)
- **Wild *H. annuus* and wild relatives**



These results suggest again *HaOr7* is originated from a wild *Helianthus*

Candidate gene

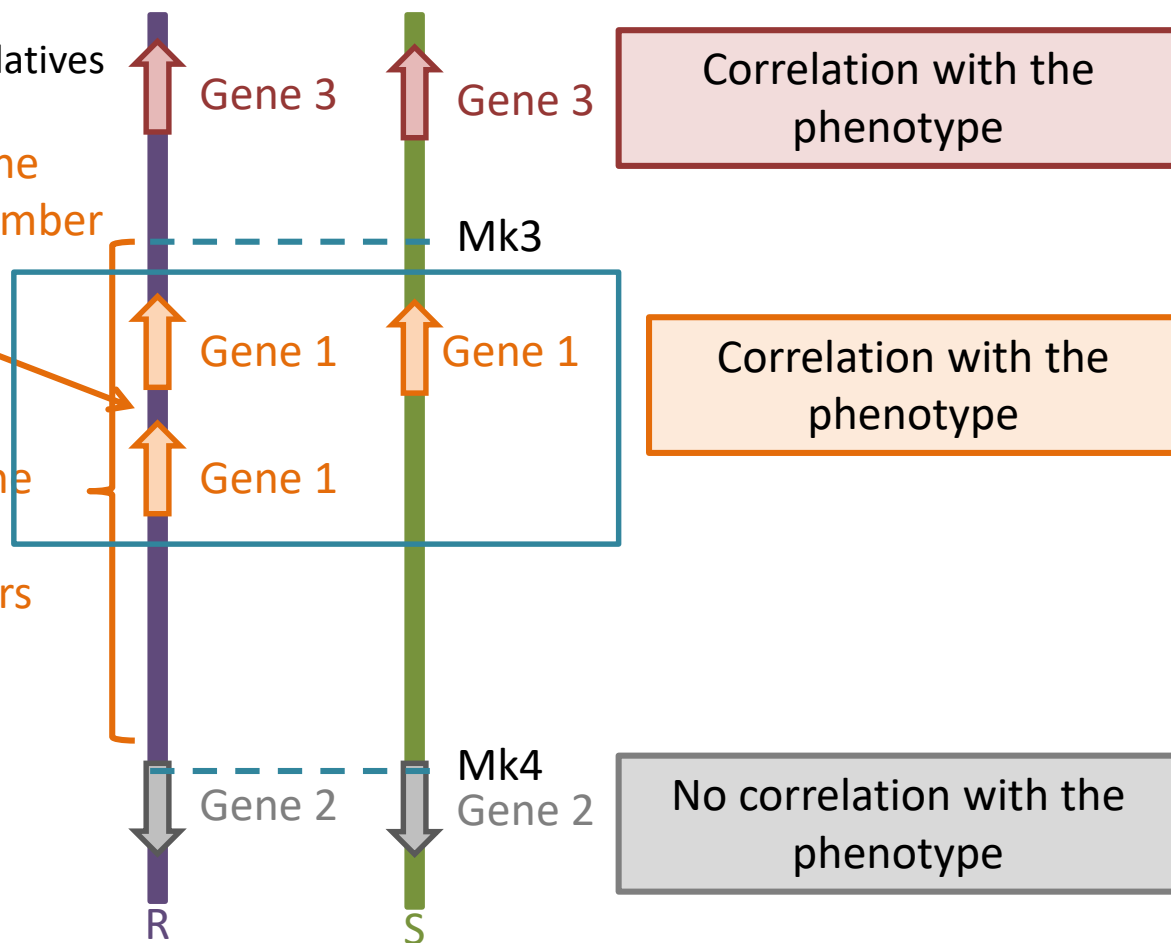
Map-based cloning of the gene

170 sunflower accessions :

- *HaOr7+* resistant cultivated lines
- *HaOr7-* cultivated lines (resistant and susceptible)
- Wild *H. annuus* and wild relatives

All *HaOr7+* lines carry the duplication (qPCR Copy Number Variant on gDNA)

All *HaOr7+* lines have the same genotype with resistant specific markers



HaOr7 is encoded by a LRR receptor-like kinase

Summary

Map-based cloning of the gene

- ✓ *HaOr7* is encoded by a Leucine-Rich-Repeat Receptor-like kinase
- ✓ *HaOr7* is duplicated (mediated by retrotransposons) on the resistant genomic sequence
- ✓ We identified a wild origin of *HaOr7* (phenotype of the wild relatives = resistant)

What is the role of the *HaOr7* gene for the resistance to race F?

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Functional characterization

Resistance is due to a fully functional Leucine-Rich-Repeat Receptor-like kinase protein compared to the truncated protein of the susceptible allele.

What is the role of the *HaOr7* gene for the resistance to race F?

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At which step of the life cycle *HaOr7* acts?

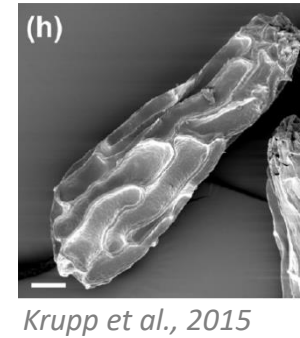
We observed in fields the resistant line has no broomrape emergences.



Tubercles development



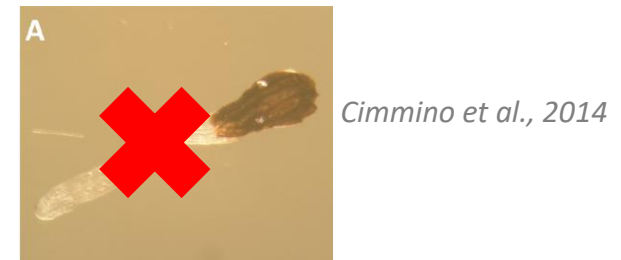
- Tubercle development?
- Attachment to the roots?
- Germination ?



Haustorium development and attachment to the root.



Germination



At which step of the life cycle *HaOr7* acts?

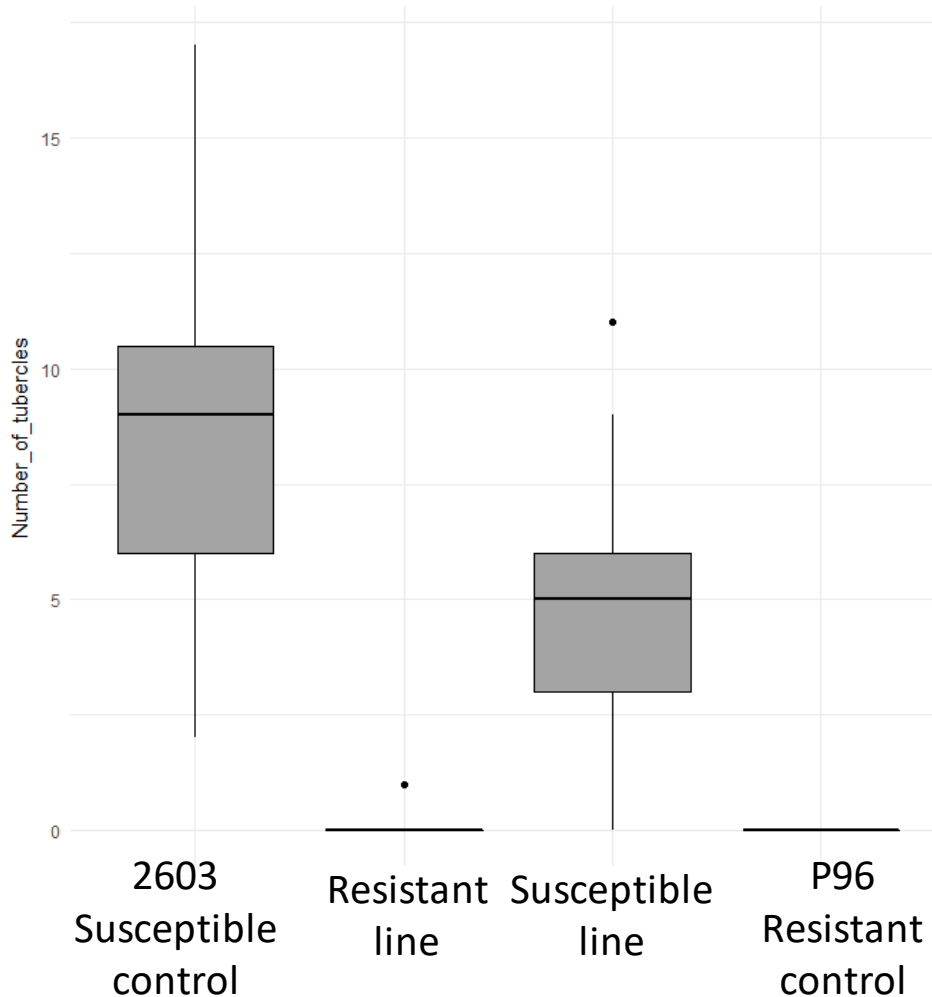
Physiological characterization

Tubercles development



Duca et al., 2013

The resistant line has no broomrape tubercles on the roots. Does *HaOr7* act before?



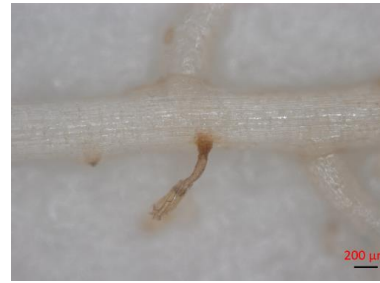
After 5 weeks of interaction.

We count the number of tubercles on sunflower roots

At which step of the life cycle *HaOr7* acts?

Physiological characterization

Haustorium development and attachment to the root.

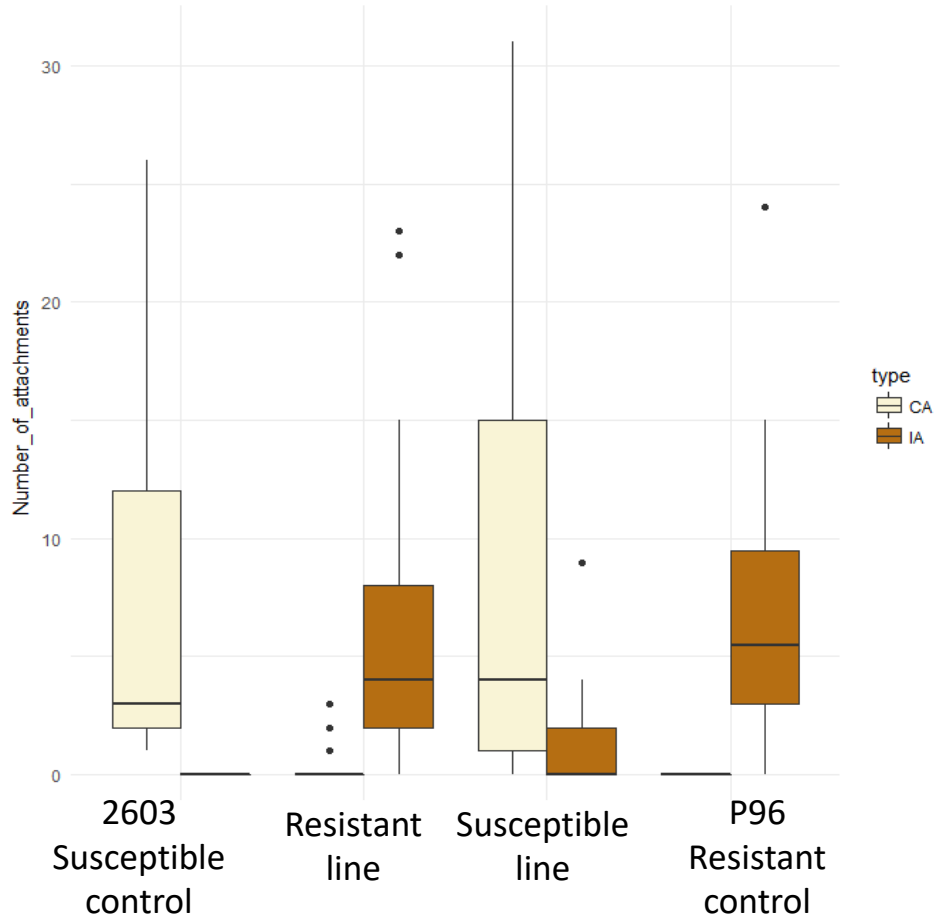
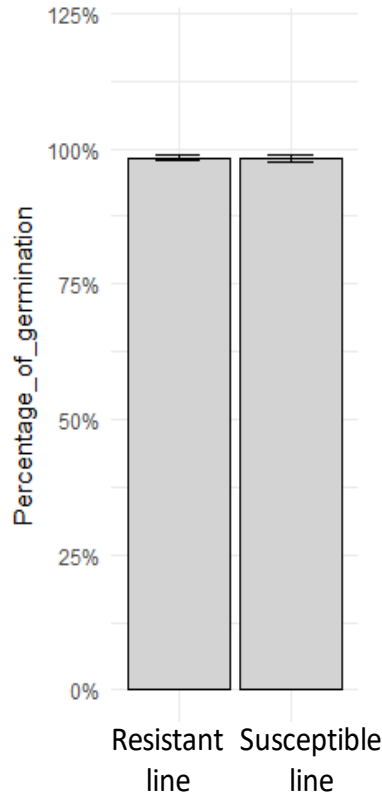


HaOr7 prevents the attachment to the root.



Compatible attachments

Incompatible attachment



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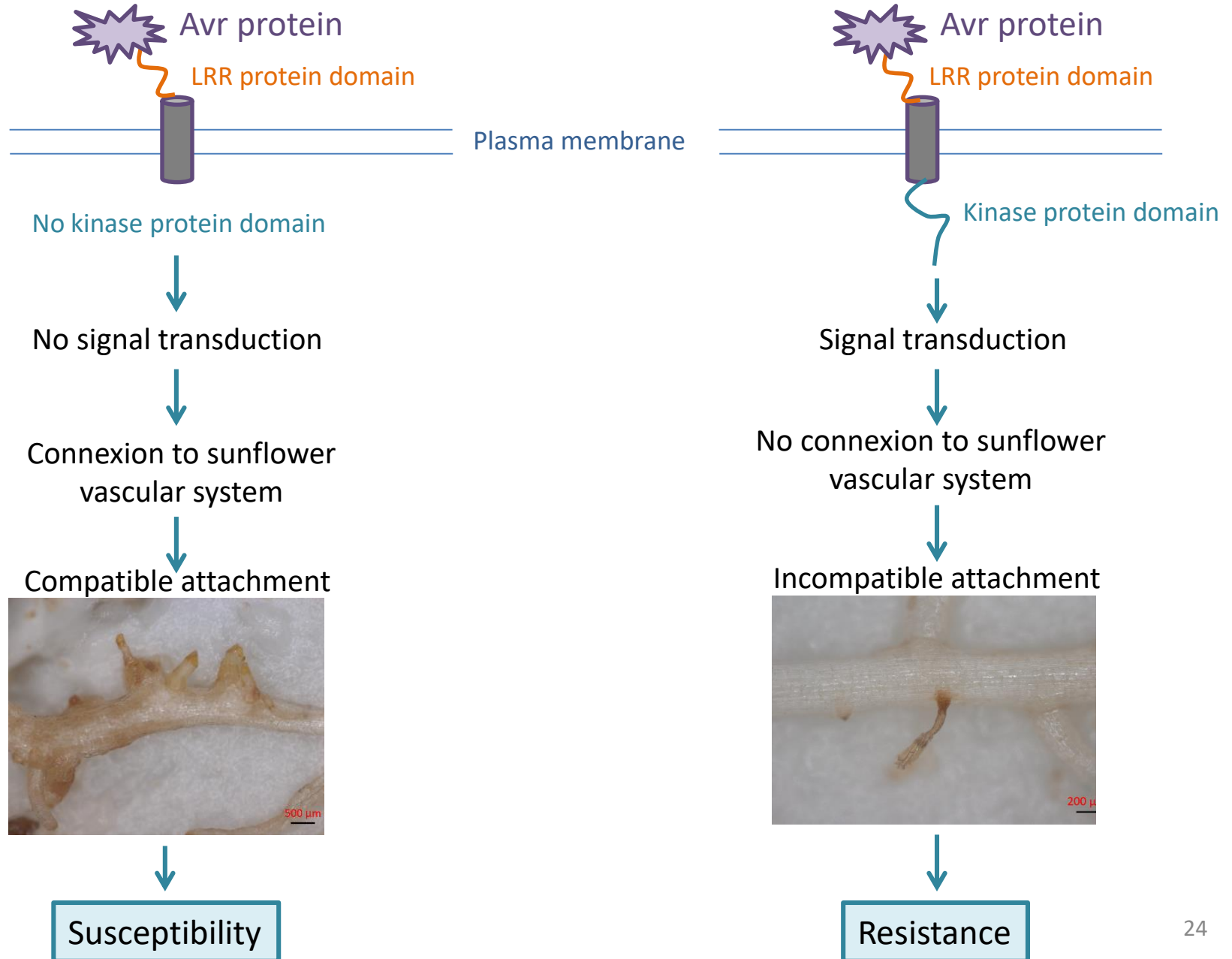
Functional characterization

Resistance is due to a fully functional Leucine-Rich-Repeat Receptor-like kinase protein compared to the truncated protein of the susceptible allele.

Physiological characterization

HaOr7 prevents the *O. cumana* connexion to the sunflower vascular system with incompatible attachments.

Model for the resistance mechanism of *HaOr7*



Duriez et al, in prep
Submission in the coming weeks...

All sequences deposited at GenBank

Thank you for your attention

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