

Resistance to *Orobanche cumana*: “We will keep on fighting until the end”

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Sunflower-Pests Interactions

Group leader: Stéphane Muñoz

June 2022 members

Mireille Chabaud (IR): cell biology, phenotyping, transformation, project coordination

Marie-Claude Boniface (TR, 50%): genetic resources, phenotyping

Alexandra Legendre (AT): genomics, functional biology

Stéphane Muñoz (IR): genomics, molecular genetics, project coordination

Permanent staff with complementary skills

Estelle Bouvier (AI): phenotyping, microscopy

Maylis Bobin (AI): phenotyping, molecular biology

Lea Mouysset (Master student, Toulouse University): functional agronomy

Camille Pubert (coll. MAS SEEDS, PhD student, Toulouse University): Genetics

O. cumana: a parasitic weed that specifically infects sunflower crop



Obligatory parasitic plant:

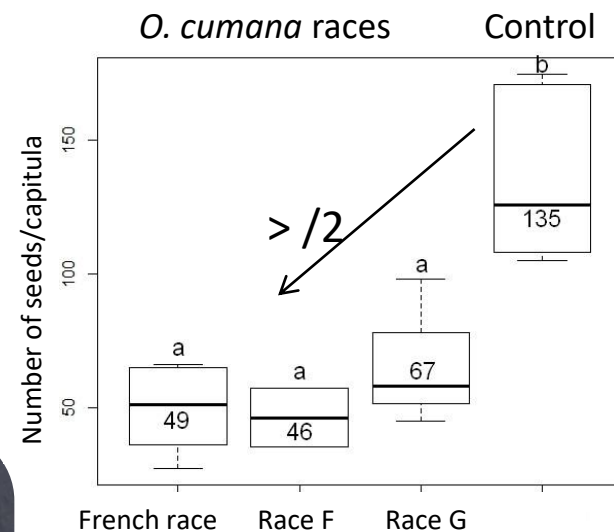
Non-photosynthetic

No roots



Control:
Susceptible line 2603

Susceptible line 2603
+ *O. cumana*



Effect on seed number/capitula

Strong Yield losses

Small seeds of *Orobanche Cumana*: rapid spreading

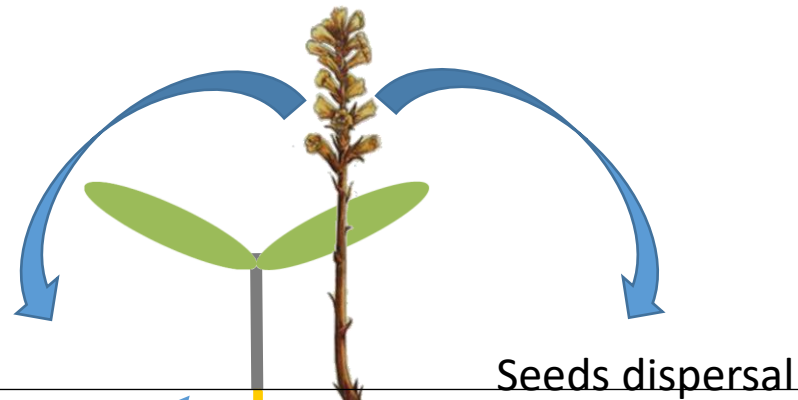
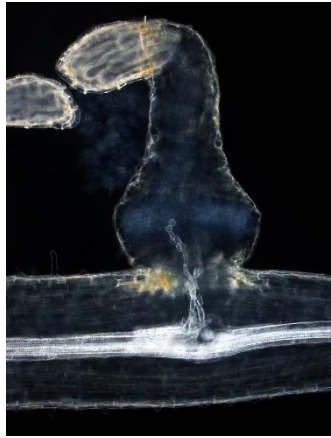


Very small seeds (0.2-0.3 mm).

One plant can produce up to 100 000 seeds.

Seeds can survive during 10 years in the soil.

Biology cycle of *Orobanche cumana*



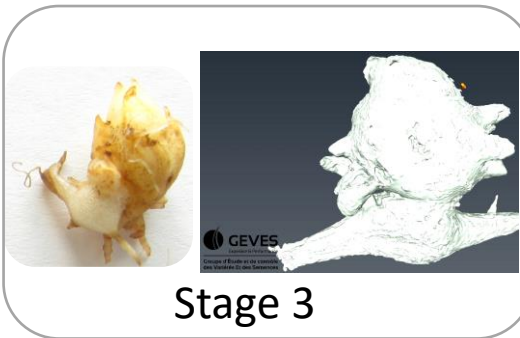
Seeds dispersal

Underground

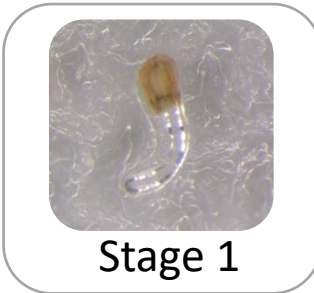


Stage 2

Tubercle development



Stage 3



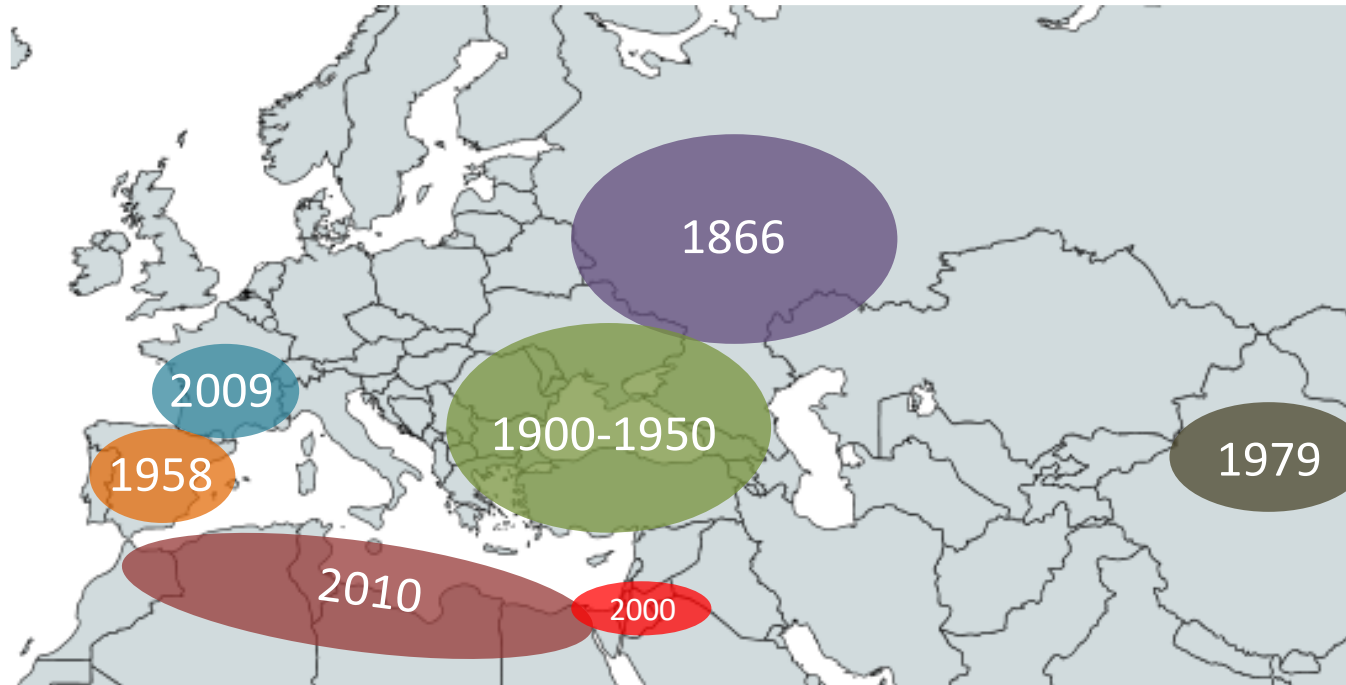
Stage 1

Fixation

Germination

From Louarn *et al.*, 2016

Orobanche cumana in the World



Virulence of *Orobanche cumana* populations

Broomrape populations differ from their virulence and aggressivity

O. cumana: a similar situation than Downy Mildew.

Their virulence (from A to H) is deduced from the resistance of a set of differential sunflower genotypes.

| Line of sunflower | Proposed codes for <i>O. cumana</i> races | | | | | | | |
|-------------------|---|-----|-----|-----|-----|-----|---------|---------|
| | 100 | 300 | 700 | 710 | 730 | 770 | 771 | 773 |
| AD66 | S ^a | S | S | S | S | S | S | S |
| K A-41 | R | S | S | S | S | S | S | S |
| J8281 | R | R | S | S | S | S | S | S |
| Record | R | R | R | S | S | S | S | S |
| S1358 | R | R | R | R | S | S | S | S |
| P1380 | R | R | R | R | R | S | S | S |
| LC1093 | R | R | R | R | R | R | S | S |
| P96 | R | R | R | R | R | R | R | S |
| Historical race | A | B | C | D | E | F | F or G? | F or G? |

^a S: susceptible, R: resistant.

Leire Molinero-Ruiz *et al.*, SJAR, 2015

The situation for races > E is unclear : F, F⁺, G, G⁺, G_{GV}, H

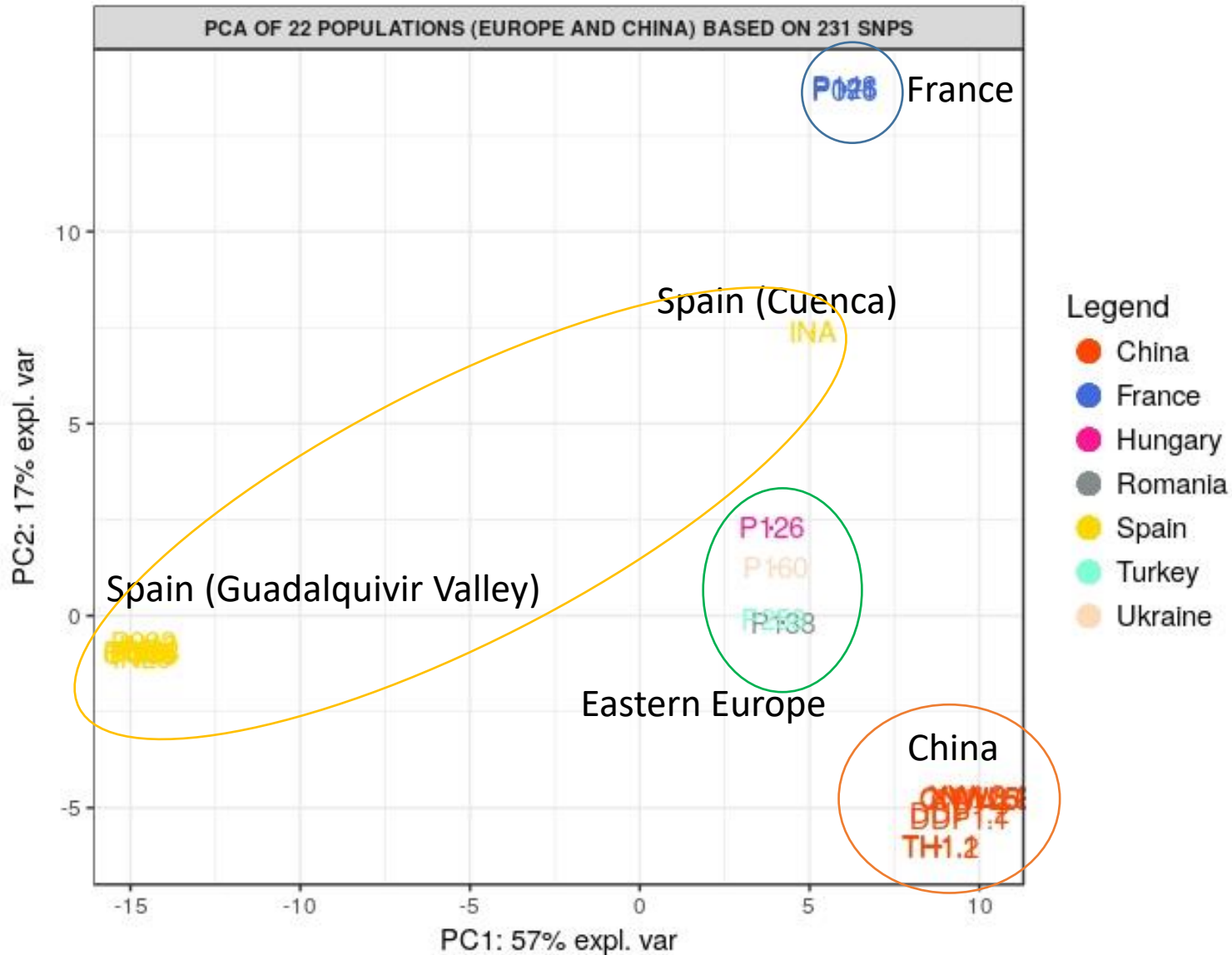
We need an universal set of differential sunflower genotypes

Molecular diversity of the worldwide broomrape populations



Luyang Hu
PhD student
Zhejiang University
INRAE (2-years stay)

Coll. Innolea



O. cumana Genomics

We sequenced the 19 chromosomes of the *O. cumana* genome (1.47 Gb) of IN23 population (homozygous population, Race F from Guadalquivir (IAS-CSIC, Spain)).

of protein coding genes 43,313

More recently we obtained high quality genomes of 4 new races from other countries.

We also re-sequenced (Illumina PE sequencing) the genomes of 21 populations

- China: 6
- France: 3
- Spain: 5 (IN23 included)
- Eastern-Europe:7

5,652,731 SNPs identified

Objectives: understand populations evolution and link polymorphisms to the virulence of the populations

How to control *O. cumana*?

1. Genetic resistances

Quantitative resistances



CrossMark

Sunflower Resistance to Broomrape (*Orobanche cumana*) Is Controlled by Specific QTLs for Different Parasitism Stages

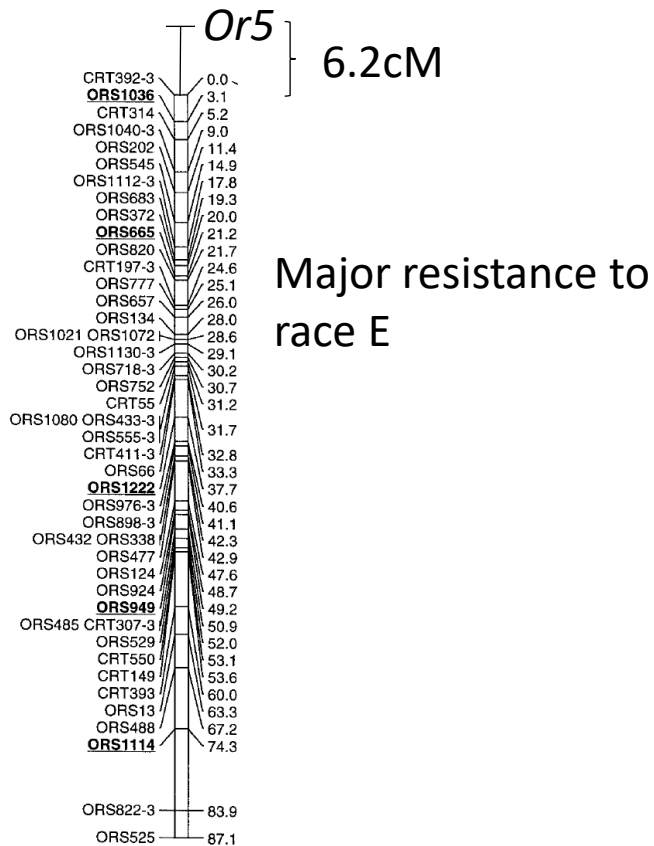
Johann Louarn¹, Marie-Claude Boniface¹, Nicolas Pouilly¹, Leonardo Velasco²,
Begoña Pérez-Vich², Patrick Vincourt¹ and Stéphane Muñoz^{1*}

¹ LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France, ² Instituto de Agricultura Sostenible-Consejo Superior de Investigaciones Científicas, Córdoba, Spain

Many QTLs but no complete resistance

Major resistance genes

Or5 mapped on LG3 (telomeric region)



System2 (chromosome 4)

Received: 28 January 2019 | Accepted: 4 November 2019 | Published online: 26 March 2020

DOI: 10.1002/csc2.20002

Crop Science

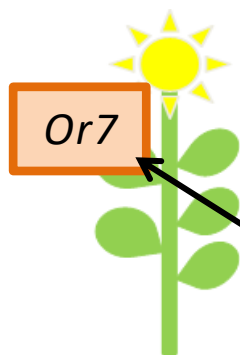
ORIGINAL RESEARCH ARTICLE

Crop Breeding & Genetics

Characterization of post-haustorial resistance to sunflower broomrape

Alberto Martín-Sanz¹ | Begoña Pérez-Vich² | Sandra Rueda¹ | José M. Fernández-Martínez² | Leonardo Velasco²

Identification of the *HaOr7* resistance gene



Pauline Duriez (former PhD student)
Now PostDoc at UBC (Loren Rieseberg's lab)



She is looking for a position

- ❖ Pioneer Hybrid
- ❖ Early 2000's
- ❖ Total resistance against race F, no emergences in field
- ❖ Gene-for-gene interaction

No information about:

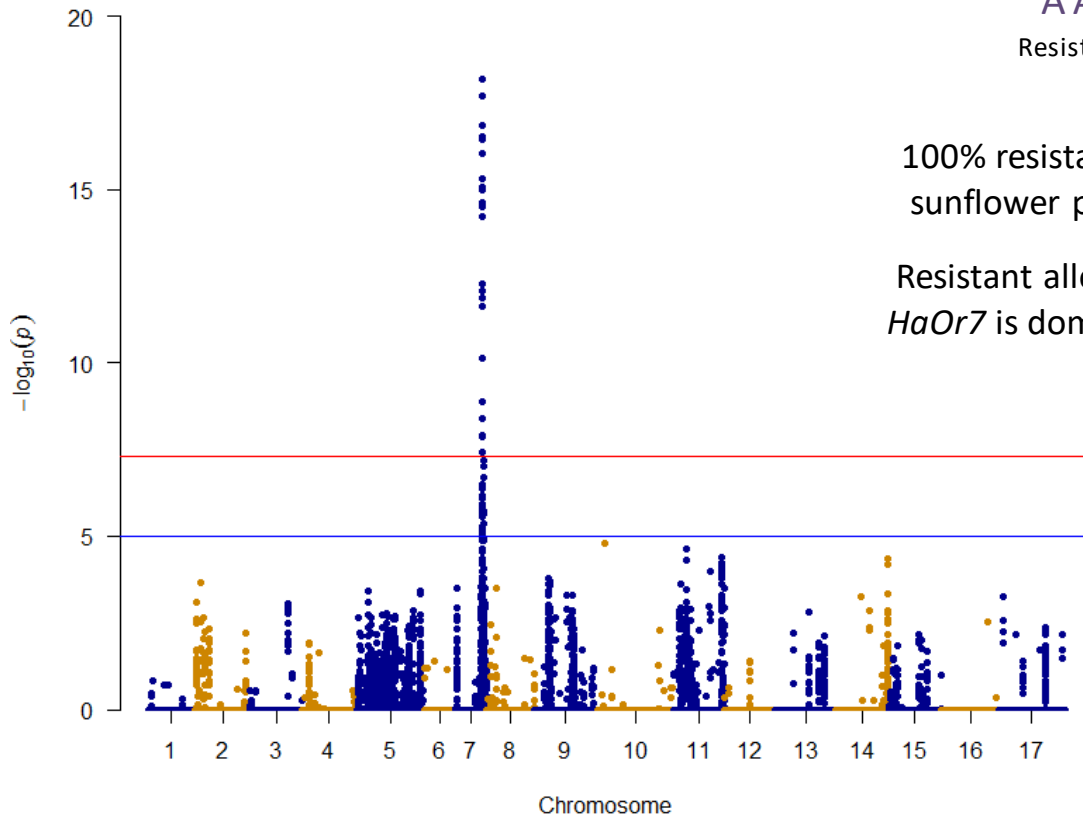
- ❖ The physiological mechanism
- ❖ The molecular characterization

Mapping

GWAS on 55 lines :

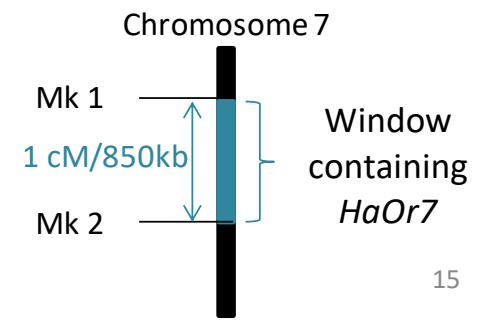
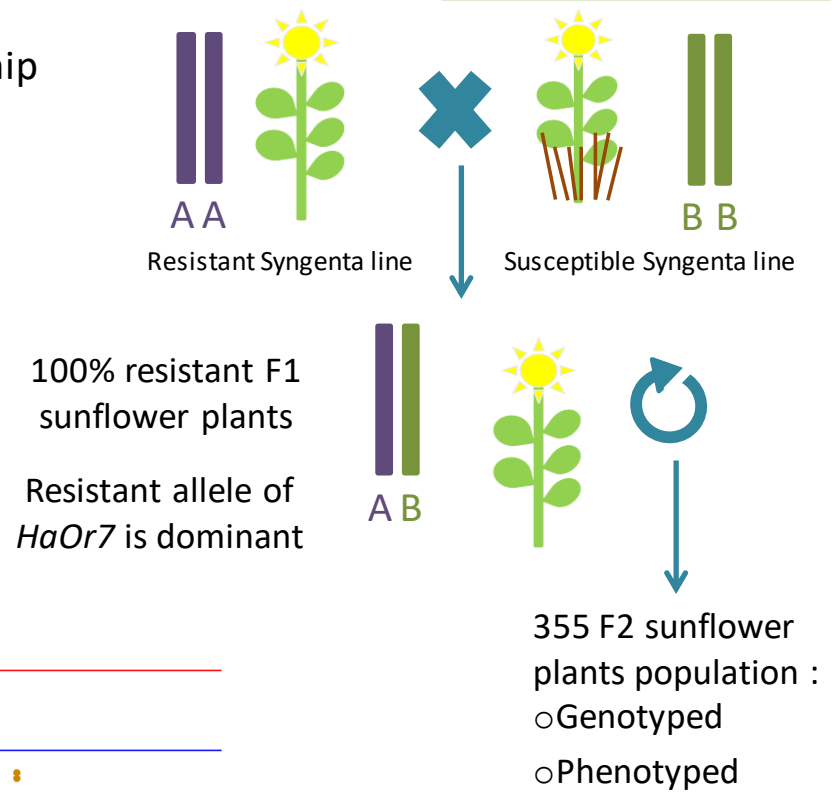
- 21 *HaOr7*- susceptible lines
 - 34 *HaOr7*+ resistant lines
- 600k SNPs AXIOM chip

With the same origin !



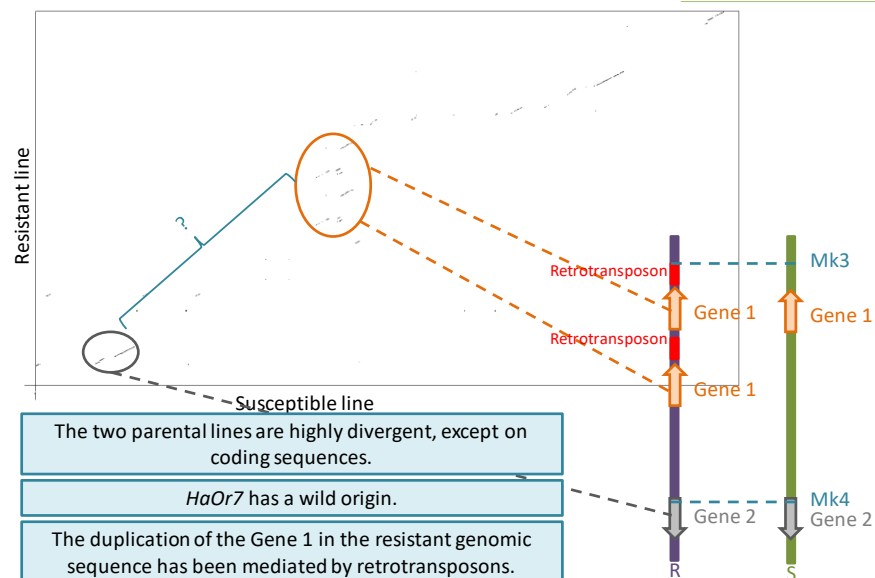
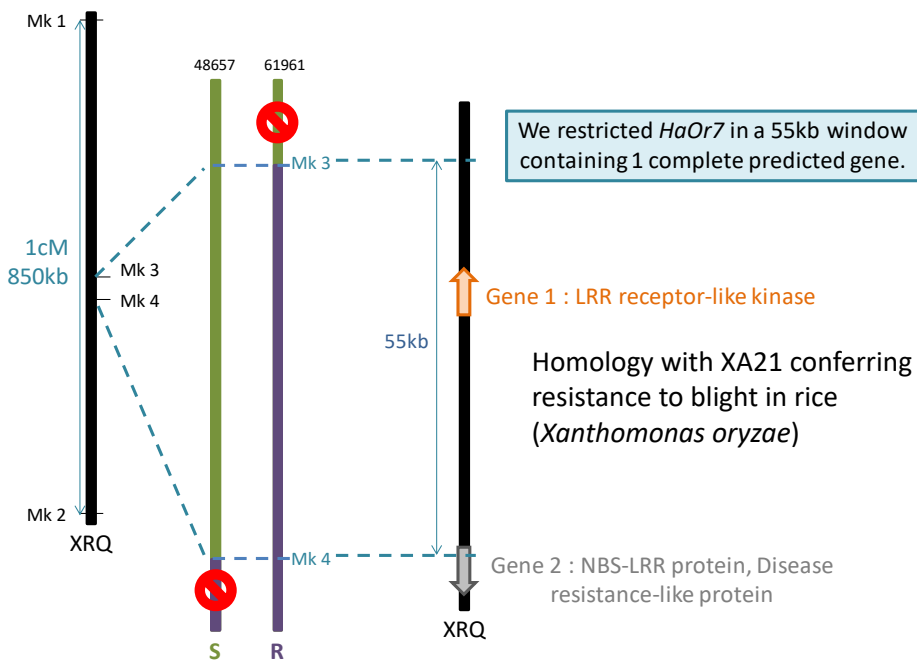
We located *HaOr7* in a 1cM/850kb window on chromosome 7

Map-based cloning of the gene

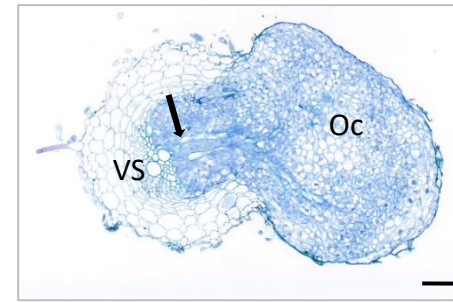
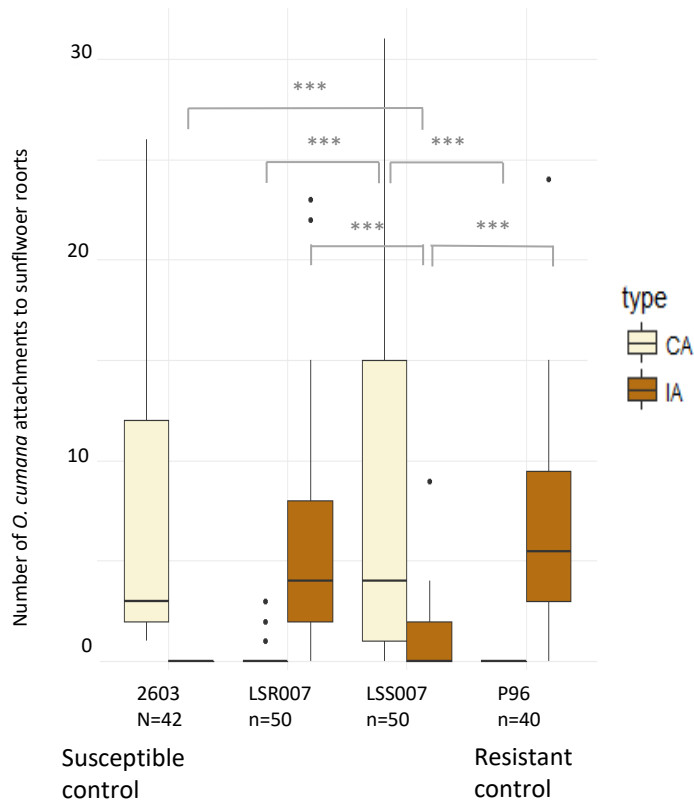


Identification of the *HaOr7* gene

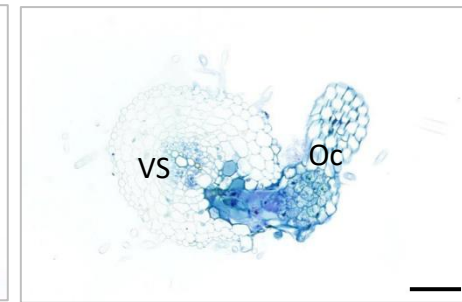
We screened by genotyping a large F2 population (14,000) and phenotype the recombinant plants during 2 years in south of Spain



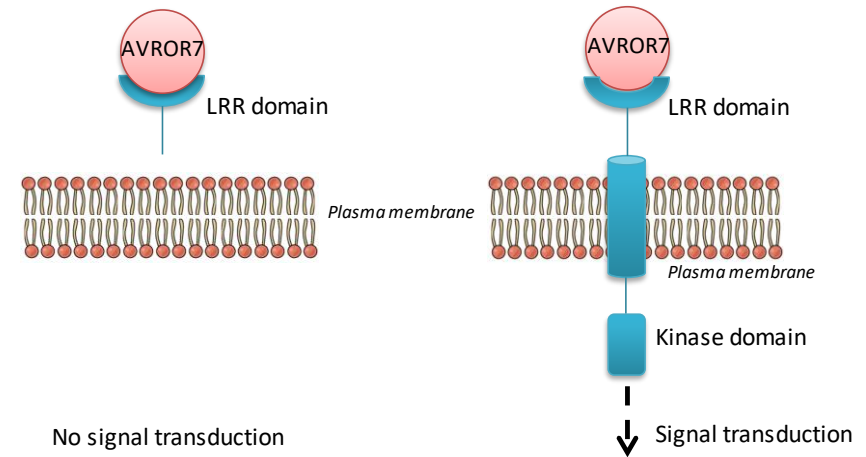
Functional characterization of the *HaOr7* gene



Compatible attachment



Incompatible attachment






Connection to the vascular system of sunflower root
Susceptibility



No connection to the vascular system of sunflower root
Resistance

Duriez *et al.* (Nature Plants, 2019)

A receptor-like kinase enhances sunflower resistance to *Orobanche cumana*

Pauline Duriez^{1,2}, Sonia Vautrin³, Marie-Christine Auriac¹, Julia Bazerque¹, Marie-Claude Boniface¹, Caroline Callot³, Sébastien Carrère ¹, Stéphane Cauet ³, Mireille Chabaud¹, Fabienne Gentou², Marta Lopez-Sendon², Clémence Paris², Prune Pegot-Espagnet¹, Jean-Christophe Rousseaux², Begoña Pérez-Vich⁴, Leonardo Velasco⁴, Hélène Bergès³, Joël Piquemal² and Stéphane Muñoz ^{1*}

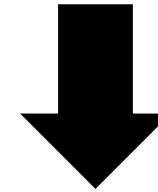
Toward the identification of the avirulence gene interacting with *HaOr7* (coll. Syngenta, LIPME, CSIC)

Mapping of the Or_{Deb2} gene

A source of resistance to race G has been identified in the annual species *H. debilis* subsp. *tardiflorus* (2011)



The resistance is monogenic and dominant



Introgressed in *H. annuus*


Mapped on chromosome 4

Theoretical and Applied Genetics
<https://doi.org/10.1007/s00122-021-03979-9>

ORIGINAL ARTICLE



Genetic and physiological characterization of sunflower resistance provided by the wild-derived Or_{Deb2} gene against highly virulent races of *Orobanche cumana* Wallr

Mónica Fernández-Aparicio¹ · Lidia del Moral¹ · Stéphane Muños² · Leonardo Velasco¹ · Begoña Pérez-Vich¹ 

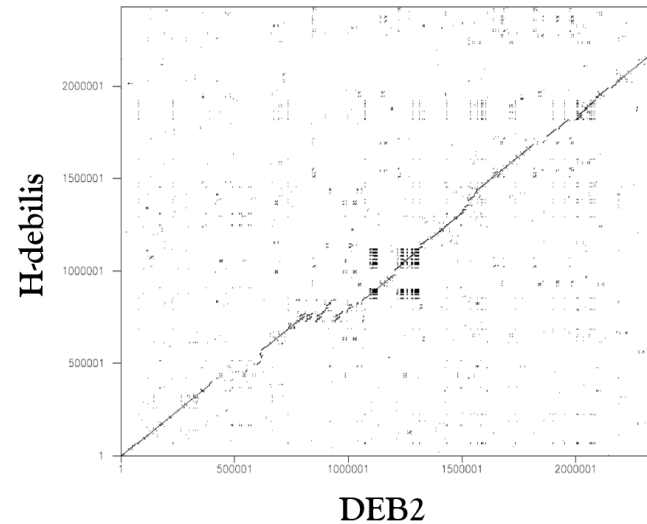
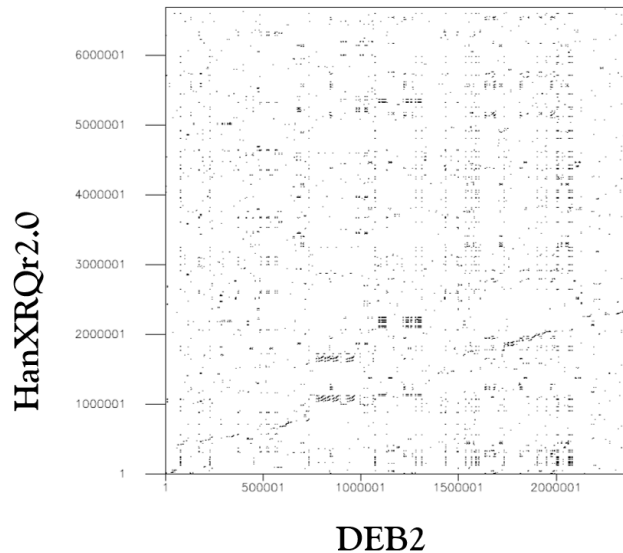
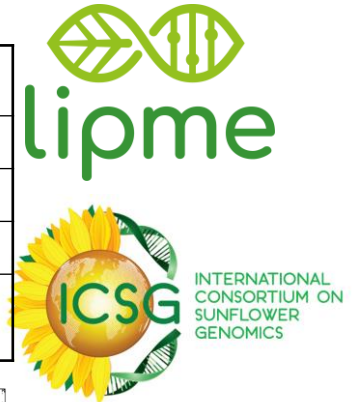
Genome sequencing of the deb2 line

Belén Fernandez-Melero (PhD student CSIC-IAS, Spain, 4 months stay in my group)

| DEB2 genome (homozygous) | |
|--------------------------|------------|
| NUM | 39 contigs |
| N50 | 176 Mbp |
| BP | 3.16 Gbp |
| Not annotated | |



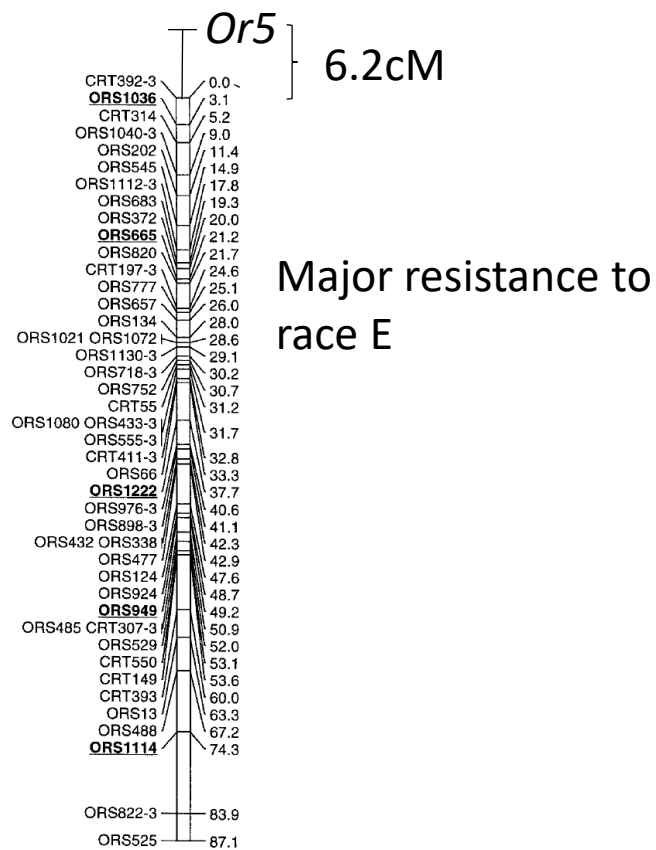
| H-debilis genome (heterozygous) | |
|-------------------------------------|--------------|
| NUM | 1417 contigs |
| N50 | 112.7 Mbp |
| BP | 4.9 Gbp |
| Annotated genes coding for proteins | 84226 |



Strong homology between the two accessions of *H. debilis* Or_{Deb2} under identification (IAS-CSIC)

The *HaOr5* gene as a model gene to better understand the molecular mechanisms of the interaction

Or5 mapped on LG3 (telomeric region)



Tang *et al.*, Crop Science, 2003

Gene-for-Gene Interaction



DOI: 10.1111/wre.12034

A dominant avirulence gene in *Orobanche cumana* triggers *Or5* resistance in sunflower

M I RODRÍGUEZ-OJEDA*, R PINEDA-MARTOS†, L C ALONSO*, J FERNÁNDEZ-ESCOBAR*, J M FERNÁNDEZ-MARTÍNEZ†, B PÉREZ-VICH† & L VELASCO†

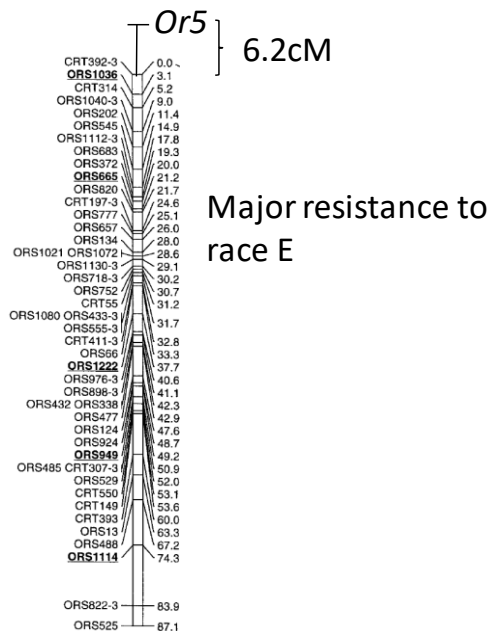
*Káipesol Semillas S.A., Sevilla, Spain, and †Instituto de Agricultura Sostenible (IAS-CSIC), Córdoba, Spain

Identification of the *HaOr5* gene

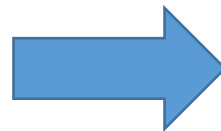
Camille Pubert, Phd student
(INRAE-MAS SEEDS)



Or5 mapped on LG3 (telomeric region)



HaOr5 provides resistance to race E



Gene mapped in a 1.5 Mb

Tang *et al.*, Crop Science, 2003

Mapped based cloning of the *HaOr5* gene

Same strategy than for HaOr7 : screening by genotyping a large F2 population and phenotyping the recombinant plants

Genomic region restricted from 1.5 Mb to 193kb

Identification of the *HaOr5* gene

Sequencing in Gentyane platform
(INRAE Clermont Ferrand, France), 2
smrt cells HiFi + optical map in CNRGV
(INRAE Toulouse, France)

Susceptible HaOr5- line:
20 scaffolds
N50 = 177Mb
Size = 3.02Gb
%N = 0,5%

LC1093:
21 scaffolds
N50 = 176Mb
Size = 3.01Gb
%N = 0,05%

→ **Good quality of the two genome sequences**

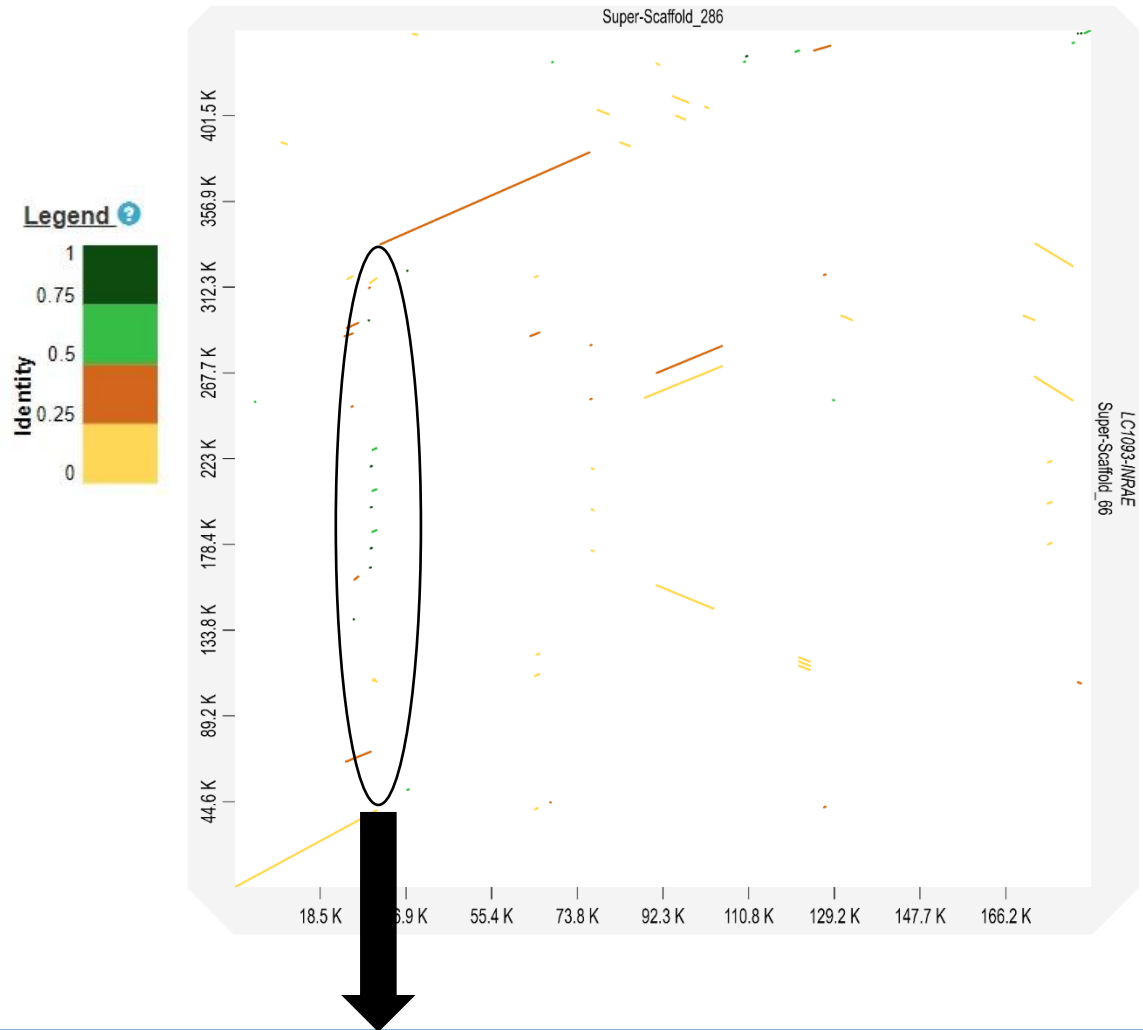
Identification of the *HaOr5* gene

Comparison of sequence
between nmk3 and nmk4
(D-genies)

193kb on XRQ

185kb on TFP4417

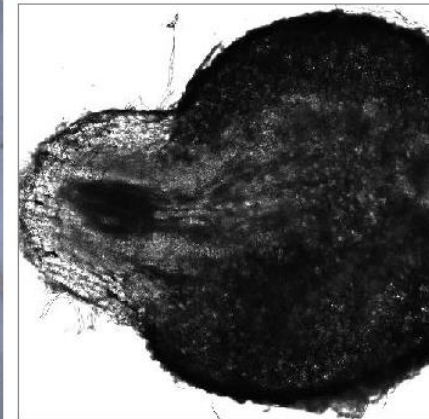
446kb on LC1093 INRAE



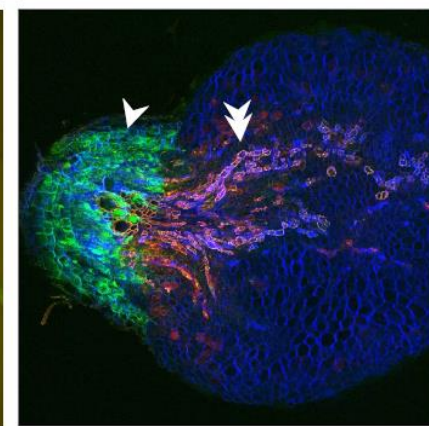
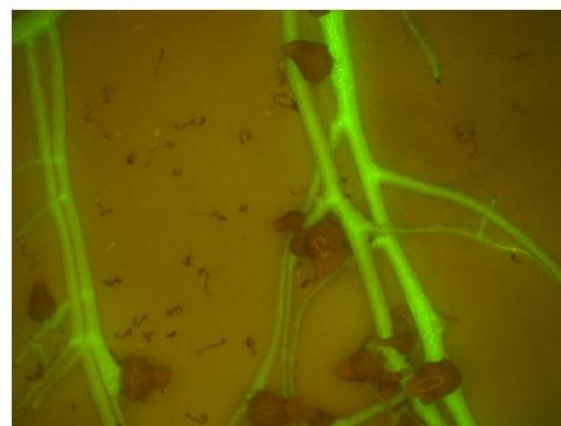
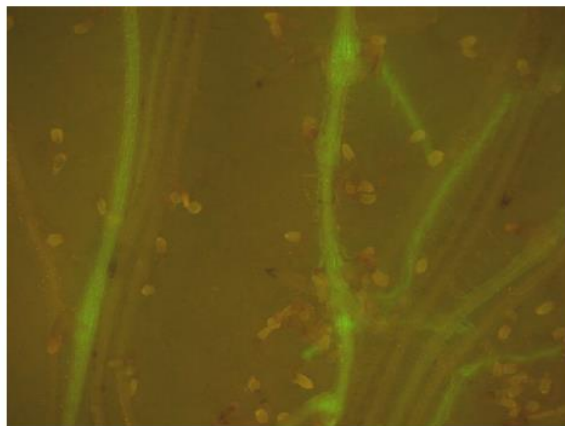
Large (> 200kb) insertion in LC1093 could contain the resistance gene

Functional validation of the major resistance genes

Sunflower root transformation using *Agrobacterium rhizogenes*



Confocal observation of 100 μ section in gelose-staining calcofluor 0,04%-staining Syto 84 0,5 μ M



GFP fluorescence expressed in sunflower transformed root cells

Calcofluor staining of cell walls
Syto84 staining of *Orobanche* tubercle xylem vessels

Mireille Chabaud (LIPME)

How to control *O. cumana*?

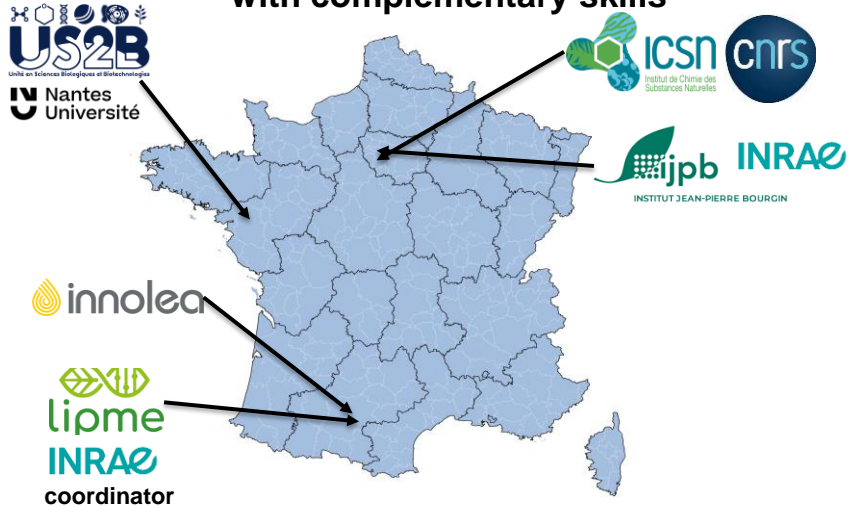
2. New control methods

A new ongoing project

The STIGO project in brief.

Deciphering the molecular mechanisms governing sunflower-specific recognition by *O. cumana*

A private-public consortium (5 partners)
with complementary skills



A PRCE project

Started: January 1st, 2022

4-years project (2022-2025)

Total budget: 2,402 k€ = ANR fundings (710 k€) + Innolea fundings (324k€) + public salaries

Three main objectives of the STIGO Project:

1. Identification of the *O. cumana* seed germination stimulants (GS) and their biosynthesis genes in sunflower

using omics, biochemistry and genetic approaches

2. Characterizing the GS receptors in *O. cumana*

-*in vitro* approaches using the KAI2 receptor proteins

-diversity of the receptors in worldwide populations

-identification of germination-inhibiting molecules (large scale screening of chemical libraries)

3. Development of new control methods

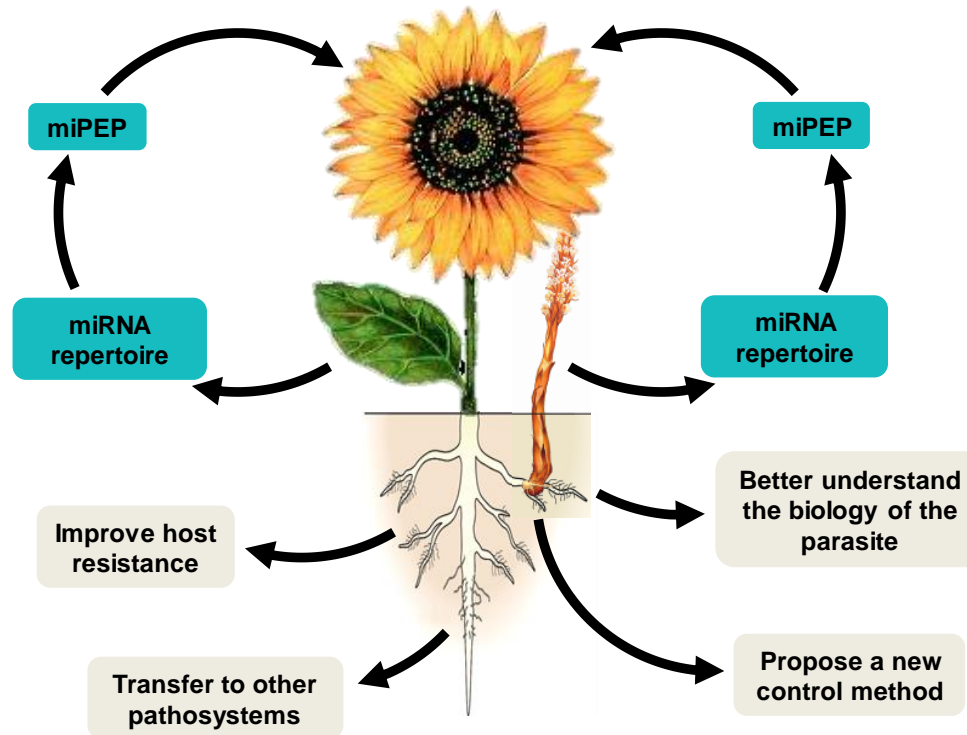
-molecular assisted selection of new sunflower resistant varieties (non-GS)

-new agro-chemical control (long-term)

Evaluation of new control methods

Mipep (micropeptides) are small peptides (10-20 amino acids) that can be synthesized or produced in biotanks

Applied on plants, they can regulate gene expression and can affect phenotypes



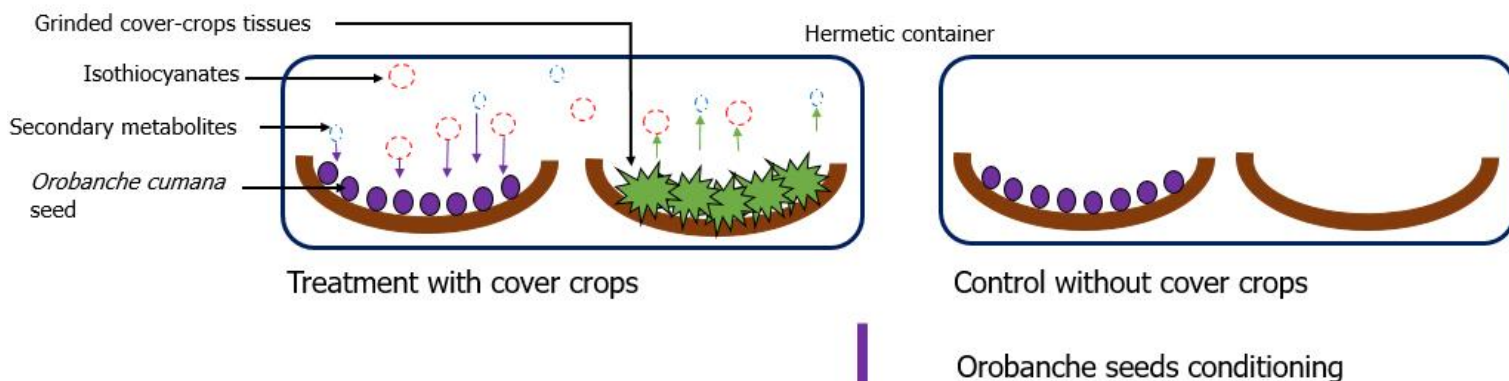
Philippe Delavault's talk is following

OMOCO project: Assessment of winter cover crops (Brassicaceae and/or Fabaceae) and biofumigation as a Control of sunflower broomrape

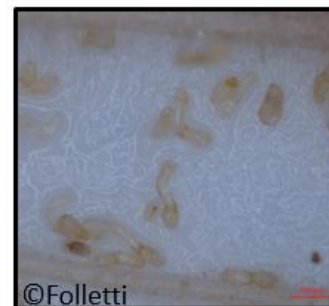
Objective : Assessing the potential of cover crops to reduce sunflower broomrape damages by modulating seeds germination

Coll. MAS SEEDS, UMR AGIR (INRAE), UMR LIPME (INRAE)

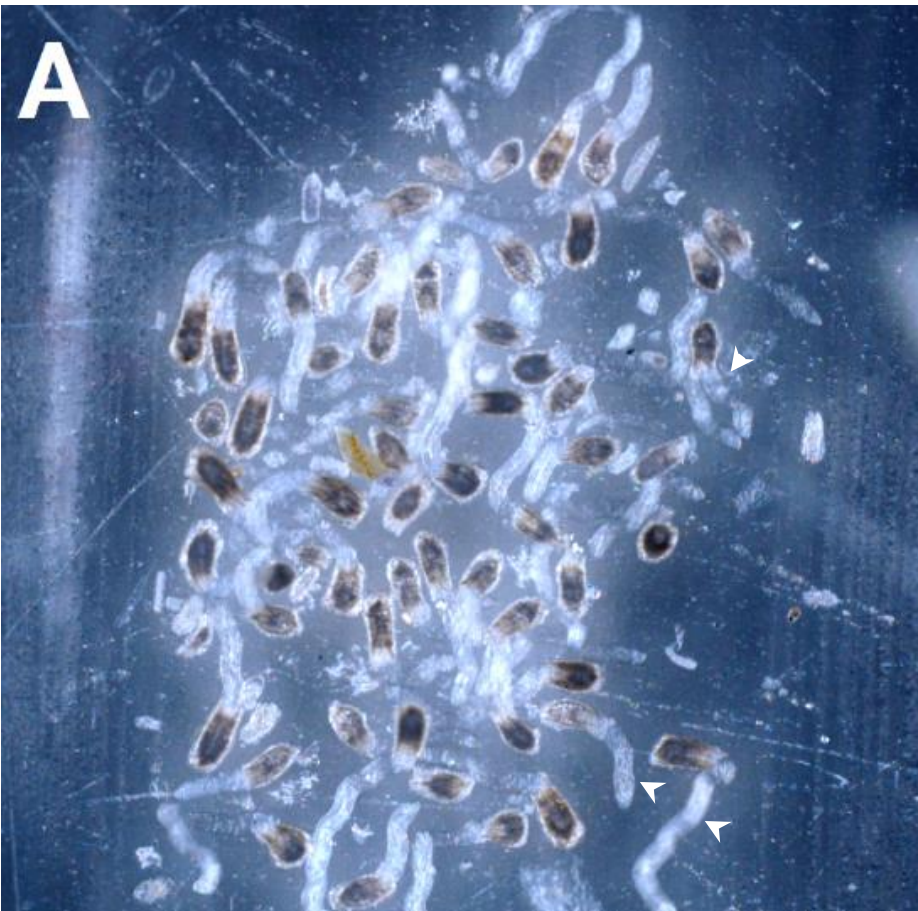
(A) : Putting in contact Orobanche and cover crops residus



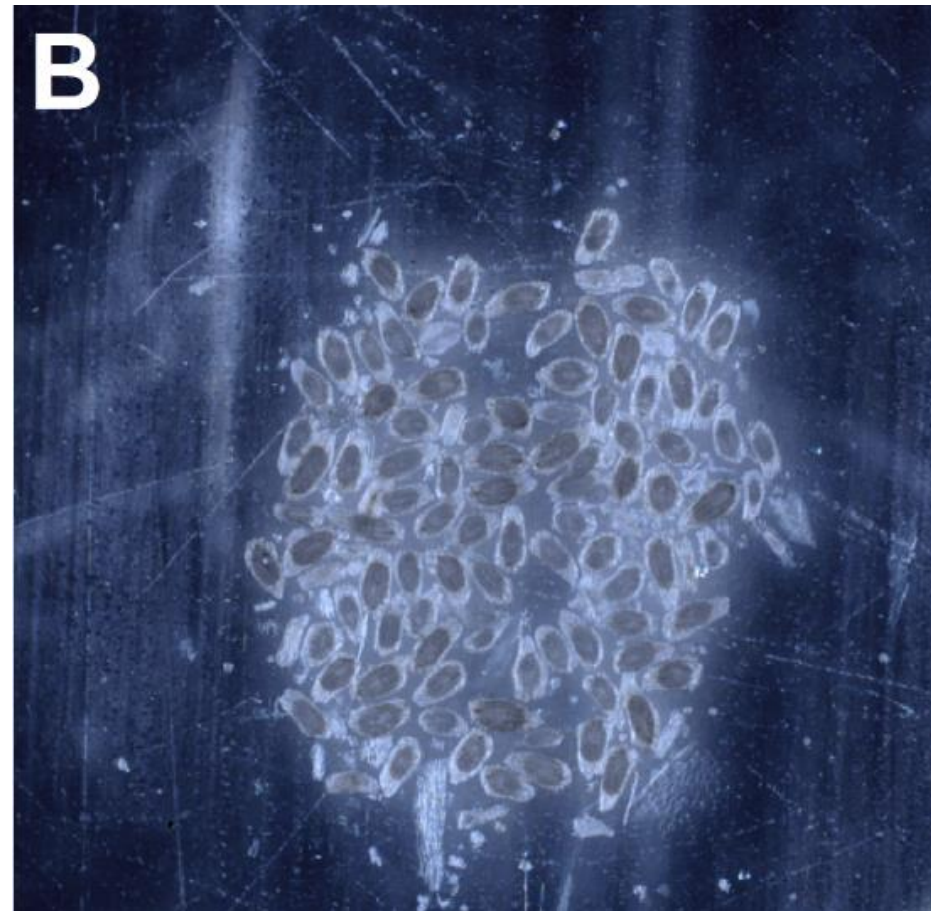
(B) : Counting germination rates



CONTROL



+ one species of covercrop



Many thanks to Collaborators

Local:

Bioinformatics LIPME
 ASTR LIPME
 CNRGV
 UMR AGIR
 TPMP
 TRI imaging
 Get-Plage
 LRSV

National:

Terres Inovia
 GEVES
 Nantes University

International:

- China: IMAU (Jun Zhao), Zhejiang University (Weijun Zhu, Luyang Hu, PhD student, 2-years stay)
- Spain: IAS-CSIC (Begoña Pérez-Vich, Leo Velasco, Belen Fernandez)
- Netherlands: Wageningen (KeyGene, Martin DeVos)
- USA: Jim Westwood (Virginia Tech University), USDA (Lora Marek)
- Canada: UBC (Loren Rieseberg)
- Japan: Satoko Yoshida

Private partners: Syngenta, Innolea, MAS SEEDS, Sofiproteol, Promosol, MicroPep



O. cumana biology



Sunflower genetics
 Genomics:
O. cumana and sunflower

First project initiated in 2013



Sunflower genetics
O. cumana genetics and diversity