



# Genetic and Biological Approaches to Decipher *O.cumana* Resistance in Sunflower Wild Relatives



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## *O. cumana* Wallr.



- Holoparasitic weed,
- from central Asia to south-eastern Europe,
- Rapid populations evolution
- **Increase level of virulence**

## Sunflower relatives



- Breeding for *O. cumana* resistance needs multigenic sources (Höniges *et al.*, 2008; Seiler and Jan, 2014),
- Sunflower **Crop Wild Relatives** are considered as a reservoir of resistance, (Velasco *et al.*, 2016).

Find and understand what are genetic determinants of *O. cumana* resistance in sunflower crop wild relatives ?



- **Combination of genetic, biological and molecular approaches to identify and characterize genetic determinants of *O. cumana* resistance in sunflower interspecific populations.**

## Genetic approach

- Evaluation of interspecific populations for *O. cumana* resistance,
- Genome Wide Association Study,
- Regions fine-mapping and positional cloning.

## Characterization of the resistance

### Biological approach

- Germination test,
- Attachment characterization,
- Field evaluation,
- Spectrum of the resistance.

### Molecular approach

- Whole genome transcription analysis.
- RT-qPCR Biomark Fluidigm®



# Interspecific populations

- Produced by INRA (Montpellier)
- Generated by crosses between sunflower wild relatives and cultivated *Helianthus annuus*
- Backcrosses & Open pollinated populations

Wild Relatives



*Helianthus annuus*

Backcross  
&  
Open  
pollination



≈ 50 families /  
population



**Results for only one interspecific population will be presented**



# Understanding of the Resistance



- **Biological control**
- **Genetic control**
- **Molecular control**



# Understanding of the Resistance



- **Biological control**
- Genetic control
- Molecular control



# Biological control of the Resistance



**Phenotyping tools available to describe the resistance conferred by interspecific populations**

Rhizotron and microscopic observation



Incompatible attachment



Tubercle development

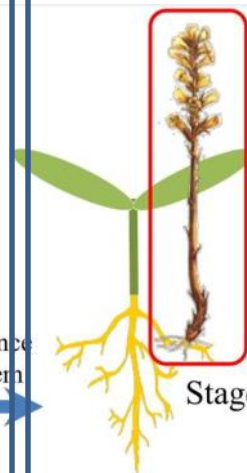
Stage 2



Tubercle necrosis

Stage 3

Growth and emergence of the flowering stem



Stage 4



Germination

Stage 1

(from Louarn *et al.* 2016)

*In vitro* *O. cumana* seeds stimulation

Oroscreen® Platform

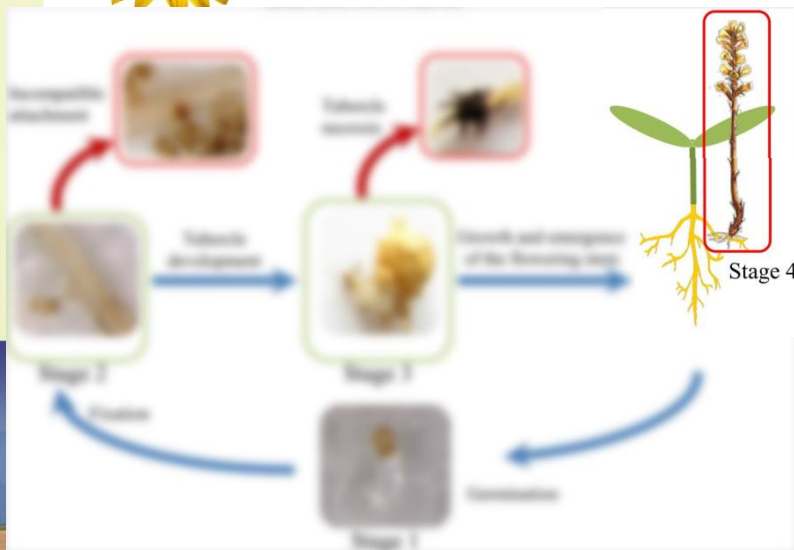
Field evaluation



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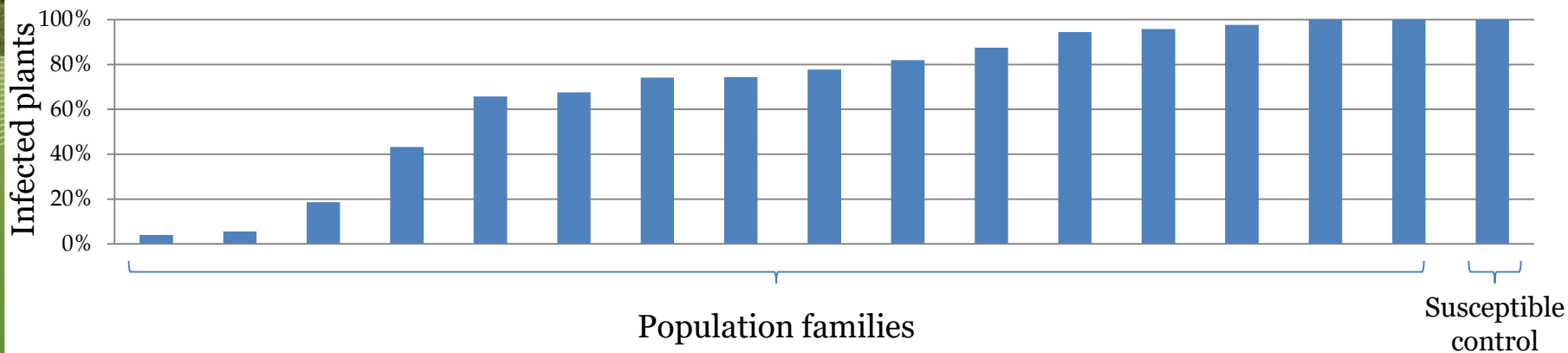
# Genetic Study in Field, Stage 4



- 2 years in South of Spain
- Race F from Guadalquivir Valley

**Selection of most resistant and most susceptible families for further phenotyping and genetic study.**

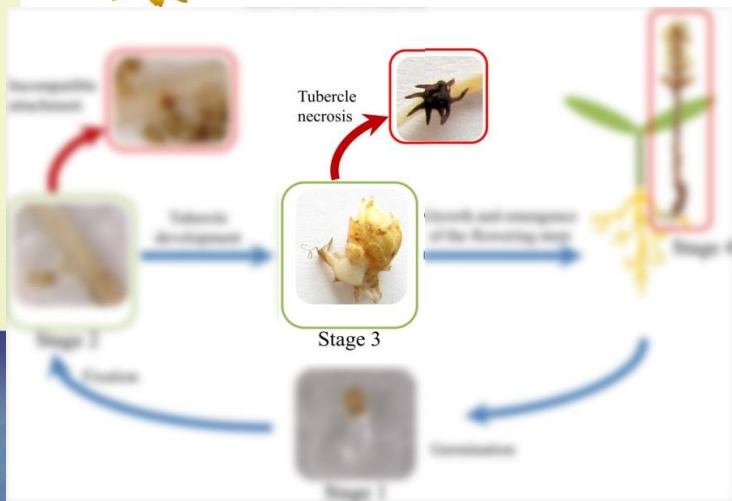
Repartition of the infection rate among the families







# Spectrum of Resistance, Stage 3



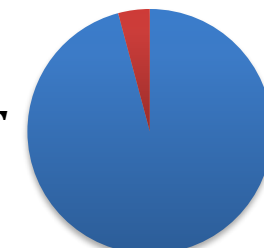
Phenotyping of R family against Spanish Race F, and two more aggressive races from Turkey and Romania

healthy Infected N=24 plants

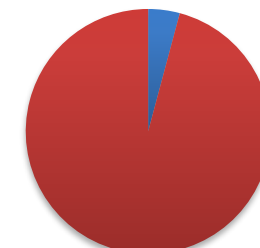
*R family*

*Control S*

*Race F*



Nb nodules ~ 0.04



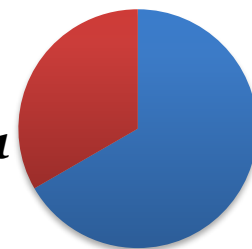
Nb nodules ~ 10

**Resistance against aggressive races, partial resistance**

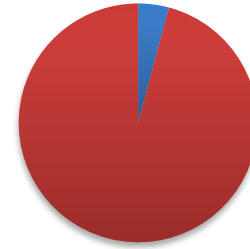
Nb nodules ~ 0.78

Nb nodules ~ 6

*Race H Romania*



Nb nodules ~ 0.33



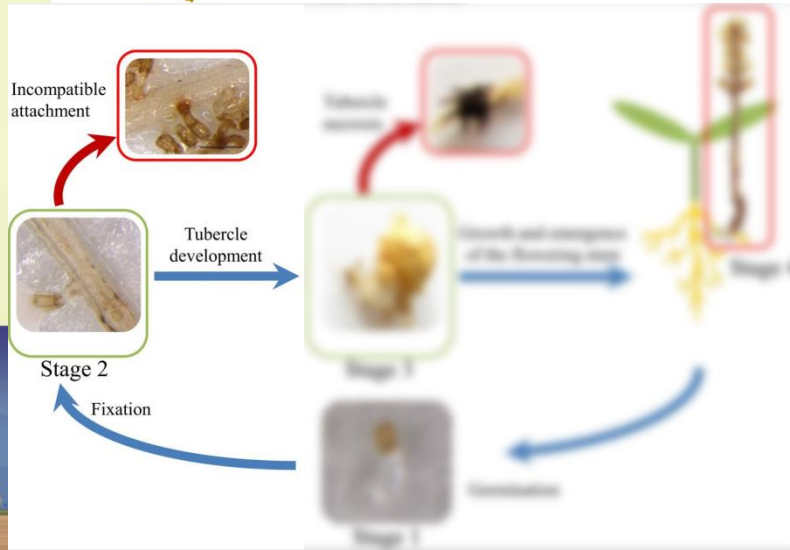
Nb nodules ~ 4



Oroscreen® platform Patent WO 2015/075131



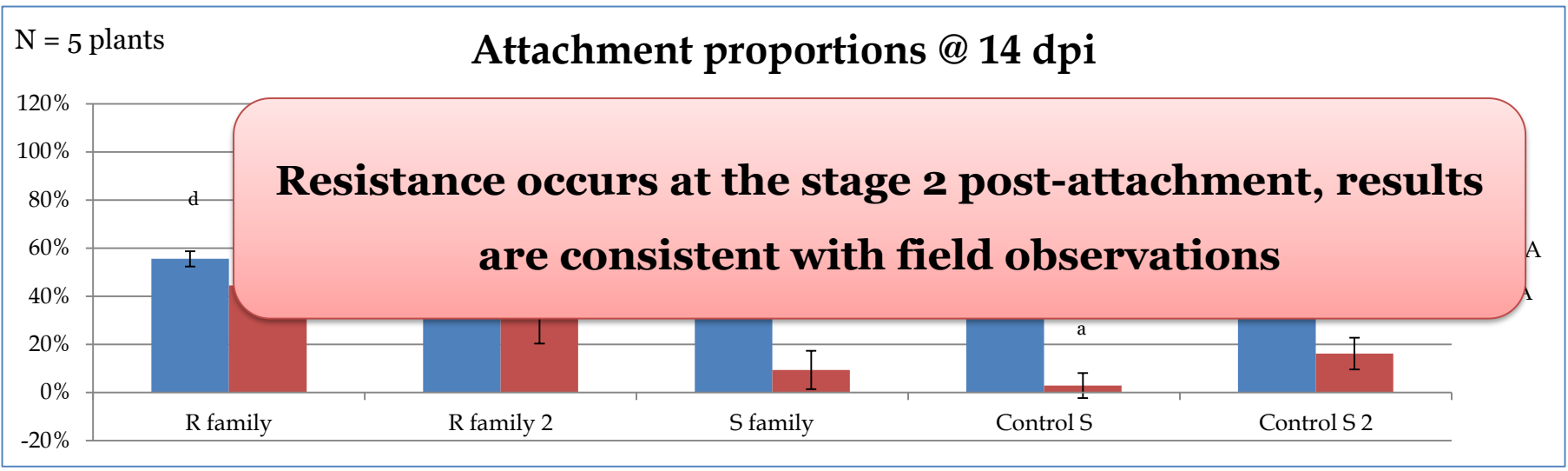
# Early infection stage, stage 2



- Rhizotron growing test, 14 days
- Observation/counting on microscope



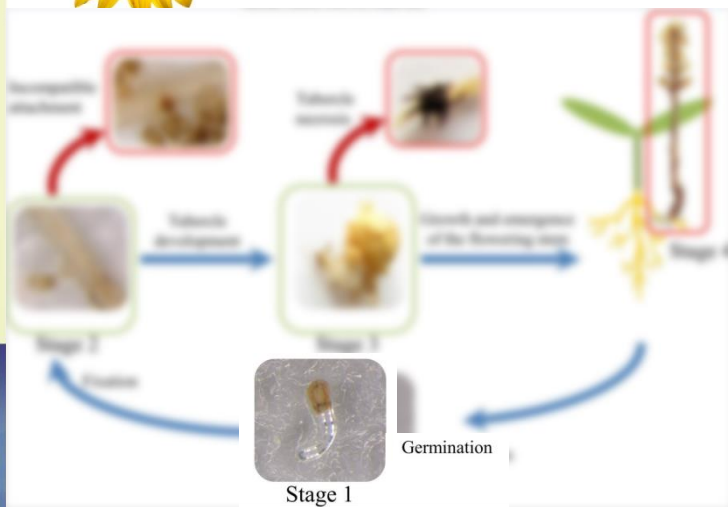
IA: Incompatible attachments.  
CA: Compatible attachments.



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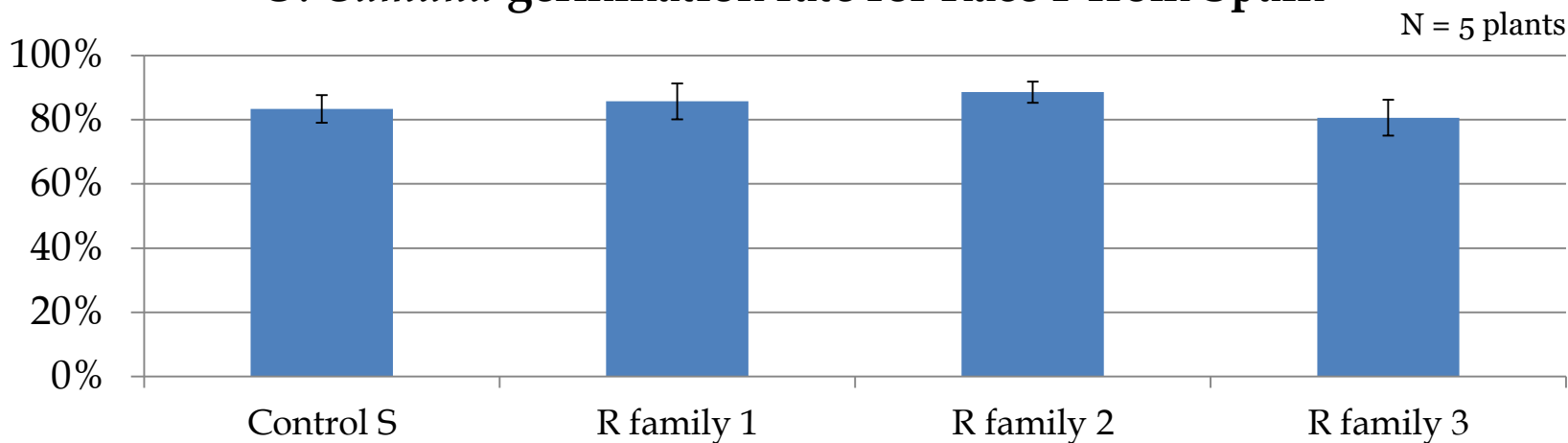
# Germination stimulation, stage 1



- In vitro test of germination rate
- Collect of root exudates
- Test of root exudates on different races

**No difference between Resistant families and Susceptible control on *O. cumana* seeds germination stimulation potential.**

*O. Cumana* germination rate for Race F from Spain





# Understanding of the Resistance

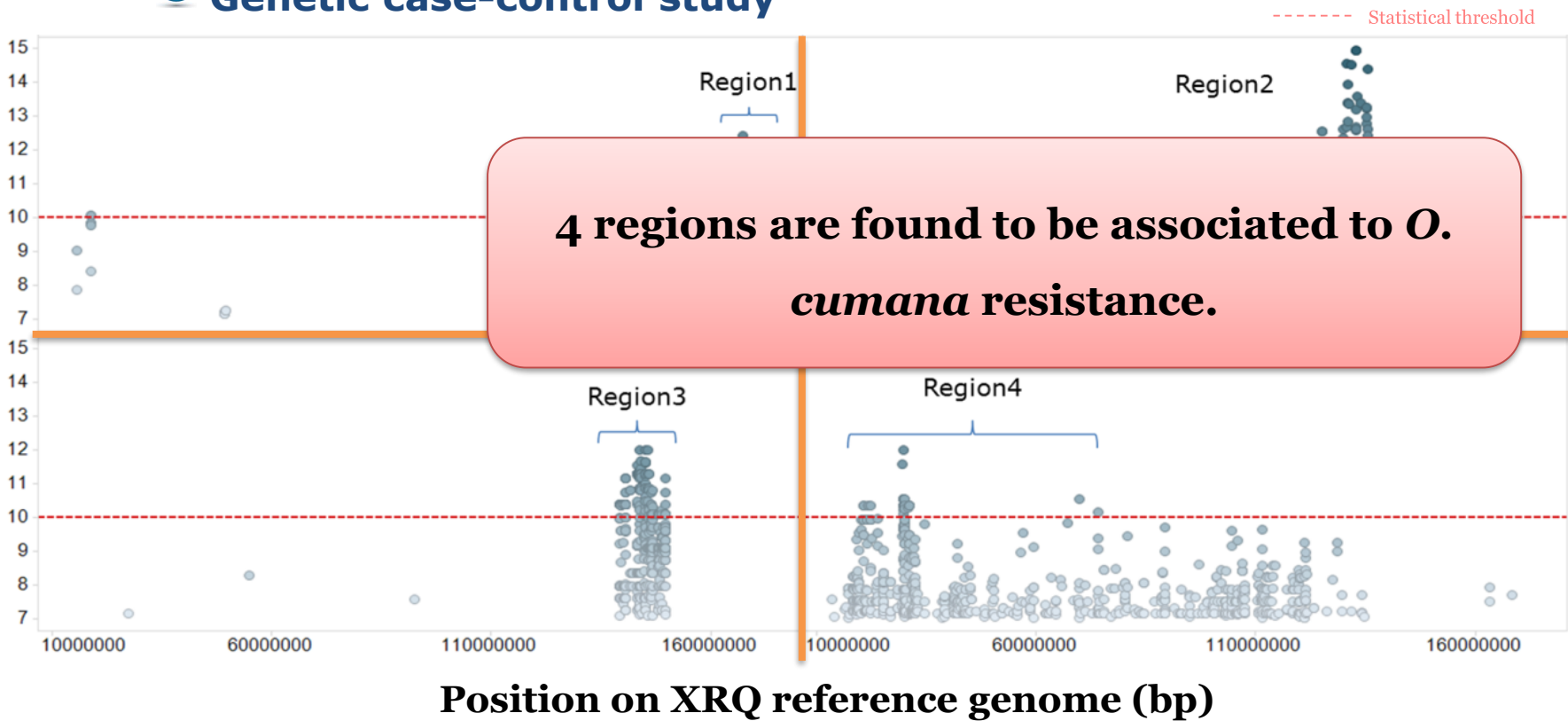


- Biological control
- **Genetic control**
- Molecular control



# Genetic Control of the Resistance

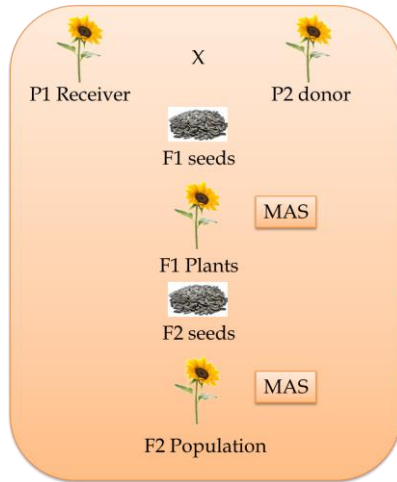
- Selection of most resistant and most susceptible families based on field evaluation,
- Exome capture to identify polymorphic SNPs (~1 Millions)
- Genetic case-control study





# Genetic Control of the Resistance

- Validation of Regions effect => Priority
- Fine mapping of the most critical regions





# Understanding of the Resistance

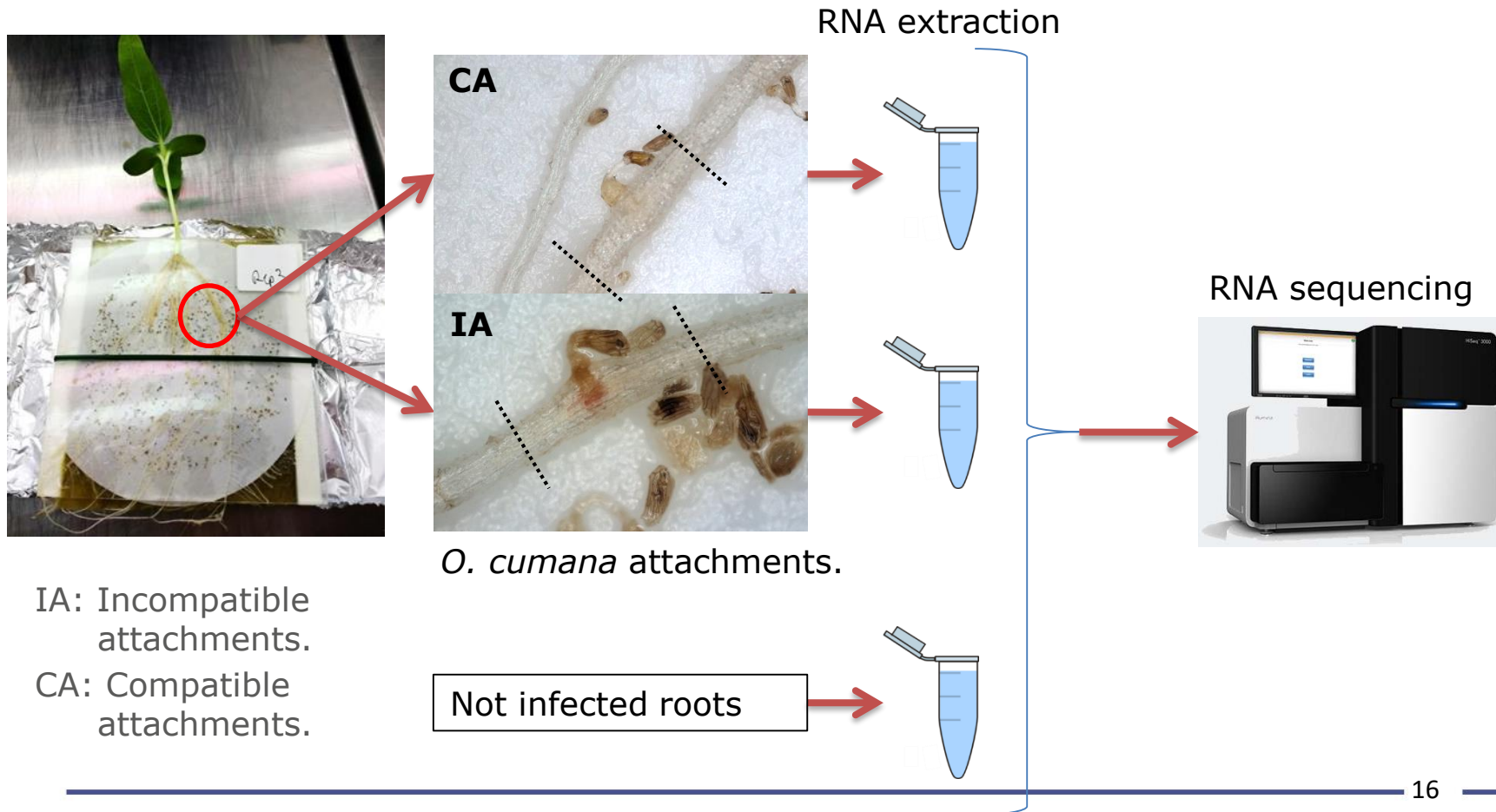


- Biological control
- Genetic control
- **Molecular control**



## Whole genome transcriptome analysis

Rhizotron experiment on most resistant family and susceptible control, sunflower roots infected by *O. cumana* Race F from Spain.



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CA: Compatible attachments.

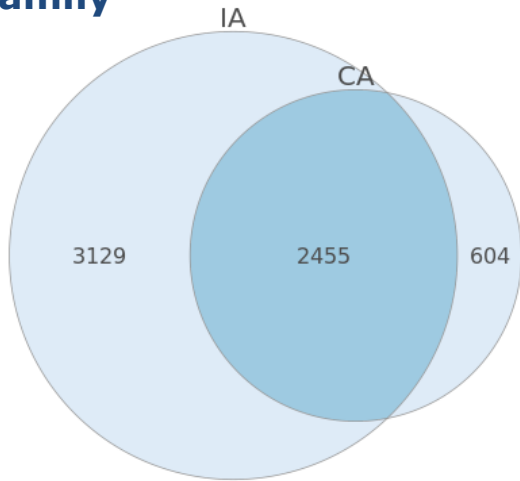
Not infected roots





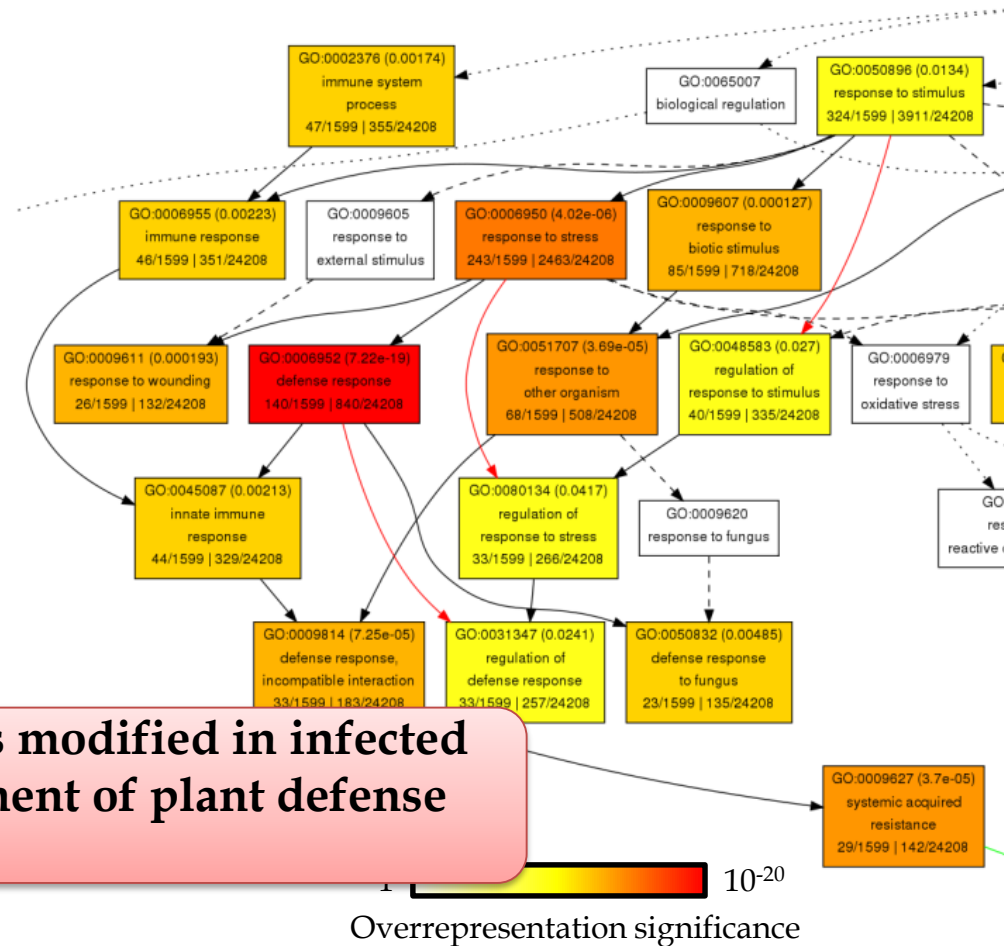
## Whole genome transcriptome analysis

- Differentially expressed genes (DEG) count in resistant family



IA: Incompatible attachments.  
CA: Compatible attachments.

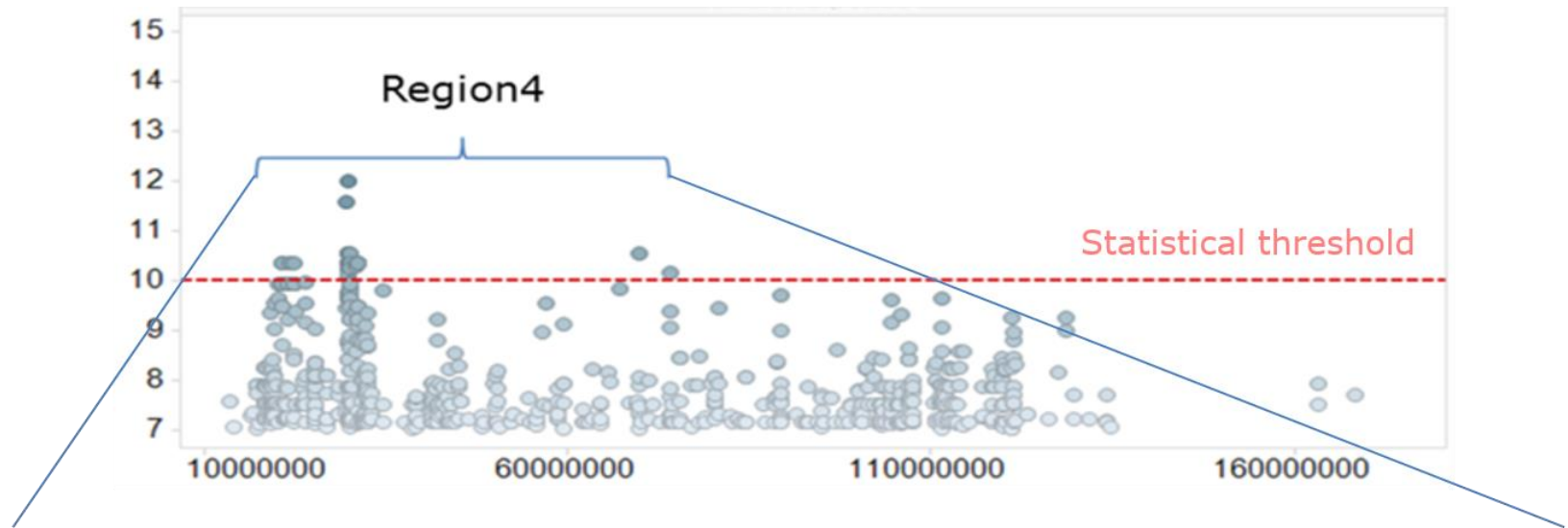
- Gene ontology enrichment on "Biological Process" category of DEG in IA condition



Transcriptome of sunflower roots is modified in infected condition, and reveals an involvement of plant defense arsenal.



## Candidate genes in associated regions



(Zhang et al., 2007)

2 LRR-Kinases

**44 differentially expressed candidate genes  
have been selected in associated regions**

2 Nodulins

Cell-death

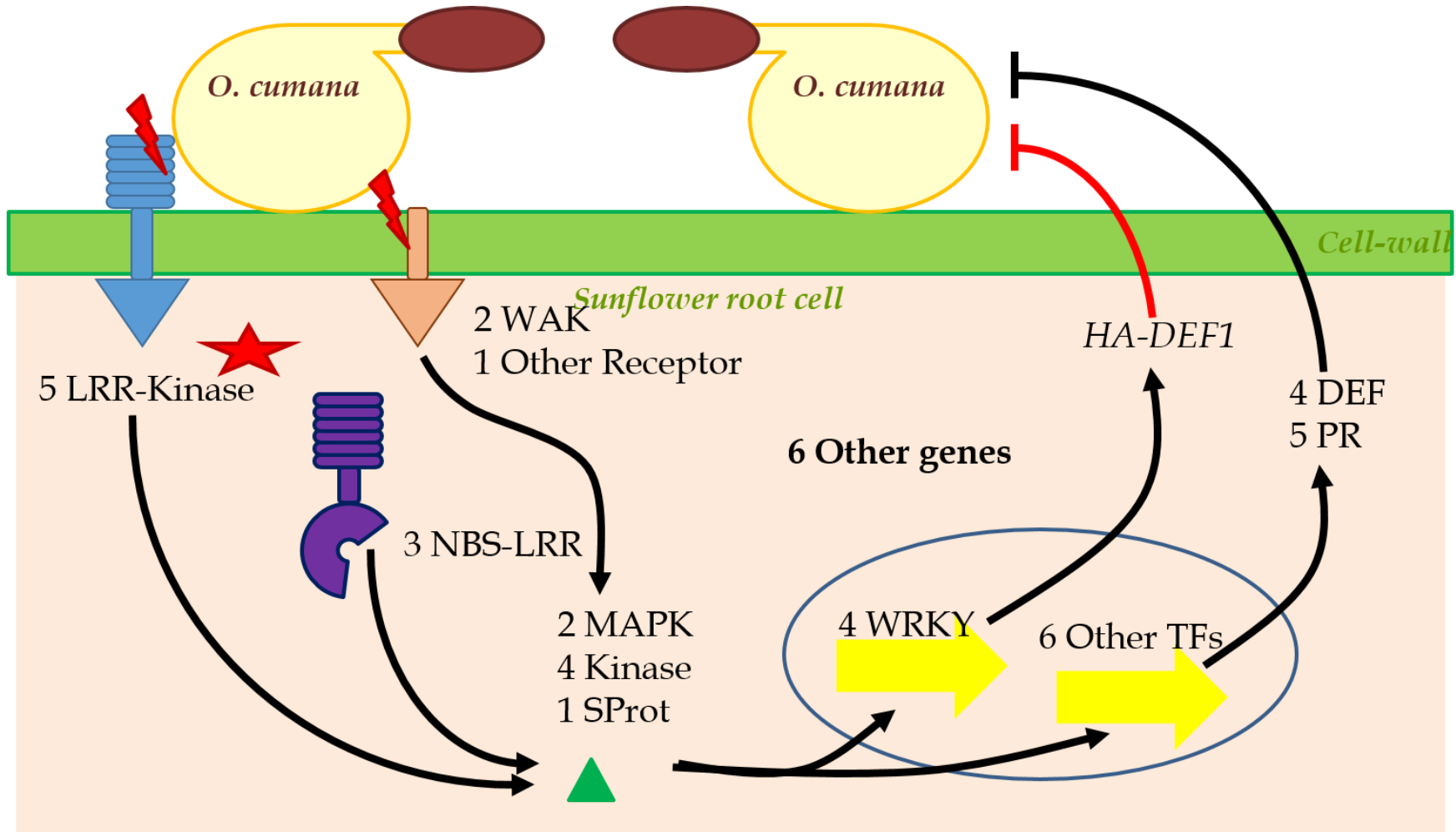
QTL\_start

Up-regulated  
Down-regulated

QTL\_end



# Hypothesis of candidate genes involvement





# Conclusions

- **Resistance is frequent in sunflower wild relative**
- **In this specific case,**
  - Good resistance/tolerance spectrum
  - Resistance mechanism is at early post-attachment stage
  - Genetic control is complex but “sustainable”
  - Gene network model
- **The understanding of the biological control of the resistance in combination of genetic and molecular approach should lead to SMART implementation of new sources of resistance into breeding program**



# Acknowledgement

