



The biology of *Phelipanche* and *Orobanche*

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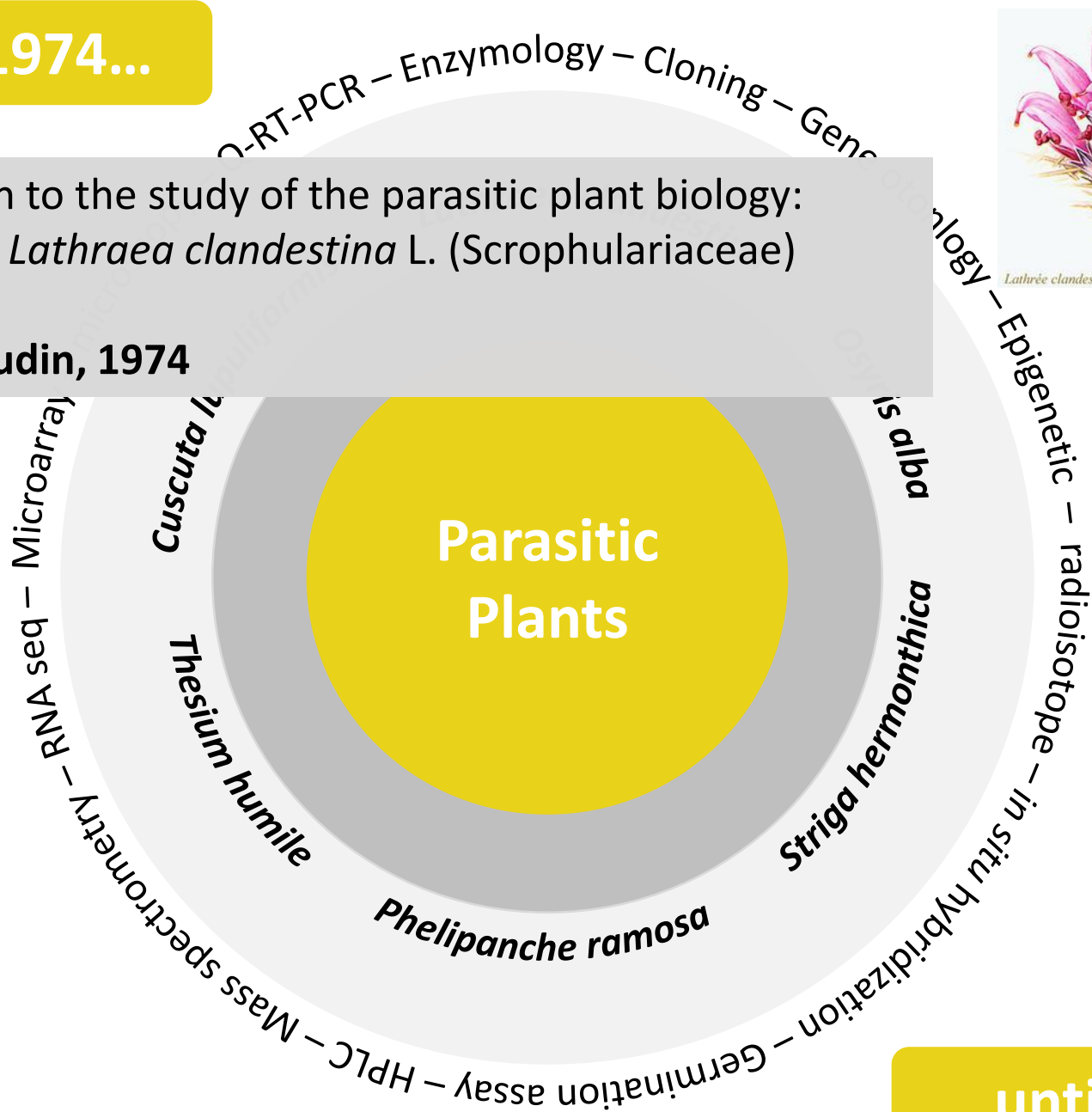
UNIVERSITÉ DE NANTES



From 1974...

Contribution to the study of the parasitic plant biology:
research on *Lathraea clandestina* L. (Scrophulariaceae)

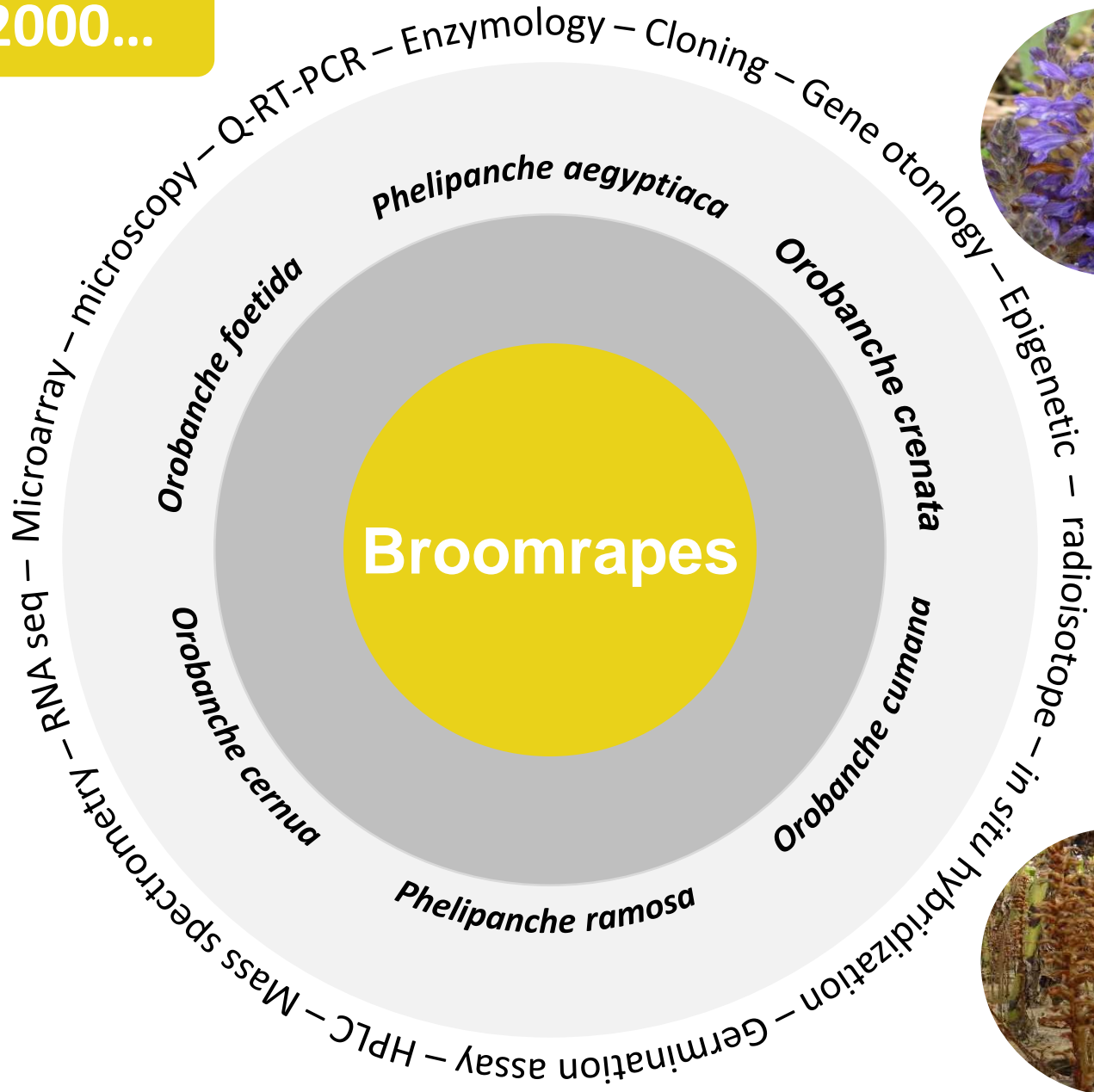
Serge Renaudin, 1974



...until today



Since 2000...



Phelipanche ramosa



Orobanche cumana



***Phelipanche ramosa*: an agronomic issue in France in oilseed rape (*Brassica napus*)**

- First infestations: . 20 years ago
- Favorable factors: . Intensive cropping of winter oilseed rape
. Absence of efficient chemicals and host resistances
. Clay and limestone soils
- Level of infestation: . Variable according to the year
. High levels with non tolerant cultivars « no yield »



Phelipanche ramosa: an agronomic issue in France in oilseed rape (*Brassica napus*)



Winter

- Significant symptoms during winter
- Growth is reduced in spring

Severe impact on yields



Spring



June



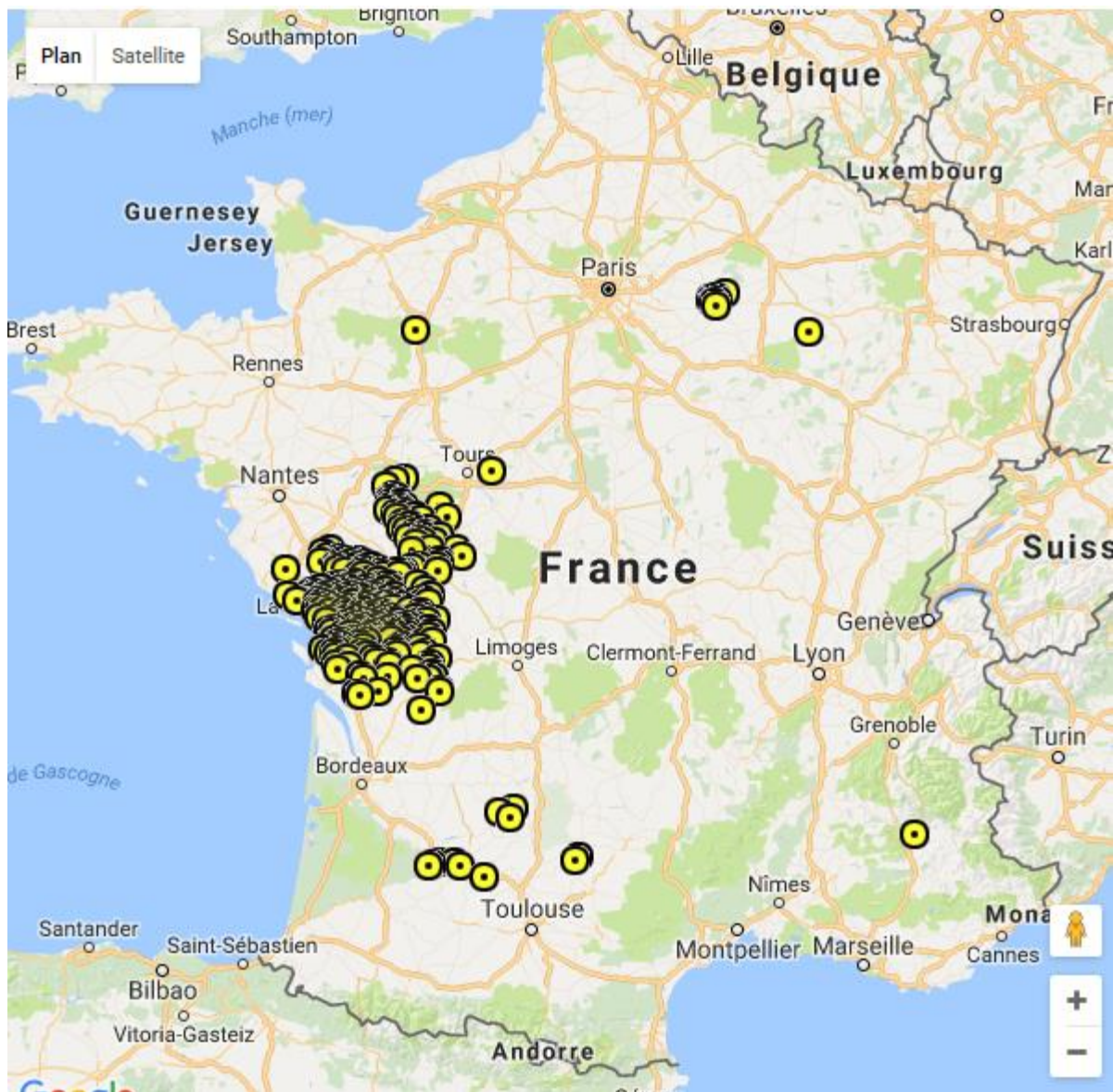
Phelipanche ramosa: an agronomic issue in France in oilseed rape (*Brassica napus*)

Mapping of infestations

Crops are under high survey by farmers and farm technicians

“to bring the information back up from the field”

Oilseed rape



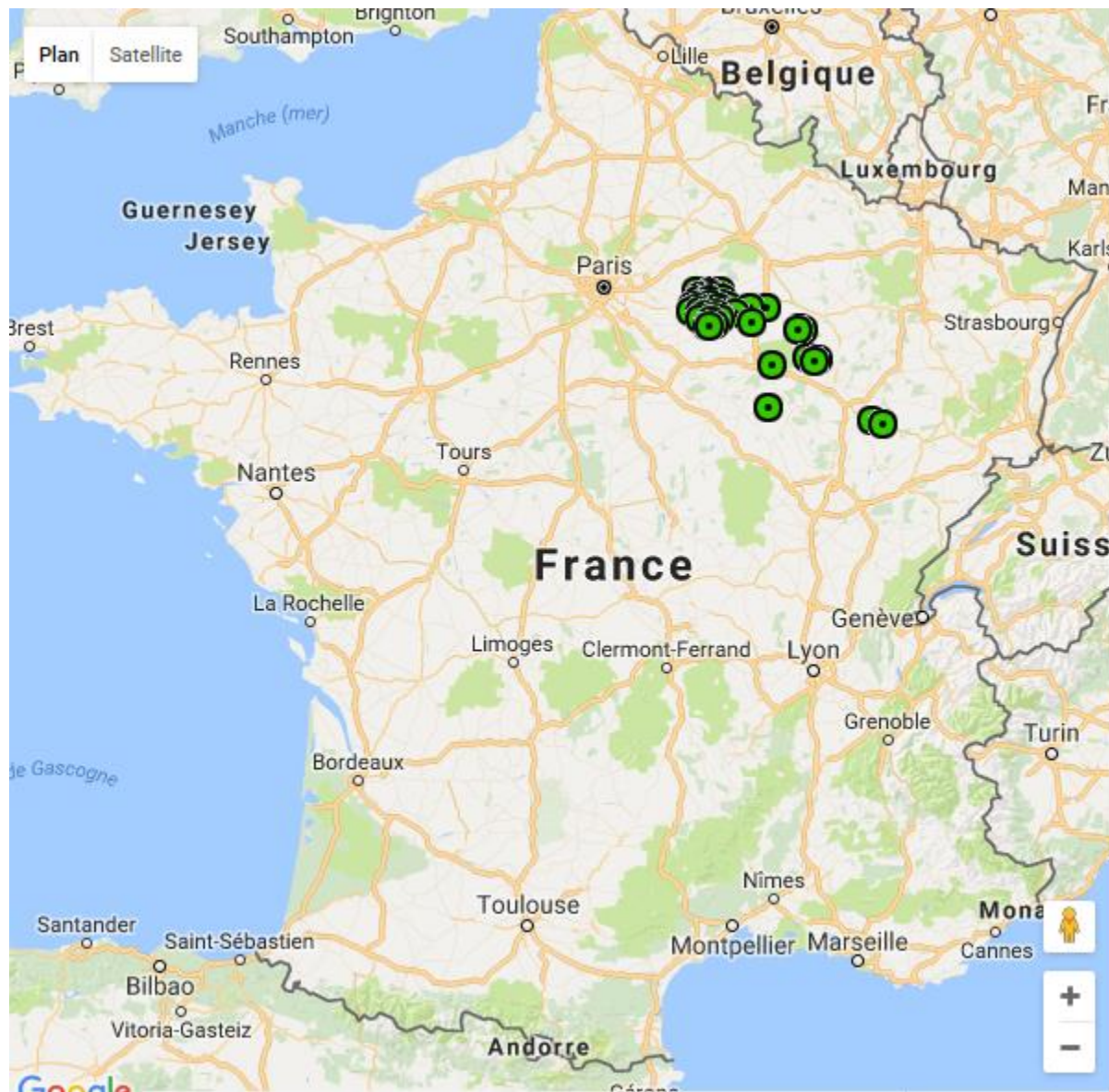
Phelipanche ramosa: an agronomic issue in France in oilseed rape (*Brassica napus*)

Mapping of infestations

Crops are under high survey by farmers and farm technicians

“to bring the information back up from the field”

Hemp



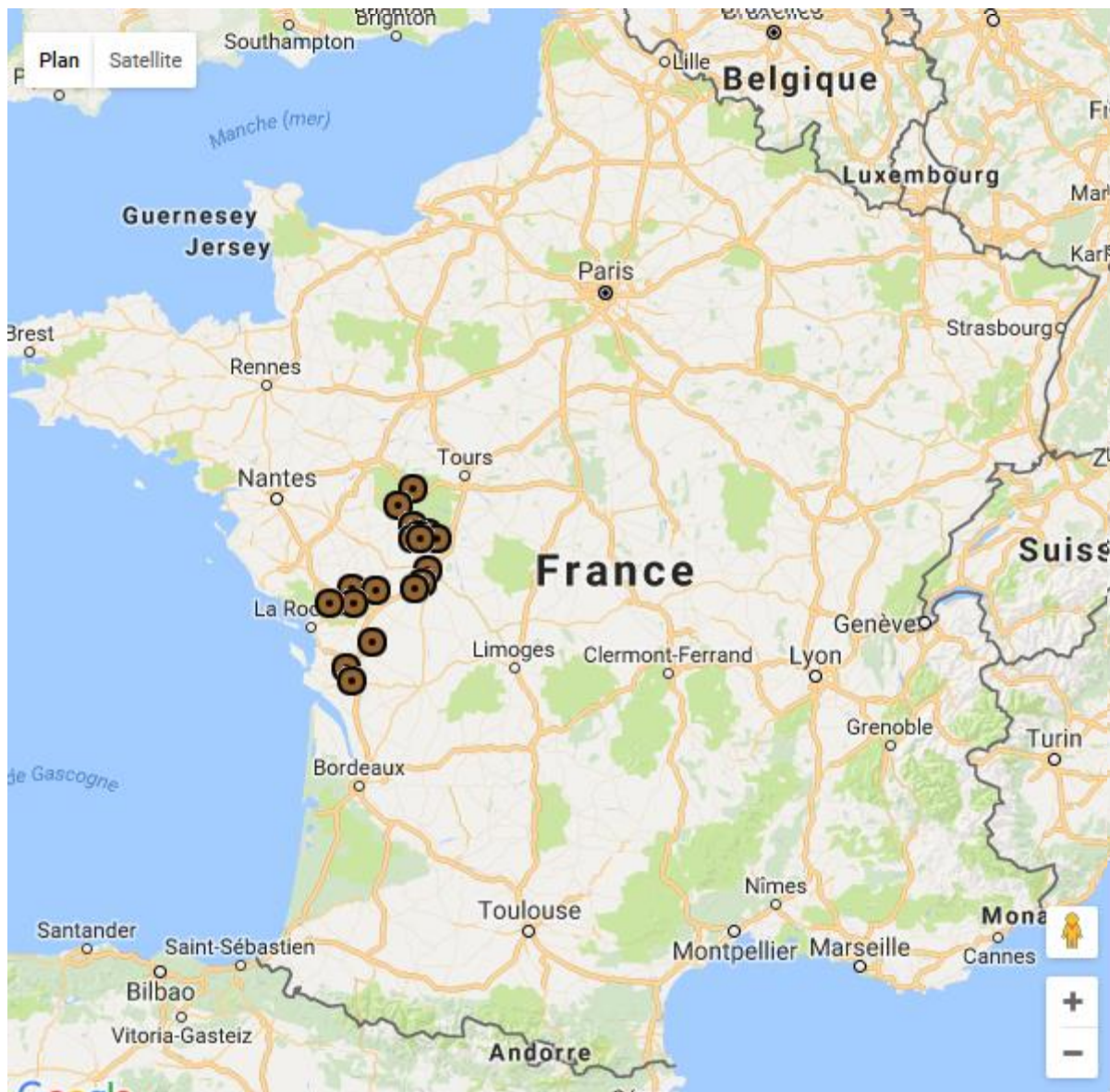
Phelipanche ramosa: an agronomic issue in France in oilseed rape (*Brassica napus*)

Mapping of infestations

Crops are under high survey by farmers and farm technicians

“to bring the information back up from the field”

Tobacco



Orobanche cumana: an agronomic issue in France in sunflower

- First infestations: . Several years ago
- Favorable factors: . Intensive cropping of sunflower
. Resistance overcomes by new races
- Level of infestation: . Variable according to the year
. High levels with non resistant varieties « no yield »



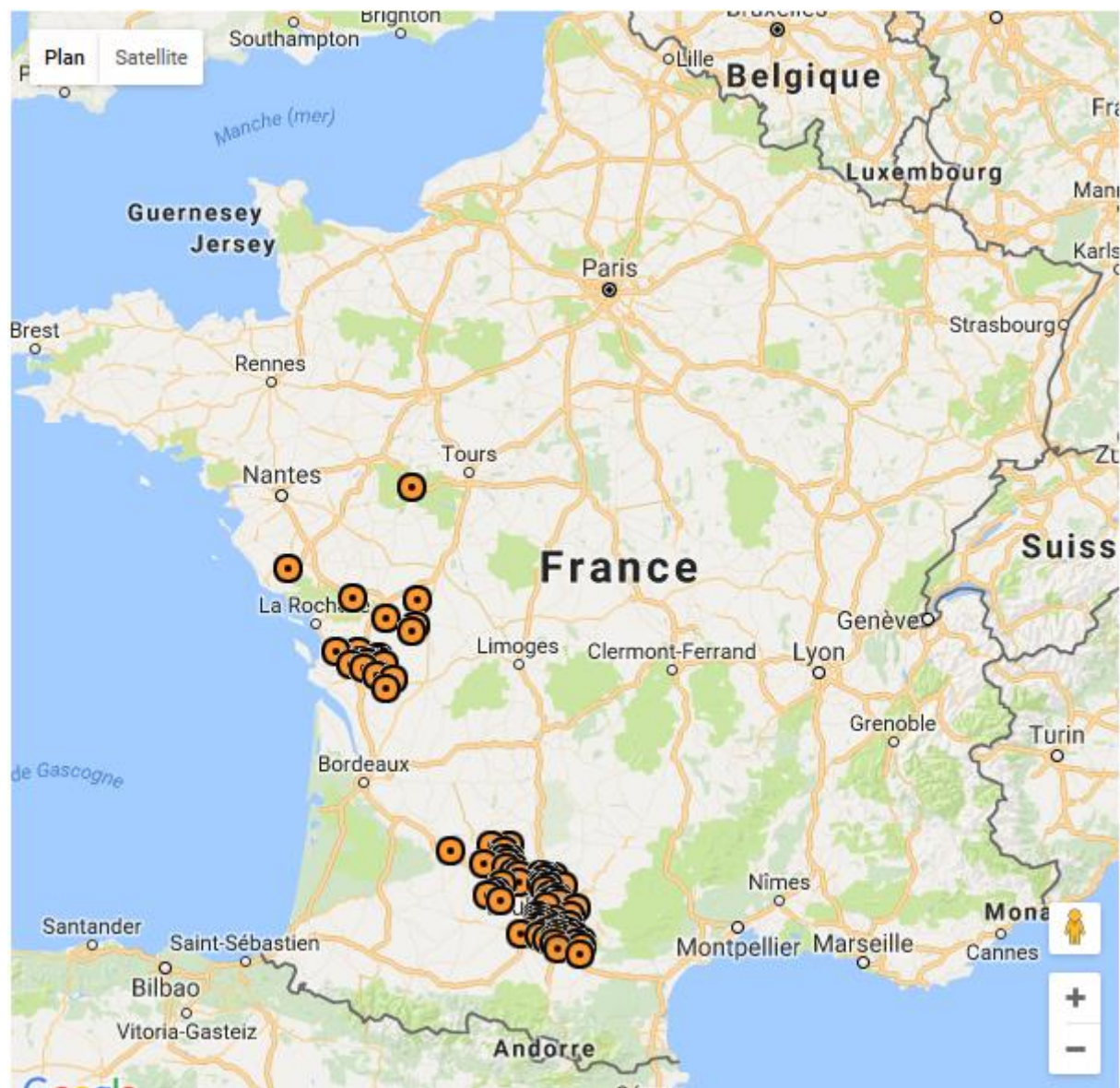
Orobanche cumana: an agronomic issue in France in sunflower

Mapping of infestations

Crops are under high survey
by farmers and farm
technicians

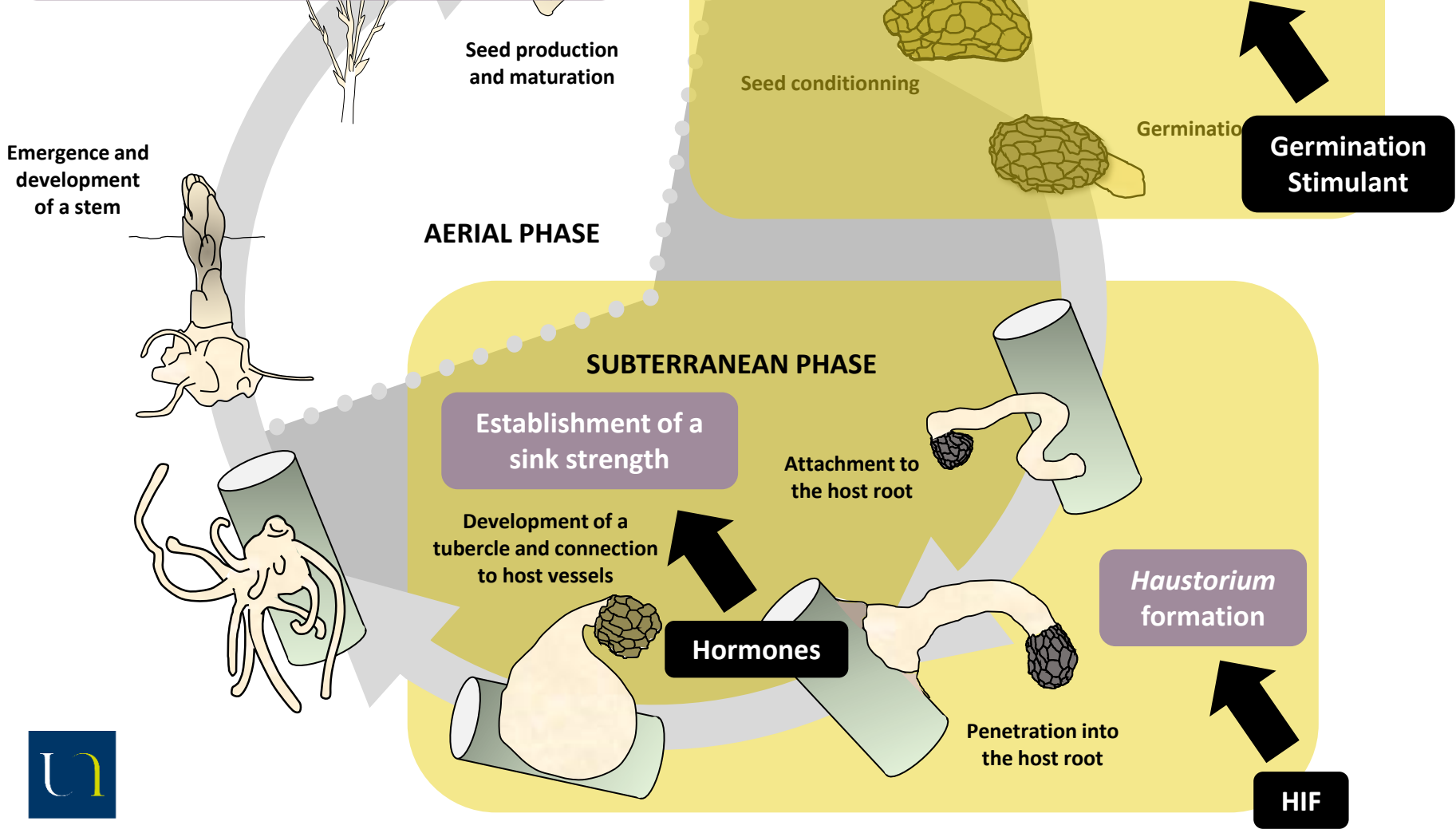
“to bring the information back
up from the field”

Sunflower

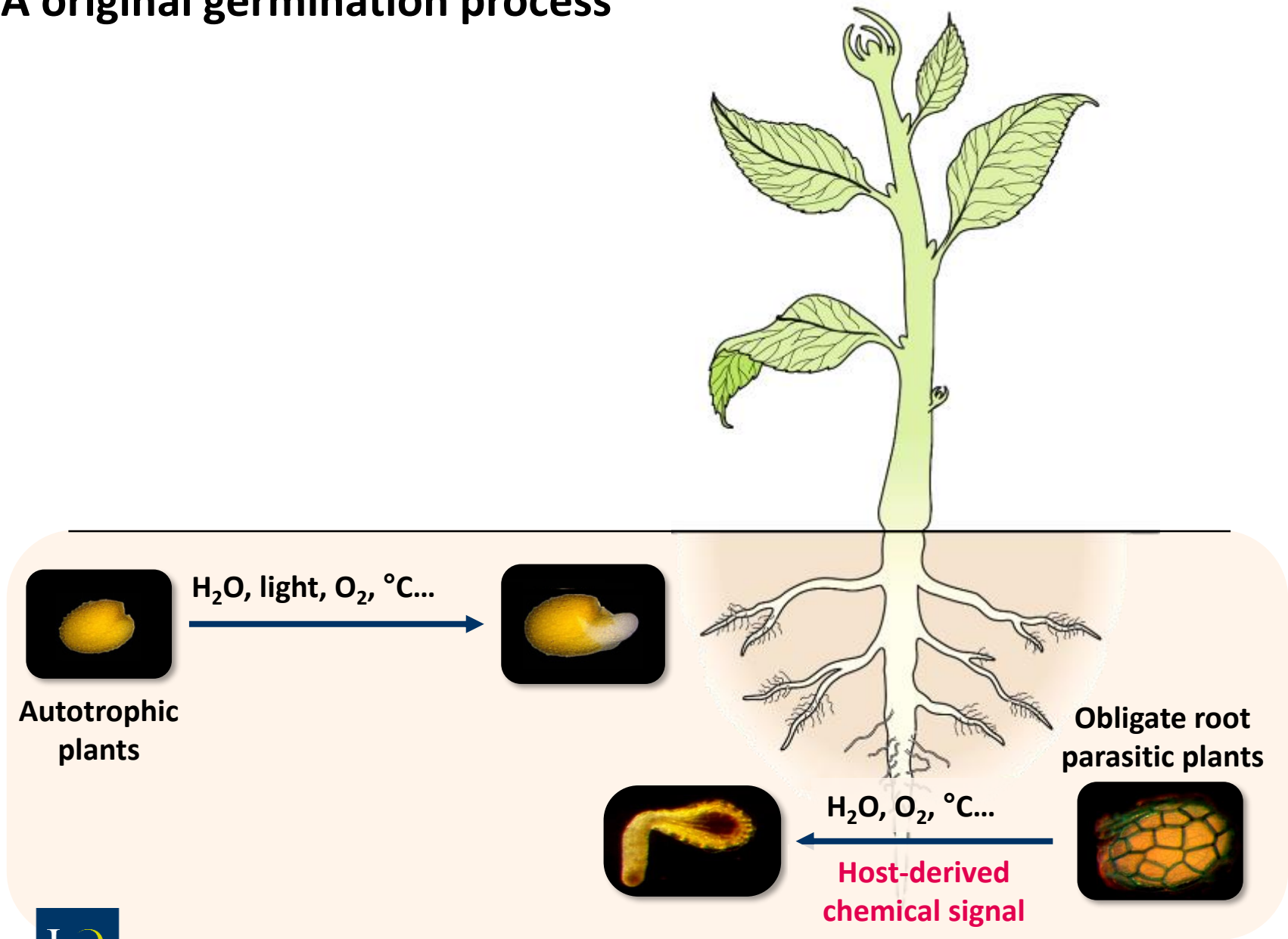


Broomrape life cycle

Identify new targets for the development of specific control methods

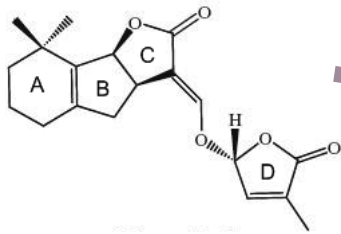


A original germination process

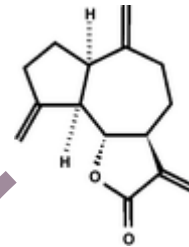


Germination stimulants

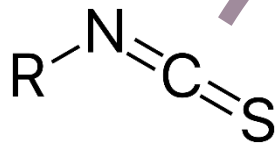
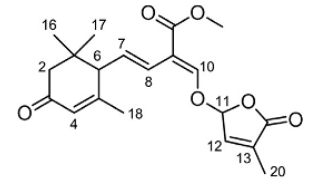
More than 15 strigolactones from various plant species (Cook *et al.*, 1966)



Sesquiterpene lactones from sunflower (Joel *et al.*, 2011; Raupp and Otmar, 2013)

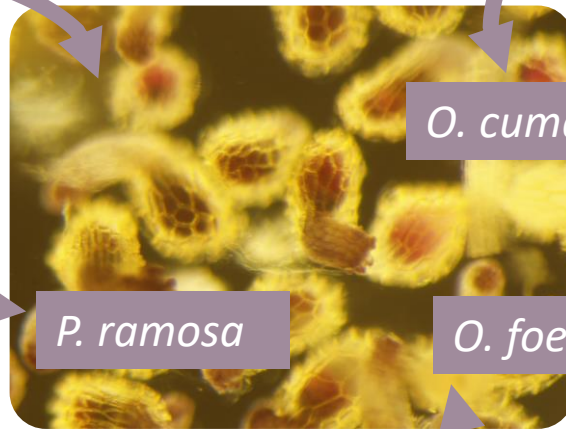


Heliolactone from sunflower (Ueno *et al.*, 2014)

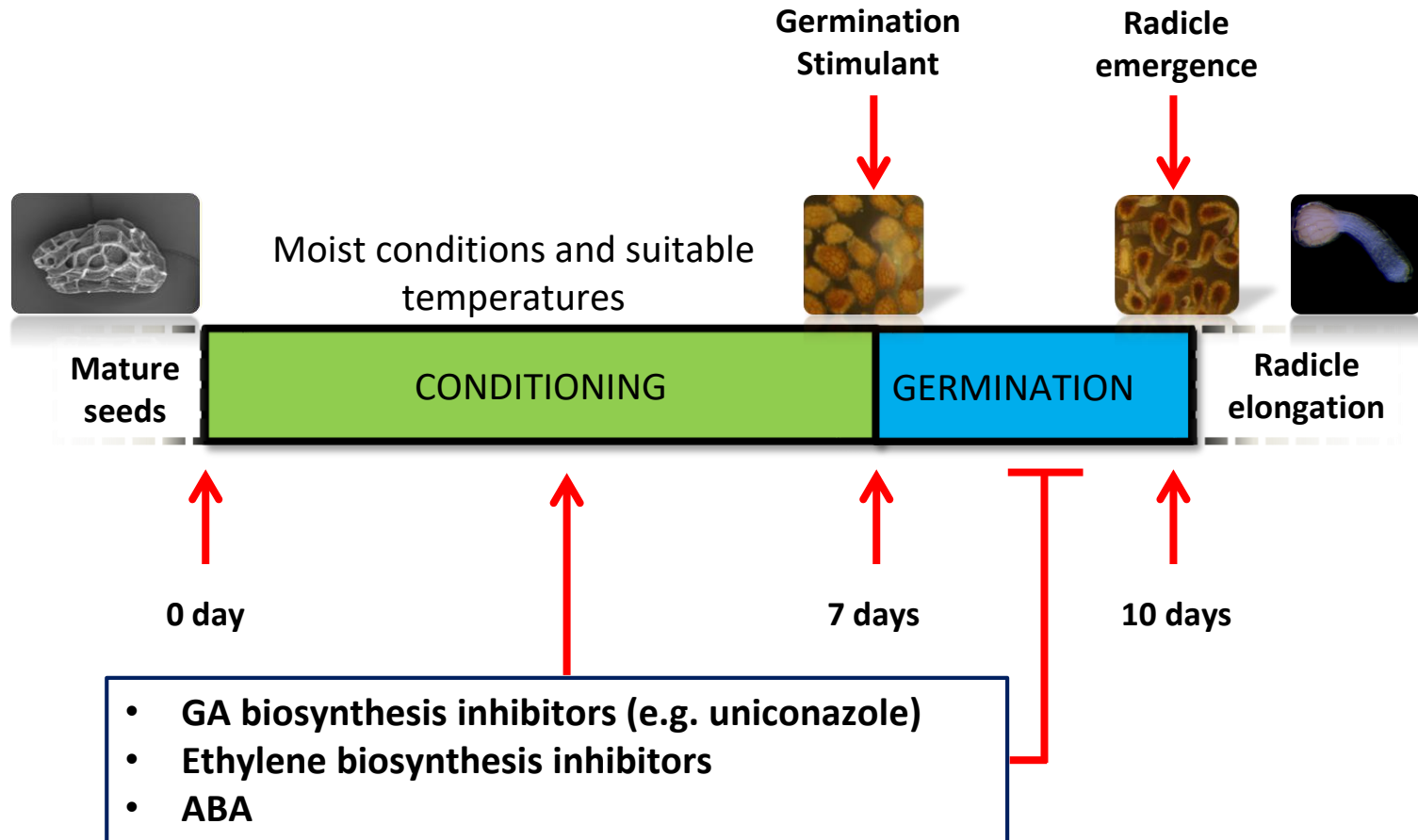


Isothiocyanates in the rhizosphere of oilseed rape (Auger *et al.*, 2012)

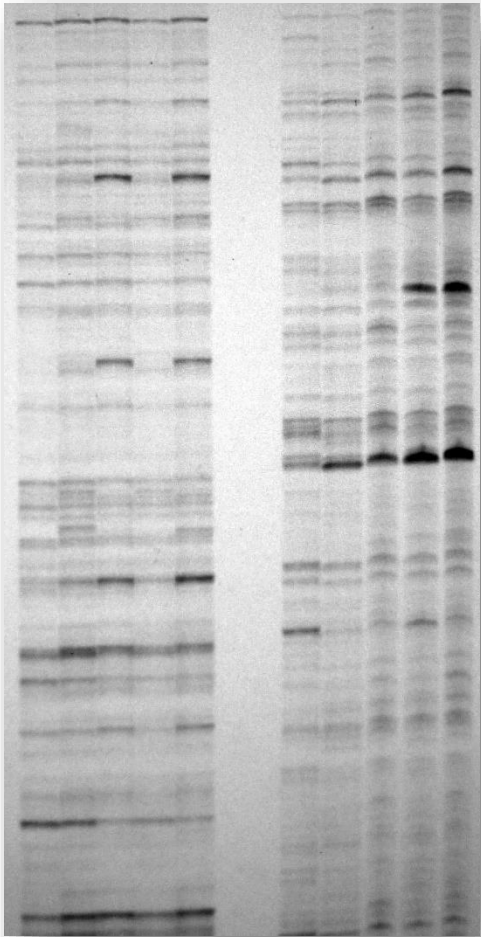
Polyphenols from common vetch (Evidente *et al.*, 2010)



Molecular actors of seed germination

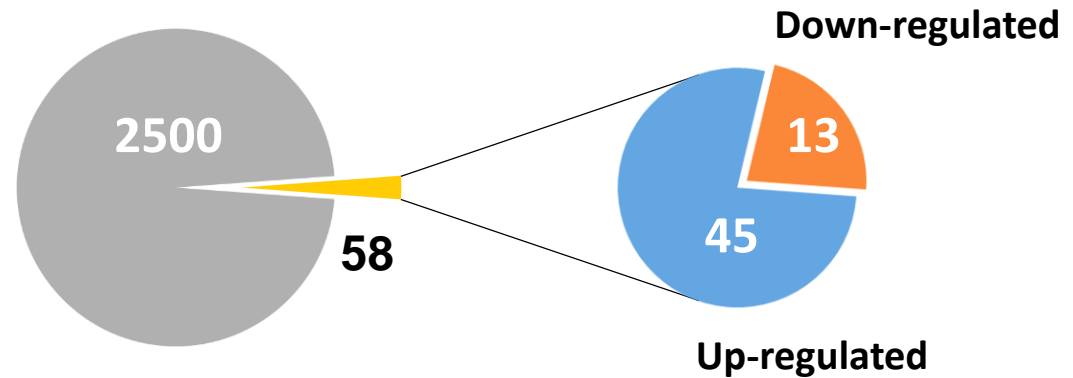


Molecular actors of seed germination



Transcriptomic approach using a cDNA-AFLP procedure on *Phelipanche ramosa* :

- 7d conditioned seeds
- 7d conditioned seeds - 2h GR24 or ITC treatment
- 7d conditioned seeds - 6h GR24 or ITC treatment



➔ **GS do not induce a massive modification of the transcriptome**

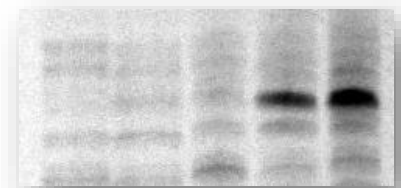
(mycorrhizal fungus *Gigaspora rosea*, Besserer *et al.*, 2008; *Arabidopsis*, Mashiguchi *et al.*, 2009 – Nelson *et al.*, 2010)



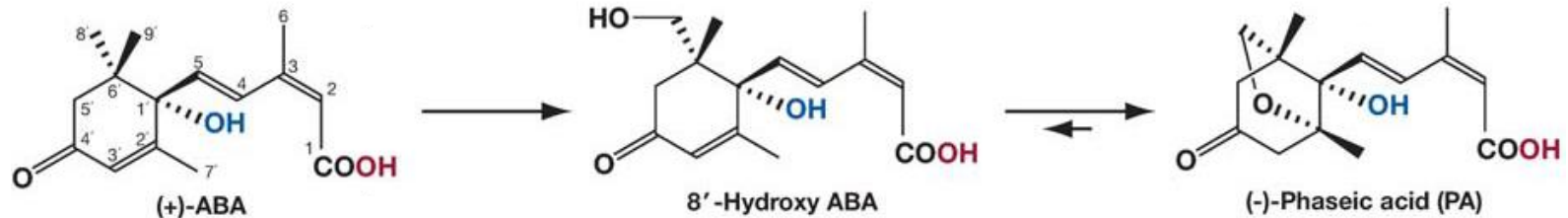
Molecular actors of seed germination

TDF #	Regulation		Best <i>Arabidopsis</i> Hit (Accession No.)	Functional category	E value
	2h	6h			
1	+	+	Sulphite reductase (NP_196079)	Oxidation-reduction processes	2.00E ⁻⁰⁸
5	+	O	Sucrose synthase 3 (NP_192137)	Carbohydrate metabolic processes	6.00E ⁻³⁰
6	-	-	High mobility group (HMG1/2) domain-containing protein (NP_565788)	Nucleotide binding	8.00E ⁻¹⁷
7	O	-	Ninja-family protein AFP3 (NP_189598)	Nucleotide binding	7.00E ⁻¹⁹
8	O	+	Peptidylprolyl isomerase ROF2 (NP_199668)	Response to stress	6.00E ⁻²²
11	O	+	Heat shock protein 81.4 (NP_200411)	Response to stress	3.00E ⁻⁶⁵
13	O	+	PPPDE putative thiol peptidase family protein (NP_187365)	Unknown	1.00E ⁻²⁴
14	O	+	Rossmann-fold NAD(P)-binding domain-containing protein (NP_175552)	Oxidation-reduction processes	1.00E ⁻¹⁶
20	+	O	Trans-cinnamate 4-monooxygenase (NP_180607)	Secondary metabolic processes	0.006
26	+	O	HIPL2 protein (NP_201069)	Carbohydrate metabolic processes	3.00E ⁻⁰⁸
28	+	+	Methionine synthase 2 (NP_001118564)	Cellular amino acid metabolic processes	3.00E ⁻²¹
30	+	+	Abscisic acid 8'-hydroxylase 1 (NP_974574)	Response to stress	0.007
31	+	O	Acetylmethine deacetylase (NP_001190758)	Protein metabolic processes	0.091
32	-	-	26S proteasome regulatory subunit 4-A (NP_194633)	Protein metabolic processes	1
33	+	+	RNA recognition motif-containing protein (NP_197436)	Nucleotide binding	2.00E ⁻²³
36	O	+	Heat shock 70kDa protein 1 (NP_195870)	Response to stress	0.073
37	+	+	Abscisic acid 8'-hydroxylase 1 (NP_974574)	Response to stress	0.001
38	O	-	BI1-like protein (NP_567466)	Unknown	3.00E ⁻¹⁶
39	+	+	Heat shock protein 81-1 (NP_200076)	Response to stress	3.00E ⁻²³
40	+	O	Putative aquaporin TIP3-2 (NP_173223)	Transport	6.00E ⁻⁵⁰
41	+	+	Beta-glucosidase 44 (NP_188436)	Carbohydrate metabolic processes	5.00E ⁻⁴¹
44	O	+	Glutathione S-transferase PM24 (NP_192161)	Response to stress	6.00E ⁻⁰⁶
45	O	+	Heat shock protein 21 (NP_194497)	Response to stress	0.02
49	+	O	Catalase 2 (NP_195235)	Response to stress	1.00E ⁻⁴⁸
50	+	O	Heat shock protein 70B (NP_173055)	Response to stress	1.00E ⁻⁷⁹
51	+	O	Phenylalanine ammonia-lyase 3 (NP_001190223)	Response to stress	6.00E ⁻¹⁰
58	O	+	Putative xyloglucan glycosyltransferase 8 (NP_180039)	Carbohydrate metabolic processes	1.00E ⁻²⁶

➔ The up-regulated transcripts 30 and 37 encode an abscisic acid 8'-hydroxylase or CYP707A, an ABA catabolic enzyme



Molecular actors of seed germination



ABA 8'-hydroxylases (CYP707A) catalyze ABA hydroxylation and produce 8'-hydroxy ABA which is then spontaneously isomerized to phaseic acid

CYP707A1 and CYP707A2, Which Encode Abscisic Acid 8'-Hydroxylases, Are Indispensable for Proper Control of Seed Dormancy and Germination in Arabidopsis

by Masanori Okamoto, Ayuko Kuwahara, Mistunori Seo, Tetsuo Kushiro, Tadao Asami, Nobuhiro Hirai, Yuji Kamiya, Tomokazu Koshiba, and Eiji Nambara

Plant Physiology, 2006, Volume 141(1):97-107

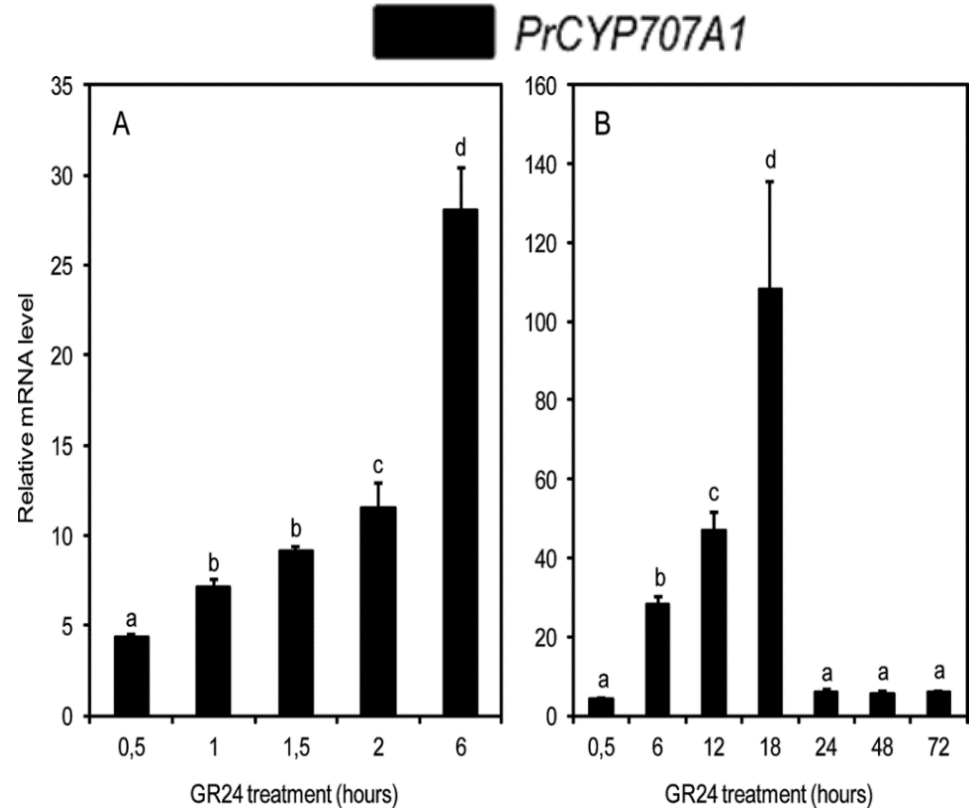


Molecular actors of seed germination

Parasitic Plant Genome Project

Home | Plants | People | Related | Blast | Search | Downloads | Data Summary

- 3 *P. aegyptiaca* *PaCYP707A* genes
- 4 *Arabidopsis* *AtCYP707A* genes
- Using RACE strategies :
 - 2 full-length *P. ramosa* cDNA
 - *PrCYP707A1*
 - *PrCYP707A2*
- Q-RT-PCR validation

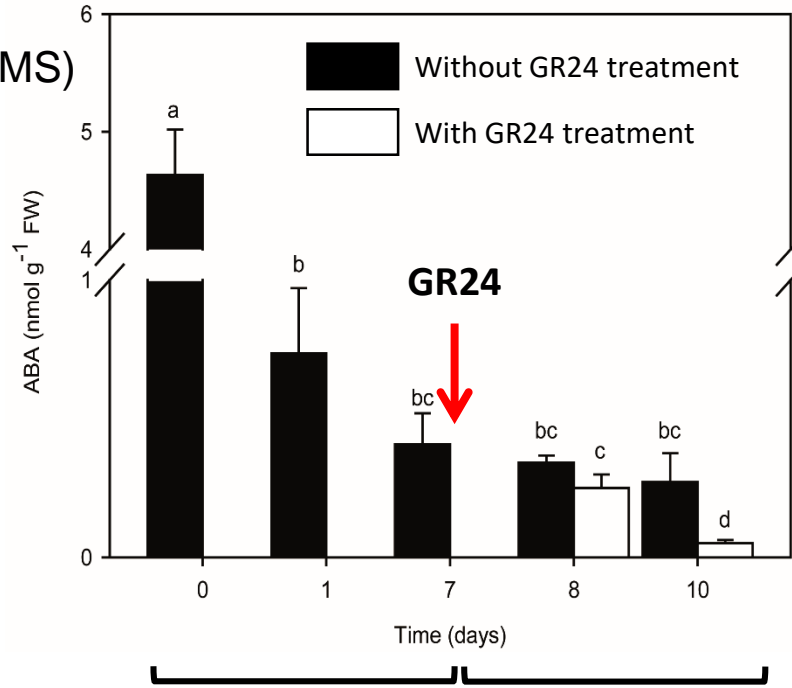


➔ ***PrCYP707A1*, an ABA 8'-hydroxylase encoding gene, is strongly up-regulated in response to GR24 and ITC**



Molecular actors of seed germination

ABA seed content (LC-MS)



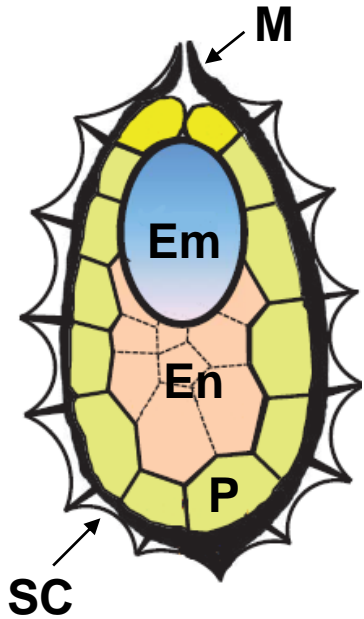
↘ [ABA] by diffusion ↘ [ABA] by PrCYP707A catabolism

➔ *P. ramosa* seeds germinate after the endogenous ABA content reaches a sufficient low level through CYP707A dependent catabolism triggered by GS

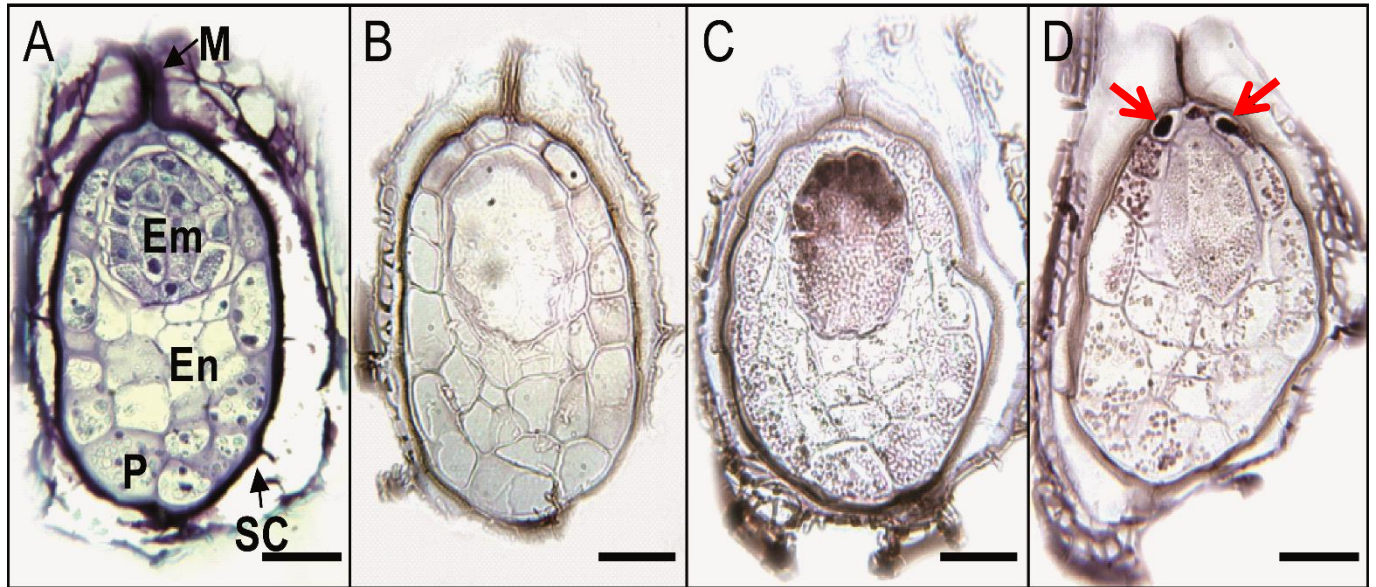


Molecular actors of seed germination

In situ localization of *PrCYP707A1* transcripts in longitudinal sections of *P. ramosa* seeds



Plakhine *et al.*, 2012



Toluidine blue staining

Negative control

7 d conditioned seed

7 d conditioned seed
+ 6 h GR24 treatment

- ➔ - SLs induce a change in the spatial localization of *PrCYP707A1* expression in seeds
- SLs induce *PrCYP707A1* mRNA accumulation in cells close to the micropyle

Putative location of germination stimulant receptors in perisperm cells?

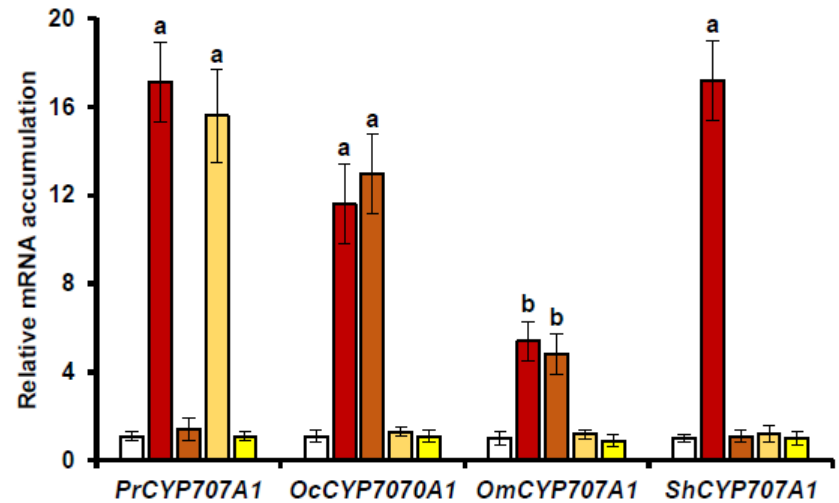
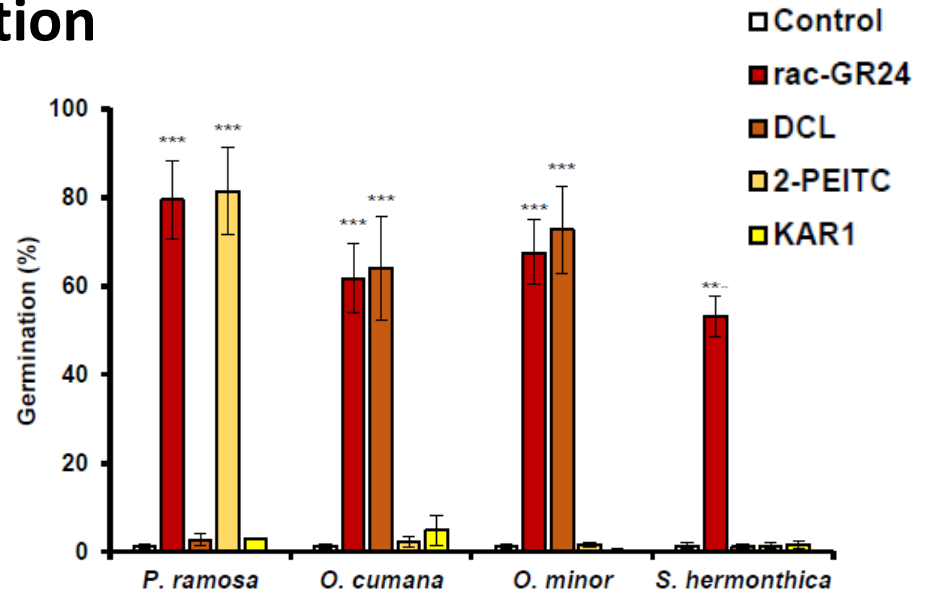


Molecular actors of seed germination

EC₅₀ :

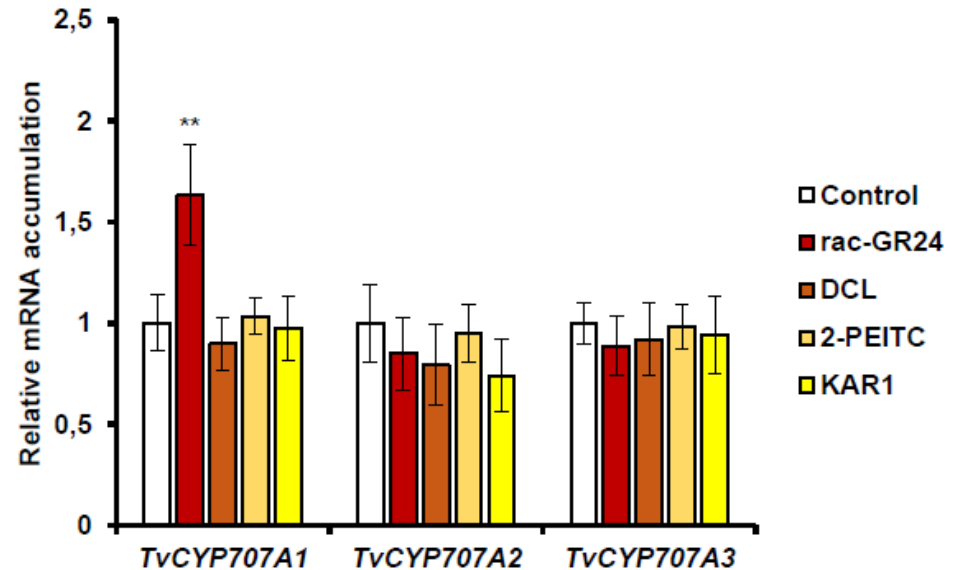
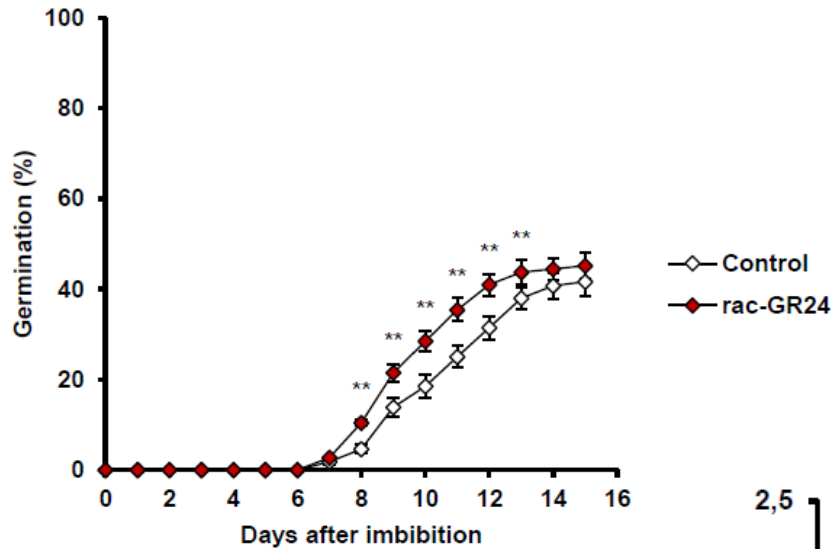
	rac-GR24	DCL	2-PEITC	KAR1
<i>P. ramosa</i>	1.10 ⁻¹²	-	1.10 ⁻⁹	-
<i>O. cumana</i>	1.10 ⁻¹¹	1.10 ⁻¹⁰	-	-
<i>O. minor</i>	1.10 ⁻⁸	1.10 ⁻⁷	-	-
<i>S. hermonthica</i>	1.10 ⁻¹⁰	-	-	-

High affinity
 Low affinity
 No activity



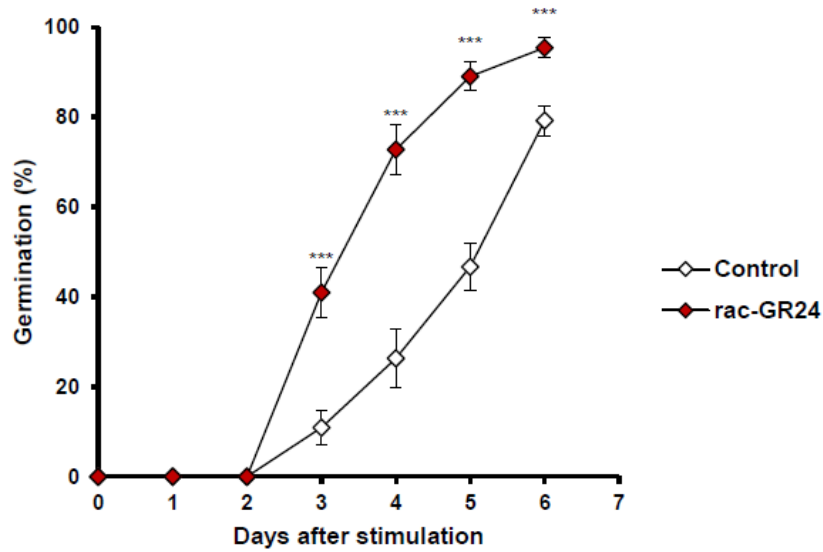
➔ Molecules that trigger the germination induce an up-regulation of a unique *CYP707A* gene in obligate root parasitic plants

Seed germination in facultative root parasitic plants

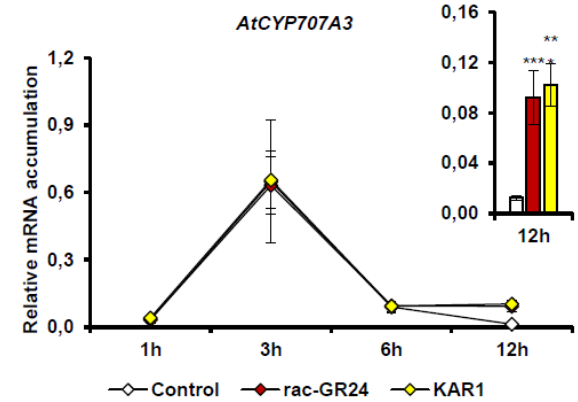
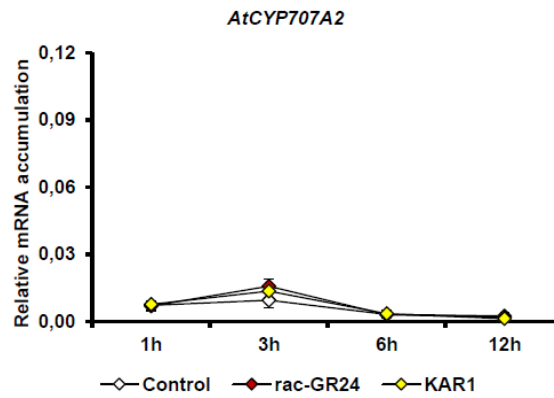
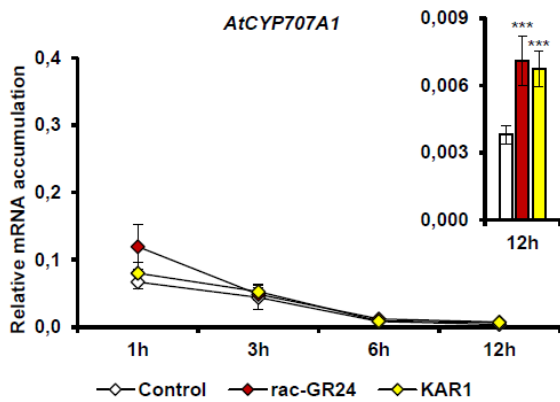


➔ GR24 can improve the seed germination of the facultative hemiparasitic plant *Triphysaria versicolor* along with an up-regulation of *TvCYP707A1*

Seed germination in autotrophic plants

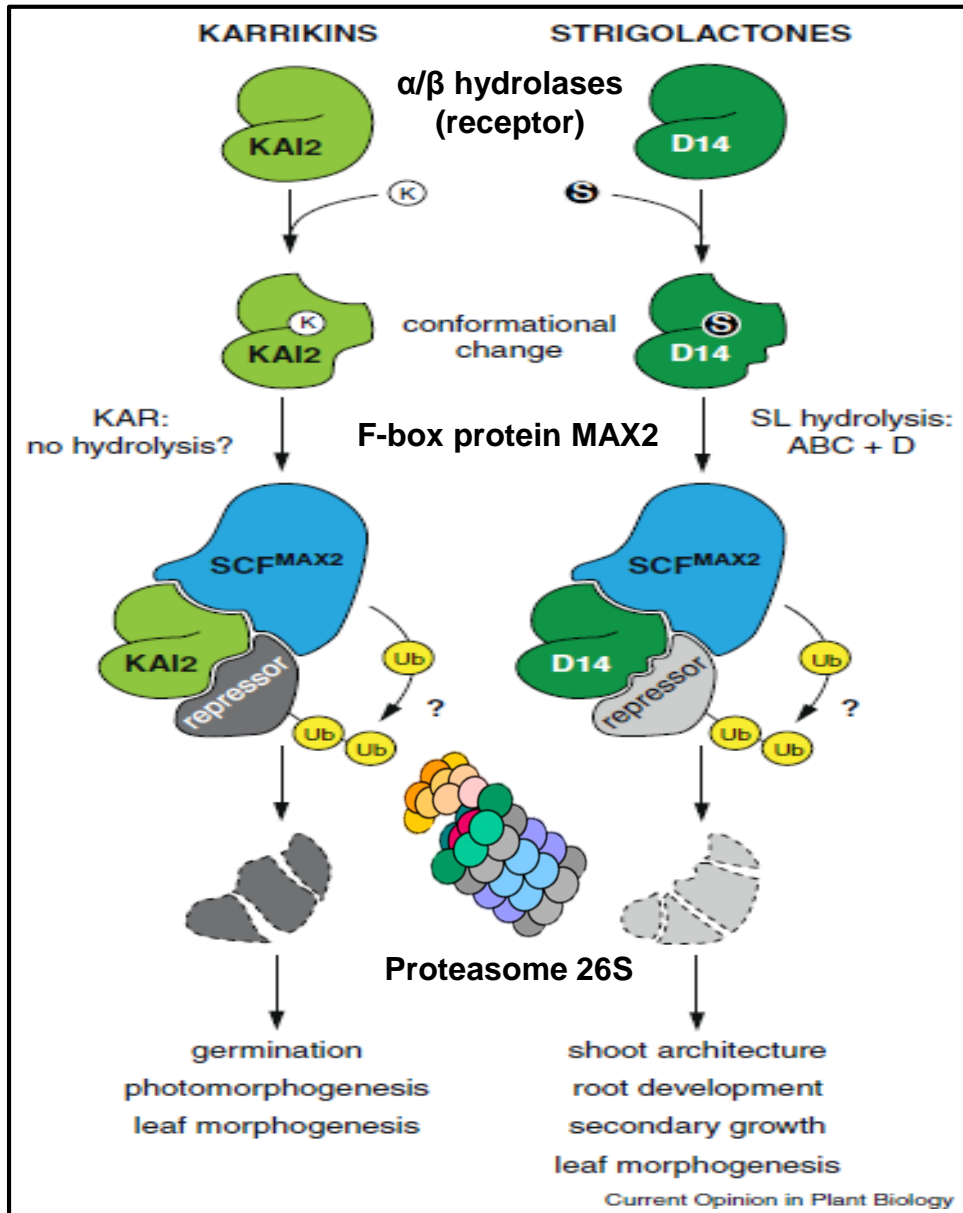


Arabidopsis thaliana

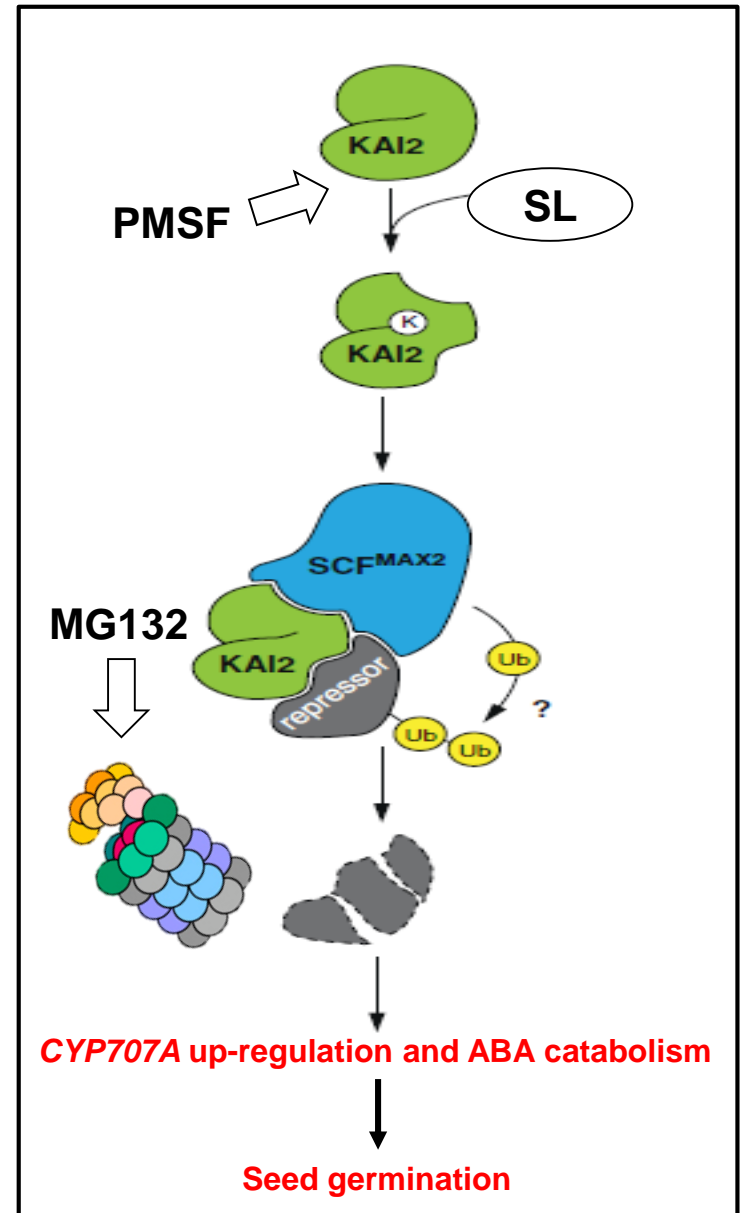


➡ **GR24 can improve the seed germination of the autotrophic plant *Arabidopsis thaliana***

Autotrophic plants



Obligate root parasitic plant



Seed germination in plants

Autotrophic plants

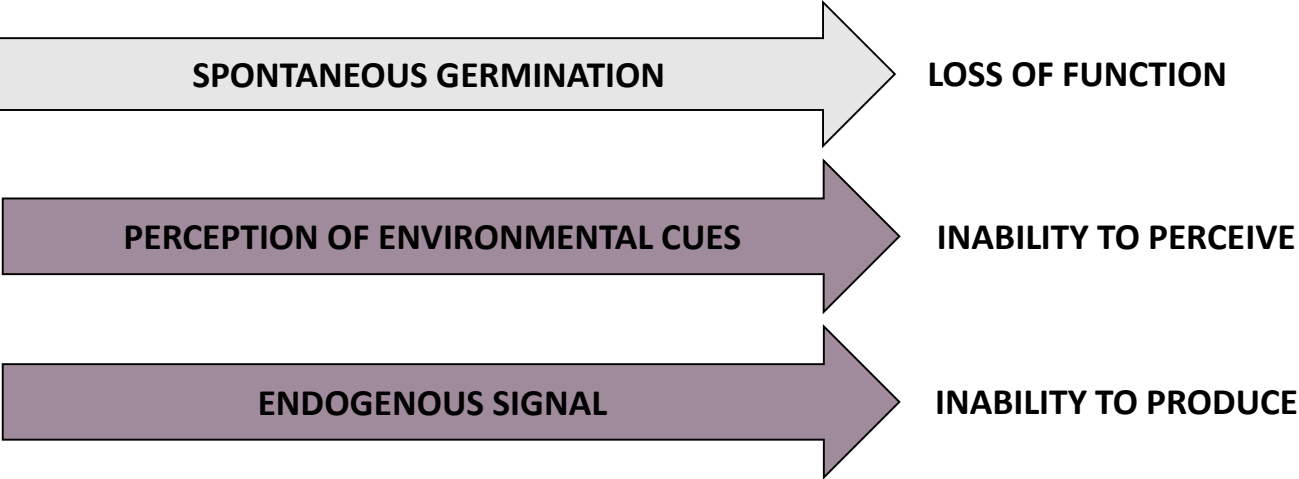
Hemiparasitic plants

Holoparasitic plants

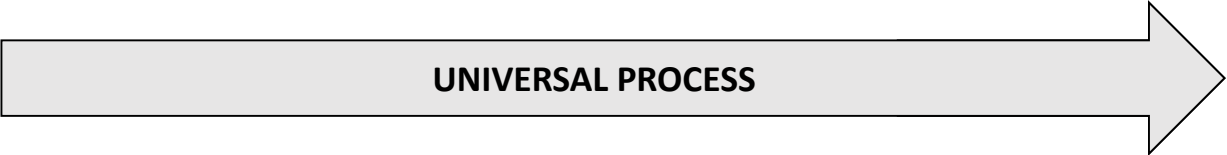
Evolution



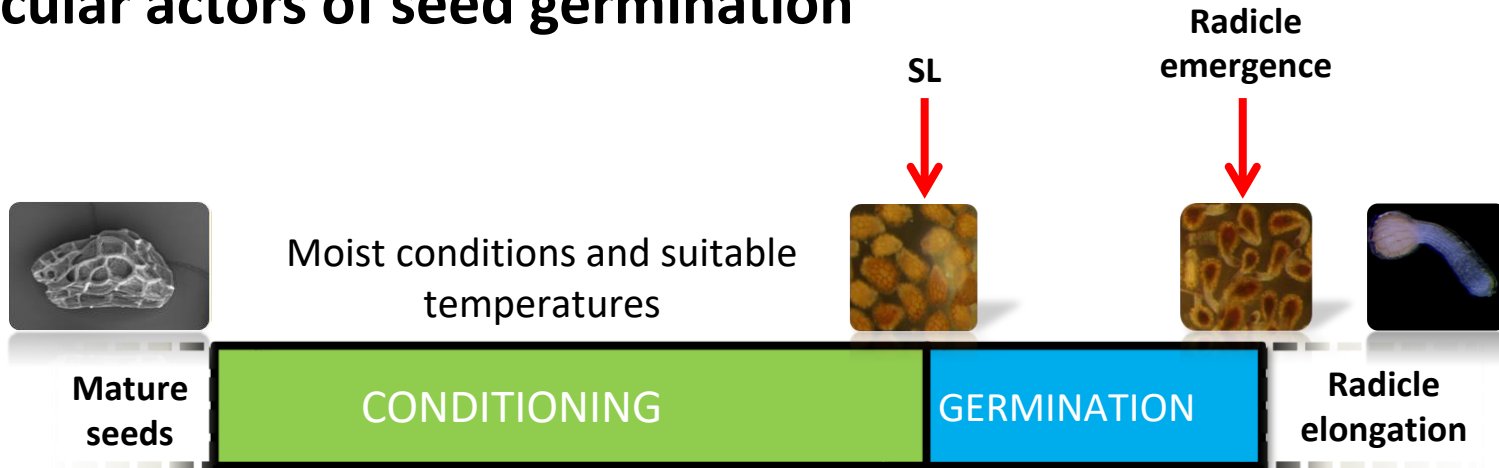
Non strigolactone-dependent seed germination



Strigolactone-dependent seed germination

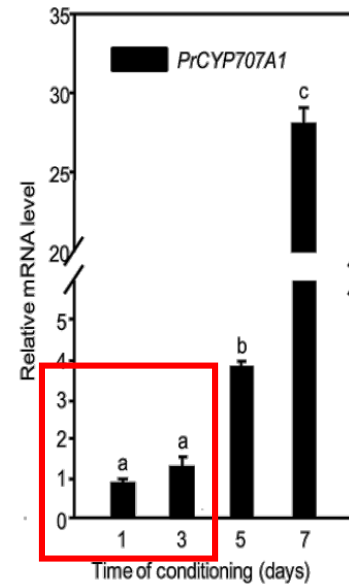
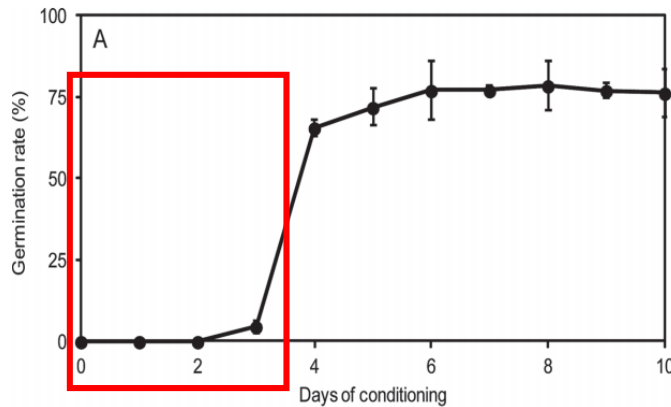


Molecular actors of seed germination



0 day

7 days

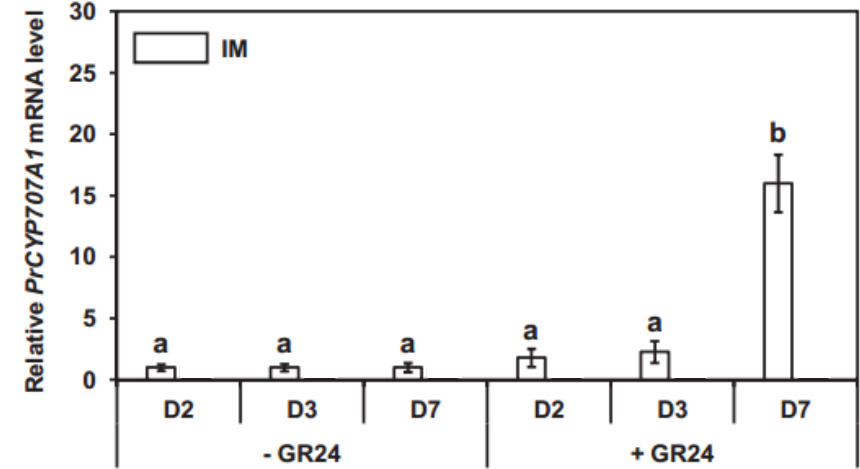
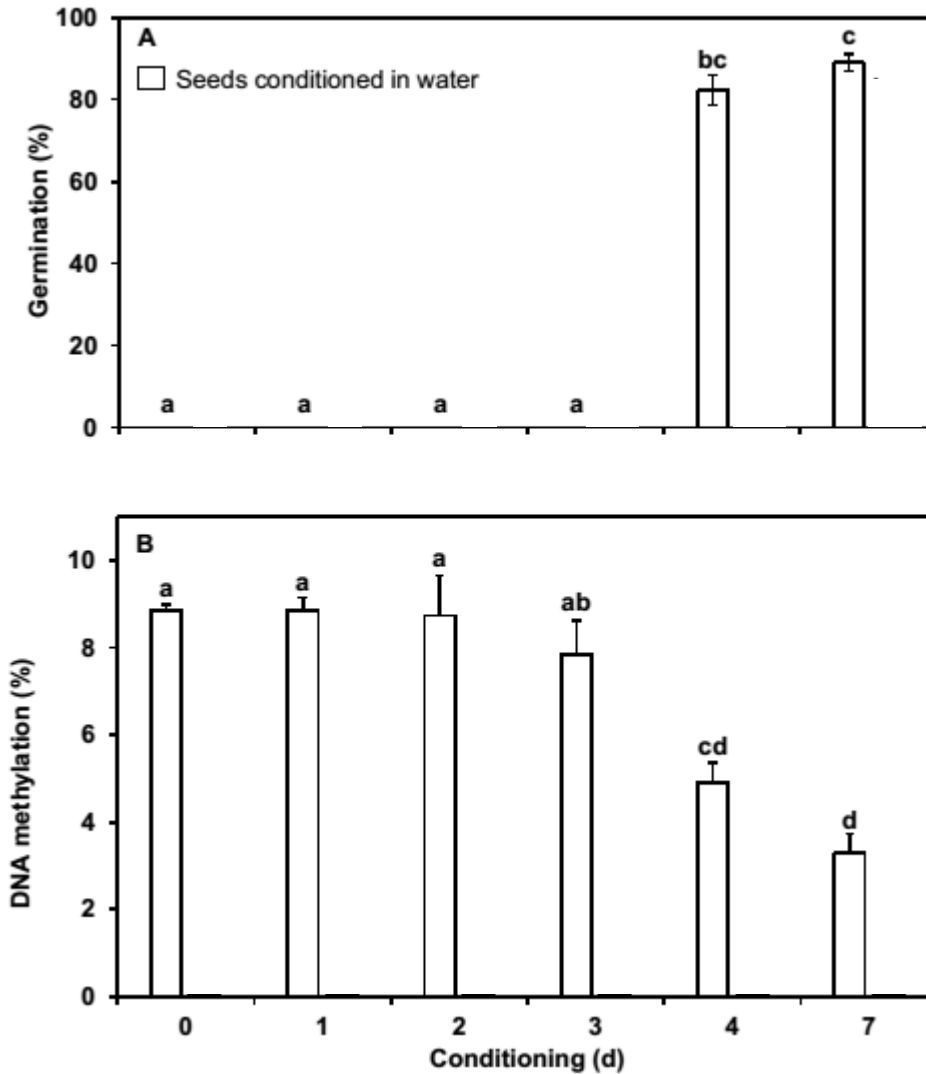


➔ A minimal 4 days conditioning period is required before SL perception and *PrCYP707A1* up-regulation.



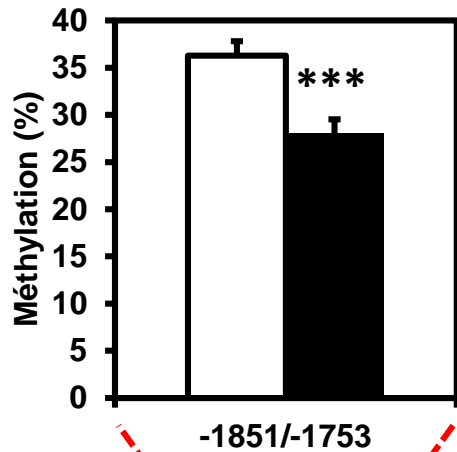
An epigenetic process: DNA methylation

DNA hypomethylating molecule : 5-azacytidine



➡ During the conditioning period a DNA demethylation process occurs which is needed for the gene expression of *CYP707A* and then the germination of the seeds.

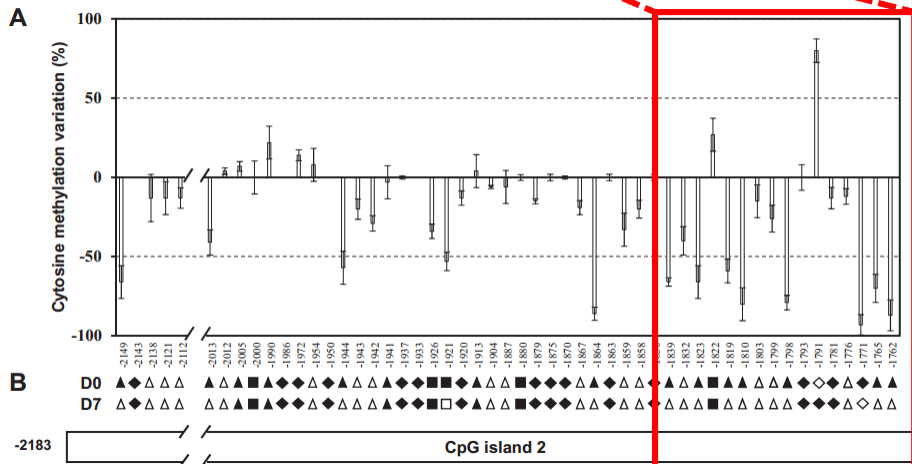
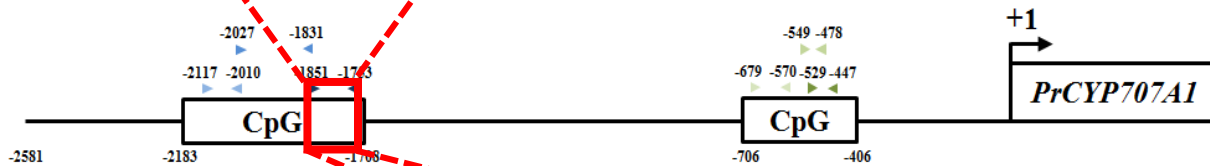




Medip Q-PCR: Methylated DNA immunoprecipitation Quantitative PCR

□ 0 day conditioning
 ■ 7 days conditioning

➔ ***Orobanche* germination requires a DNA demethylation of a 78 nucleotide region in the *PrCYP707A1* promoter during conditioning**

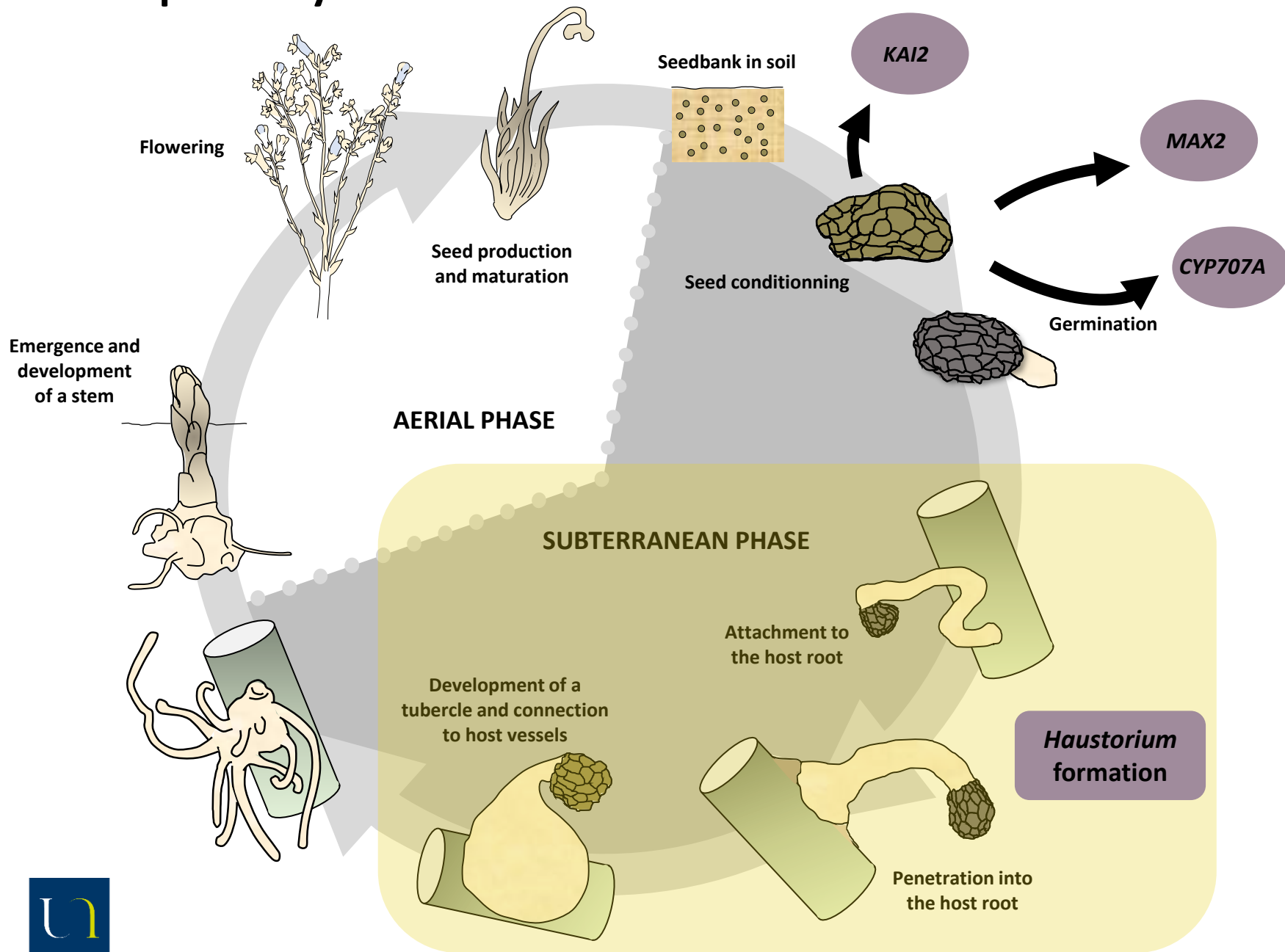


Bisulfite sequencing

➔ **Other epigenetic processes (Histone deacetylase)? (Brun *et al.*, unpublished results)**



Broomrape life cycle

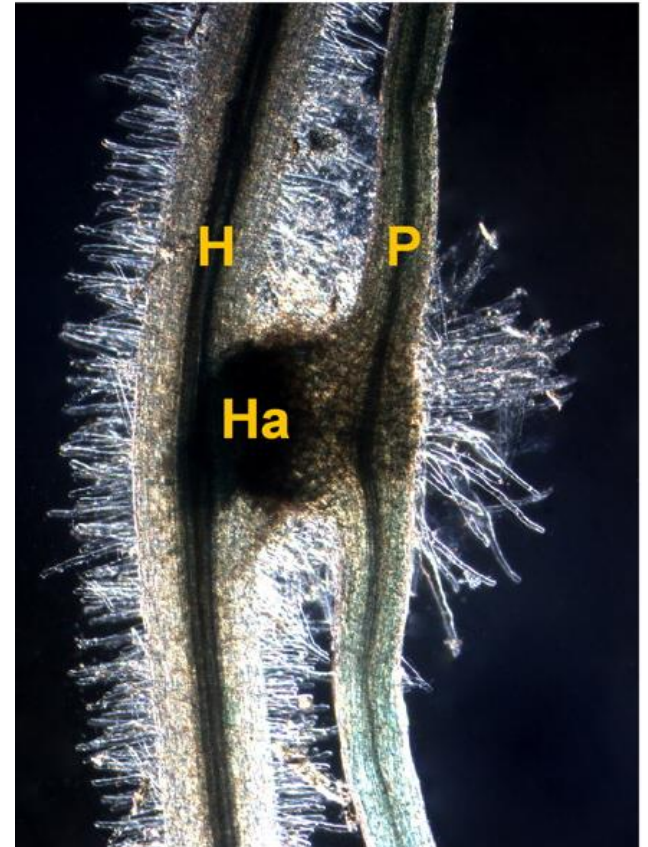


What makes a vascular plant a parasite?

The presence of a HAUSTORIUM,
a specialized invasive organ in parasitic plants

From the Latin word “haurire” meaning
"to draw"

- Attachment
- Penetration
- Connection with host vascular system



Hemiparasite

Holoparasite

Facultative

Obligate

Phtheirospermum japonicum

Triphysaria versicolor

Striga hermonthica

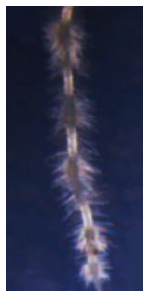
Orobanche cumana



Lateral haustorium

Terminal haustorium

Cui et al., 2016



Jamison and Yoder, 2001



Joel and Losner-Goshen, 1994



Early Haustorium Structures (EHS)

Auxin

Haustorial hairs

Cytokinins

TvQR1

Pirin

YUCC3

HIF = *Haustorium* Inducing Factor

Papillae

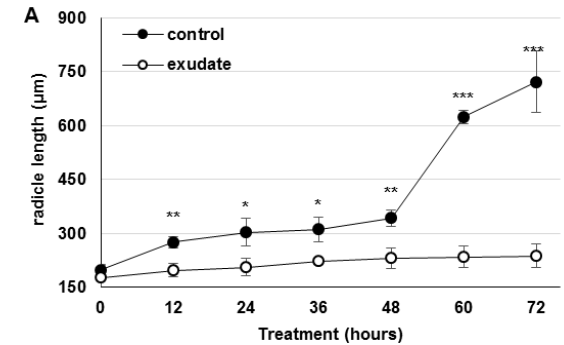
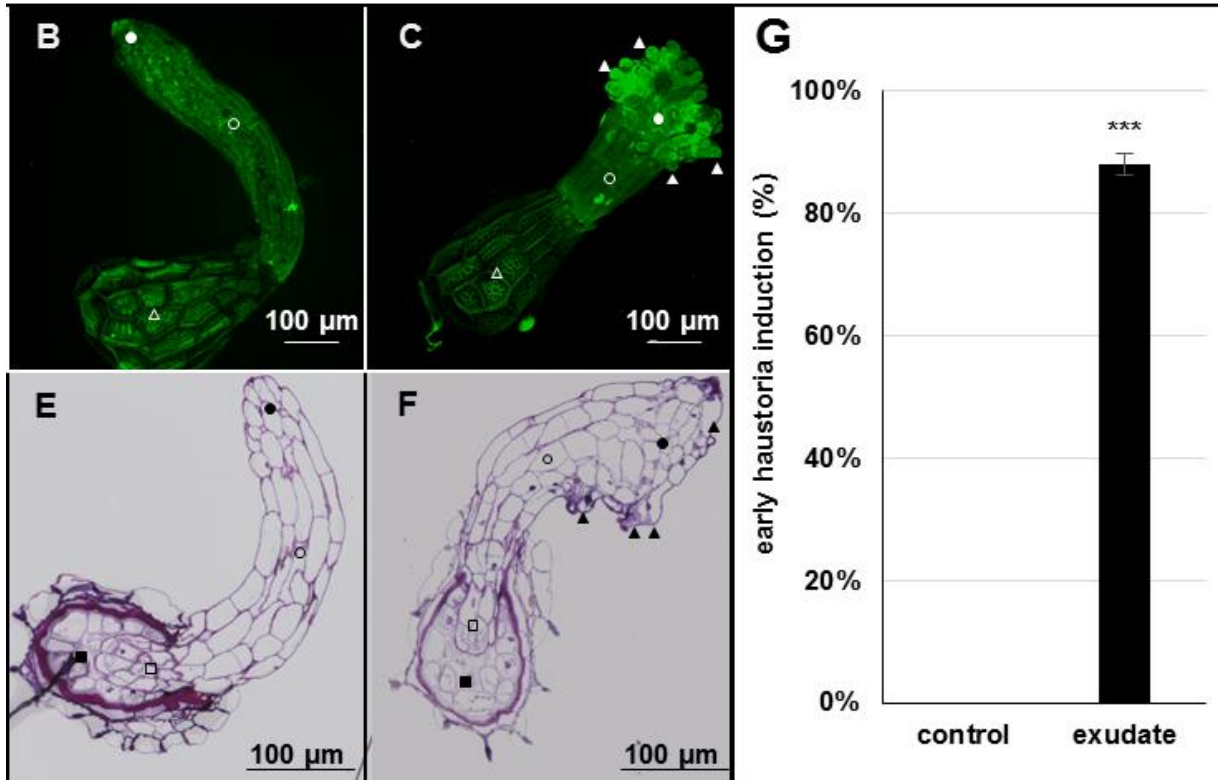
HIF ?

?

?

Simple phenolic acids (syngic acid, vanillic acid), quinones (DMBQ), and flavonoids (peonidin)

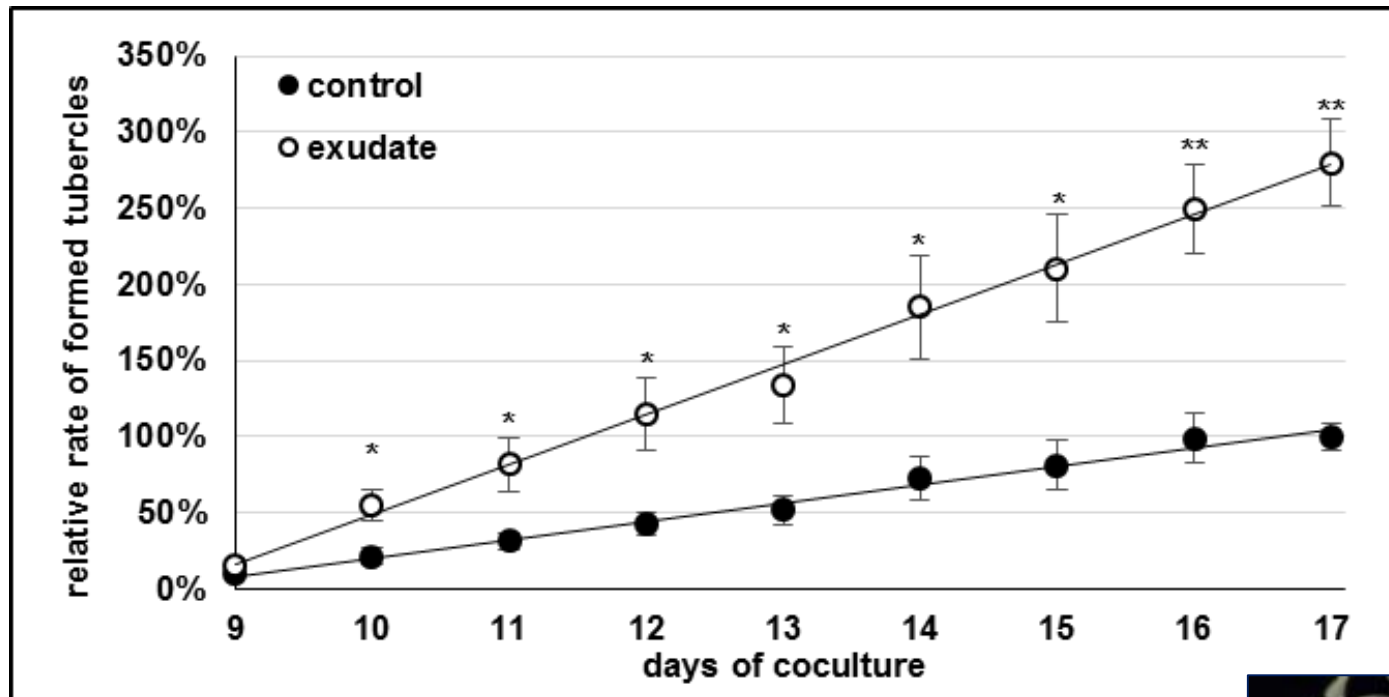
Brassica napus root exudates induce Early Haustorium Structures (EHS) at the apex of *Phelipanche ramosa* radicle

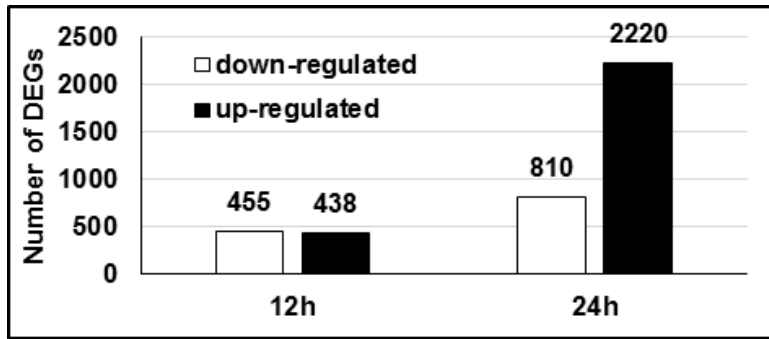


72hrs in control
buffer

72hrs in *B. napus*
root exudates

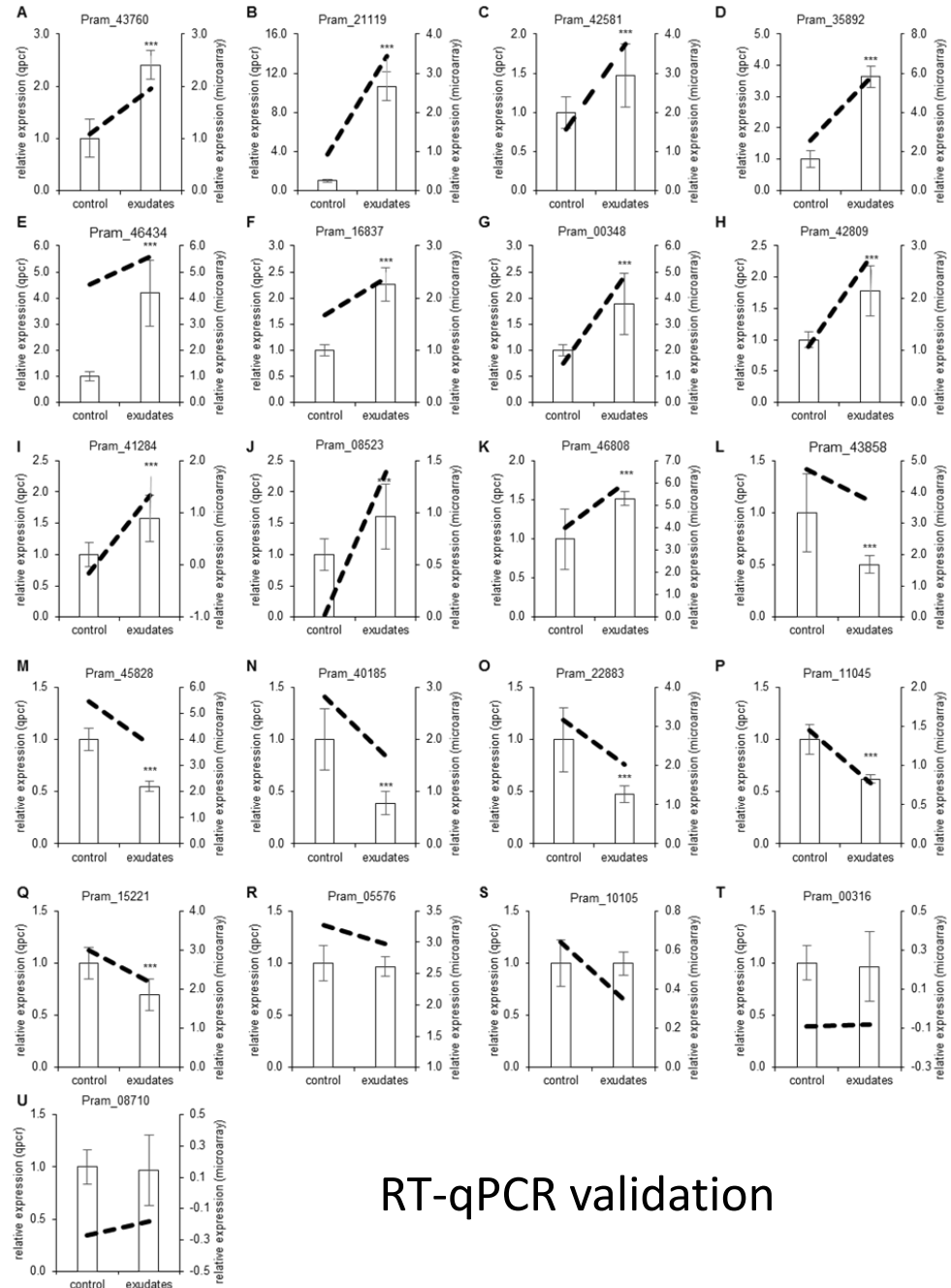
➔ Induction of EHS enhances aggressiveness of *P. ramosa* germinated seeds





Microarray: control vs 12 or 24 h treatment with *B. napus* root exudates

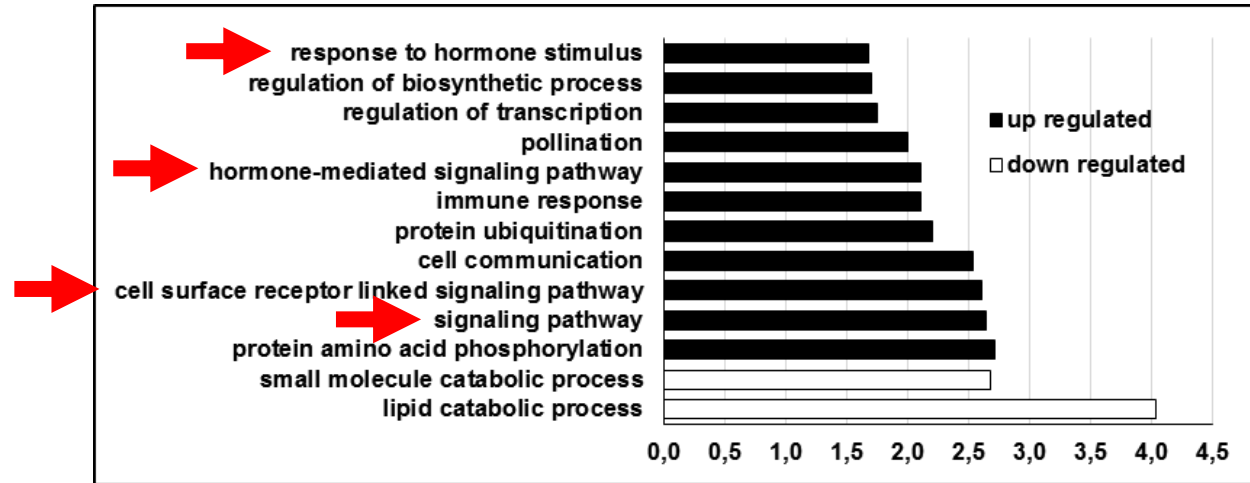
cluster	seq number with hit blast <i>A. thaliana</i>	average $\text{Log}_2(\text{FC})$
pool up-regulated	974	1.4 ± 0.7
pool down-regulated	720	-0.8 ± 0.3



RT-qPCR validation



Gene Ontology term enrichment analysis



Selected significant GO terms for pooled annotated DEGs

➔ Upregulated genes were notably enriched for terms corresponding to response to hormones and signaling pathway



Response to hormones

compound	early haustoria induction (%)						
	10 ⁻⁴ M	10 ⁻⁵ M	10 ⁻⁶ M	10 ⁻⁷ M	10 ⁻⁸ M	10 ⁻⁹ M	/
GR24	NI	NI	NI	NI	NI	NI	
Brassinosteroid	NI	NI	NI	NI	NI	NI	
Gibberellin (GA3)	NI	NI	NI	NI	NI	NI	
ABA	NI	NI	NI	NI	NI	NI	
Auxin (NAA)	NI	NI	NI	NI	NI	NI	
Ethylene (ACC)	NI	NI	NI	NI	NI	NI	
Jasmonate	NI	NI	NI	NI	NI	NI	
Cytokinin	NI	NI	NI	82 ± 4	81 ± 1	NI	
exudate	/	/	/	/	/	/	88 ± 2

Response to HIF

A compound	early haustoria induction (%)						
	10 ⁻⁴ M	10 ⁻⁵ M	10 ⁻⁶ M	10 ⁻⁷ M	10 ⁻⁸ M	10 ⁻⁹ M	/
DMBQ	NI	NI	NI	NI	NI	NI	
syringic acid	NI	NI	NI	NI	NI	NI	
vanilic acid	NI	NI	NI	NI	NI	NI	
vanillin	NI	NI	NI	NI	NI	NI	
p-coumaric acid	NI	NI	NI	NI	NI	NI	
coniferyl alcohol	NI	NI	NI	NI	NI	NI	
quercetin	NI	NI	NI	NI	NI	NI	
isorhamnetin	NI	NI	NI	NI	NI	NI	
kaempferol	NI	NI	NI	NI	NI	NI	
exudates	/	/	/	/	/	/	88 ± 2

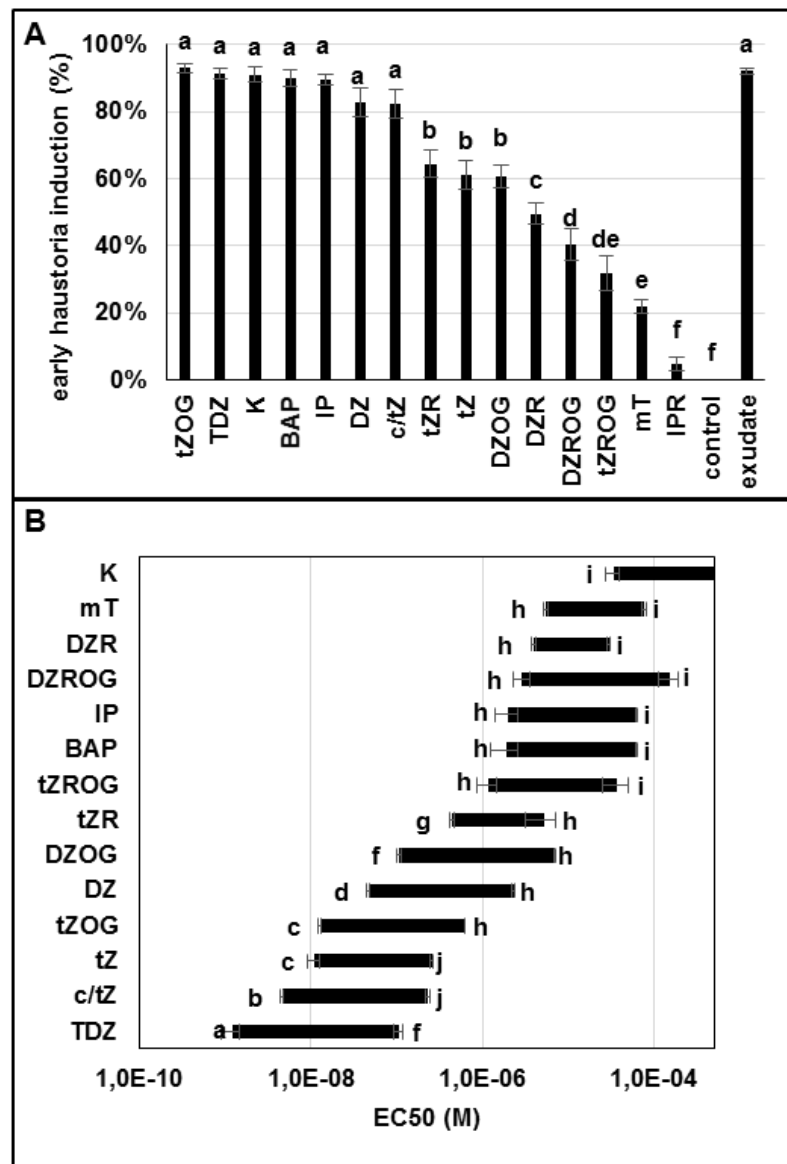
➔ Cytokinin triggers EHS formation in *P. ramosa*



Effect of exogenous treatments with cytokinins on EHS induction

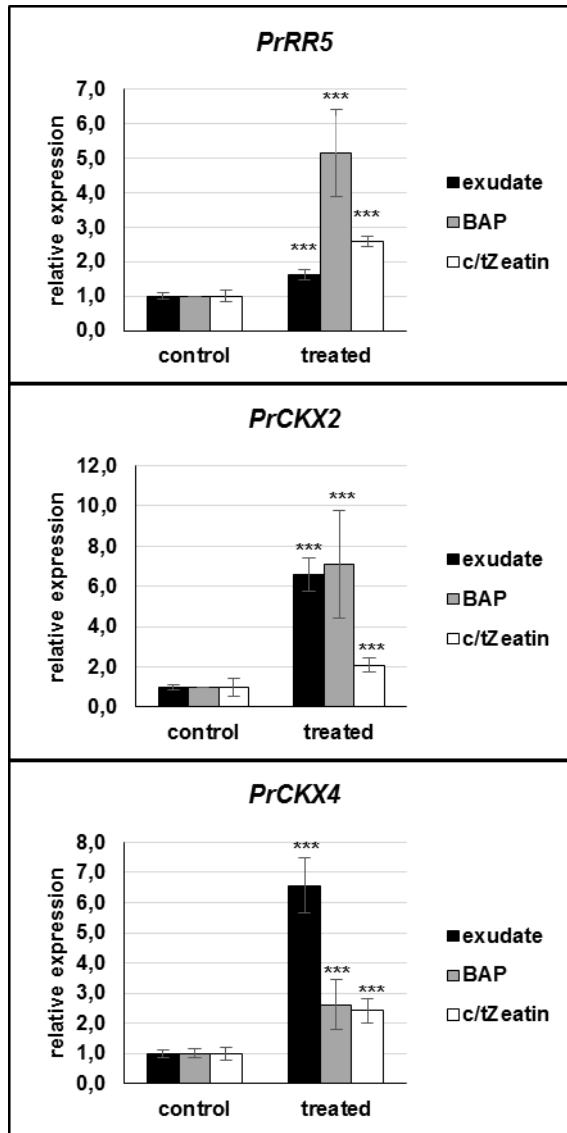
cis/trans zeatin	c/tZ
trans zeatin	tZ
trans zeatin riboside	tZR
trans zeatin o glucoside	tZOG
trans zeatin o glucoside riboside	tZROG
dihydrozeatin	DHZ
dihydrozeatin riboside	DHZR
dihydrozeatin o glucoside	DHZOG
dihydrozeatin o glucoside riboside	DHZROG
isopentenyl adenine	IP
isopentenyl adenosine	IPR
meta-topolin	mT
kinetin	K
6-benzylaminopurine	BAP
thidiazuron	TDZ

TDZ, Thidiazuron



➔ Cytokinin treatments induce EHS at the apex of *P. ramosa* radicle

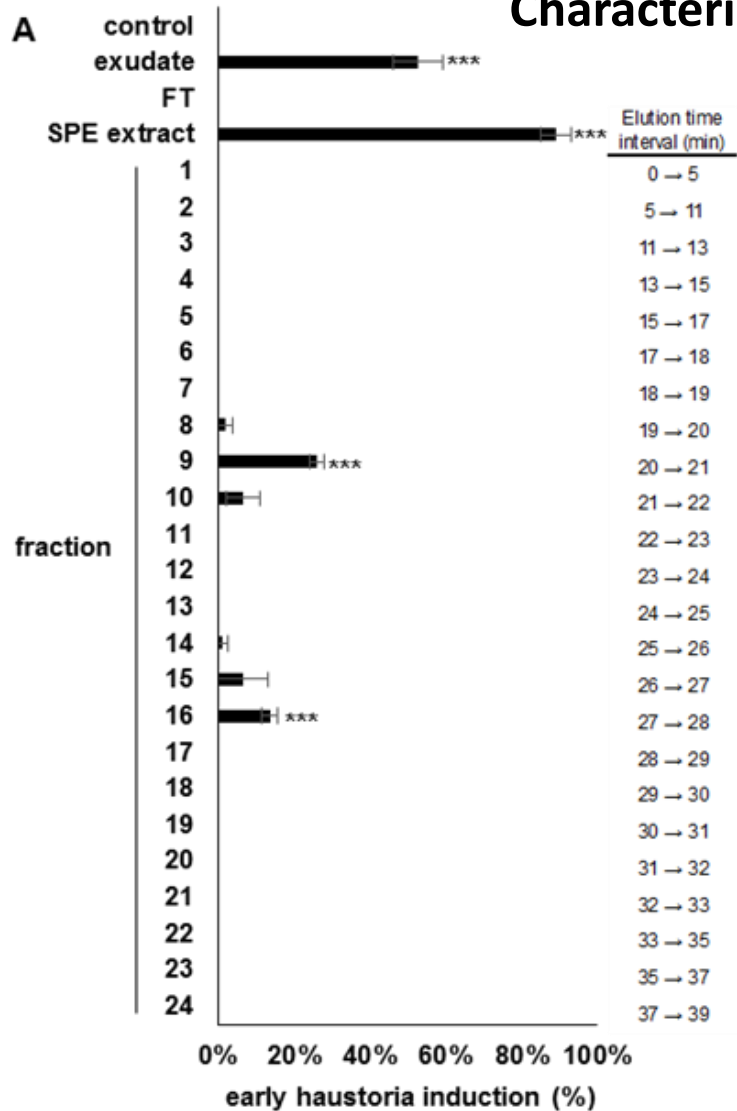
Expression levels of cytokinin responsive genes (*PrRR5*, *PrCKX2* and *PrCKX4*)



➔ Root exudates induce a cytokinin response patterns

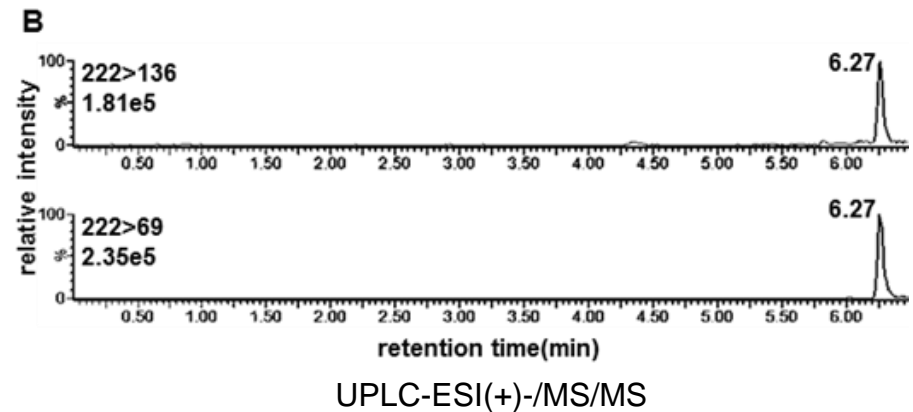


Characterization of HIFs released by *Brassica napus* roots



SPE extraction and separation by RP-HPLC

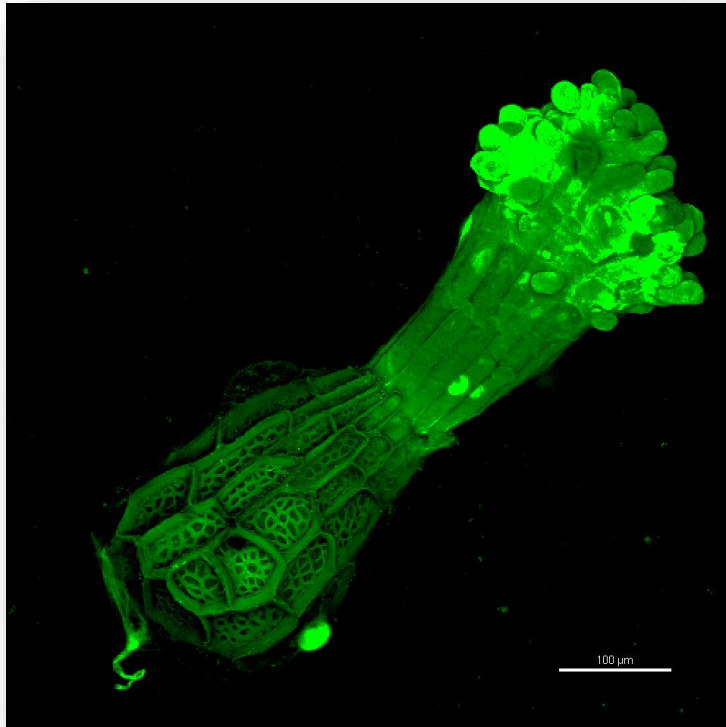
- Fraction 9 co-eluted with different CK standards displaying EHS induction, including *c/tZ*, *tZ*, *tZR*, *tZROG*, *DHZ*, *DHZR*, and *DHZROG*



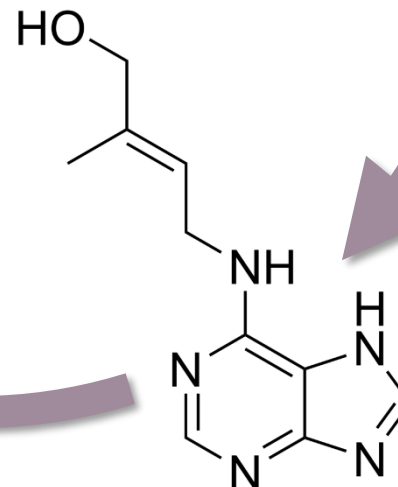
- 2 transitions of m/z 222 > 136 and 222 > 69 normally associated with dihydrozeatin (*DHZ*) were detected in fraction 9 at a retention time of 6.27 min



➔ The active fraction 9 contains a CK which shares a dihydrozeatin (*DHZ*) structure



Which HIF in broomrapes?



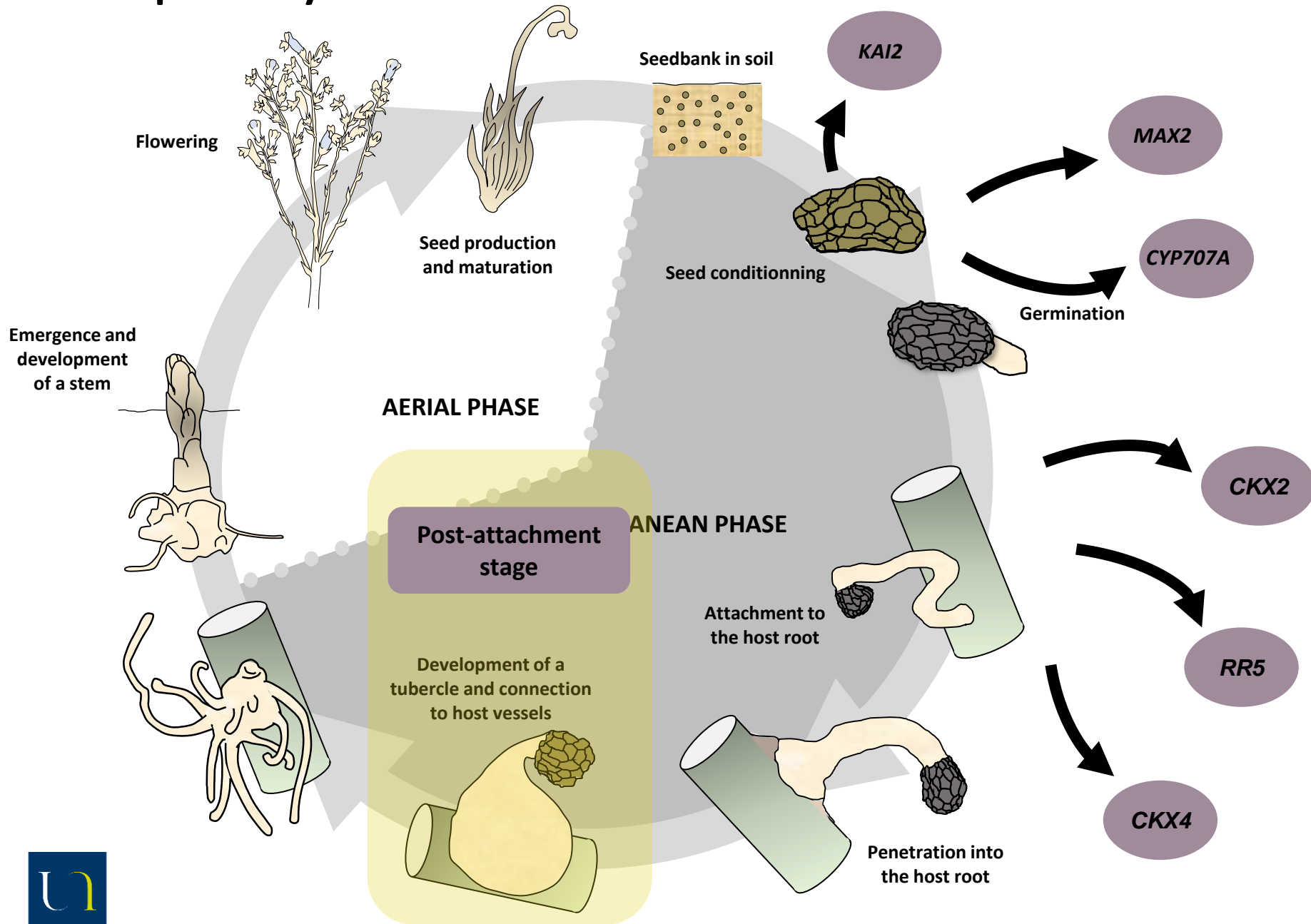
Cytokinin

Haustorium initiation in the obligate parasitic plant *Phelipanche ramosa* involves a host-exudated cytokinin signal.

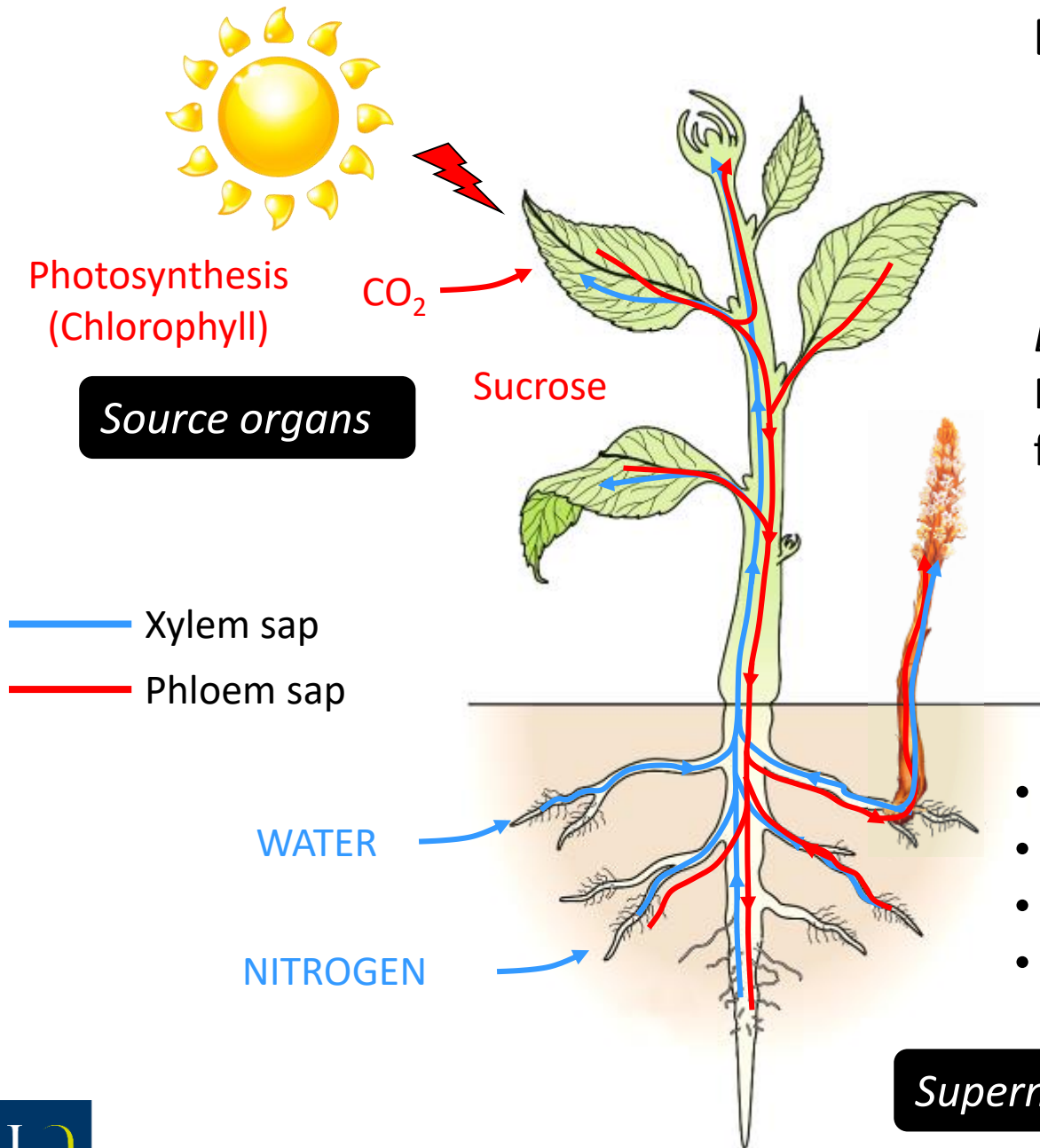
Goyet et al., 2017. *Journal of Experimental Botany* 68: 5539–5552.



Broomrape life cycle



Broomrape nutrition

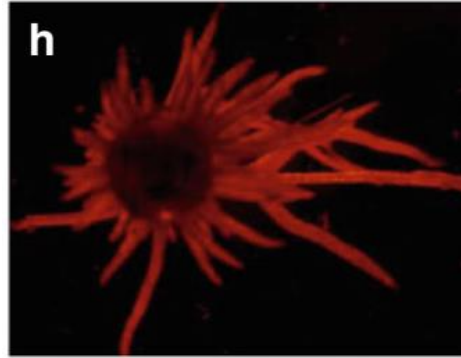
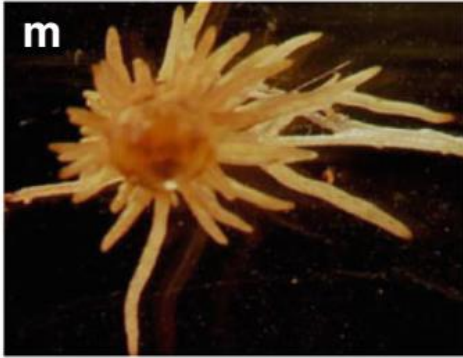


Broomrapes :
No chlorophyll and no functional roots

- Nitrogen (amino acids)
- Carbohydrates (sucrose)
- Water and minerals
- Growth regulators

Supernumerary sink organs





Broomrapes are xylem-feeder...

Texas Red-labeled dextrans: xylem-mobile markers
(Aly *et al.*, 2011)

...but the trophic spoliation is mainly done *via* the phloem (“phloem-feeder”) thanks to a strong sink strength



6-Carboxyfluorescein: a phloem-mobile marker
(Péron, 2010)



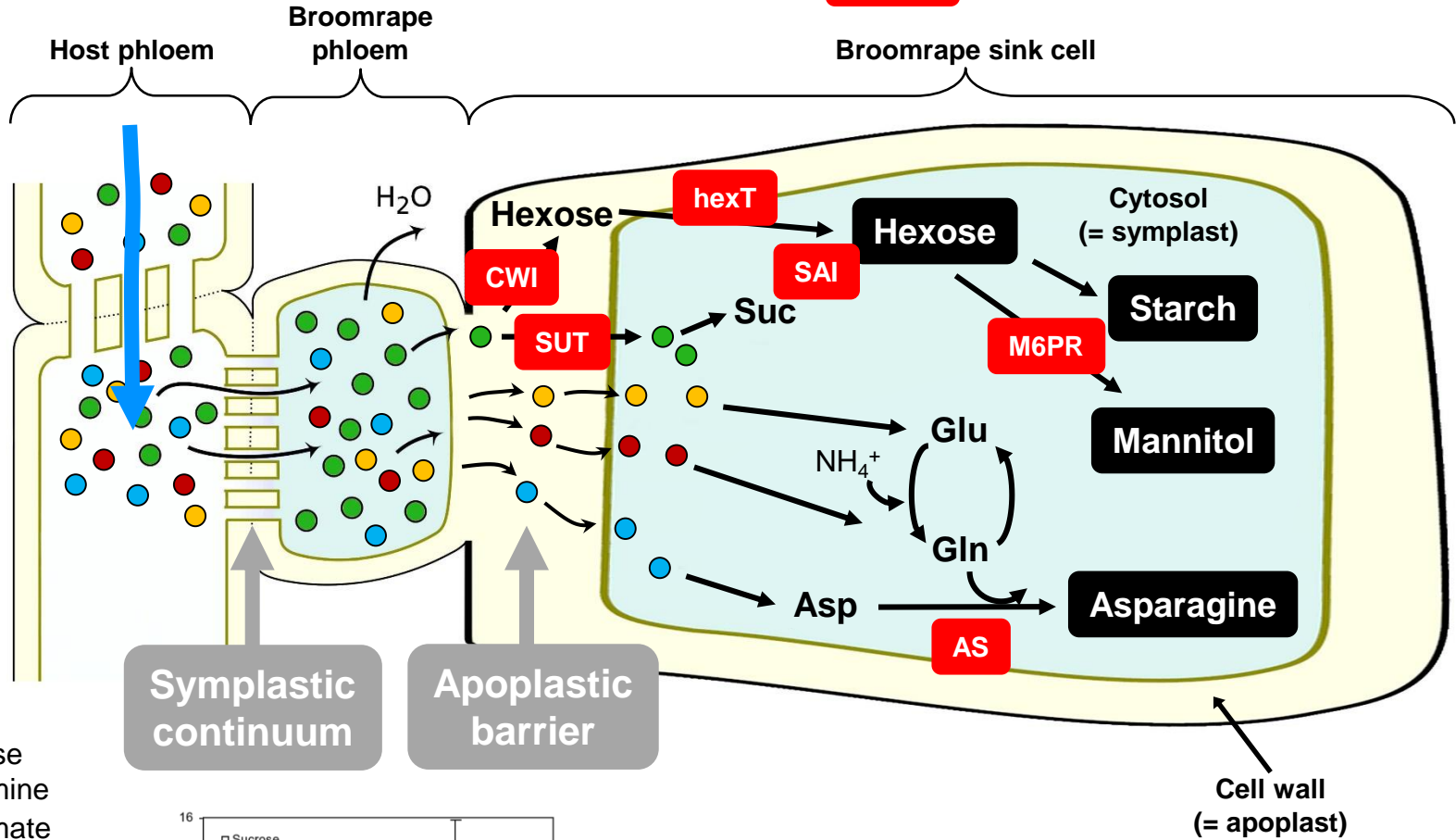
AS Asparagine Synthase (Gaudin, 2013)

CWI Cell Wall Invertase (Péron, 2010)

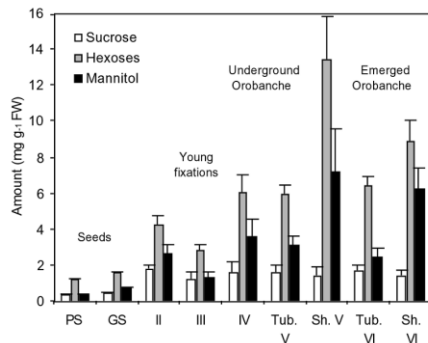
hexT **SUT** Hexose – Sucrose Transporters (Péron, 2010)

SAI Soluble Acid Invertase (Draie et al, 2012)

M6PR Mannose 6P Reductase (Delavault et al, 2002)

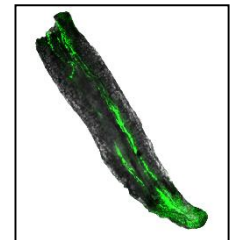
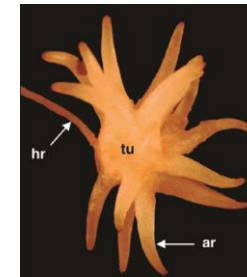


- Sucrose
- Glutamine
- Glutamate
- Aspartate

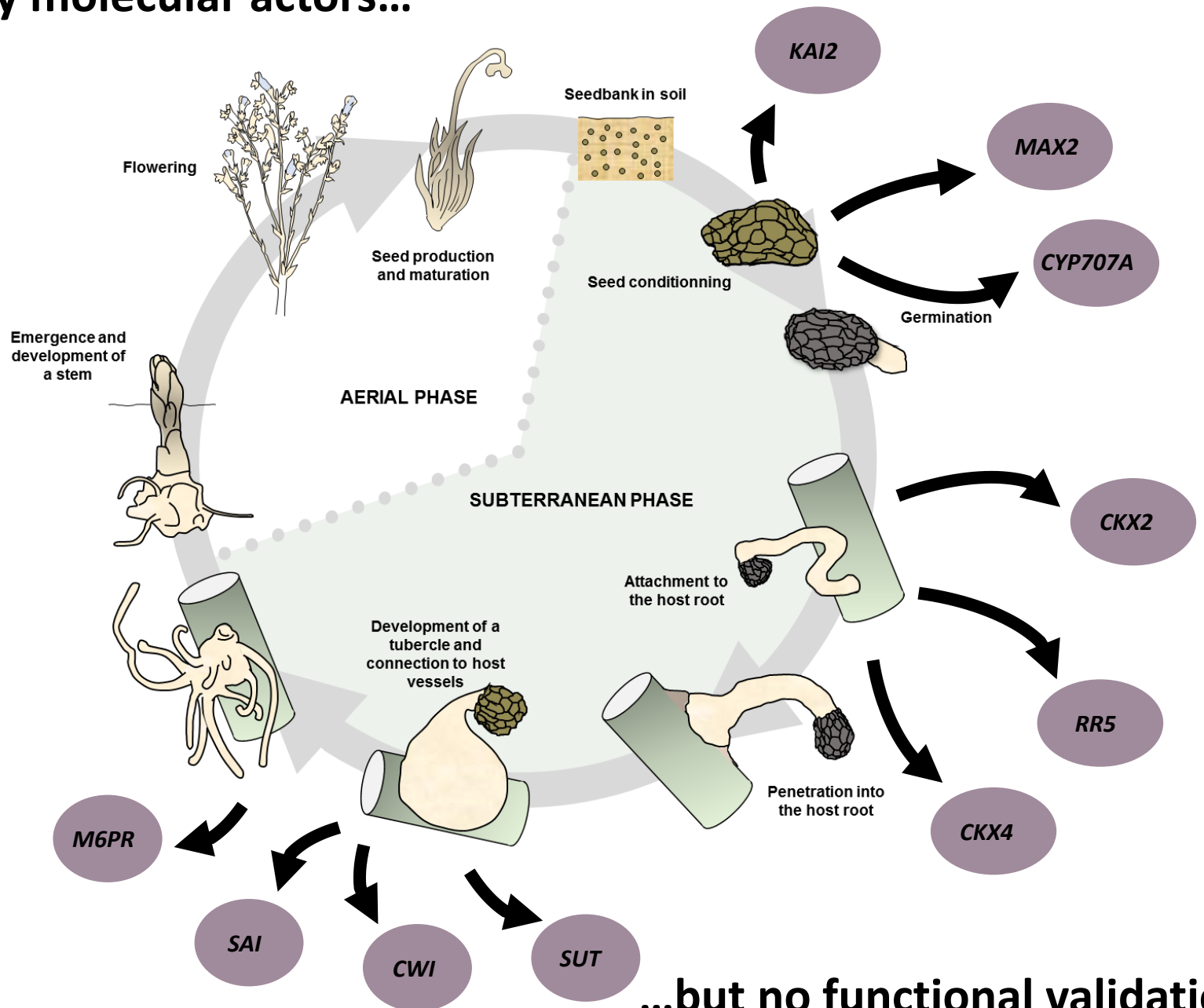


```

1  MATTSLAFTTQPERAARYAFIPAGPSAARQFRFTKLLASISFSBFFLLALILFIANQOQFQPKLDDLTQT
25  100  125  150
PSASADFFPARFPFEPFRGVAQGVSEKVFRRVGGGNSLFTNMLSHQRDATHLFCDFNMLHDEDFLYLN
175  200  225
GRYHLFYQYDPSAVMGNITWQVAVMDELKMLPLVVFQDMIDMWSGSSTILFDGRIIMLYTGDYDQV
250  275
QQQLAYFANLSDPILLAWVQVDFVLPVFFQIOMQFPSTFANEGVQVWRTIGKRVQDIDLVYDQVQ
300  325
FVYNLLEQYLNAVFTQVQMCIEDYFABLIENGLQTSANQOQIKVHGKALDEEDNDYALGTYDFINR
350  375
DQFLMAYDQIYDVKYKASFPFDQKQRKILMHWLHETFAKGLSRRVQVQIIPKELVQDSSDQV
400  425
DQFLMAYDQIYDVKYKASFPFDQKQRKILMHWLHETFAKGLSRRVQVQIIPKELVQDSSDQV
450  475
GVESLSDSDVDFHDFVLRPSGVTPILKVGASQDLQVASFIDRHSYVAIVGELSTOYDCPTSGQAMRGVL
500  525
GFPQVVLADRLTGLSFTLVPYFAKQKQKQSTFPCALLSRRAQDQVGVYVSSVPLDQVIGTISAVDQV
550  575
600  625
650  675
LSTFADQDQVITIRIYPTKATDGGARVPLFRMATQIGVTAQVKIKHNSGADIRFPFLDQ
    
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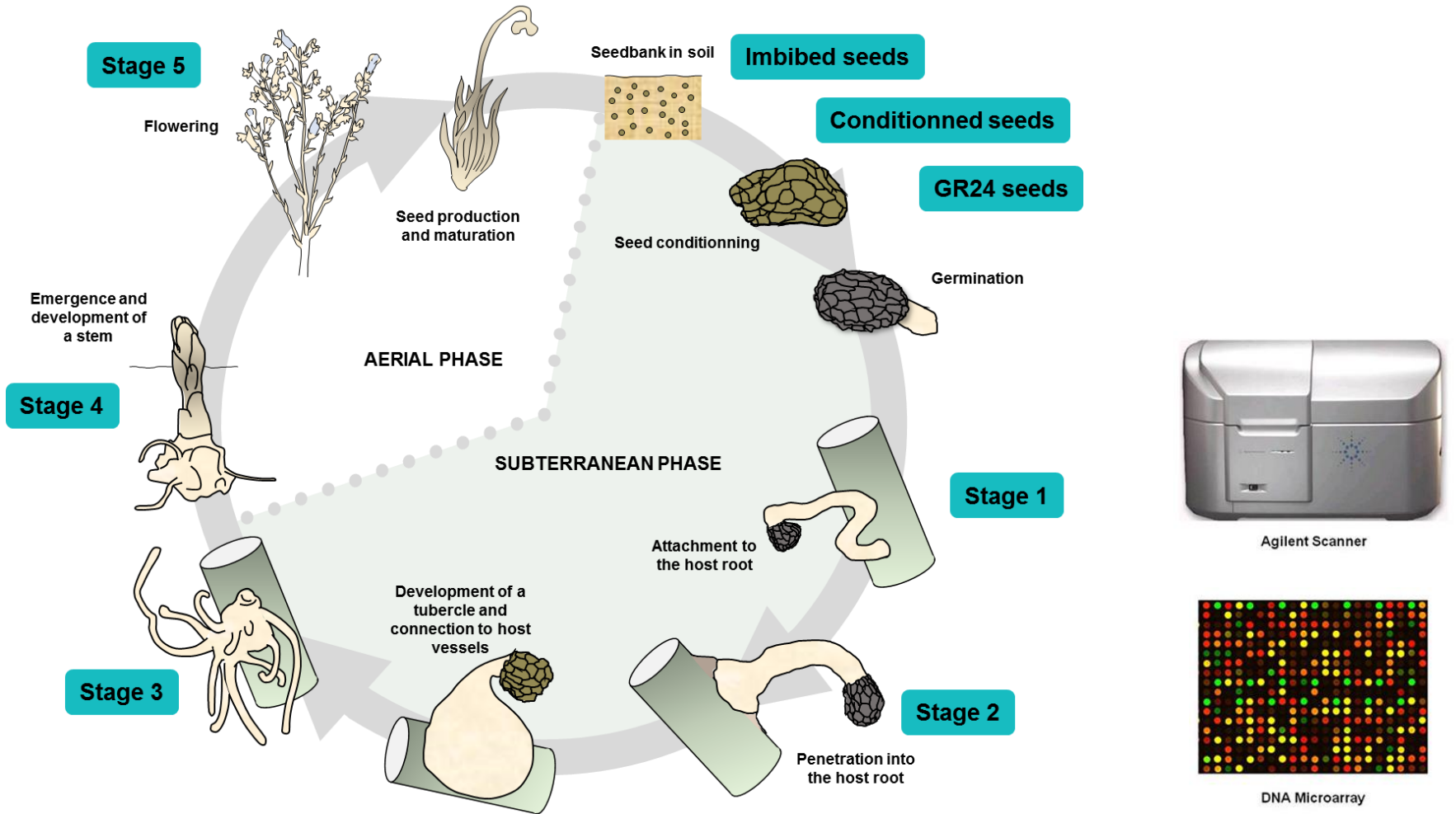
Few key molecular actors...



...but no functional validation



de novo assembly of *P. ramosa* transcriptome



- de novo transcriptome assembly was used to design 60-mer probes and fixed on Agilent 4 X 180K microarray slides
- Transcriptome data and microarray design can be provided upon request



RESOROBANCHE Project (2015-2017)

*Transcriptomic analysis of *O. cumana* – sunflower interaction*



- **Laboratory of Plant Biology and Pathology** (LBPV, University of Nantes) - P Delavault



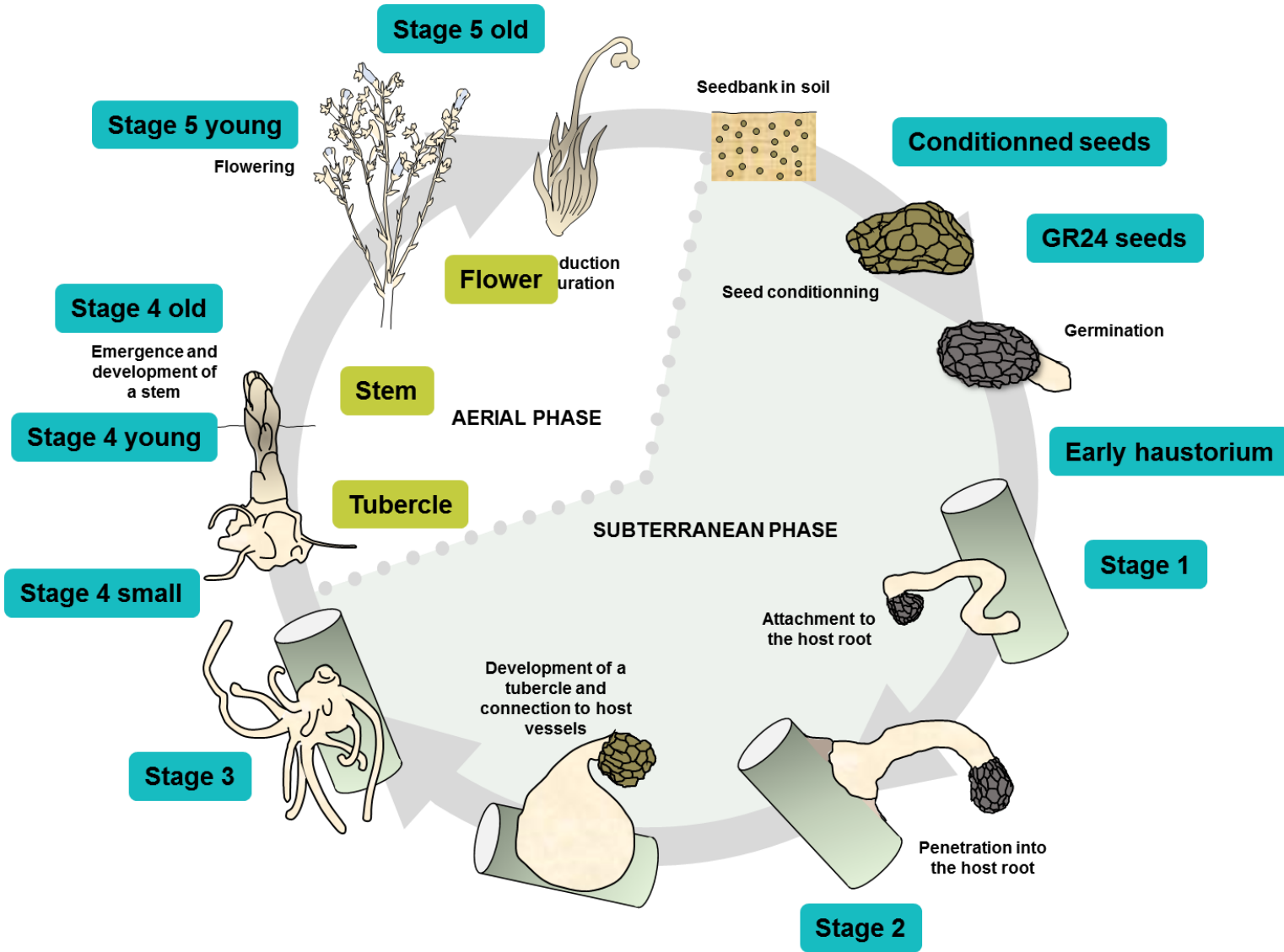
- **Laboratory of Plant-Microbe Interactions** (LIPM, UMR CNRS-INRA 2594/441; Toulouse) "Sunflower Genetics and Genomics" team - Stéphane Muños

Outputs

- Reference transcriptome of *O. cumana* (*race F*)
- Quantitative transcriptomic profiling during broomrape development
- Annotation of *O. cumana* genome sequence



Orobanche cumana transcriptome



20 Samples (different stages and organs) - 3 repetitions for each



The HeliOr project

The complete genome sequence of *Orobanche cumana* (sunflower broomrape)

Jérôme Gouzy¹, Nicolas Pouilly¹, Marie-Claude Boniface¹, Olivier Bouchez², Sébastien Carrère¹, Olivier Catrice¹, Stéphane Cauet³, Clotilde Claudel⁴, Ludovic Cottret¹, Sébastien Faure⁴, Álvaro Calderón González⁵, Xavier Grand^{1,4}, Luyang Hu¹, Céline Jézioriski³, Ludovic Legrand¹, Johann Louarn¹, Nicolas Ribière⁴, Erika Sallet¹, Philippe Simier⁶, Leonardo Velasco Varo⁵, Cécile Donnadiou², Christophe Jestin⁷, Philippe Delavault⁶, Hélène Bergès³, Marie Coque⁴, Begoña Pérez-Vich⁵, Stéphane Muñoz¹

stephane.munos@inra.fr

1. Laboratoire des Interactions Plantes-Microorganismes, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France
2. GeT-PlaGe, Genotoul, INRA, Castanet-Tolosan, France
3. Centre National de Ressources Génomiques Végétales, INRA, Castanet-Tolosan, France
4. Biogemma, Mondonville, France
5. Instituto de Agricultura Sostenible, CSIC, Córdoba, Spain
6. Laboratoire de Biologie et Pathologie Végétales, Université de Nantes, France
7. Terres Inovia, France



miPEPiTO Project (2017-2019)

Use of miPEPs to control the sunflower - orobanche interaction

miPEP : miRNA-encoded peptide



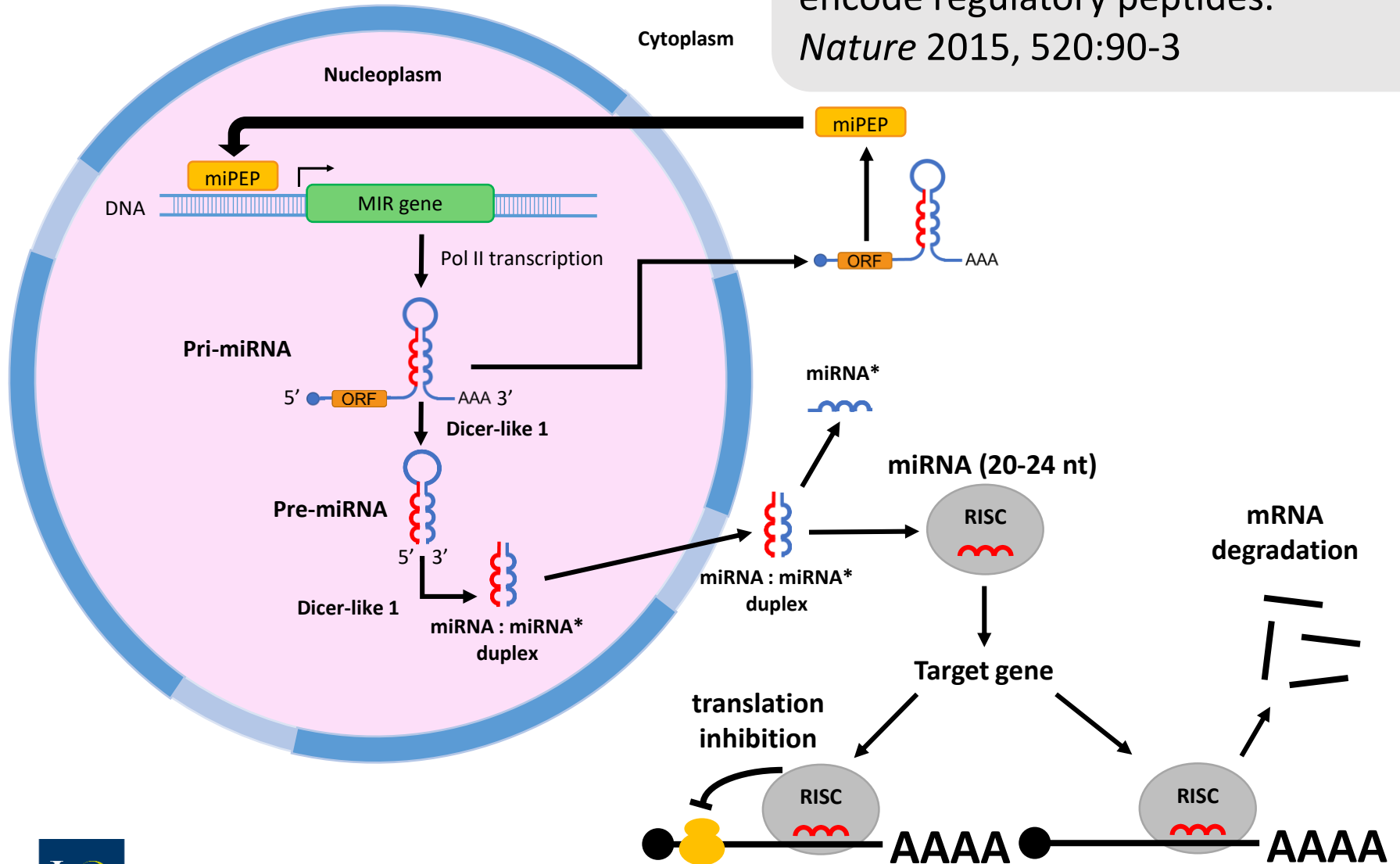
UNIVERSITÉ DE NANTES

- **Laboratory of Plant Biology and Pathology** (LBPV, University of Nantes) - P Delavault
- **Research Laboratory on Plant Sciences** (LRSV, UMR 5546 UPS-CNRS; Toulouse) "Peptide and Small RNAs" team - JP Combier
- **Laboratory of Plant-Microbe Interactions** (LIPM, UMR CNRS-INRA 2594/441; Toulouse) "Sunflower Genetics and Genomics" team - Stéphane Muñoz
- **MicroPEP Technologies** (Toulouse) - Thomas Laurent



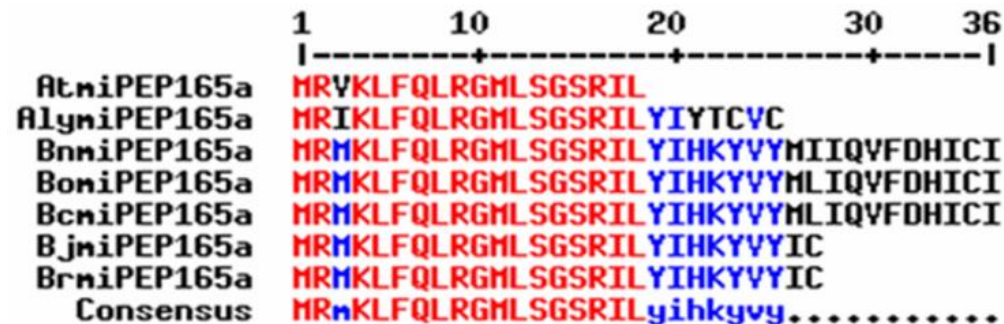
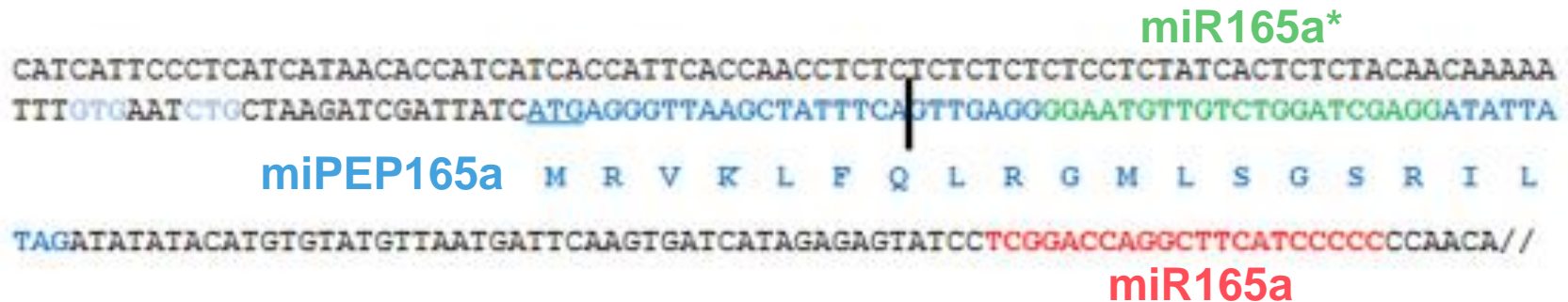
miPEP, miRNA, and targets

Lauressergues *et al.*
Primary transcripts of microRNAs
encode regulatory peptides.
Nature 2015, 520:90-3



Lauressergues *et al.*
 Primary transcripts of microRNAs
 encode regulatory peptides.
Nature 2015, 520:90-3

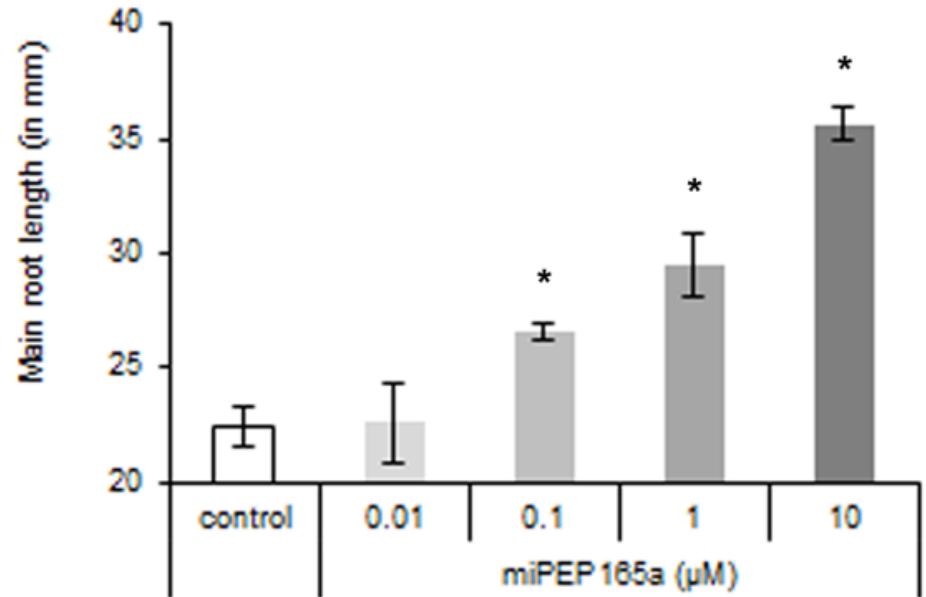
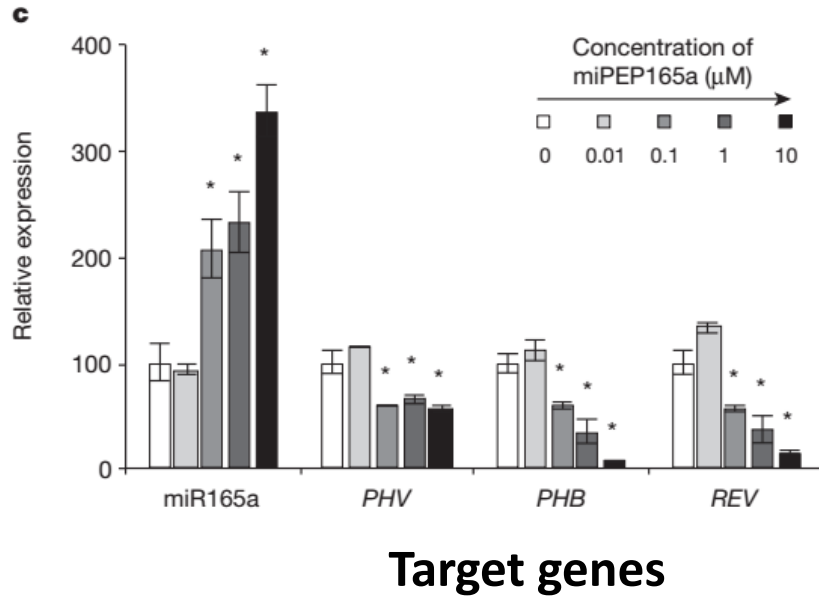
- Species: *Arabidopsis*
- miRNA: **miR165a**
- miPEP: **miPEP165a**
- Target genes: PHAVOLUTA (PHV), PHABOLUSA (PHB), and REVOLUTA (REV) which are transcription factors regulating the formation of lateral roots



Alignment of miPEP165a amino acid sequences of 7 species of Brassicaceae. At, *Arabidopsis thaliana*; Aly, *Arabidopsis lyrata*; Bn, *Brassica napus*; Bo, *Brassica oleracea*; Bc, *Brassica carinata*; Bj, *Brassica juncea*; Br, *Brassica rapa*.



Laouressergues *et al.*
 Primary transcripts of microRNAs
 encode regulatory peptides.
Nature 2015, 520:90-3



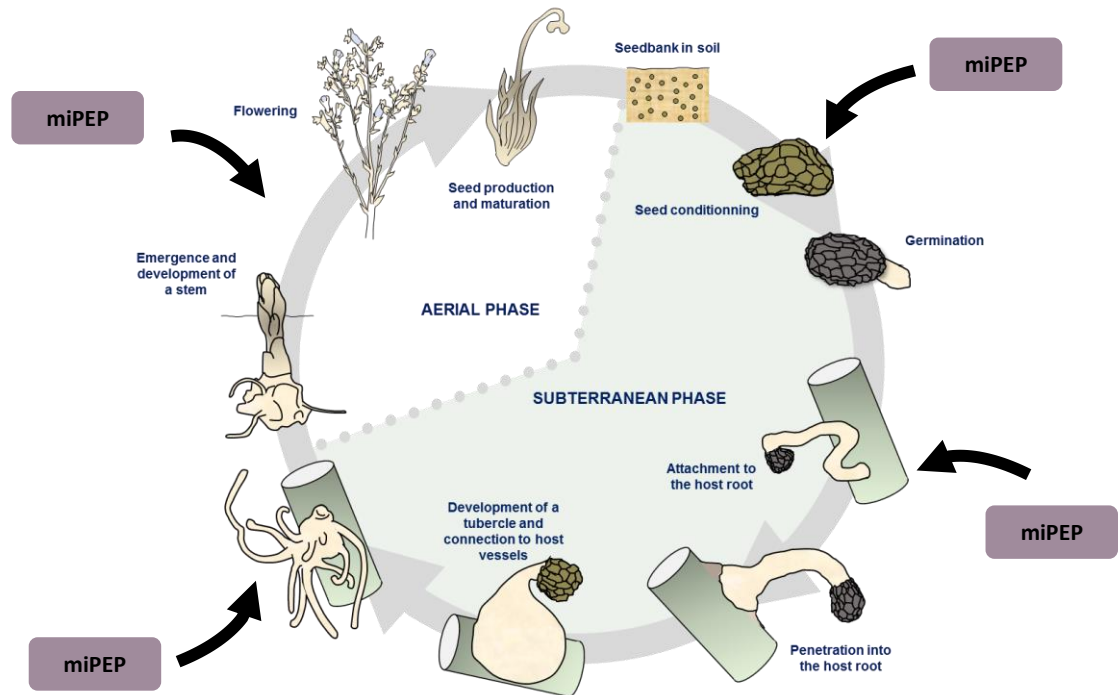
Conclusion: It is possible to control the expression of a gene and thus a phenotype by simple application of a miPEP



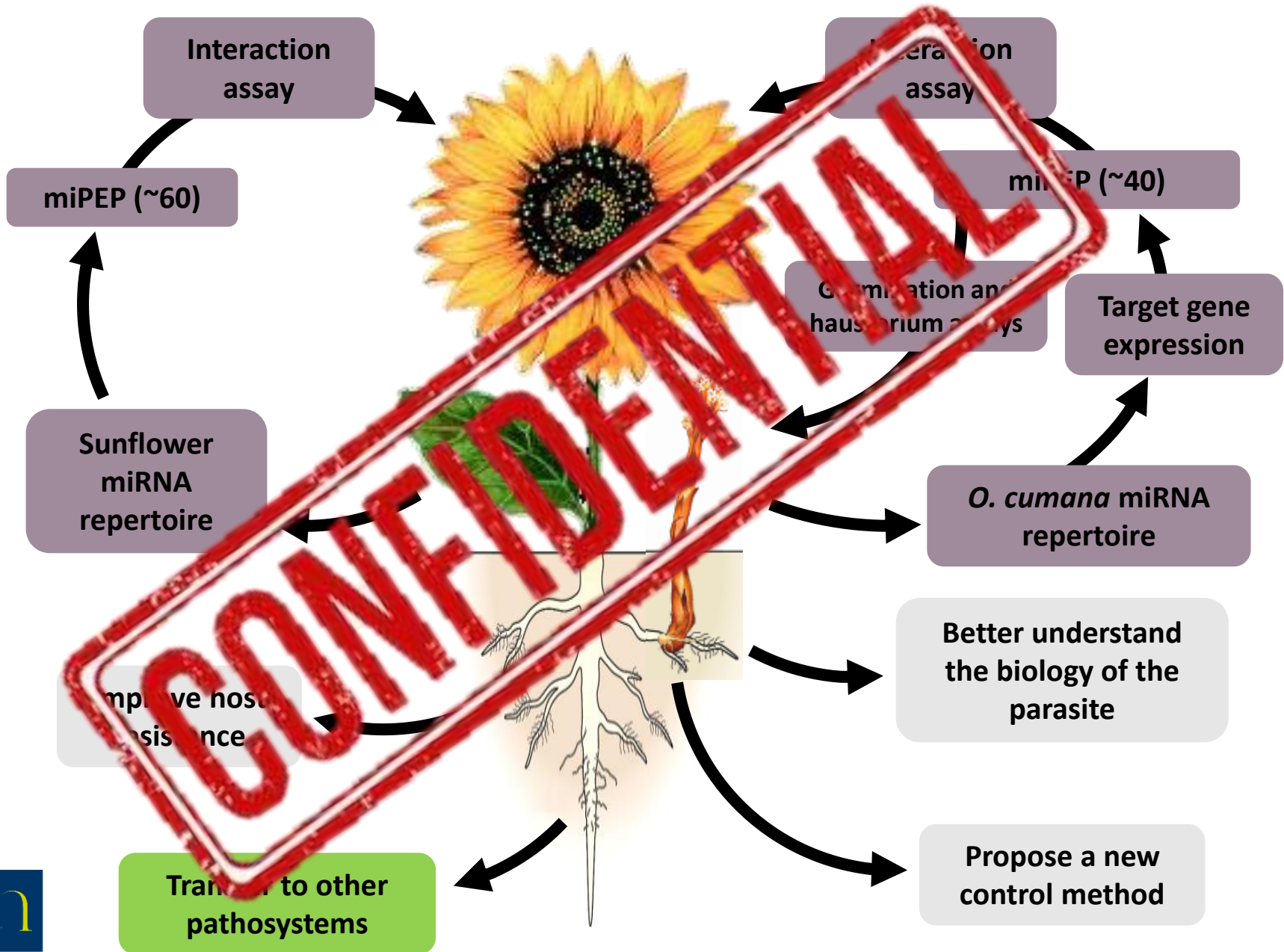
miPEPiTO Project (2017-2019)

Use of miPEPs to control the sunflower - orobanche interaction

“The use of peptides (miPEPs) as natural molecules to modulate specific gene expression (without genetic transformation or mutation) both in the parasite and in the host plant for the biocontrol of *Orobanche*, *Phelipanche*, and *Striga* parasitism is a completely new and cutting-edge technology”



miPEPiTO project objectives



miPEPitO PROJECT : A NEW STRATEGY TO STUDY AND CONTROL THE SUNFLOWER – *O. cumana* INTERACTION

Sabine TOURNEUR¹, Joséphine GIBERT¹, Jean-Philippe COMBIER², Stéphanie...³, Thomas LAURENT⁴, Philippe DELAVAUULT¹

¹Laboratory of Plant Biology and Pathology, University of Nantes, France; ²Laboratory of Plant Pathology, UMR 5546 UPS-CNRS, Toulouse; ³Laboratory of Plant-Microbe Interactions, UMR CNRS-INRA 2594/441, Toulouse; ⁴MicroPEP Technologies, Toulouse

Objectives

Orobanche cumana is an achlorophyllous root parasitic plant that depends on its host sunflower, for water and nutrients. *O. cumana* causes important losses in sunflower production in countries surrounding the Black Sea, in Southern Europe, and in growing areas of the world. Unfortunately, sustainable and efficient control methods against this noxious weed are currently unavailable. The miPEPitO project objectives are to develop a new biocontrol method using small peptides (miPEP, miRNA-encoded peptide) aiming to increase sunflower resistance and to inhibit the parasitic plant development. miPEPs with effect on *O. cumana* development (germination and haustorium formation) are also used to study the biology of this parasitic plant.

Orobanche cumana life cycle

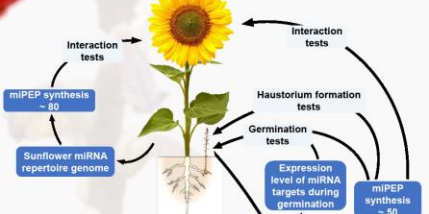


miPEP, miRNA, and target



miRNAs (miRNA) are small non-coding RNA molecules that regulate gene expression. They participate in the regulation of numerous biological processes in plants like stress responses and in many developmental processes. A number of previously identified miPEPs are encoded by pri-miRNAs and have different target sequences. miPEPs specifically and specifically their encoding MIR gene. miPEPs were identified by bio-informatic analysis of *O. cumana* and sunflower genomes and synthesized by chemical methods.

Methods



Expression level study of miRNA targets and miRNA during germination
 Identification of miRNAs involved in *O. cumana* germination
 Selection of corresponding miPEPs
 miPEP molecular and phenotypic germination tests
 Selection of miPEPs with effect on germination : study of miPEP regulation pathway (University of Nantes) and development of miPEP-associated biocontrol method (MicroPEP Technologies)
 (same approach on *O. cumana* haustorium formation)

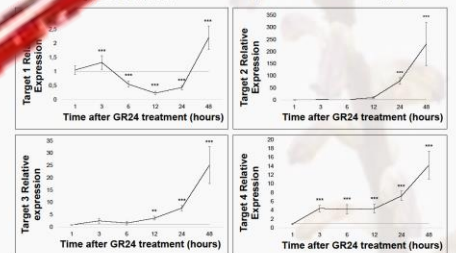
Bibliography

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- Lauresergues D., Cougou J.-M., San Clemente H., Martinez Y., Dunand C., Bécard G., and Combie J.-P. (2015) Primary transcripts of microRNAs encode regulatory peptides. *Nature*. DOI : 10.1038/nature14346
- Pouvreau J.-B., Gaudin Z., Auger B., Lechat M.-M., Gauthier M., Delavaul P., and Simier P. (2013) A high-throughput seed germination assay for root parasitic plants. *Plant Methods*. DOI : 10.1186/1745-4719-9-32
- Lechat MM, Pouvreau J.-B., Féron T., Gauthier M., Montiel G., Veronési C., Todoroki Y., Le Bizec B., Monteau F., Machere J., Simier P., Thoirion S., and Delavaul P. (2012) PCY707A1, an ABA catabolic gene, is a key component of *Phalipanche ramosa* seed germination in response to the strigolactone analogue GR24. *J Exp Bot*. DOI : 10.1093/jxb/err119
- Goyet V., Billard E., Pouvreau J.-B., Lechat M.-M., Pelletier S., Bahut M., Monteau F., Spichal L., Delavaul P., Montiel G., and Simier P. (2017) Haustorium initiation in the obligate parasitic plant *Phalipanche ramosa* involves a host-exuded cytokinin signal. *J Exp Bot*. DOI : 10.1093/jxb/erw359



Sabine Tourneur
 PhD student
 University of Nantes

Preliminary results (confidential) • 42 miPEPs were synthesized based on 16 miRNAs with several variants per miRNA
 • Expression of 40 miRNA target genes



qRT-PCR expression levels of four targets of the miRNAx during *O. cumana* seed germination after GR24 treatment, a synthetic strigolactone analog. The mean values and standard errors were calculated based on the results of three biological replicates. Data were normalized to the UB19 reference gene and negative control (solvent treatments) for each germination kinetic time. The threshold value control is set at 1, above this value the gene is up-regulated and below the gene is down-regulated. One, two and three asterisks represent significance level $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.



Belaen Céline
Benharrat Hocine
Brun Guillaume
Delagrange Sabine
Lemaitre Anne-Sophie
Montiel Grégory
Poulin Lucie
Pouvreau Jean-Bernard
Schmidt Johannes
Simier Philippe
Thoiron Séverine
Tourneur Sabine
Véronési Christophe

