GENES EXPRESSION MEASUREMENT BY USING THE GENOME SEQUENCES OF *O. CUMANA* AND SUNFLOWER

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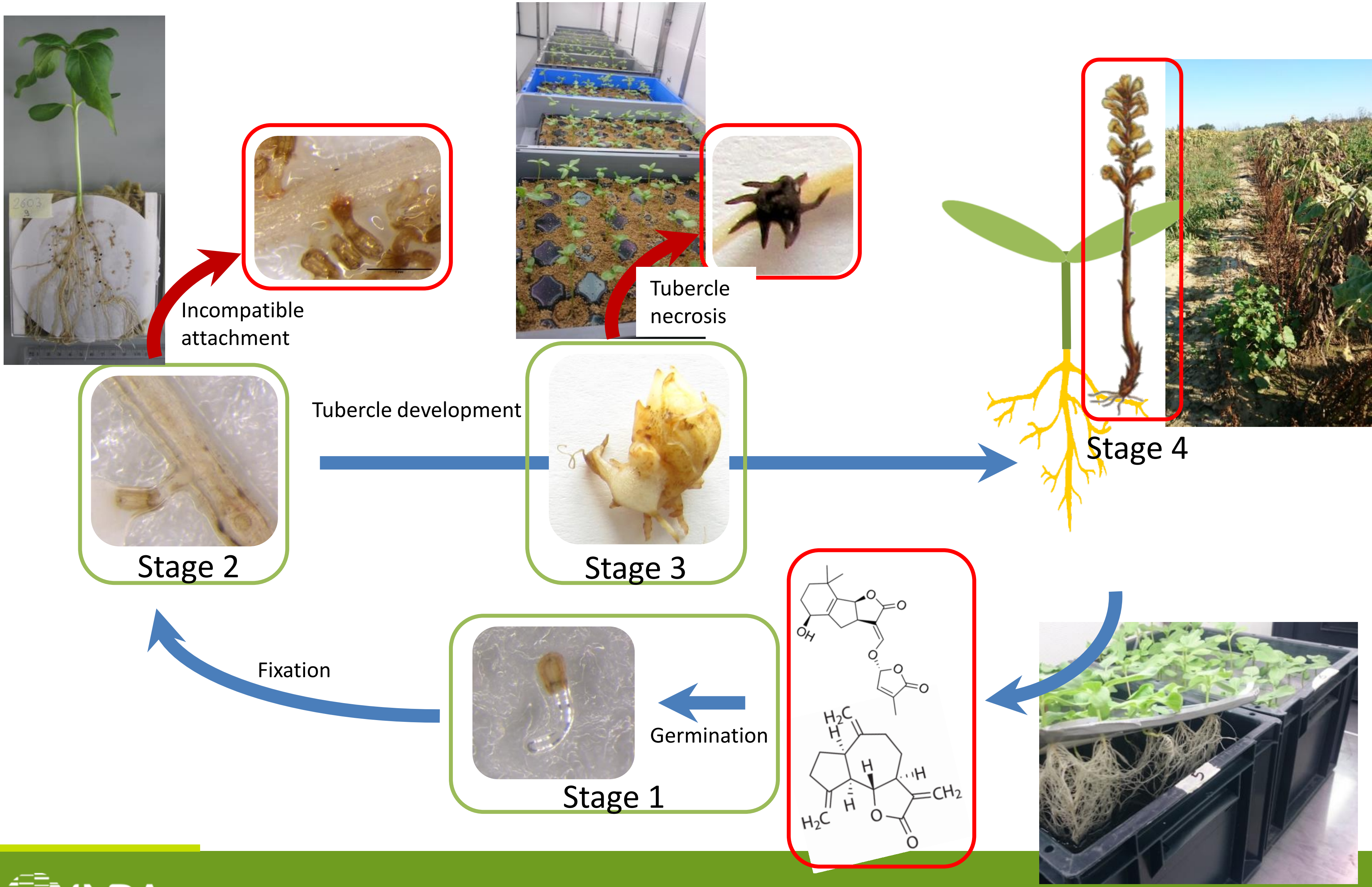
INRA Occitanie-Toulouse

ISBS, July 3rd, 2018, Bucharest

Biology cycle of *Orobanche Cumana and resistance mechanisms*



Incompatible attachment

Tuberche necrosis

Tubercle development

Stage 2

Stage 3

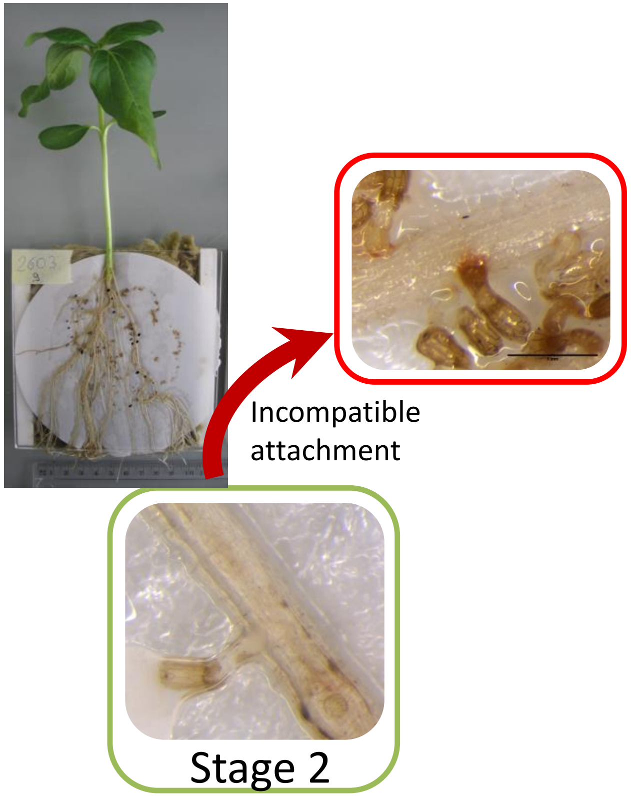
Stage 4

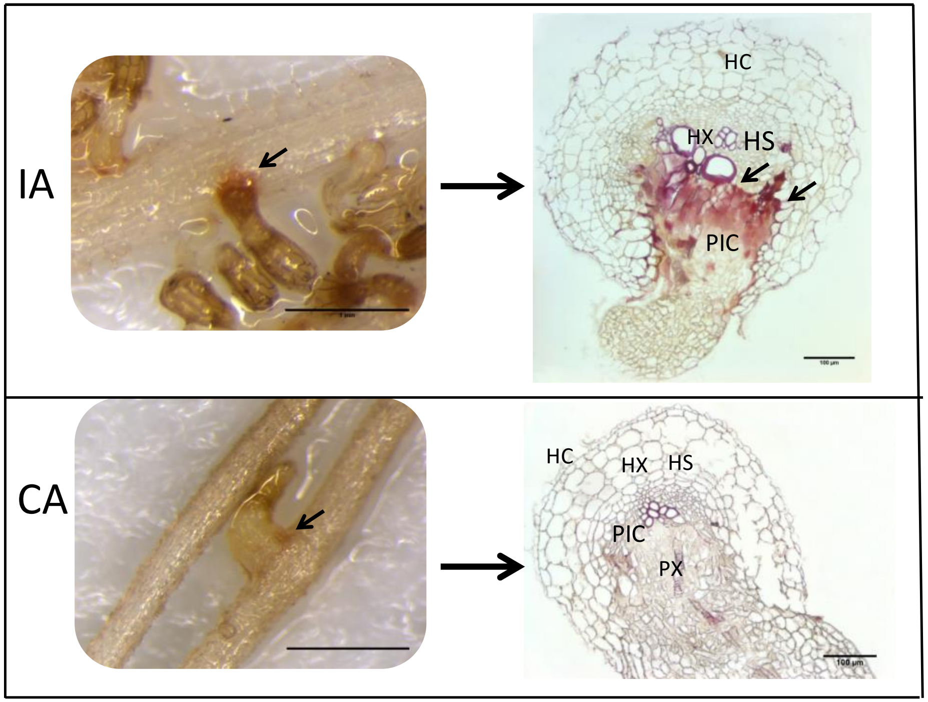
Fixation

Germination

Stage 1

Prevent connection to the vascular system in sunflower root : *a key resistance mechanism*





Barrier formation: **no connection** to the vascular system of the host

Connection to the vascular system of the host

Cell wall modifications seem to be involved both compatible and incompatible attachments.

Which genes are involved?

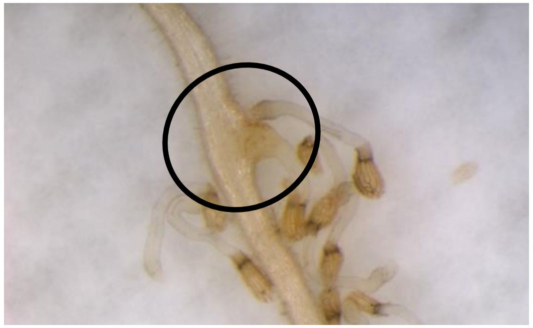
Understanding the molecular and cellular mechanisms involved in the incompatible attachments could help in identifying new candidate genes for the resistance

Genotype	IA rate	# of Healthy tubercles	# of necrotic tubercles	Total number of tubercles
LR1	**66.5**	2.4	0	2.4
HA89	**7.1**	3.4	0	3.4
RIL334	**73.4**	0.2	0.2	0.4
2603	**0**	13	0	13

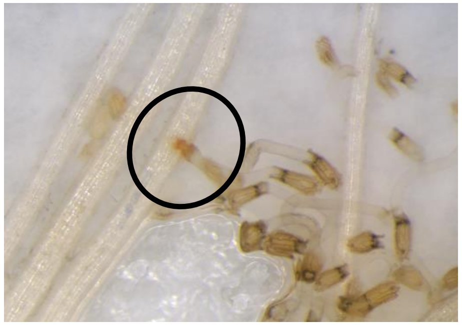
O. cumana race F used for infection

Sampling for transcriptomic analysis

Compatible attachment (CA)

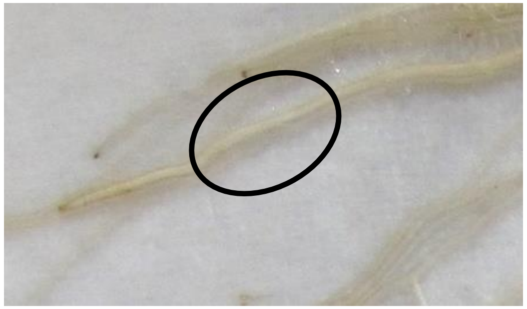


Incompatible attachment (IA)



Systemic roots
Non infected roots of infected plant

Control roots
Roots of non infected plant

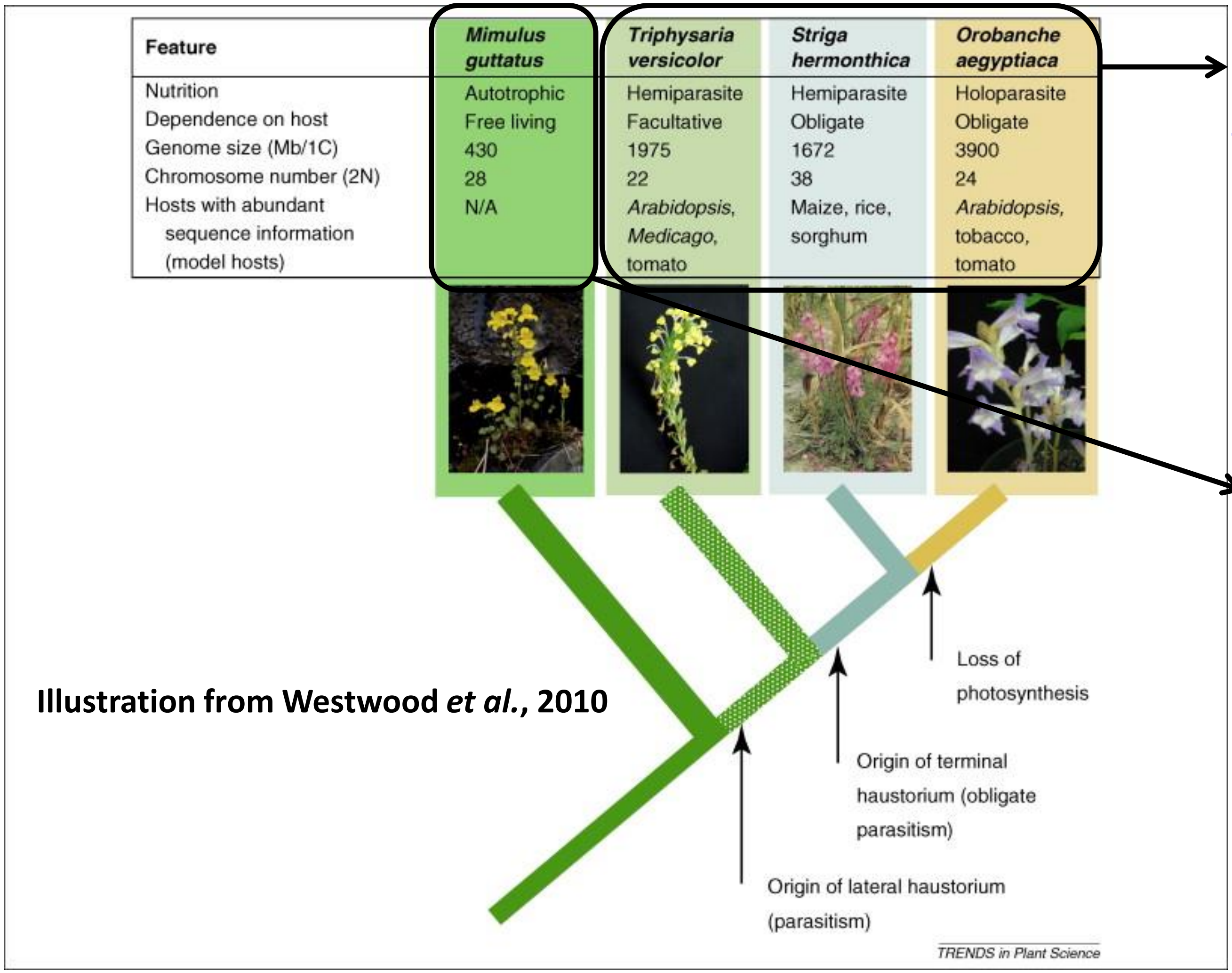


Genes from both broomrape and sunflower are expressed in the attachments samples

RNASeq experiment is the main method to measure the expression of thouthands of genes simultaneously.

But reference sequences of all genes are needed

Genomics of parasitic plants and of their hosts



Feature	*Mimulus guttatus*	*Triphysaria versicolor*	*Striga hermonthica*	*Orobanche aegyptiaca*
Nutrition	Autotrophic	Hemiparasite	Hemiparasite	Holoparasite
Dependence on host	Free living	Facultative	Obligate	Obligate
Genome size (Mb/1C)	430	1975	1672	3900
Chromosome number (2N)	28	22	38	24
Hosts with abundant sequence information (model hosts)	N/A	*Arabidopsis*, *Medicago*, tomato	Maize, rice, sorghum	*Arabidopsis*, tobacco, tomato

Loss of photosynthesis
Origin of terminal haustorium (obligate parasitism)
Origin of lateral haustorium (parasitism)
TRENDS in Plant Science

Illustration from Westwood *et al.*, 2010

Parasitic Plant Genome Project
http://ppgp.huck.psu.edu

Transcriptome libraries using 454 sequencing (Roche)

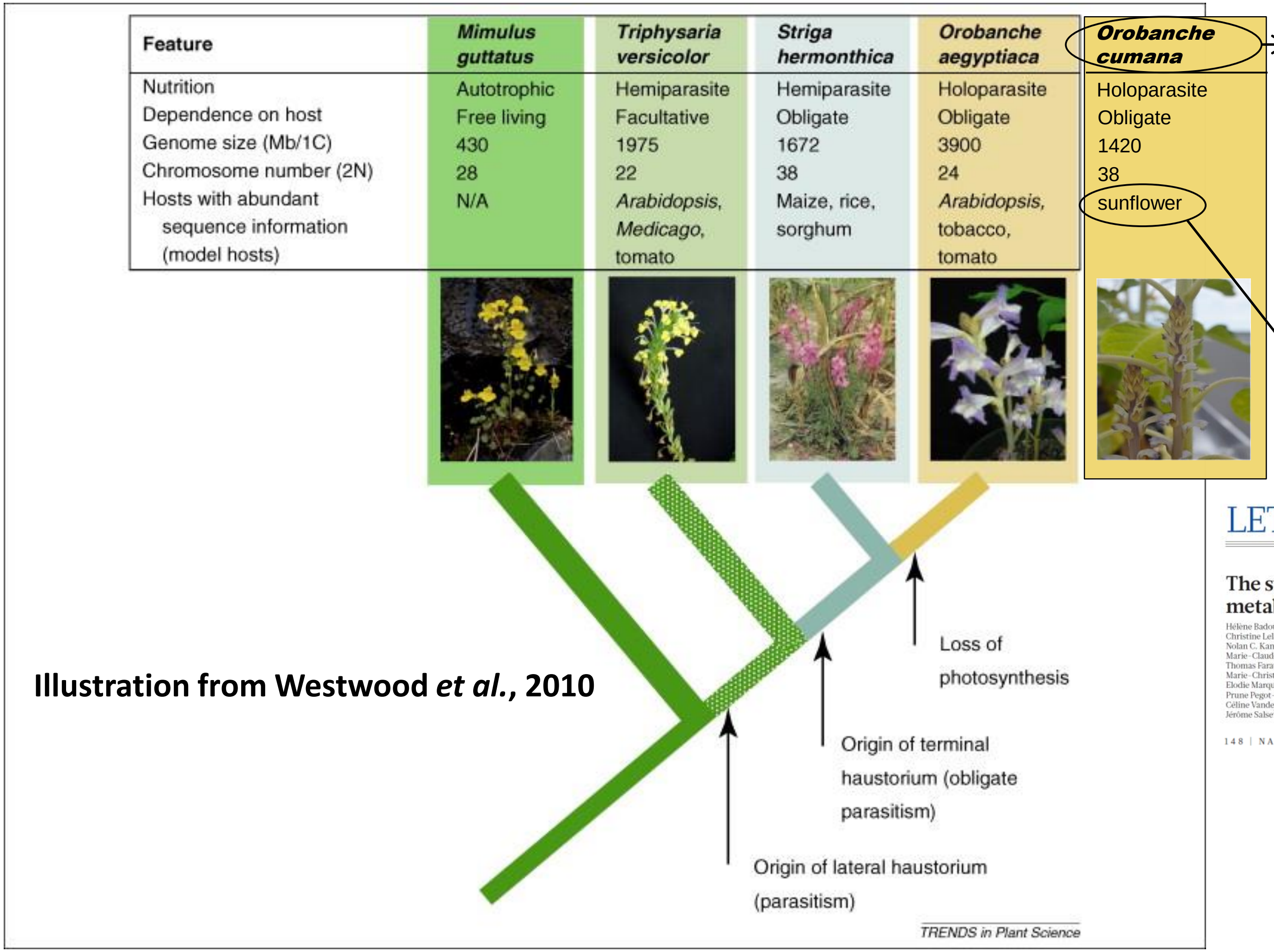
Fully sequenced (Hellsten *et al.*, 2013)
https://phytozome.jgi.doe.gov

Sanger shotgun sequencing

# scaffolds: 1507
Sequence size total: 312.7 Mb (72.7% of the genome, 7.3% N)
N50: 21.2Mb (7 scaffolds)

33573 protein-coding transcripts

Genomics of parasitic plants and their hosts



Feature	*Mimulus guttatus*	*Triphysaria versicolor*	*Striga hermonthica*	*Orobanche aegyptiaca*	***Orobanche cumana***
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Loss of photosynthesis
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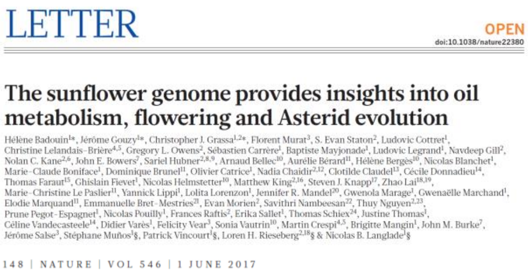
TRENDS in Plant Science

Illustration from Westwood *et al.*, 2010

→Fully sequenced

Fully sequenced



LETTER

OPEN

doi:10.1038/nature22380

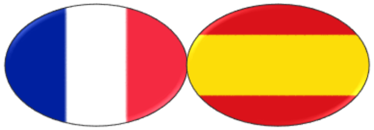
The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution

Hélène Badouin[1*], Jérôme Gouzy[1*], Christopher J. Grassa[1,2*], Florent Murat[3], S. Evan Staton[2], Ludovic Cottret[1], Christine Lelandais-Brière[4,5], Gregory L. Owens[2], Sébastien Carrère[1], Baptiste Mayjonade[1], Ludovic Legrand[1], Navdeep Gill[2], Nolan C. Kane[2,6], John E. Bowers[7], Sariel Hubner[2,8,9], Arnaud Bellec[10], Aurélie Bérard[11], Hélène Bergès[10], Nicolas Blanchet[1], Marie-Claude Boniface[1], Dominique Brunel[11], Olivier Catrice[1], Nadia Chaidir[2,12], Clotilde Claudel[13], Cécile Donnadieu[14], Thomas Faraut[15], Ghislain Fievet[1], Nicolas Helmstetter[10], Matthew King[2,16], Steven J. Knapp[17], Zhao Lai[18,19], Marie-Christine Le Paslier[11], Yannick Lippi[1], Lolita Lorenzon[1], Jennifer R. Mandel[20], Gwenola Marage[1], Gwenaëlle Marchand[1], Elodie Marquand[11], Emmanuelle Bret-Mestries[21], Evan Morien[2], Savithri Nambeesan[22], Thuy Nguyen[2,23], Prune Pegot-Espagnet[1], Nicolas Pouilly[1], Frances Raftis[2], Erika Sallet[1], Thomas Schiex[24], Justine Thomas[1], Céline Vandecasteele[14], Didier Varès[1], Felicity Vear[3], Sonia Vautrin[10], Martin Crespi[4,5], Brigitte Mangin[1], John M. Burke[7], Jérôme Salse[3], Stéphane Muños[1]§, Patrick Vincourt[1]§, Loren H. Rieseberg[2,18]§ & Nicolas B. Langlade[1]§

148 | NATURE | VOL 546 | 1 JUNE 2017

Genomic resources available make *O. cumana*–sunflower a unique pathosystem

The HeliOr project



A public-private joint French-Spanish consortium

INRA SCIENCE & IMPACT
LIPM Laboratoire Interactions Plantes Micro-organismes

**Sunflower team:*
Sunflower genetics and genomics
O. cumana diversity
**Bioinformatics team:*
Genome assembly and annotation
SNP calling
Informatic tools for biologists

UNIVERSITÉ DE NANTES LBP NANTES

O. cumana biology

GeT Génome et Transcriptome genotoul

Sequencing platform

biogemma

Sunflower genetics
O. cumana diversity

CNRGV PLANT GENOMIC CENTER

BAC libraries
Genomic tools (optical map…)

Terres Inovia l'agronomie en mouvement

Advices to farmers
List of infected fields in France

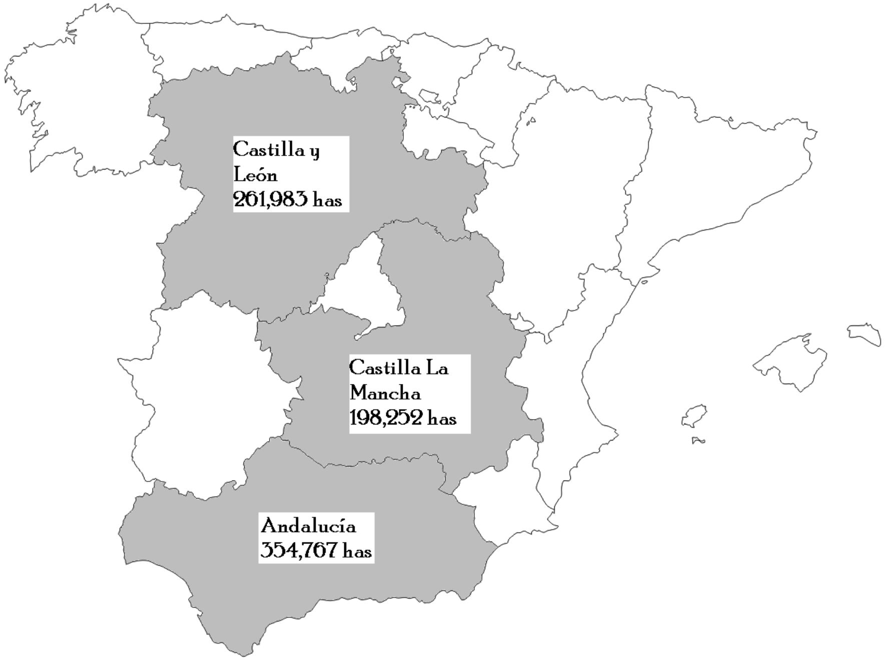
CSIC CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
INSTITUTO DE AGRICULTURA SOSTENIBLE IAS

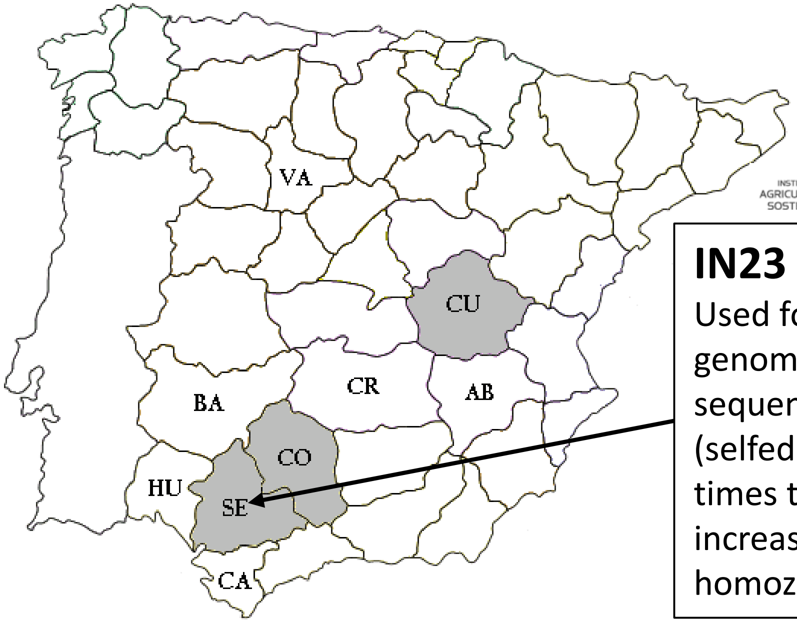
Sunflower genetics
O. cumana genetics and diversity

Fundings: SOFIPROTEOL L'engagement durable

Sunflower and *Orobanche cumana* in Spain

- **Race F has been the most virulent race until recently in both main areas of occurrence of sunflower broomrape: Cuenca (CU) and the Guadalquivir Valley: two distinct genetic pools**

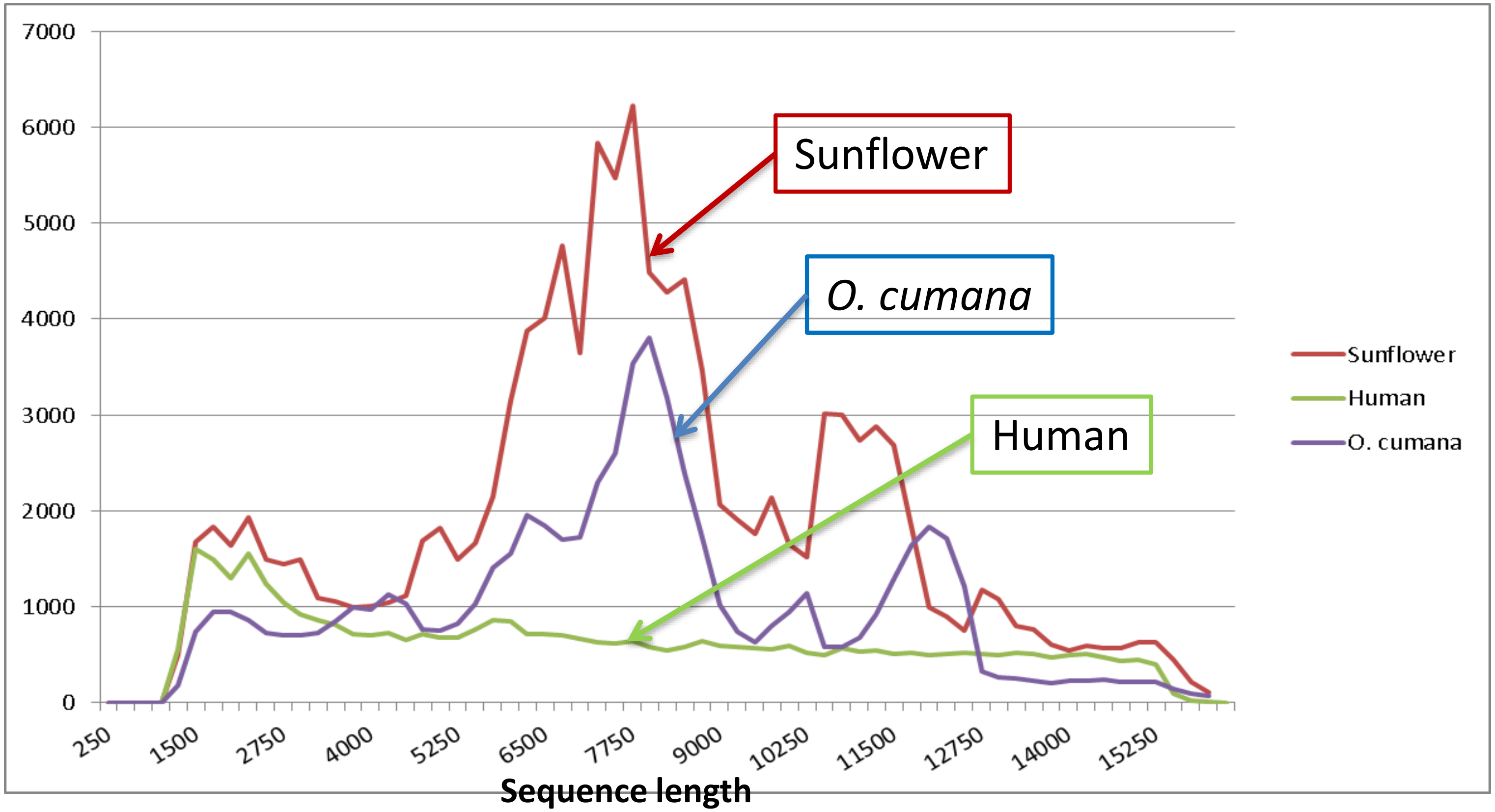




IN23
Used for genome sequencing (selfed three times to increase homozygosity)

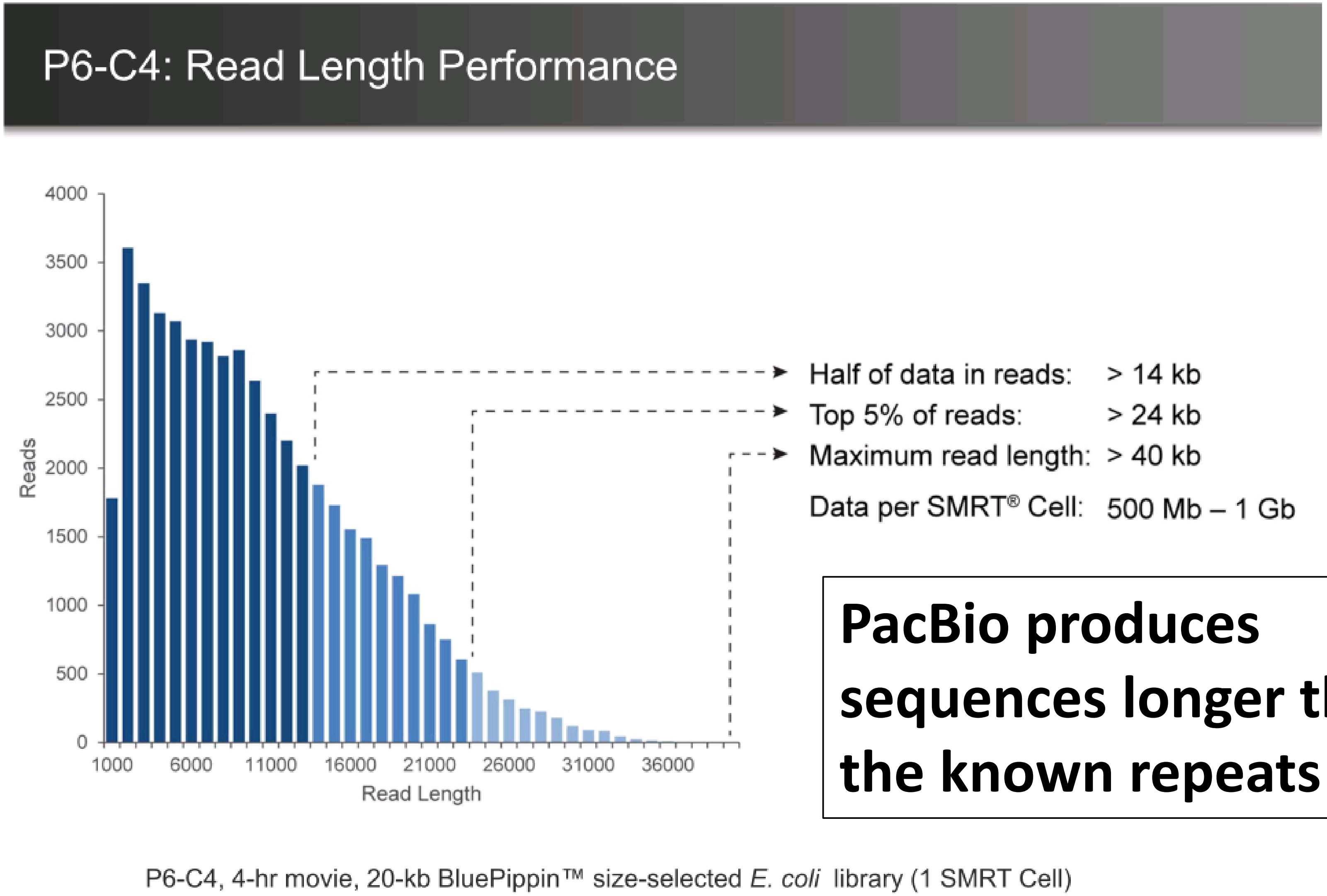
O.cumana: a nightmare not only for sunflower. For bioinformaticians too!!!

J. Gouzy



A lot of repeats (like sunflower, i.e. 33% of the genome) but longer than in sunflower

Long read sequencing using PacBio RSII (Pacific Biosciences)



P6-C4: Read Length Performance

Half of data in reads:	> 14 kb
Top 5% of reads:	> 24 kb
Maximum read length:	> 40 kb
Data per SMRT® Cell:	500 Mb – 1 Gb (in 4 hours)

PacBio produces sequences longer than the known repeats

P6-C4, 4-hr movie, 20-kb BluePippin™ size-selected *E. coli* library (1 SMRT Cell)

PacBio data

N. Pouilly

- All data produced at GeT-Plage (INRA)

- Produced from October 2015 to February 2016
  - 100X depth expected
  - 126 SMRT Cells (mean: 1.19 Gb/SMRT Cell)

Statistics of contig sequences after assembly of the raw data

Steps	NUM	MAX (Mp)	N50 (Mp)	NUM >=N50	MEAN (bp)	MEDIAN	Total (Gb)
Raw data (subreads) 126 SMRT Cells	13.2M	85.05					**149.9**
Corrected reads (CANU)	7.04M	55.53	13.98	2.01M	10651	9777	**75.06**
	NUM	**MAX (Mb)**	**N50 (Mb)**	**NUM >=N50**	**MEAN (Mb)**	**MEDIAN (Mb)**	**Total (Gb)**
Genome assembly (CANU)	905	16.88	3.57	107	1.53	6.49	1.388
Remove spurious + Sequence based Scaffolding + polishing (QUIVER)	**793**	16.98	**4.21**	96	1.74	7.43	**1.380**

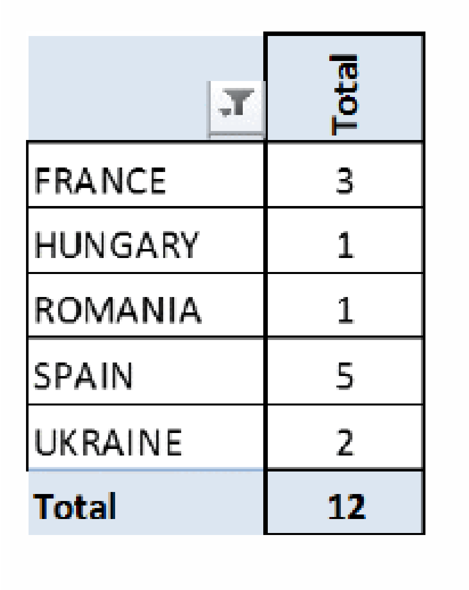
/2

From contigs to chromosomes sequences!

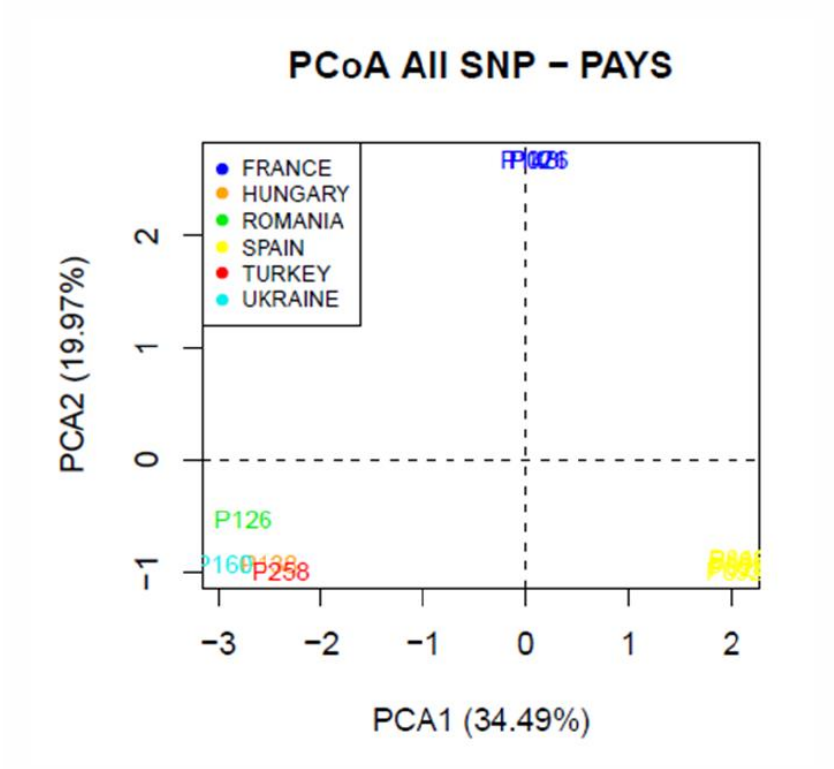
Using genetic map and optical map!

Diversity analysis in *O. cumana*

M. Coque



	Total
FRANCE	3
HUNGARY	1
ROMANIA	1
SPAIN	5
UKRAINE	2
Total	**12**



PCoA All SNP – PAYS

Exome capture from the 12 populations : 362285 SNPs

1536 SNPs selected to maximize the diversity of the whole set

Fundings: SOFIPROTEOL L'engagement durable

A segregating population for the first genetic of *O. cumana*

B. Pérez-Vich L. Velasco

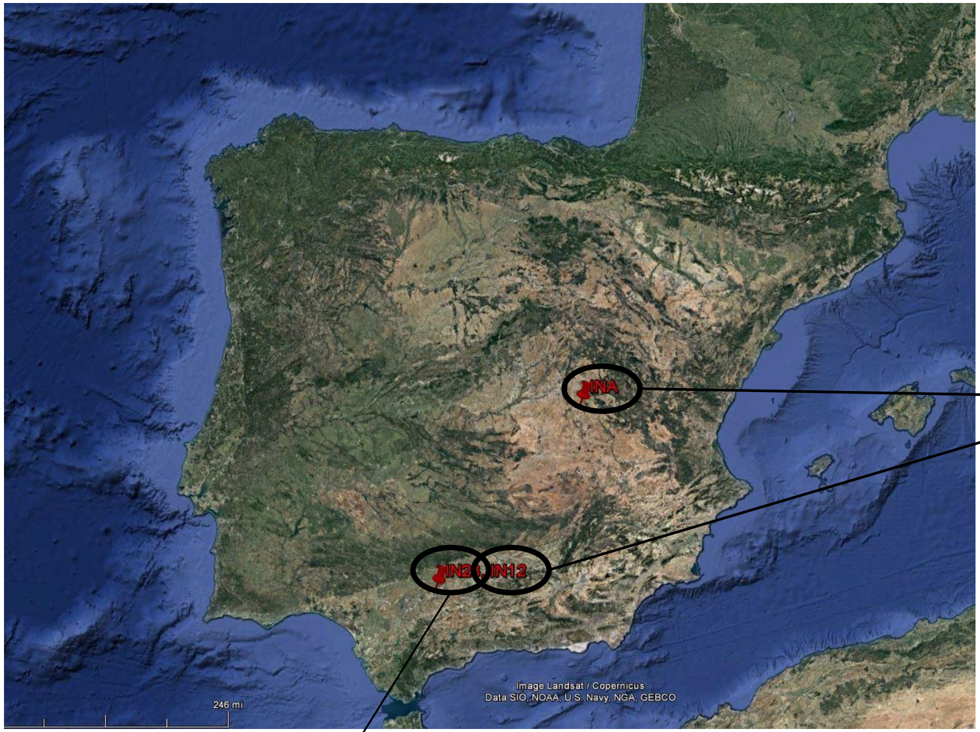
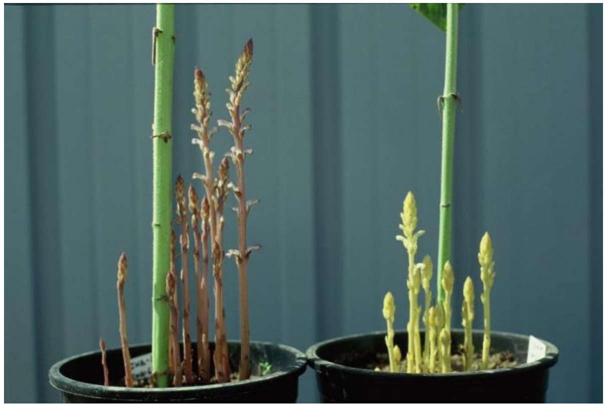
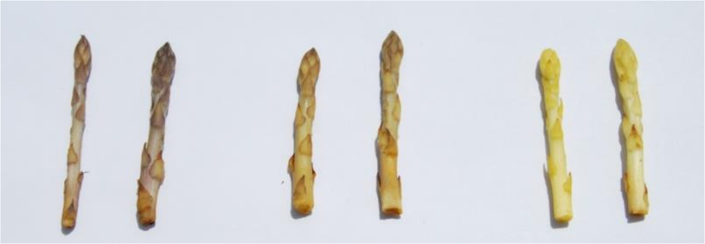


Image Landsat / Copernicus
Data SIO, NOAA, U.S. Navy, NGA, GEBCO



IN12 INA



IN12 F1 INA

IN23 used for genome sequencing

The first genetic map of *O. cumana*

X. Grand

1536 SNPs + 168 SSR x 91 F2 and parental lines have been genotyped

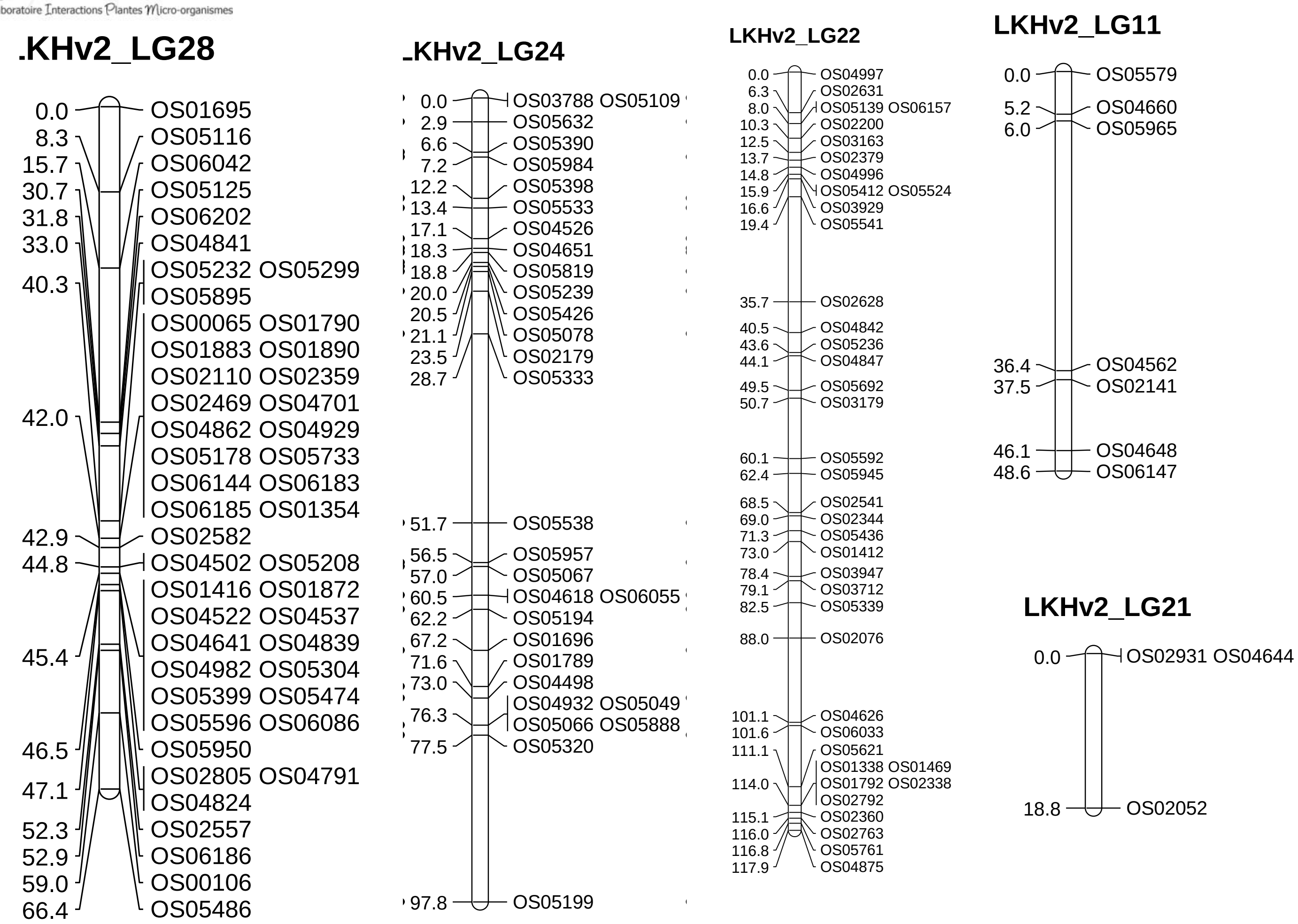
509 SNPs + 18 SSR were polymorphic and did not show any distortion of their segregation in the full population

Genetic map built using CarthaGène software (INRA) with a high stringency

⬇

1479cM
28 linkage groups for the 19 chromosomes

The first genetic map of *O. cumana*



Colinearity between the genetic map and the genome

Almost perfect colinearity between physical map and genetic map.

But strong variations between physical distances and genetic distances according to the regions.

This genetic map enabling the anchoring of 95 contigs (593Mb) from the 256 contigs representing 90% of the genome assembly.

161 contigs remain unmapped to anchor 90% of the genome!

ReSequencing the 2 parental lines of the segregating population

A. Calderon

The genome of the 2 parental lines used to produce the F2 segregating populations have been resequenced (HiSeq, Illumina)

⬇

40 912 accurate polymorphic SNPs between the two parental lines

Improving the genetic map

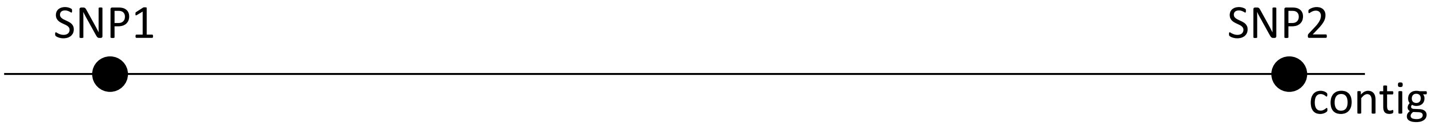
L. Hu

Not enough contigs anchored to the genetic map (95 contigs)

40 912 polymorphic SNPs between the two parental lines

13511 SNPs located on 145 contigs from the 161 contigs that remain unmapped

278 SNPs will be genotyped in the full segregating population to anchor the 145 more contigs



All these data should anchor 90% of the genome assembly

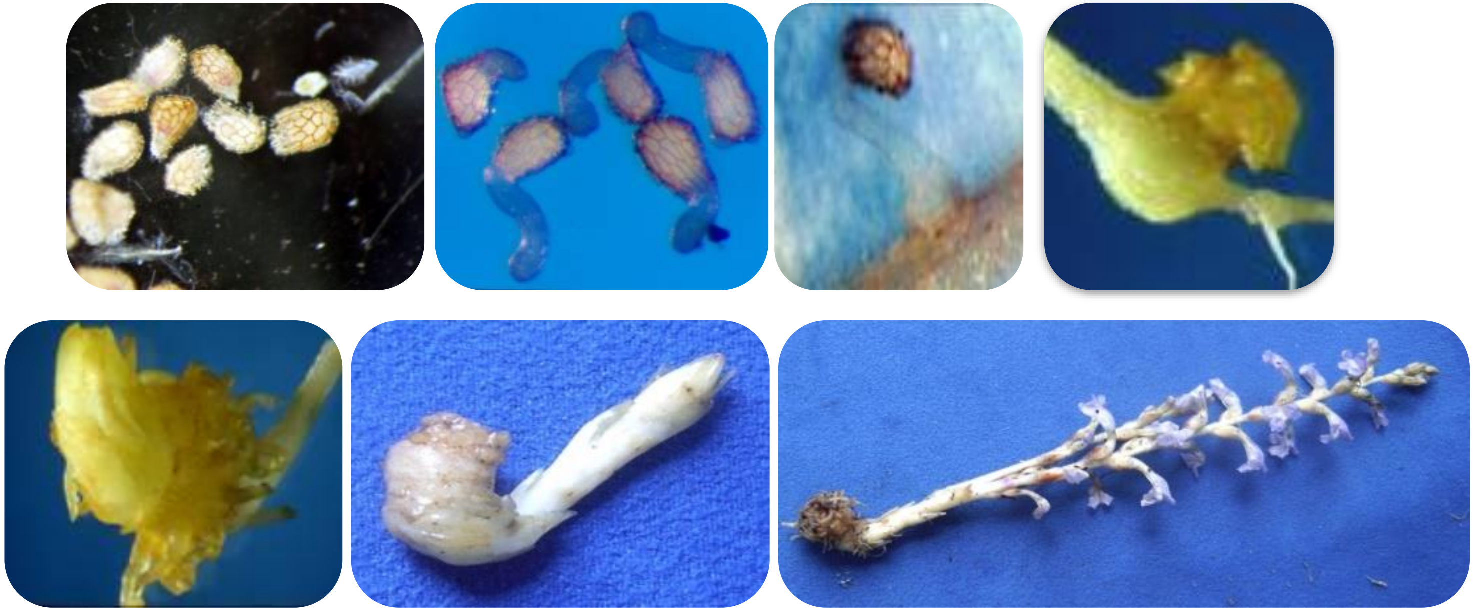
Transcriptomic data to annotate the *genome*

P. Delavault

Fundings P.R.O.M.O.S.O.L.
ASSOCIATION
POUR LA PROMOTION
DE LA SÉLECTION
DES PLANTES OLÉAGINEUSES

60 RNASeq libraries sequenced (data obtained on 26 April 2016)

Corresponding to **20 broomrape development stages** from seeds to flowering (3 replicates/stage)



E. Sallet

Use of an automatic annotation pipeline

- EuGene Plant pipeline (egn-ep)

Total number of genes: **55726**

Number of protein coding genes: 46447
Mean gene length (bp): 3568.75
Per cent genes with introns : 63 **genes with 5’ UTR: 82% genes with 3’ UTR: 83%**
- Exons Mean number per gene: 3.63 Mean length (bp): 472.95 GC %: 40.94
- Introns Mean number per gene: 2.63 Mean length (bp): 706.35 GC % 35.40
-CDS Mean length (bp): 732.32 GC % 45.25

Experimental expression evidence added!

Number of non protein coding genes 9279

* Tephra – repeat annotation - https://github.com/sestaton/tephra

11 456 genes (8 256 protein coding + 3 200 ncRNA) are fully included in repeat_region annotated by tephra

Assessment of the annotation quality using « BUSCO » (a set of conserved genes in plants)

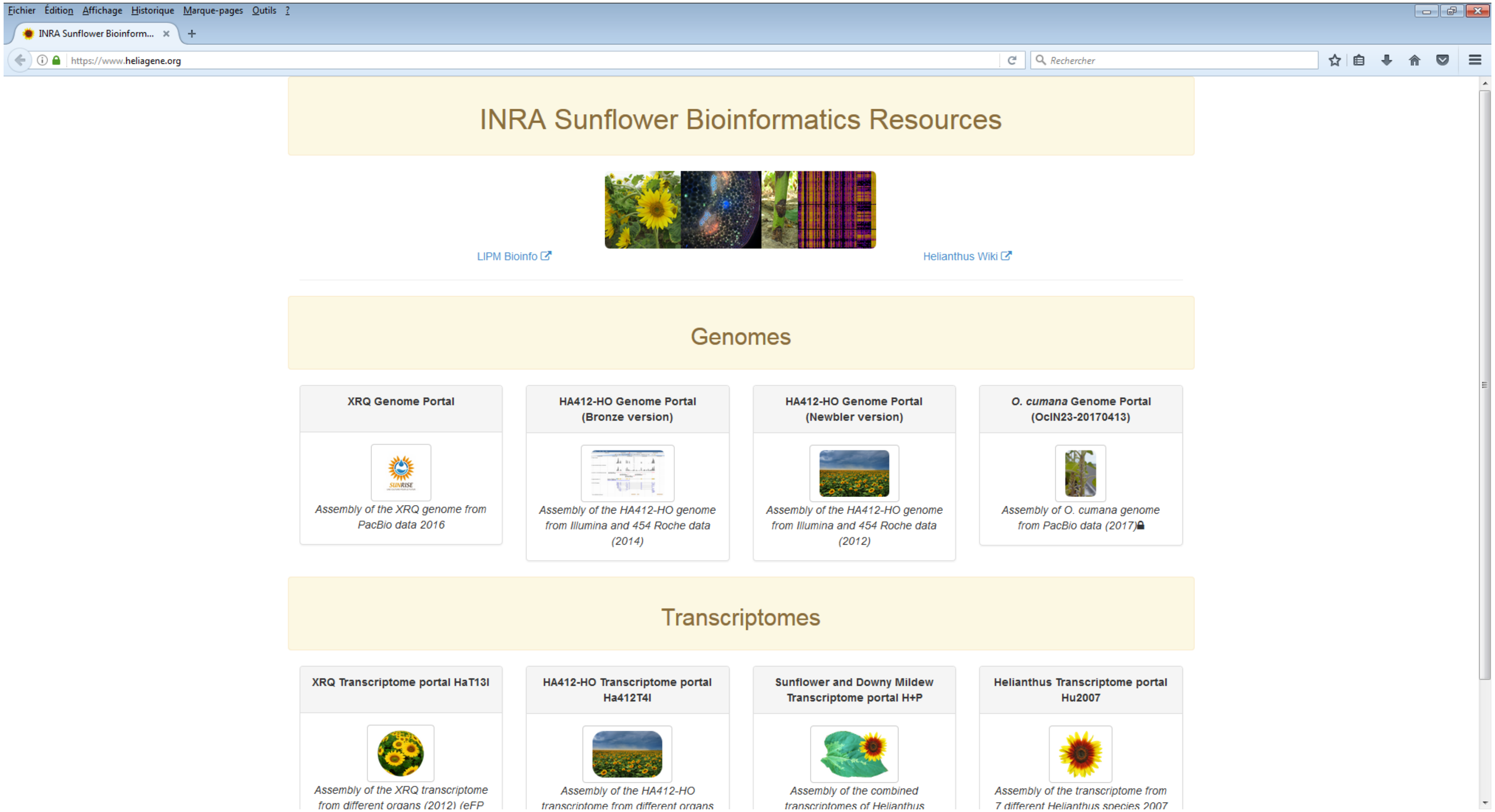
	Complete and unique	Complete but Duplicated	% complete	partial	Missing
Sunflower	1153	196	93.7%	21	70
O. cumana	999	47	**72.7%**	72	322

A lot of missing genes!
Due to the biology of *O. cumana*?

All genome information are available in a Genome Browser

https://www.heliagene.org

S. Carrère


Fichier Édition Affichage Historique Marque-pages Outils ?
INRA Sunflower Bioinform...
https://www.heliagene.org
Rechercher

INRA Sunflower Bioinformatics Resources

LIPM Bioinfo

Helianthus Wiki

Genomes

XRQ Genome Portal
Assembly of the XRQ genome from PacBio data 2016

HA412-HO Genome Portal (Bronze version)
Assembly of the HA412-HO genome from Illumina and 454 Roche data (2014)

HA412-HO Genome Portal (Newbler version)
Assembly of the HA412-HO genome from Illumina and 454 Roche data (2012)

***O. cumana* Genome Portal (OcIN23-20170413)**
Assembly of O. cumana genome from PacBio data (2017)🔒

Transcriptomes

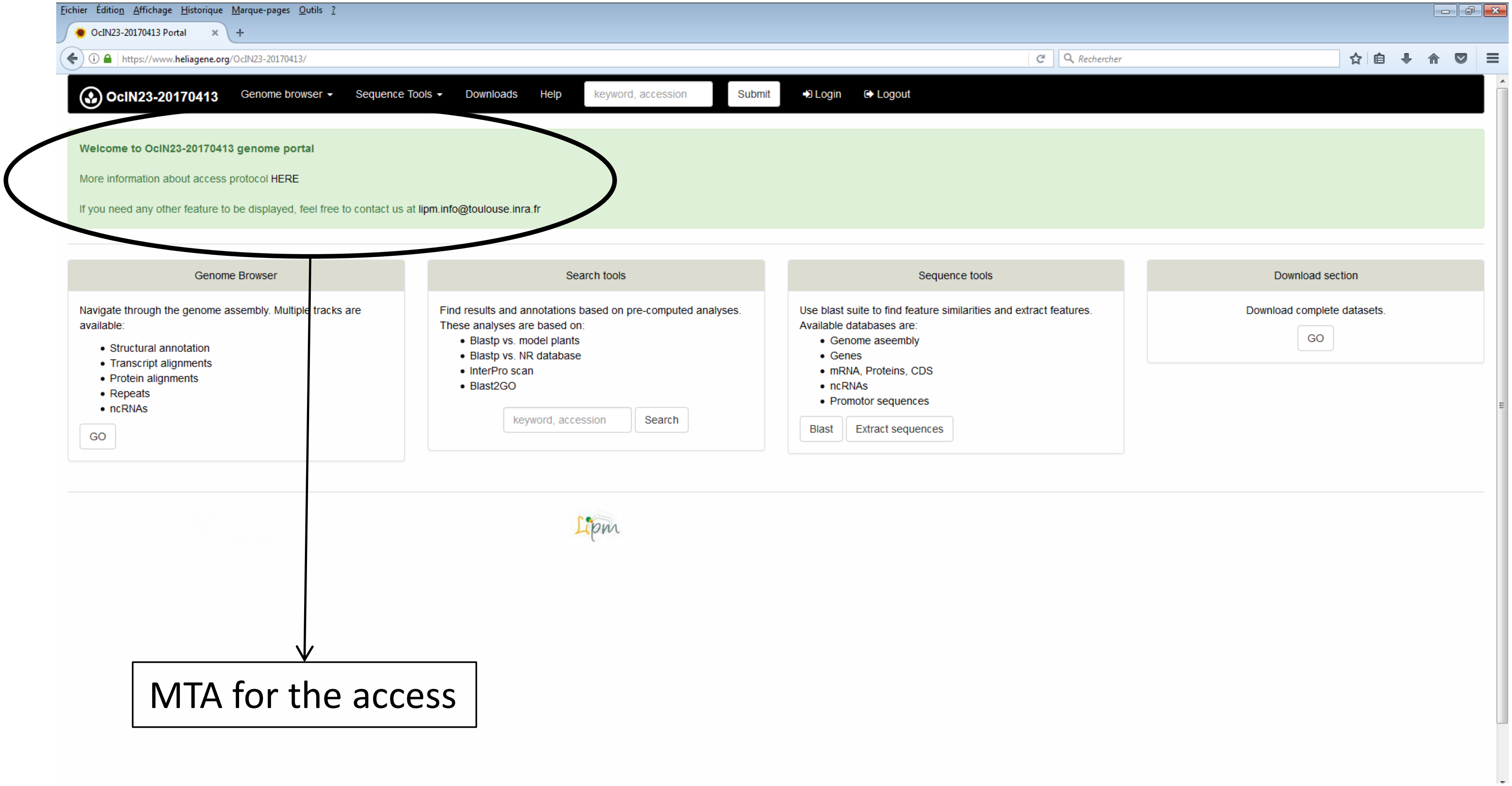
XRQ Transcriptome portal HaT13l
Assembly of the XRQ transcriptome from different organs (2012) (eFP

HA412-HO Transcriptome portal Ha412T4l
Assembly of the HA412-HO transcriptome from different organs

Sunflower and Downy Mildew Transcriptome portal H+P
Assembly of the combined transcriptomes of Helianthus

Helianthus Transcriptome portal Hu2007
Assembly of the transcriptome from 7 different Helianthus species 2007

All genome information are available in a Genome Browser



Welcome to OcIN23-20170413 genome portal

More information about access protocol HERE

If you need any other feature to be displayed, feel free to contact us at lipm.info@toulouse.inra.fr

Genome Browser

Navigate through the genome assembly. Multiple tracks are available:

- Structural annotation
- Transcript alignments
- Protein alignments
- Repeats
- ncRNAs

GO

Search tools

Find results and annotations based on pre-computed analyses. These analyses are based on:

- Blastp vs. model plants
- Blastp vs. NR database
- InterPro scan
- Blast2GO

keyword, accession Search

Sequence tools

Use blast suite to find feature similarities and extract features. Available databases are:

- Genome aseembly
- Genes
- mRNA, Proteins, CDS
- ncRNAs
- Promotor sequences

Blast Extract sequences

Download section

Download complete datasets.

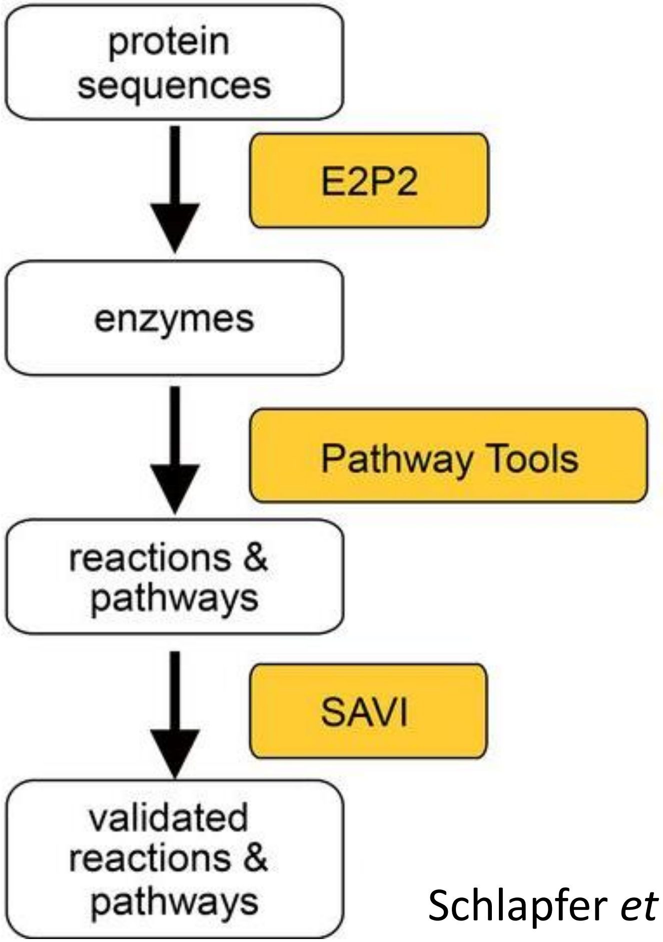
GO

MTA for the access

Biochimical informations from the genome sequence

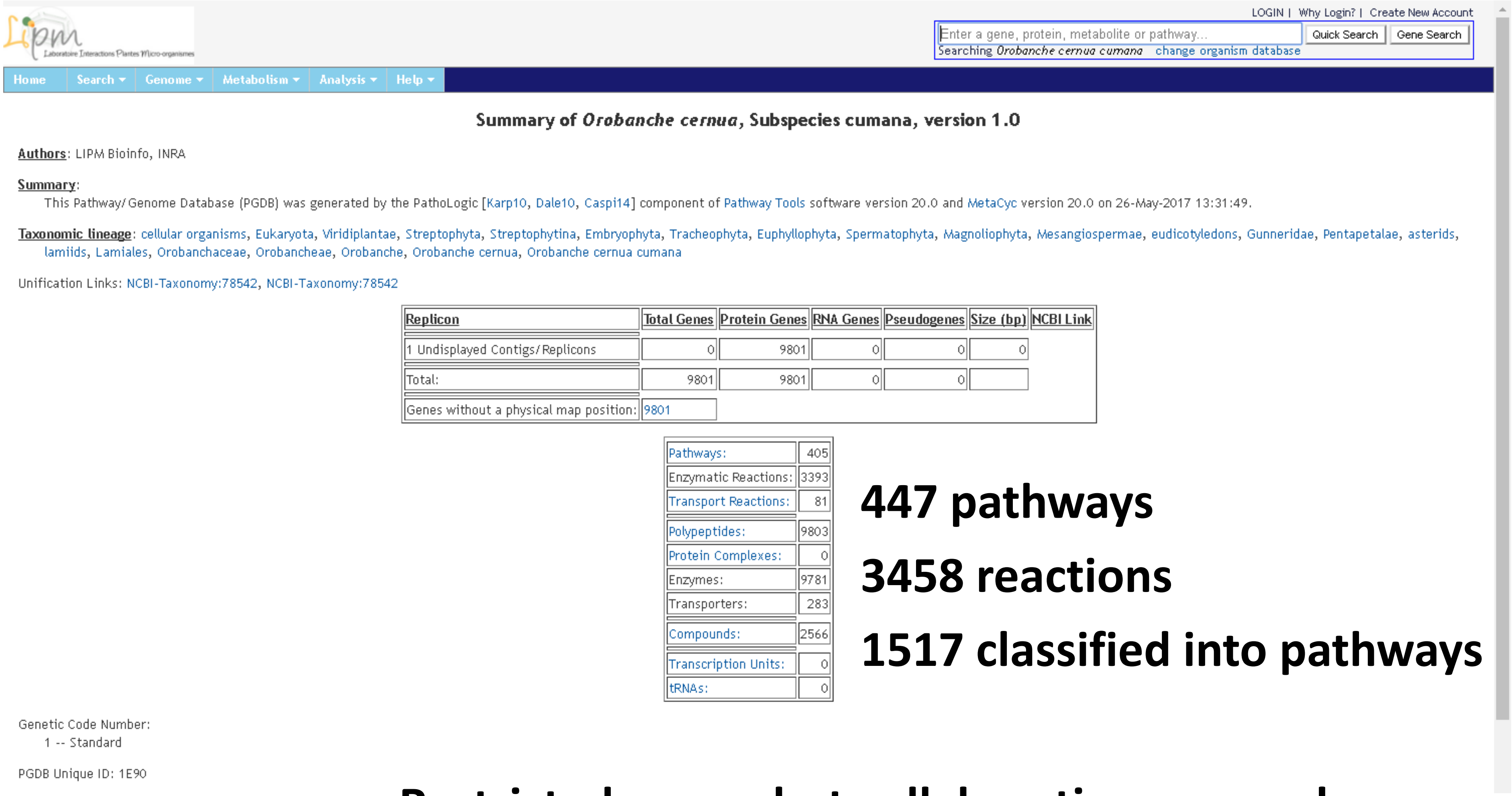
L. Cottret

Automatic process to build the metabolic database



Schlapfer *et al.,* Plant Physiology. 2017.

Metabolic database



LIPM
Laboratoire Interactions Plantes Micro-organismes
LOGIN | Why Login? | Create New Account
Enter a gene, protein, metabolite or pathway...
Quick Search
Gene Search
Home
Search ▾
Genome ▾
Metabolism ▾
Analysis ▾
Help ▾

Searching *Orobanche cernua cumana* change organism database

Summary of *Orobanche cernua*, Subspecies cumana, version 1.0

Authors: LIPM Bioinfo, INRA

Summary:
This Pathway/Genome Database (PGDB) was generated by the PathoLogic [Karp10, Dale10, Caspi14] component of Pathway Tools software version 20.0 and MetaCyc version 20.0 on 26-May-2017 13:31:49.

Taxonomic lineage: cellular organisms, Eukaryota, Viridiplantae, Streptophyta, Streptophytina, Embryophyta, Tracheophyta, Euphyllophyta, Spermatophyta, Magnoliophyta, Mesangiospermae, eudicotyledons, Gunneridae, Pentapetalae, asterids, lamiids, Lamiales, Orobanchaceae, Orobancheae, Orobanche, Orobanche cernua, Orobanche cernua cumana

Unification Links: NCBI-Taxonomy:78542, NCBI-Taxonomy:78542

Replicon	Total Genes	Protein Genes	RNA Genes	Pseudogenes	Size (bp)	NCBI Link
1 Undisplayed Contigs/Replicons	0	9801	0	0	0	
Total:	9801	9801	0	0		
Genes without a physical map position:	9801					

Pathways:	405
Enzymatic Reactions:	3393
Transport Reactions:	81
Polypeptides:	9803
Protein Complexes:	0
Enzymes:	9781
Transporters:	283
Compounds:	2566
Transcription Units:	0
tRNAs:	0

Genetic Code Number:
1 -- Standard

PGDB Unique ID: 1E90

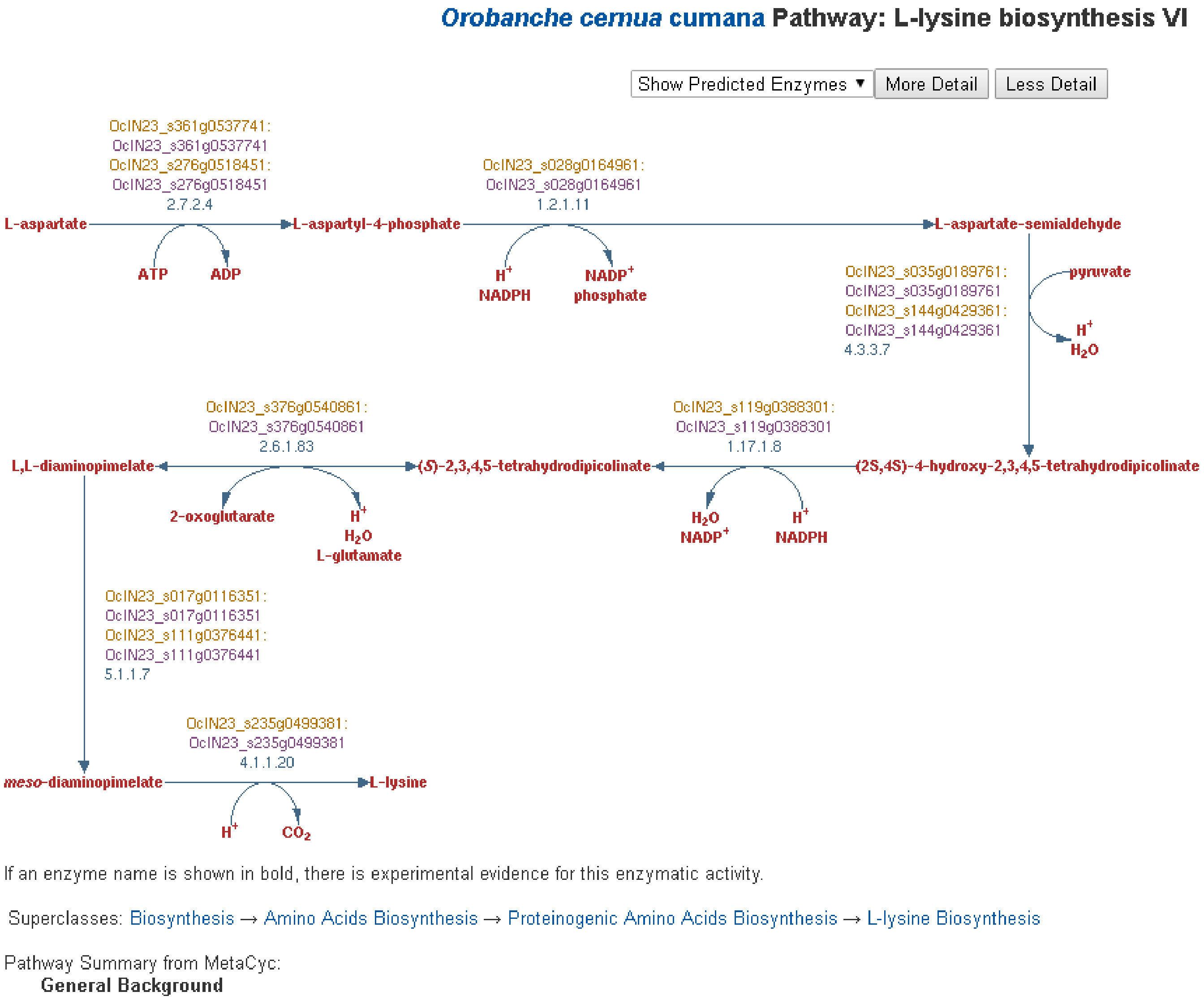
447 pathways

3458 reactions

1517 classified into pathways

Restricted access but collaborations are welcome

Link between pathways, reactions, enzymes and genes



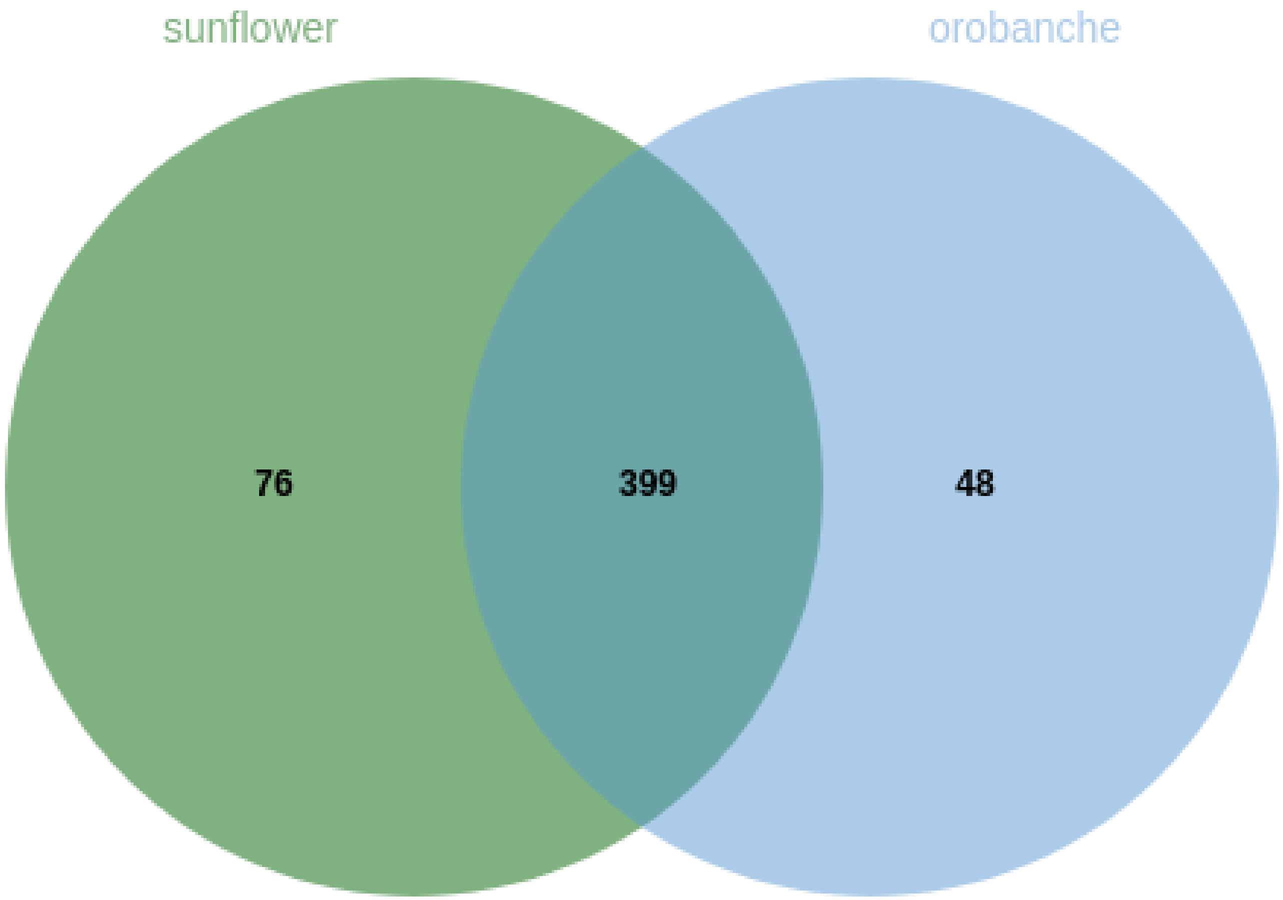
If an enzyme name is shown in bold, there is experimental evidence for this enzymatic activity.

Superclasses: Biosynthesis → Amino Acids Biosynthesis → Proteinogenic Amino Acids Biosynthesis → L-lysine Biosynthesis

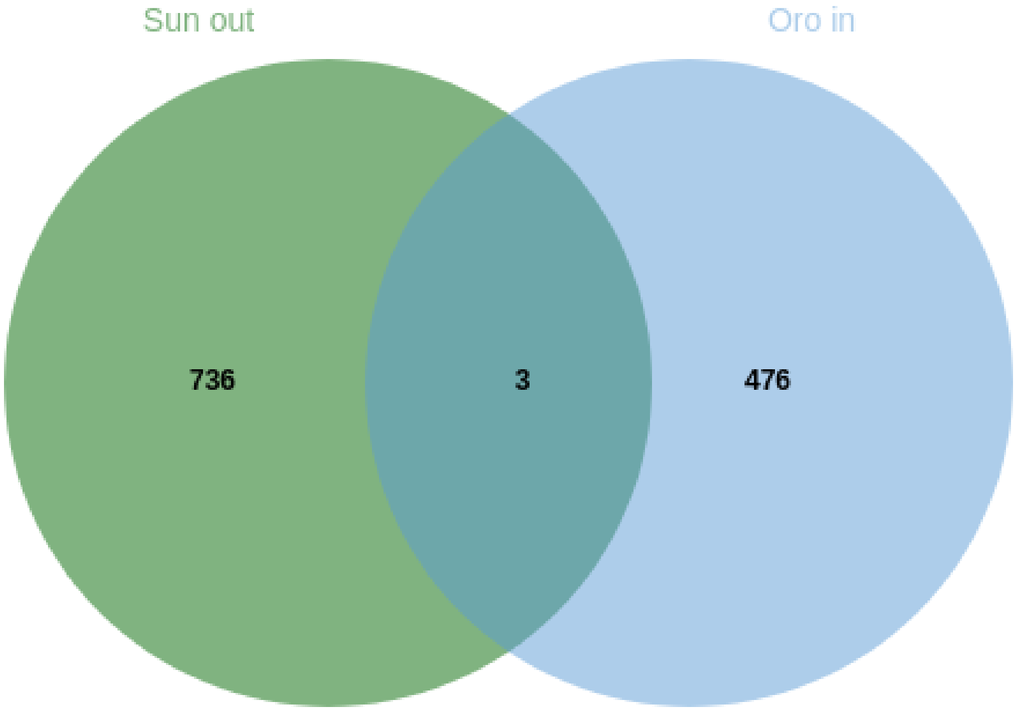
Pathway Summary from MetaCyc:

General Background

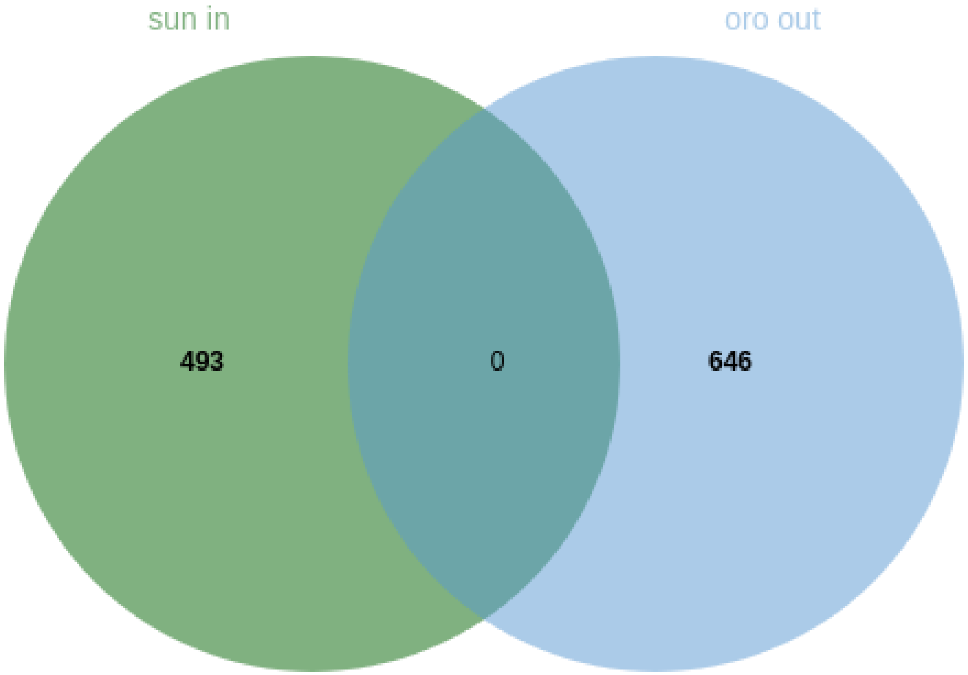
Comparison of sunflower and *O. cumana* pathways



Intersection between Orobanche input metabolites and sunflower output metabolites



Intersection between broomrape output metabolites and sunflower input metabolites



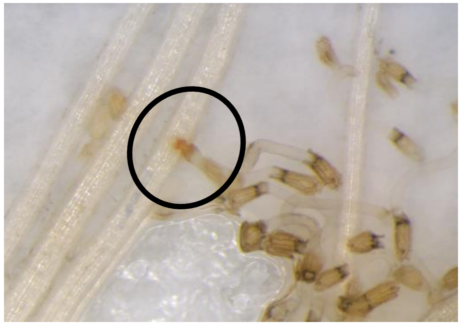
Use of the annotated genome for RNASeq analysis

We used the annotated mRNA sequences from XRQ (sunflower) and IN23 (*O. cumana*) to map the RNASeq data

Preliminary results, more analysis need to be performed to mak then more accurate!

Sunflower DEG during incompatible attachment

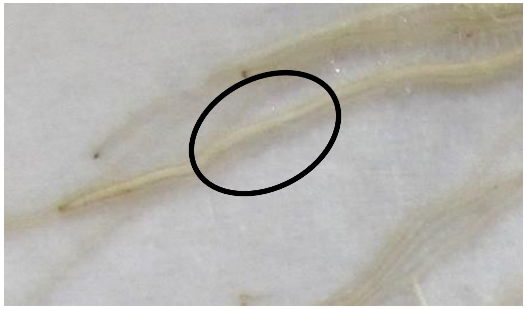
Incompatible Attachments



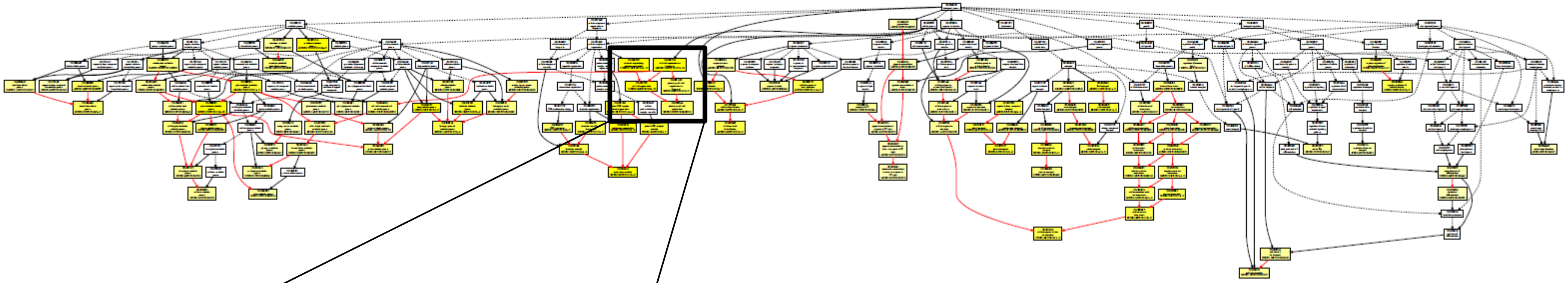
LR1: 3444 DEG

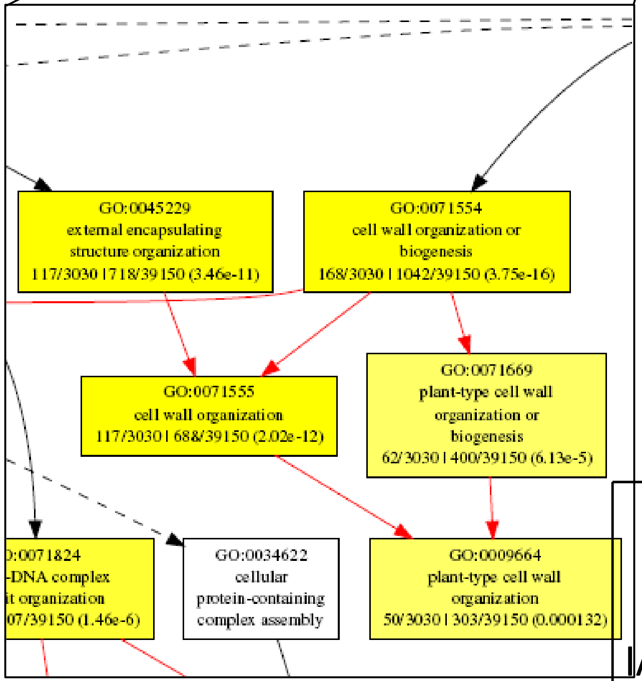
↓

Control



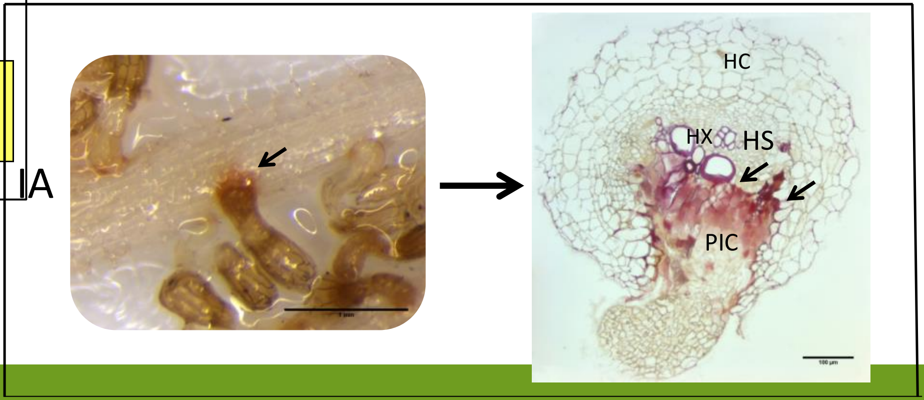
Gene Ontology terms enrichment analysis using GOEAST





A more complex response during incompatible attachment

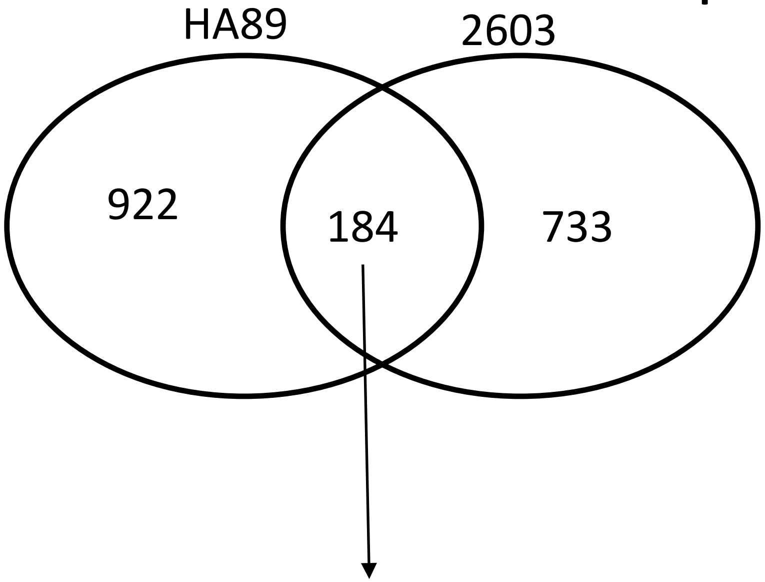
DEG involved in cell wall biogenesis are enriched to prevent connection of the vascular system of the host



Barrier formation: **no connection** to the vascular system of the host

Sunflower DEG during compatible attachment

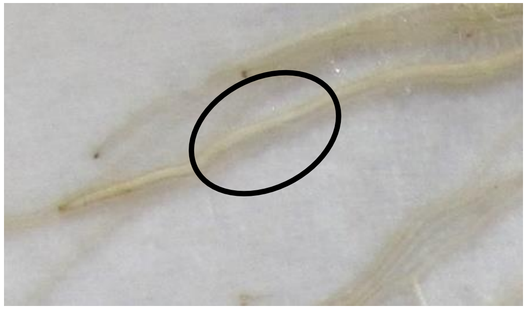
HA89: 1106 DEG
CA 2603: 917 DEG

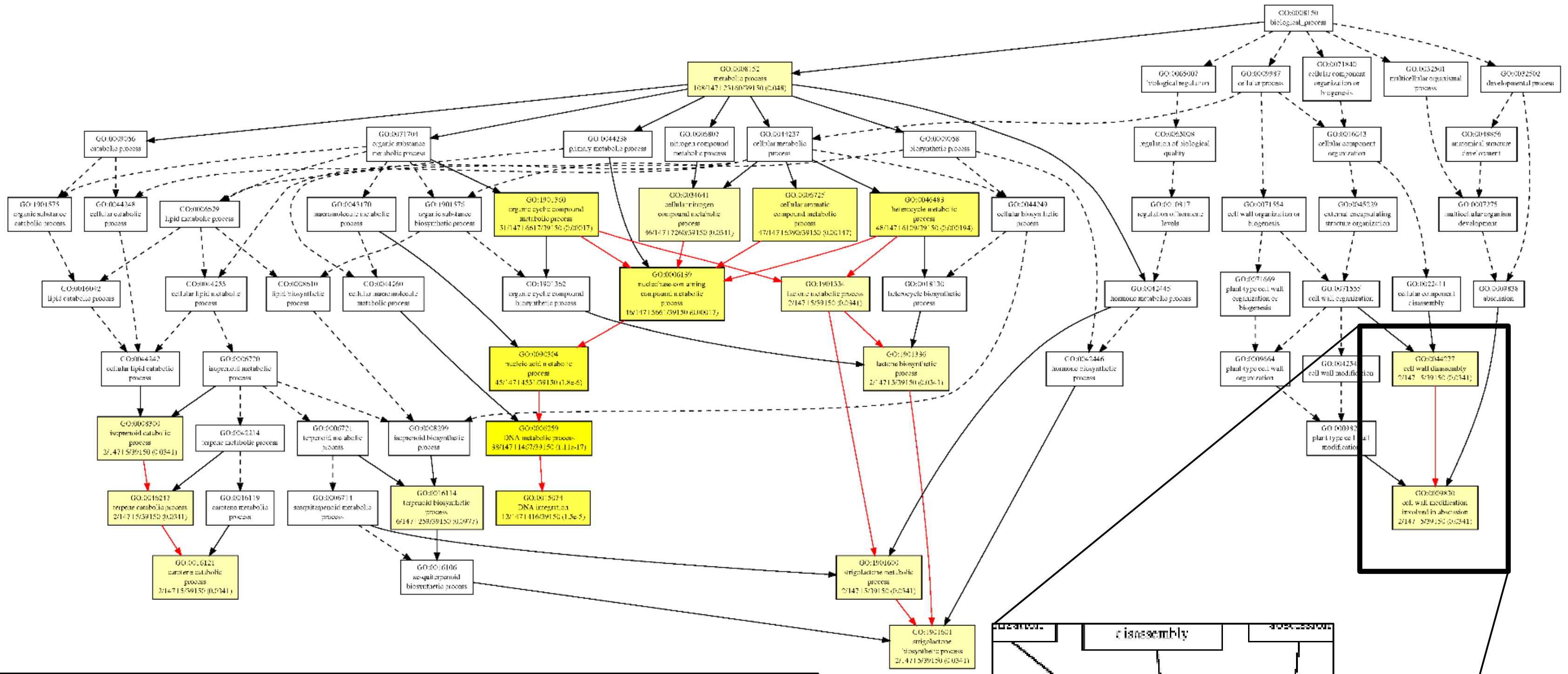


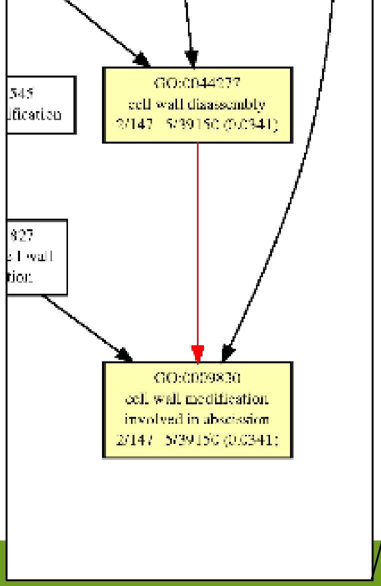
Gene Ontology terms enrichment analysis using GOEAST

Compatible Attachments

Control

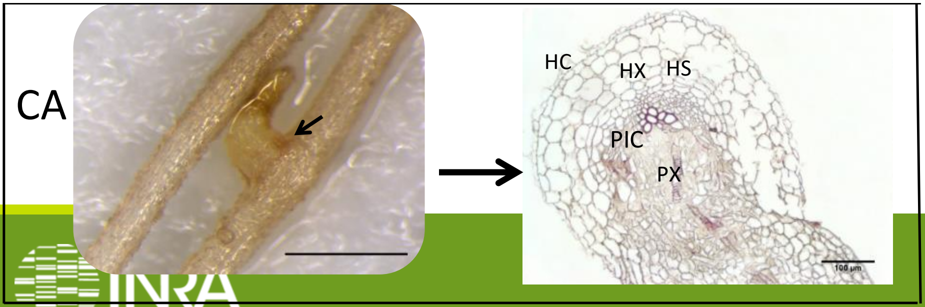



GO:0008150
biological_process
GO:0008152
metabolic process
108/147 | 23180/39150 (0.048)
GO:0065007
biological regulation
GO:0009987
cellular process
GO:0071840
cellular component organization or biogenesis
GO:0032501
multicellular organismal process
GO:0032502
developmental process
GO:0009056
catabolic process
GO:0071704
organic substance metabolic process
GO:0044238
primary metabolic process
GO:0006807
nitrogen compound metabolic process
GO:0044237
cellular metabolic process
GO:0009058
biosynthetic process
GO:0065008
regulation of biological quality
GO:0016043
cellular component organization
GO:0048856
anatomical structure development
GO:1901575
organic substance catabolic process
GO:0044248
cellular catabolic process
GO:0006629
lipid metabolic process
GO:0043170
macromolecule metabolic process
GO:1901576
organic substance biosynthetic process
GO:1901360
organic cyclic compound metabolic process
31/147 | 6617/39150 (0.00017)
GO:0034641
cellular nitrogen compound metabolic process
46/147 | 7260/39150 (0.0341)
GO:0006725
cellular aromatic compound metabolic process
47/147 | 6390/39150 (0.00147)
GO:0046483
heterocycle metabolic process
48/147 | 6109/39150 (0.000194)
GO:0044249
cellular biosynthetic process
GO:0010817
regulation of hormone levels
GO:0071554
cell wall organization or biogenesis
GO:0045229
external encapsulating structure organization
GO:0007275
multicellular organism development
GO:0016042
lipid catabolic process
GO:0044255
cellular lipid metabolic process
GO:0008610
lipid biosynthetic process
GO:0044260
cellular macromolecule metabolic process
GO:1901362
organic cyclic compound biosynthetic process
GO:0006139
nucleobase-containing compound metabolic process
16/147 | 5661/39150 (0.00017)
GO:1901334
lactone metabolic process
2/147 | 5/39150 (0.0341)
GO:0018130
heterocycle biosynthetic process
GO:0042445
hormone metabolic process
GO:0071669
plant-type cell wall organization or biogenesis
GO:0071555
cell wall organization
GO:0022411
cellular component disassembly
GO:0009838
abscission
GO:0044242
cellular lipid catabolic process
GO:0006720
isoprenoid metabolic process
GO:0090304
nucleic acid metabolic process
45/147 | 4551/39150 (1.8e-6)
GO:1901336
lactone biosynthetic process
2/147 | 5/39150 (0.0341)
GO:0042446
hormone biosynthetic process
GO:0009664
plant-type cell wall organization
GO:0042545
cell wall modification
GO:0044277
cell wall disassembly
2/147 | 5/39150 (0.0341)
GO:0008300
isoprenoid catabolic process
3/147 | 5/39150 (0.0341)
GO:0042214
terpene metabolic process
GO:0006721
terpenoid metabolic process
GO:0008299
isoprenoid biosynthetic process
GO:0006259
DNA metabolic process
38/147 | 1467/39150 (1.11e-17)
GO:0009827
plant-type cell wall modification
GO:0016217
terpene catabolic process
2/147 | 5/39150 (0.0341)
GO:0016119
carotene metabolic process
GO:0006714
sesquiterpenoid metabolic process
GO:0016114
terpenoid biosynthetic process
6/147 | 259/39150 (0.0977)
GO:0015074
DNA integration
13/147 | 416/39150 (1.5e-5)
GO:0009830
cell wall modification involved in abscission
2/147 | 5/39150 (0.0341)
GO:1901600
strigolactone metabolic process
2/147 | 5/39150 (0.0341)
GO:0016121
carotene catabolic process
2/147 | 5/39150 (0.0341)
GO:0016106
sesquiterpenoid biosynthetic process
GO:1901601
strigolactone biosynthetic process
2/147 | 5/39150 (0.0341)



DEG involved in Cell wall disassembly are enriched.

The interaction (*O. cumana?*) seems to target cell wall degradation gene to enable *O. cumana* to connect to the vascular system of the host

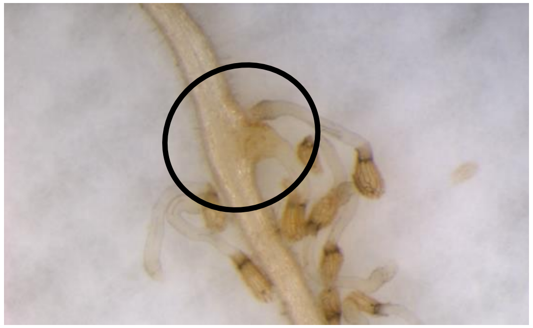
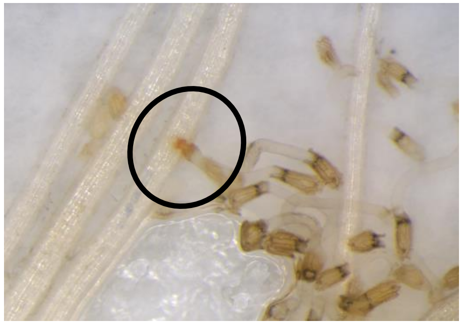
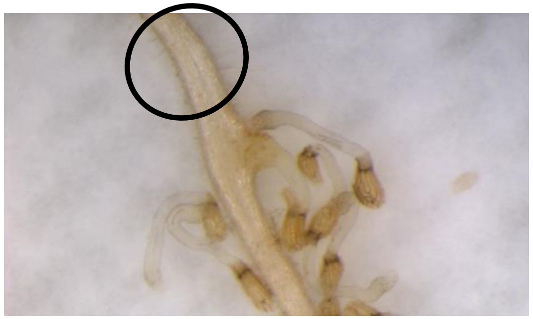
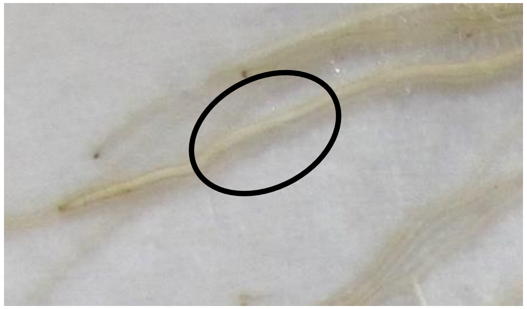


Connection to the vascular system of the host

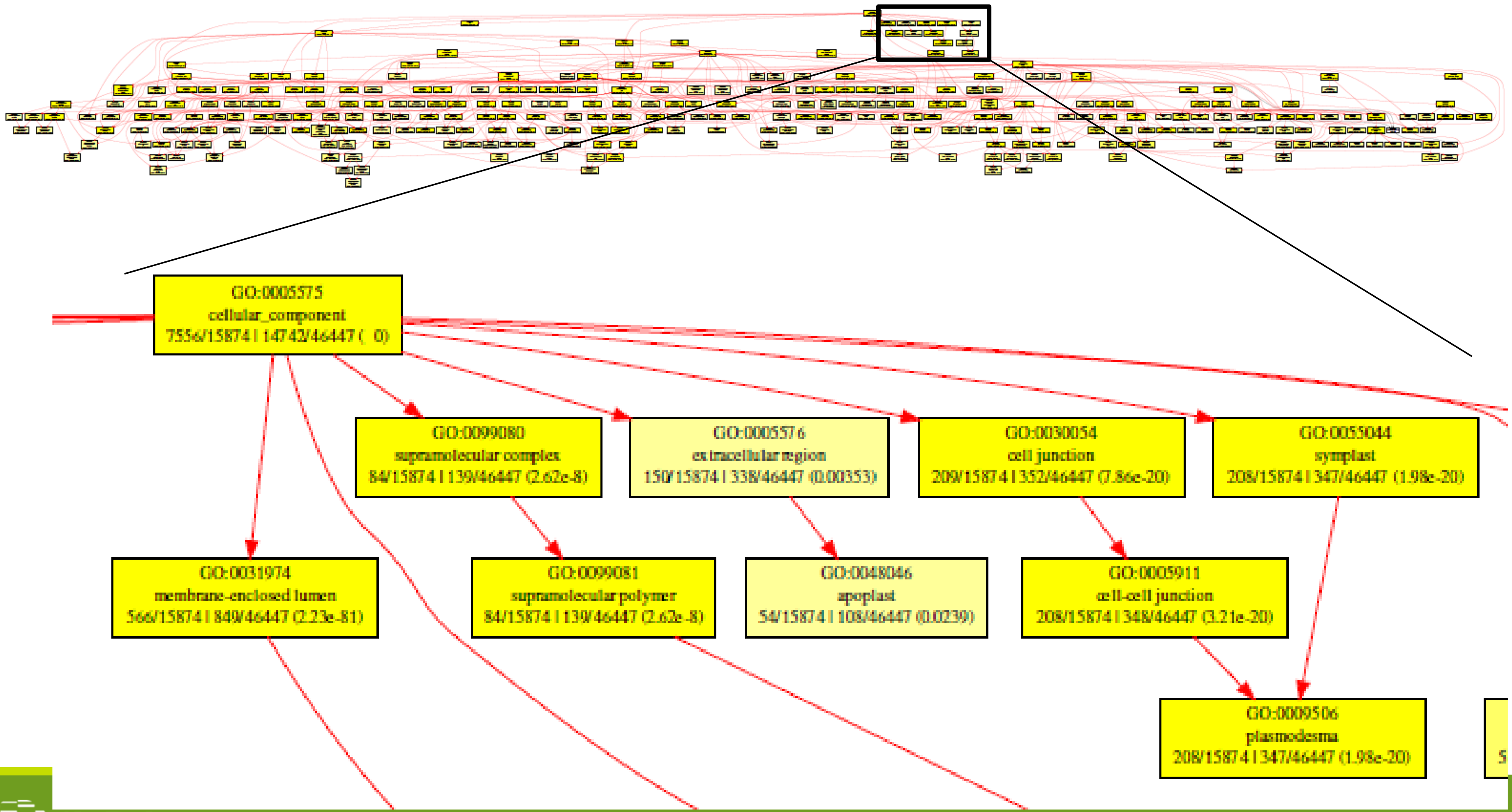
O. cumana DEG

IA vs CA LR1: 15874

IA vs CA Other Resistant line : 742

Compatible attachment (CA)	Incompatible attachment (IA)
	
	

O. cumana DEG


GO:0005575
cellular_component
7556/15874 | 14742/46447 (0)
GO:0099080
supramolecular complex
84/15874 | 139/46447 (2.62e-8)
GO:0005576
extracellular region
150/15874 | 338/46447 (0.00353)
GO:0030054
cell junction
209/15874 | 352/46447 (7.86e-20)
GO:0055044
symplast
208/15874 | 347/46447 (1.98e-20)
GO:0031974
membrane-enclosed lumen
566/15874 | 849/46447 (2.23e-81)
GO:0099081
supramolecular polymer
84/15874 | 139/46447 (2.62e-8)
GO:0048046
apoplast
54/15874 | 108/46447 (0.0239)
GO:0005911
cell-cell junction
208/15874 | 348/46447 (3.21e-20)
GO:0009506
plasmodesma
208/15874 | 347/46447 (1.98e-20)
5

Summary

A high quality genome sequence of *O. cumana* produced

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Usefull for functional and genetic analysis

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L'engagement durable

Terres Inovia
l'agronomie en mouvement

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SCIENCE & IMPACT

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POUR LA PROMOTION
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DES PLANTES OLÉAGINEUSES

biogemma

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