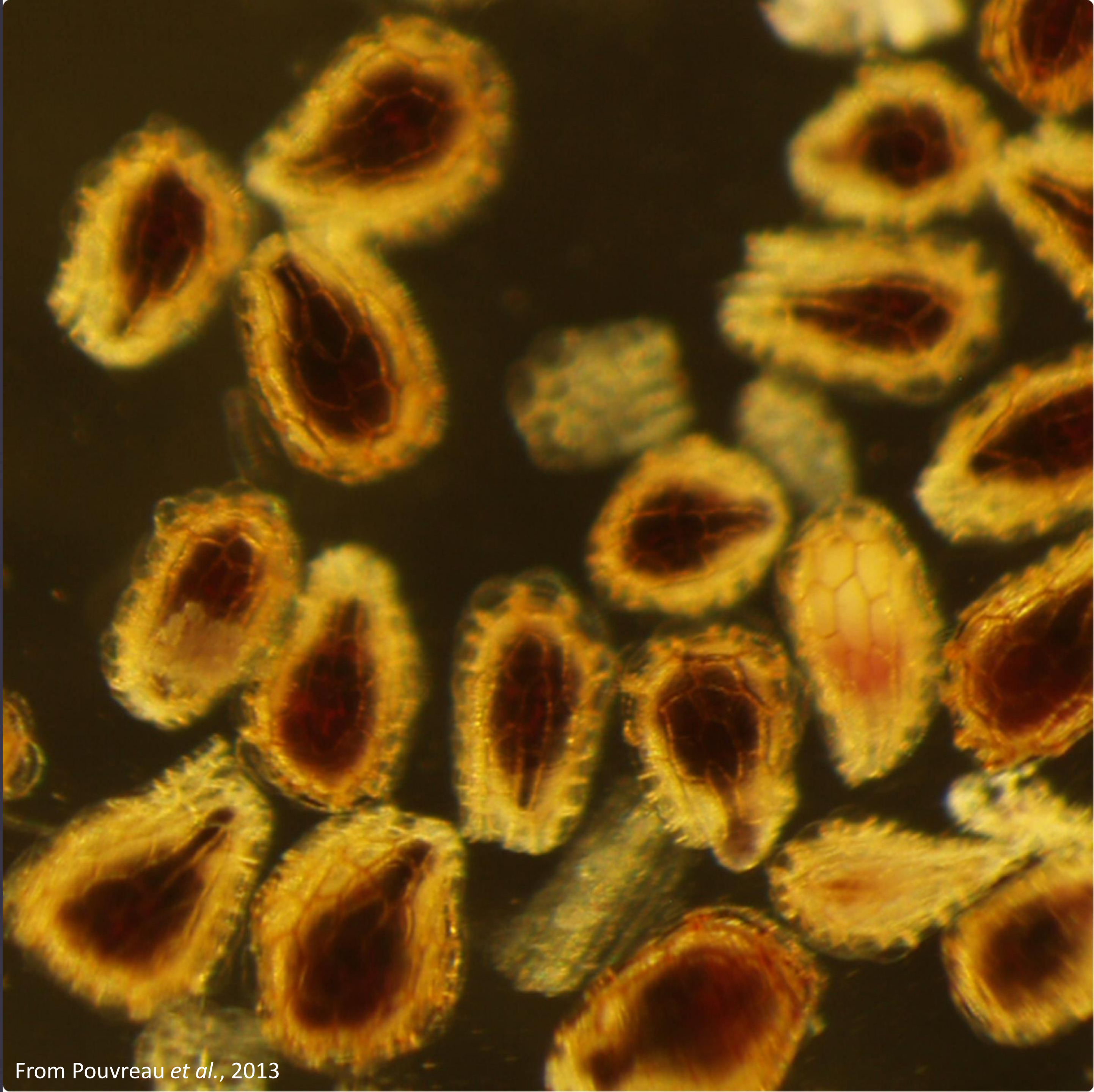

From Pouvreau *et al.*, 2013

An overview of assorted *Phelipanche ramosa* seeds:

Genotype, Phenotype & Microbiota Profiling

Sarah HUET
M2 BV GSP
14th June 2019

Supervised by:
Lucie POULIN
Jean-Bernard POUVREAU

Microbiota

Microscopy of a human gut microbiota. Photo : Martin Oeggerli

Plant & microbiota

Biomass accumulation

- Sugiyama *et al.*, 2012

Flowering time

- Panke-Buisse *et al.*, 2015

Drought tolerance

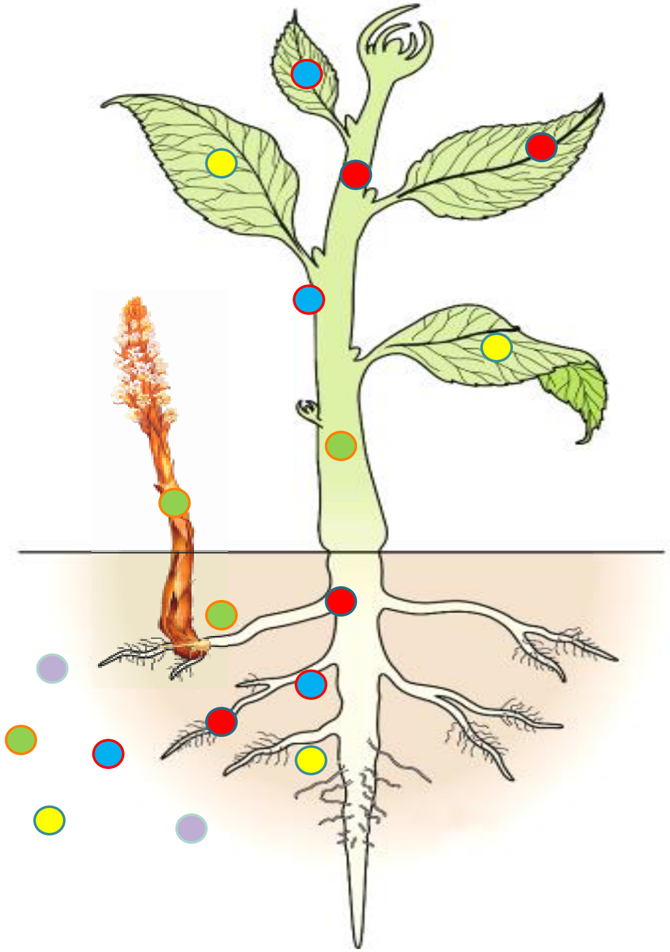
- Lau & Lennon, 2012

Resistance against pathogens

- Mendes *et al.*, 2011
- Santhanam *et al.*, 2015
- Busby *et al.*, 2016

Plant Growth Promoting Rhizobacteria & PGP Fungi

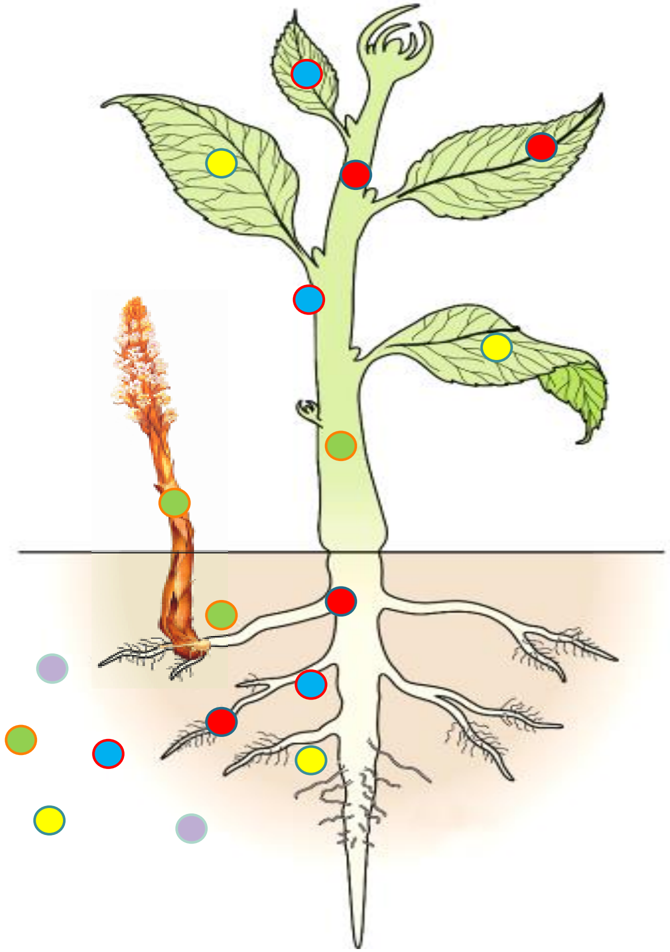
- Spaepen *et al.*, 2009
- Trillas & Segarra, 2009
- De Vleesschauwer & Höfte, 2009



Plant & microbiota & parasitic plant

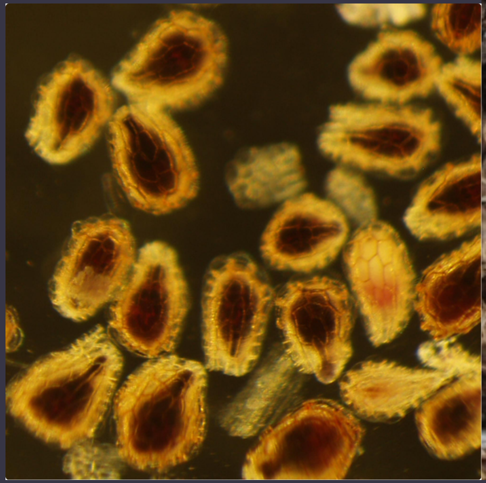
Phelipanche ramosa

Phelipanche ramosa. Credits: MOrDOr



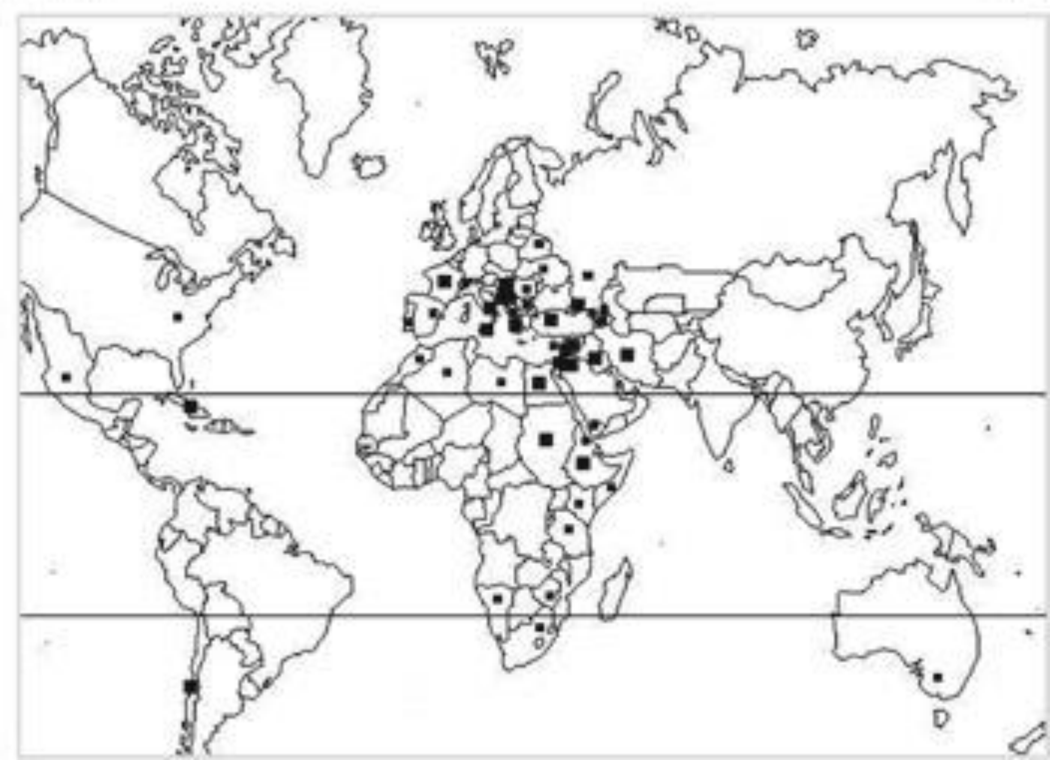
Phelipanche ramosa

- Epirhize holoparisite (achlorophytic root parasite)
- Wide host range
  - Hemp, oilseed rape, tobacco, tomato, cabbage …
- Yield losses from 30 to 100% on oilseed rape
- Seeds
  - 10^4 à 10^5 / floral scape
  - Diameter < 200 µm
  - Viability > 10 years in soil



Phelipanche ramosa. Credits: MOrDOr

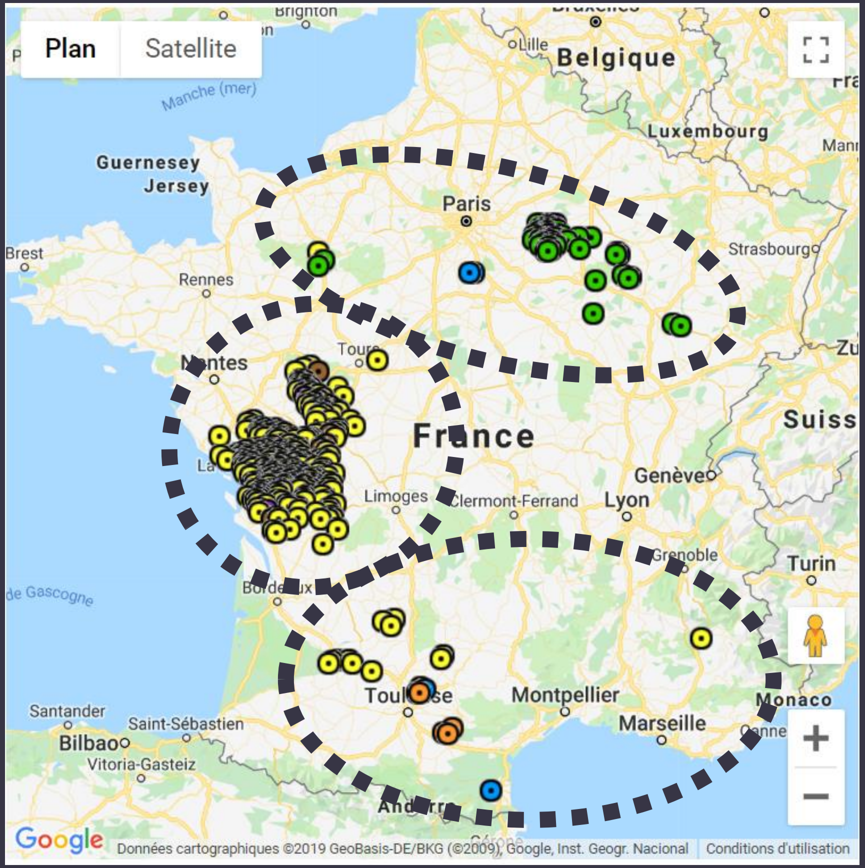
Phelipanche ramosa



Phelipanche ramosa world distribution. From Parker, 2013

Phelipanche ramosa. Credits: MOrDOr

Phelipanche ramosa



Repartition of *Phelipanche ramosa* parasite in different host fields in France. Yellow: oilseed rape, green: hemp, brown: tobacco (not up--to--date), orange: sunflower, purple: several crops, blue: others. (From Terres Inovia)

From Stojanova *et al.*, 2019

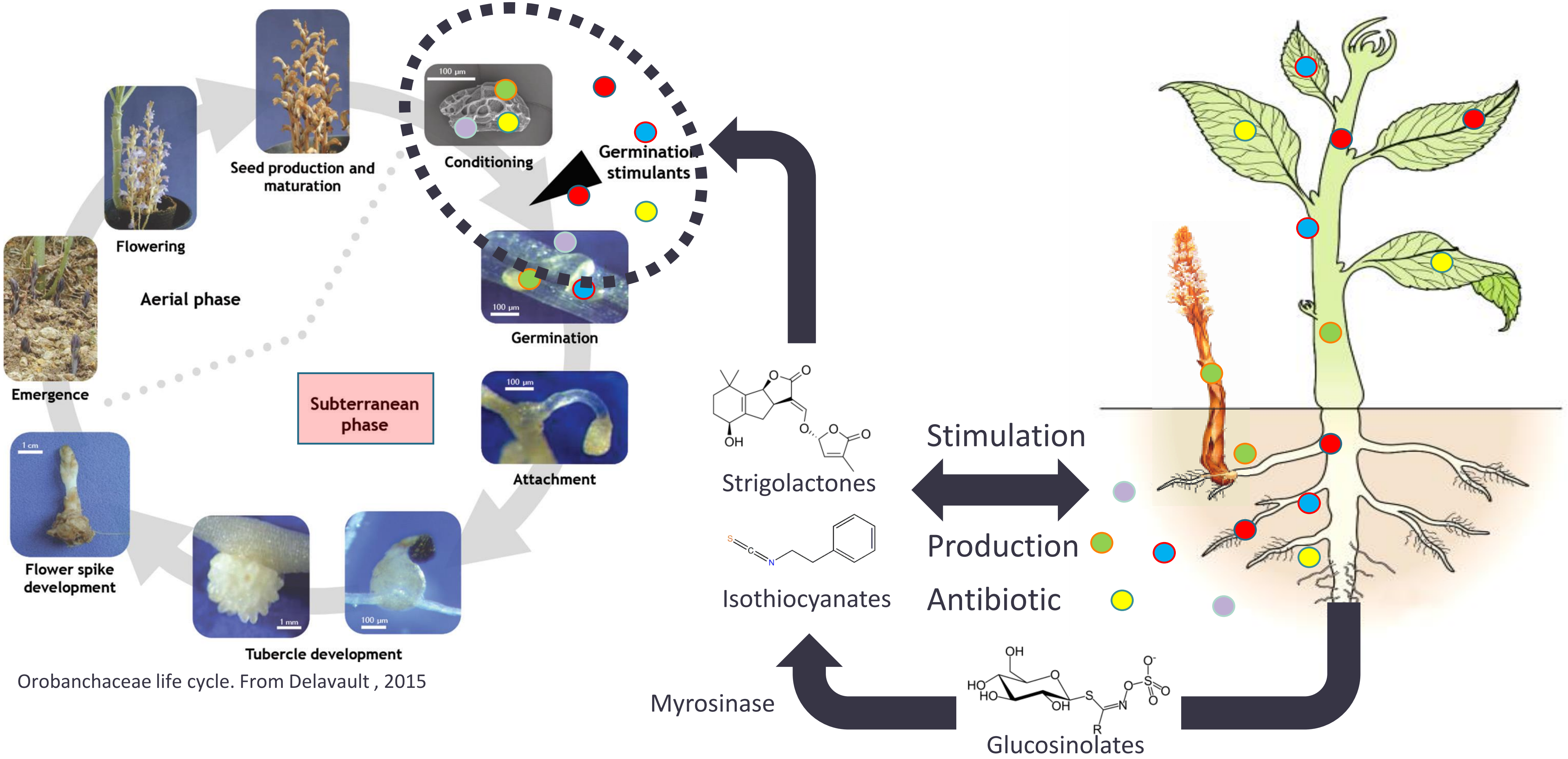
Genotype	Preferential host	Area
G 1	Oilseed rape	Western France
G 2a	Hemp	Eastern France
G 2b	Tobacco	Southern France

Hemp

Oilseed rape

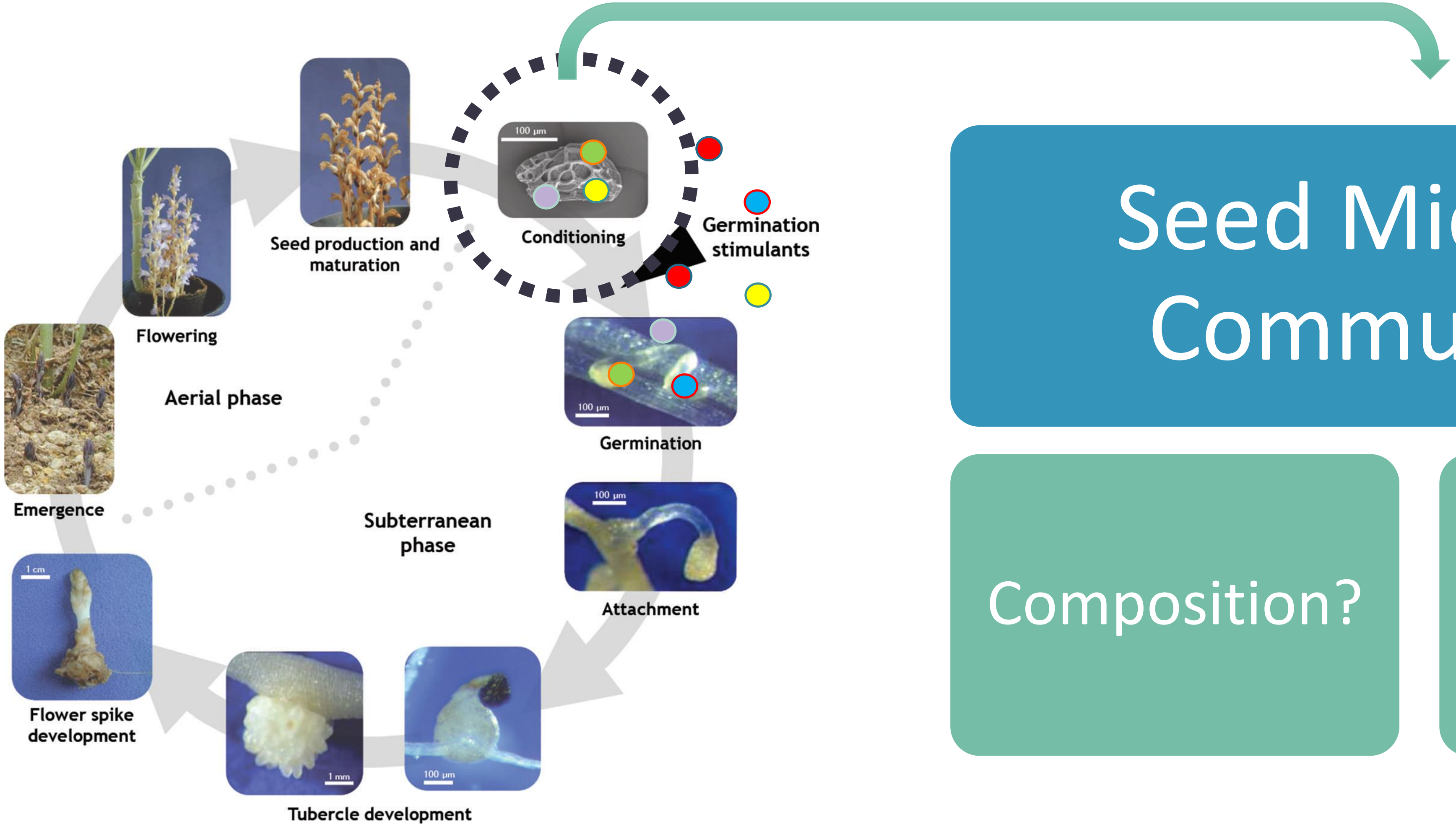
Host specialization

Plant & microbiota & parasitic plant



Orobanchaceae life cycle. From Delavault , 2015

Plant & microbiota & parasitic plant



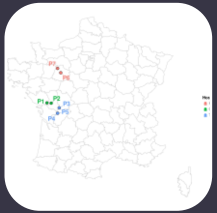
Orobanchaceae life cycle. From Delavault , 2015

Seed Microbial Communities

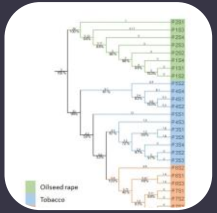
Composition?

Influencing factors?

MOrdOr Project

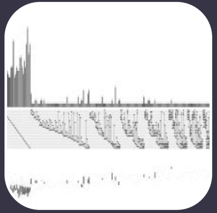


Sampling campaign



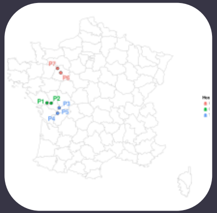
Genotype

Phenotype



Microbiota

MOrdOr Project



Sampling campaign

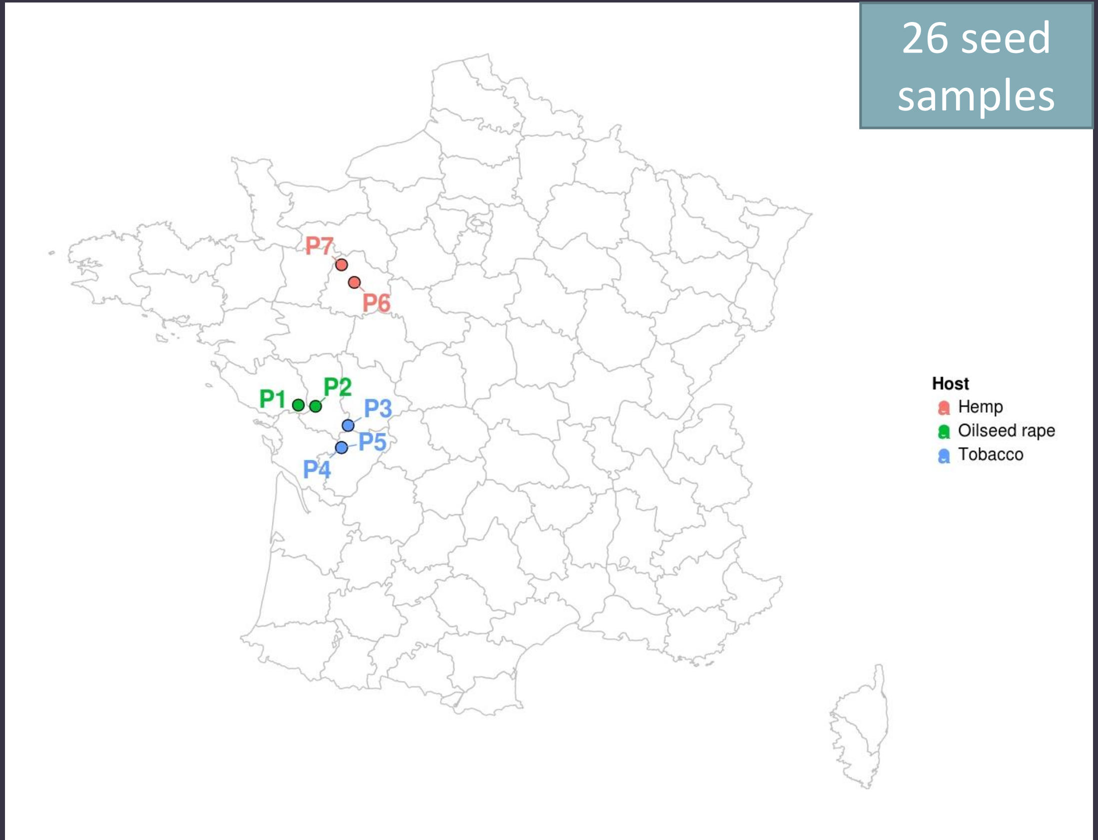
Genotype

Phenotype

Microbiota

Sampling campaign

26 seed samples

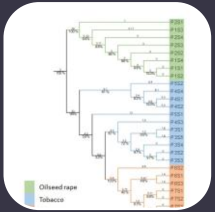


Infested plot by *P. ramosa* where samples were collected

Phelipanche ramosa in a tobacco field. Credits: MOrDOr

MOrdOr Project

Sampling campaign

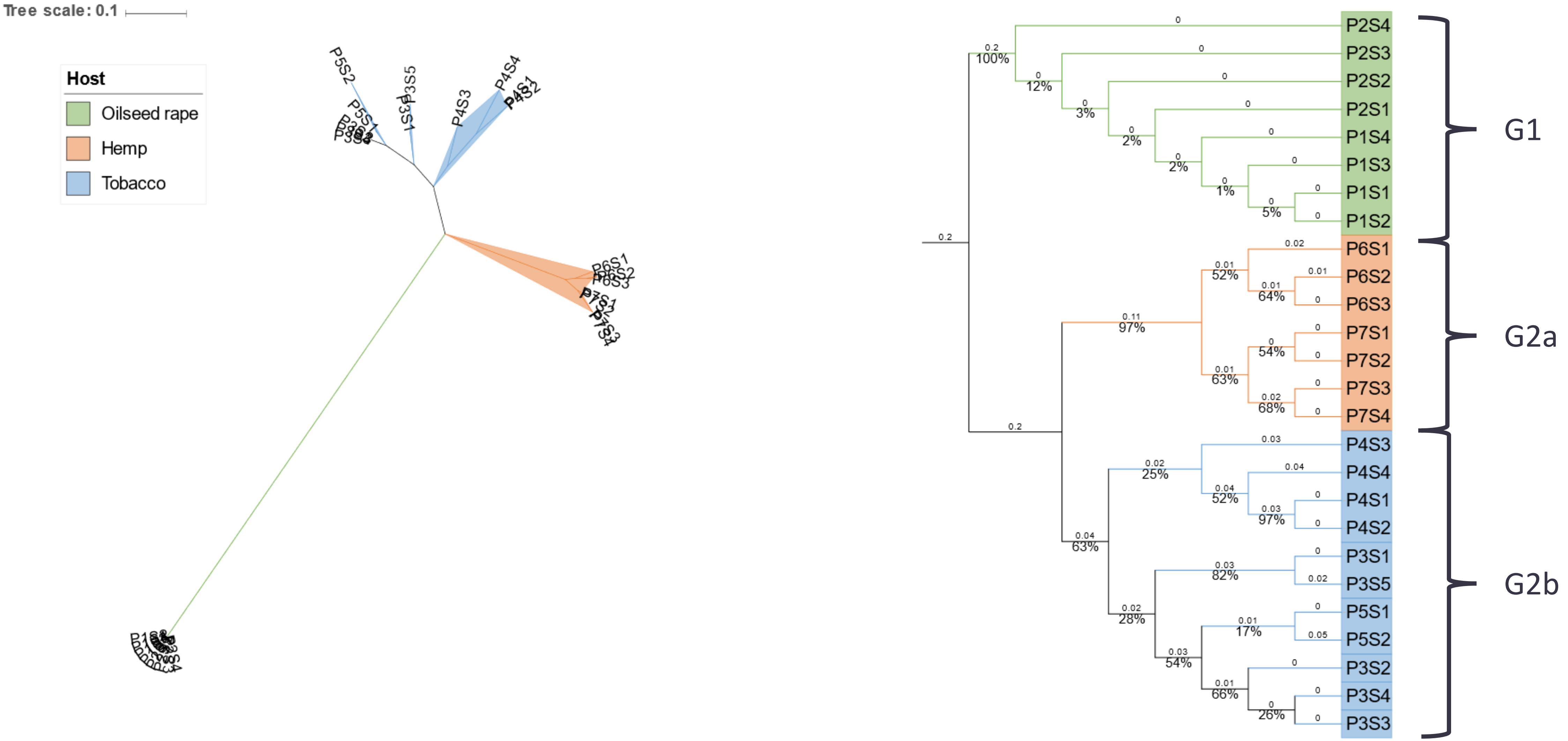


Genotype

Phenotype

Microbiota

Genotyping



Bruvo’s distance between 20 microsatellites and built by Neighbor Joining (999 bootstraps)

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Sampling campaign

Genotype

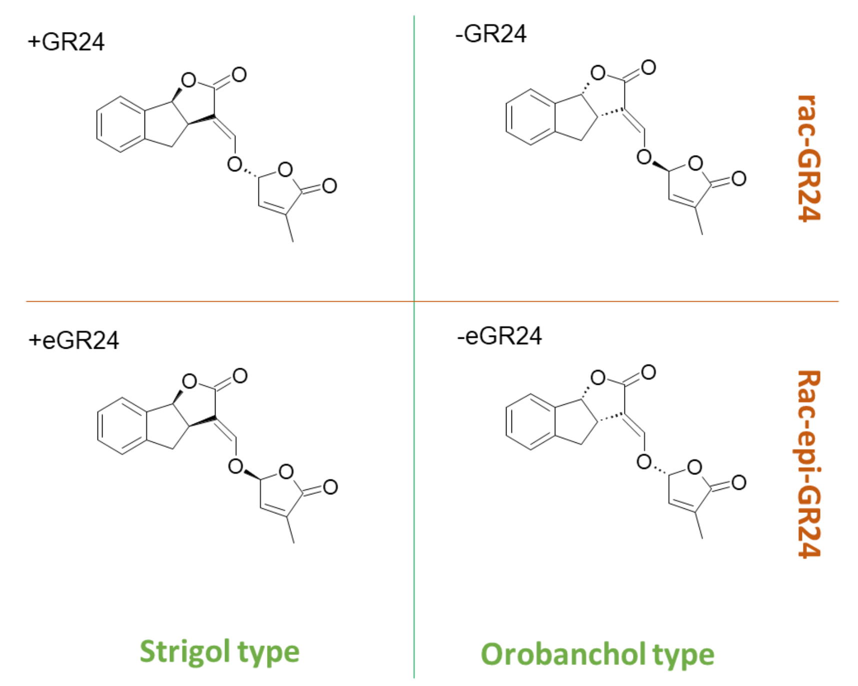
Phenotype

Microbiota

Germination assay

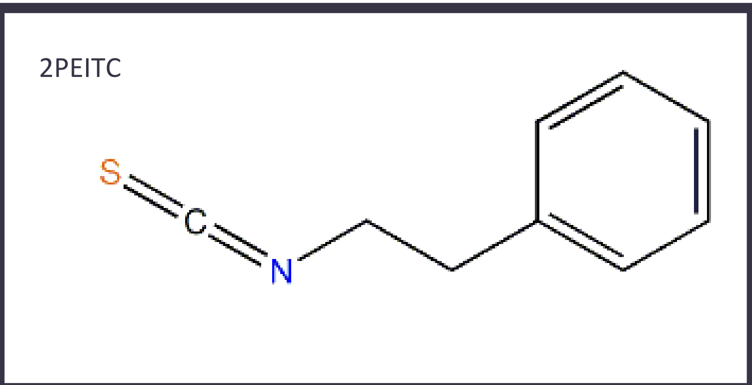
Strigolactones

GR24
(4 enantiomers)



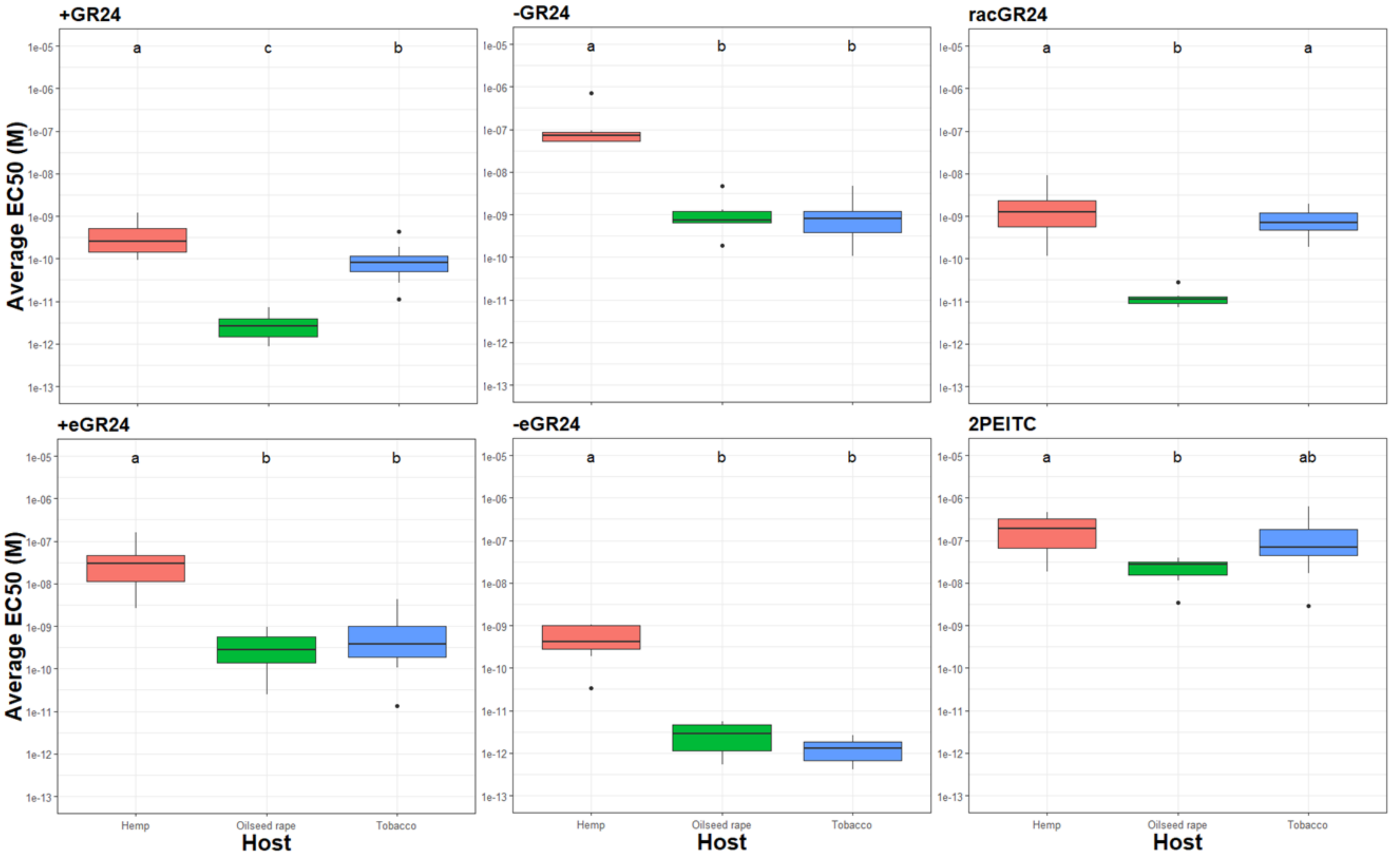
Isothiocyanate

2PEITC



EC50
=
Seed
sensitivity

Seed sensitivity to GS



🟥 Hemp
🟩 Oilseed rape
🟦 Tobacco

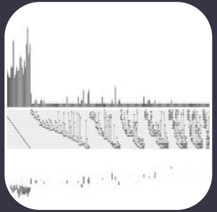
Average EC50 according to the originating host (color)

MOrdOr Project

Sampling campaign

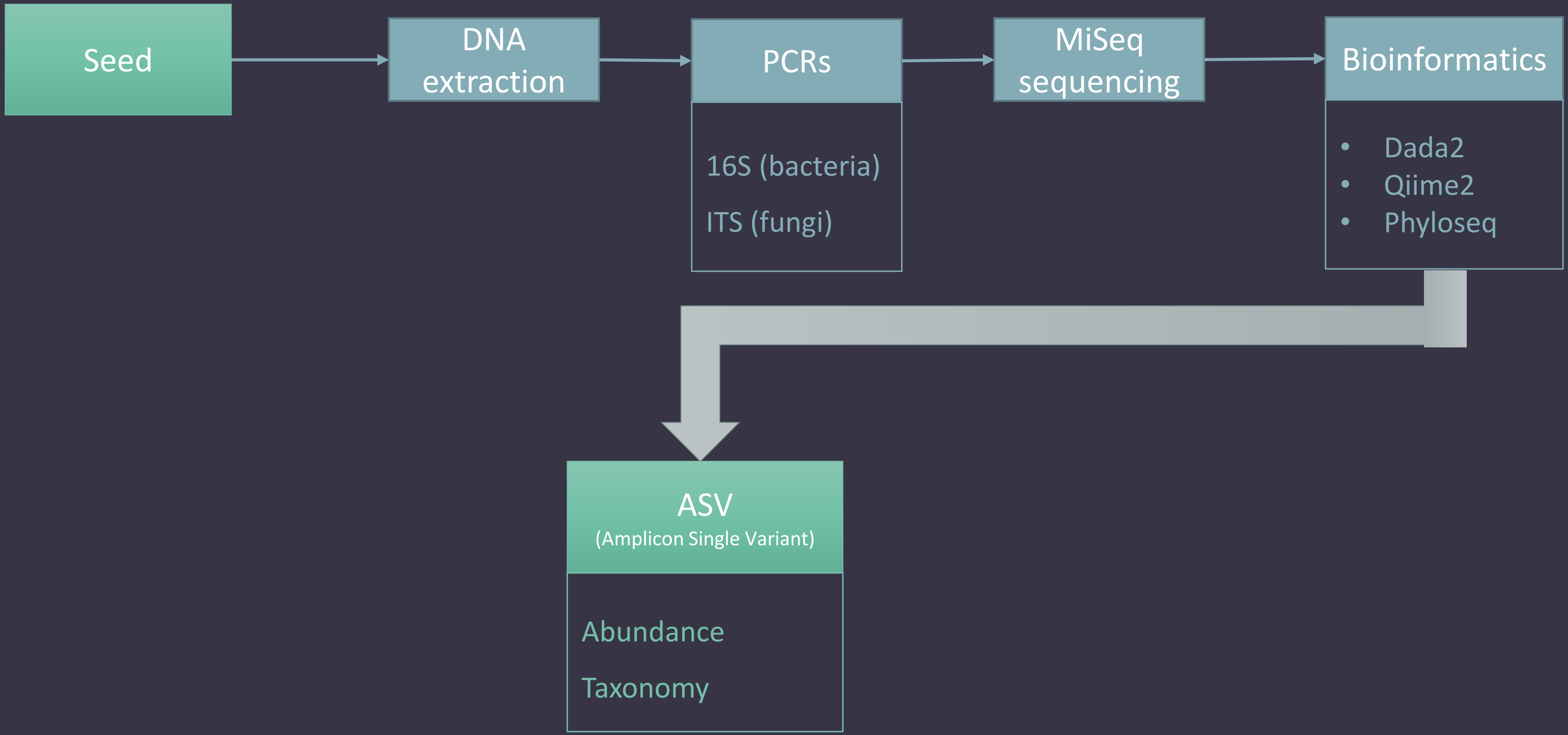
Genotype

Phenotype



Microbiota

Microbiota profiling



Seed

DNA extraction

PCRs

16S (bacteria)

ITS (fungi)

MiSeq sequencing

Bioinformatics

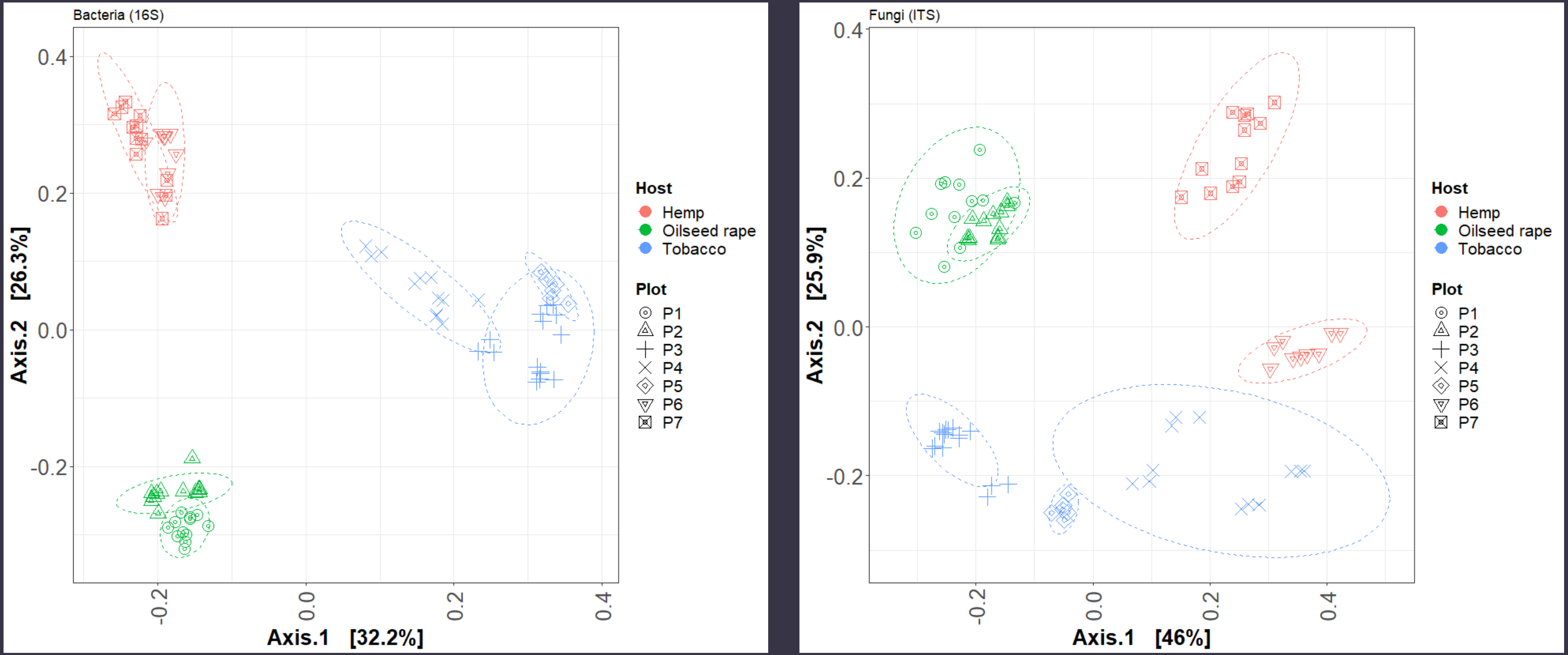
- Dada2
- Qiime2
- Phyloseq

ASV
(Amplicon Single Variant)

Abundance

Taxonomy

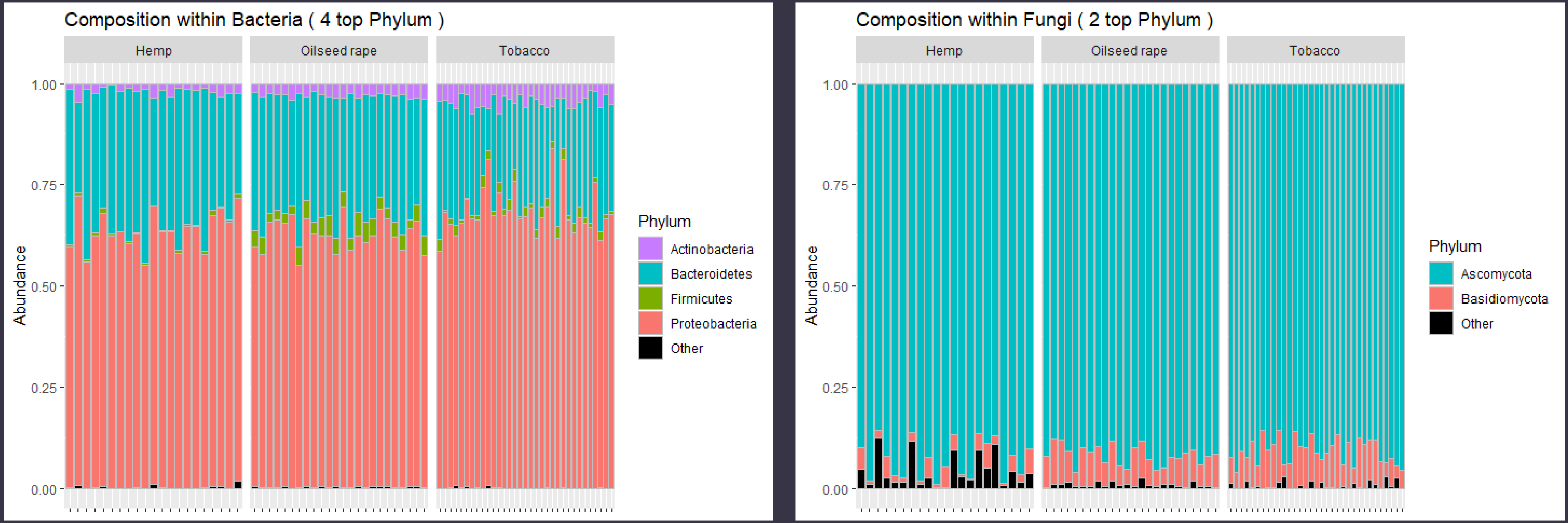
Seed β-diversity



Seed samples diversity based on Bray-Curtis distances and depending on the sample originating host (color) and field (shape)

Taxonomic composition - Phylum

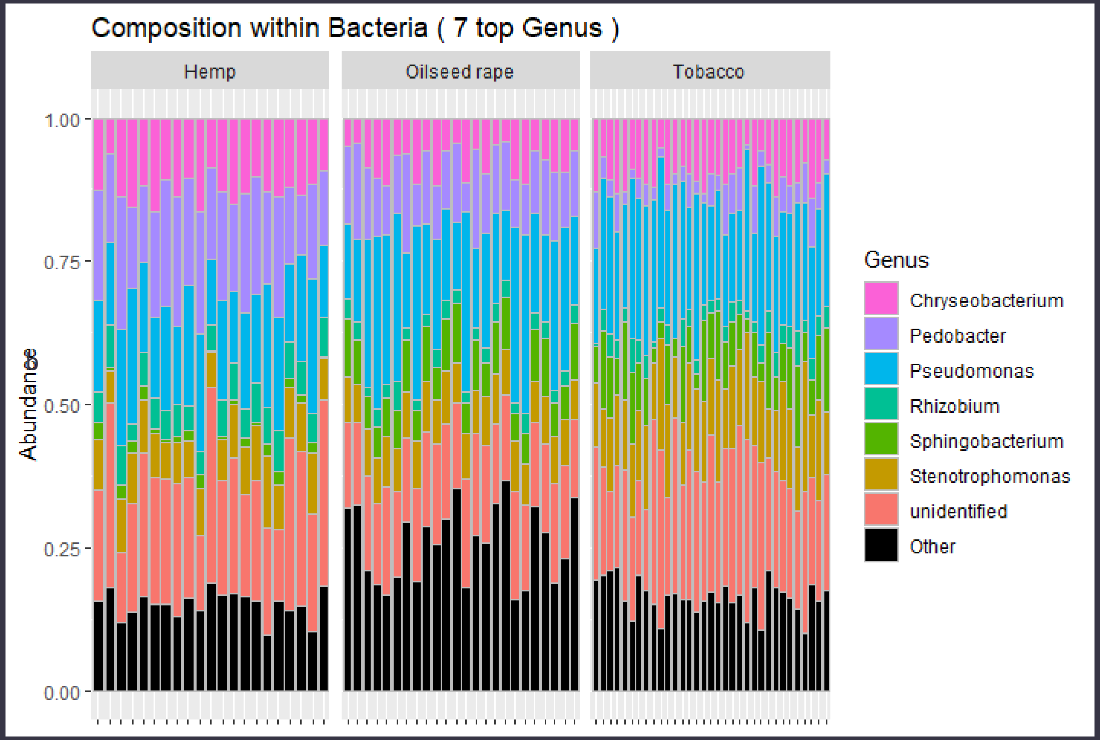
	Bacteria (16S)	Fungi (ITS)
Main phyla	4	2
Total ASV	90%	84%
Total abundance	99%	98%

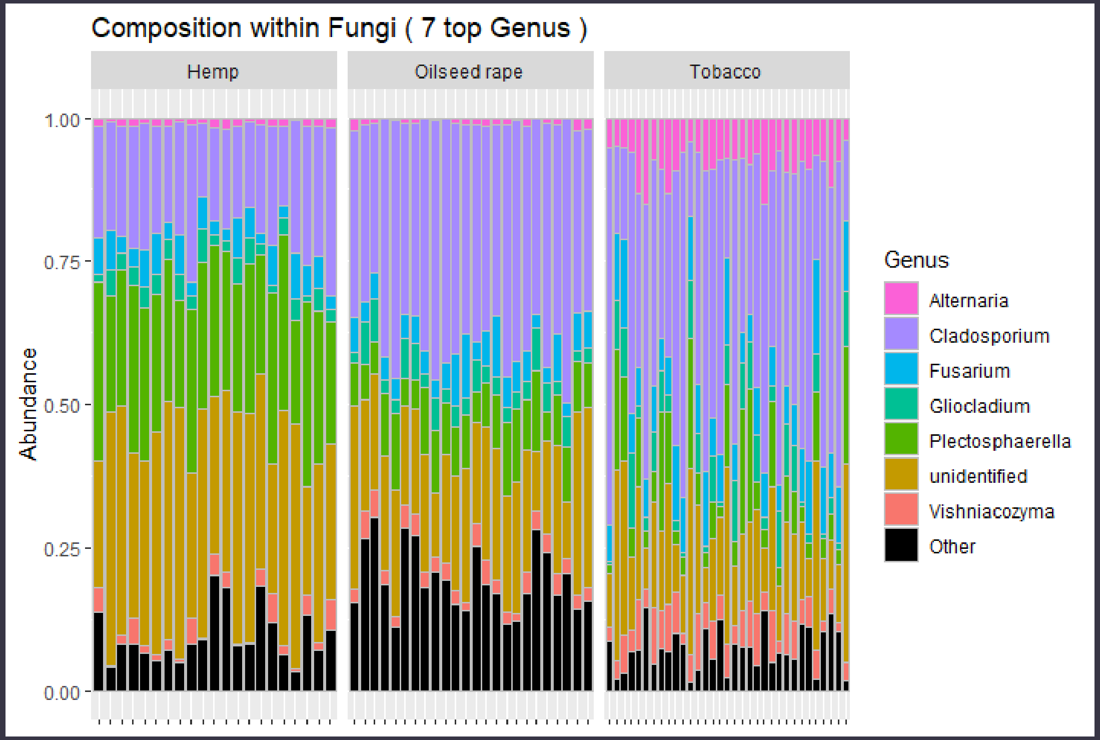


Taxonomic composition of the bacterial (left) and fungal (right) seed microbiota regarding the most abundant phyla

Taxonomic composition - Genus

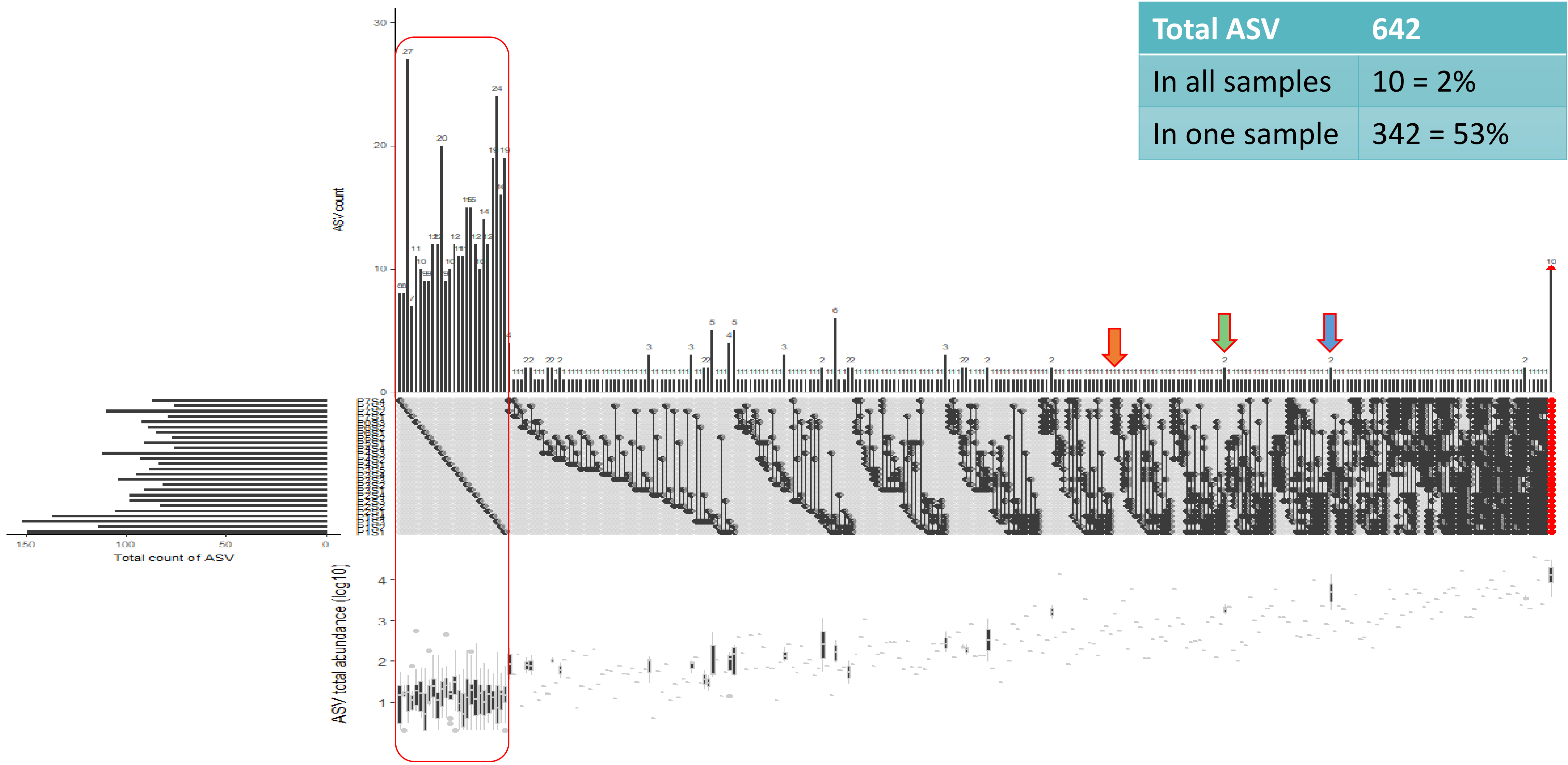
	Bacteria (16S)	Fungi (ITS)
Main genera	7	6
Total ASV	23%	11%
Total abundance	63%	64%





Taxonomic composition of the bacterial (up) and fungal (down) seed microbiota regarding the most abundant genera

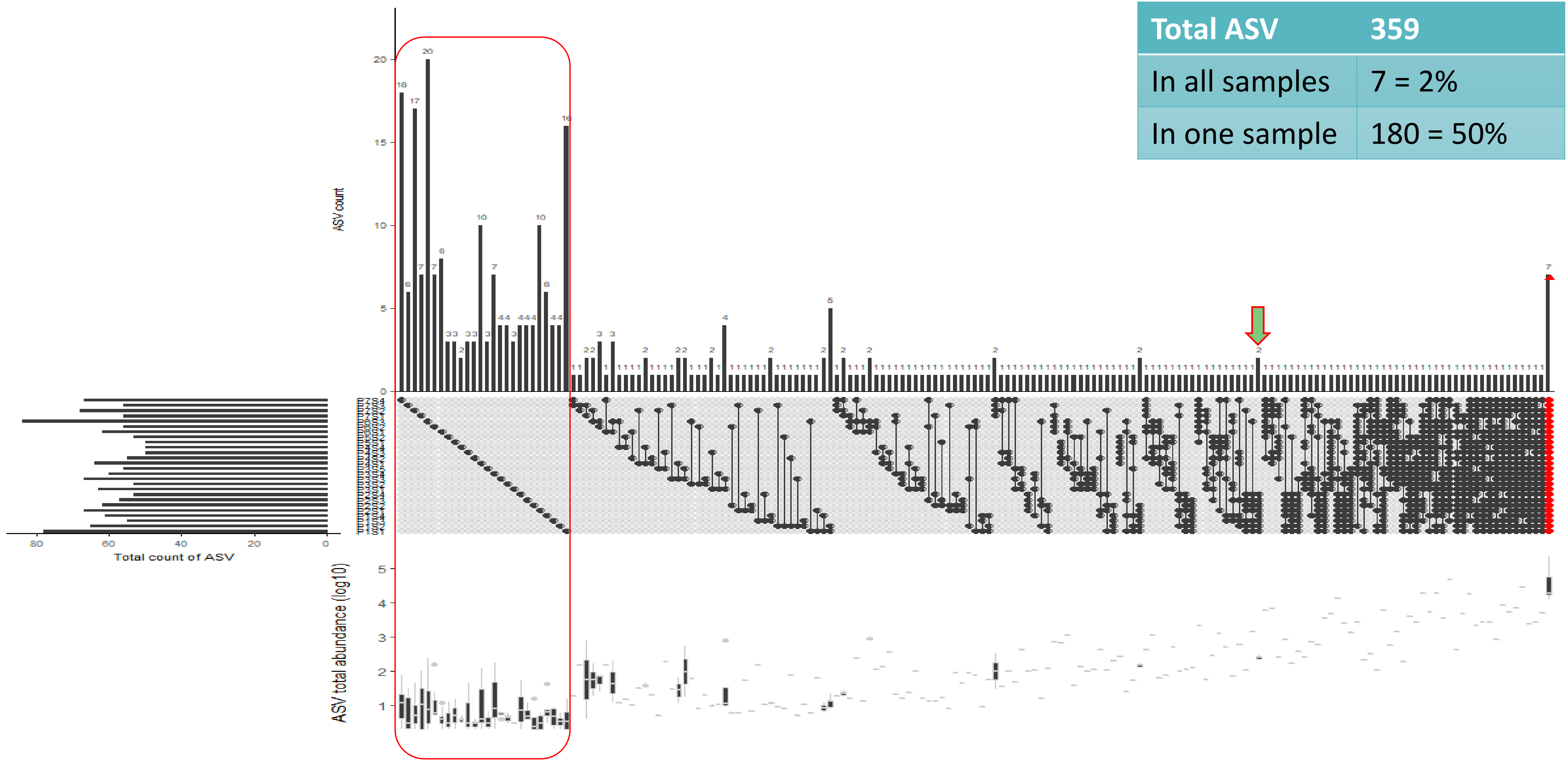
Taxonomic composition – Bacterial ASV (16S)



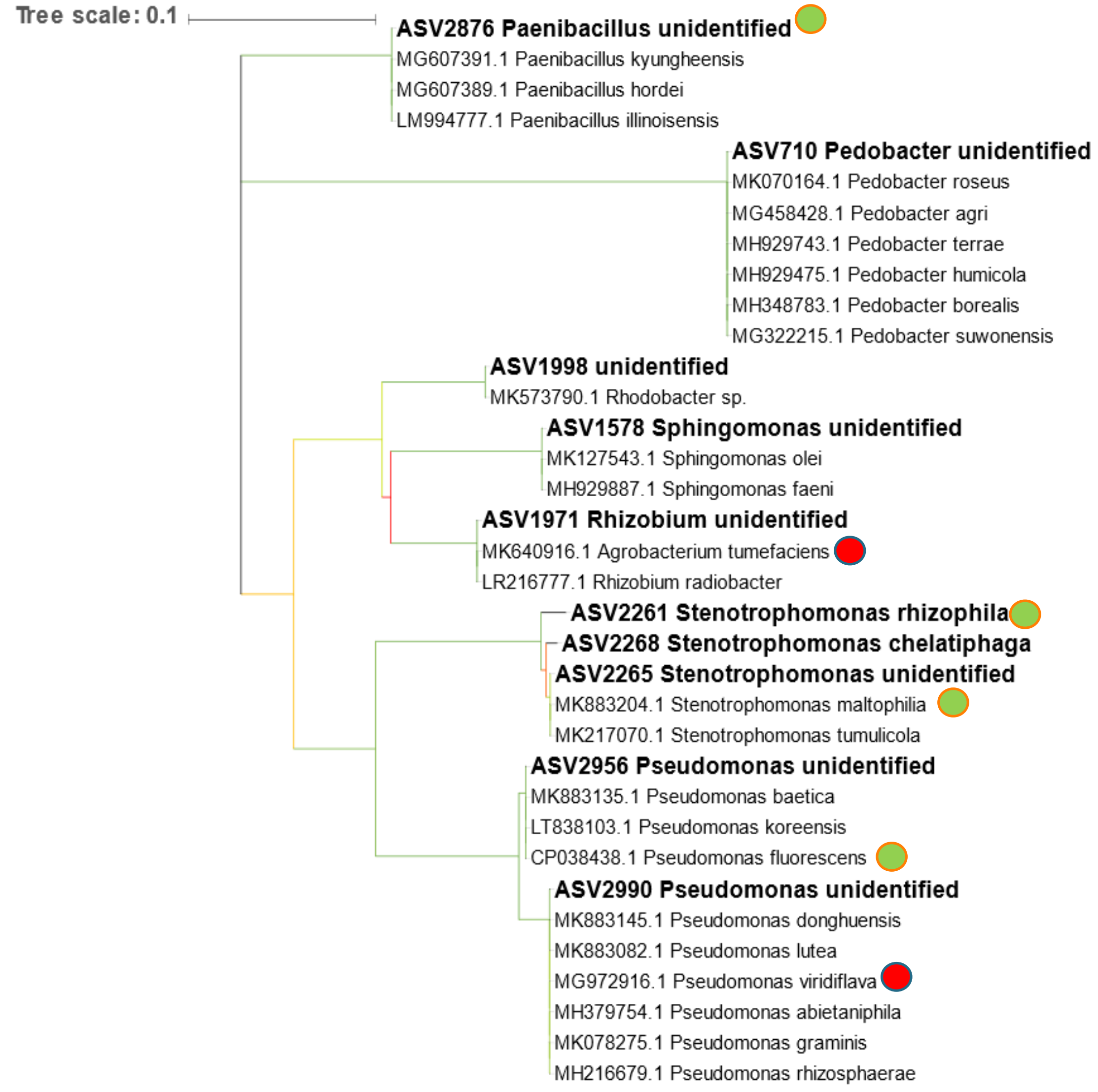
Total ASV	**642**
In all samples	10 = 2%
In one sample	342 = 53%

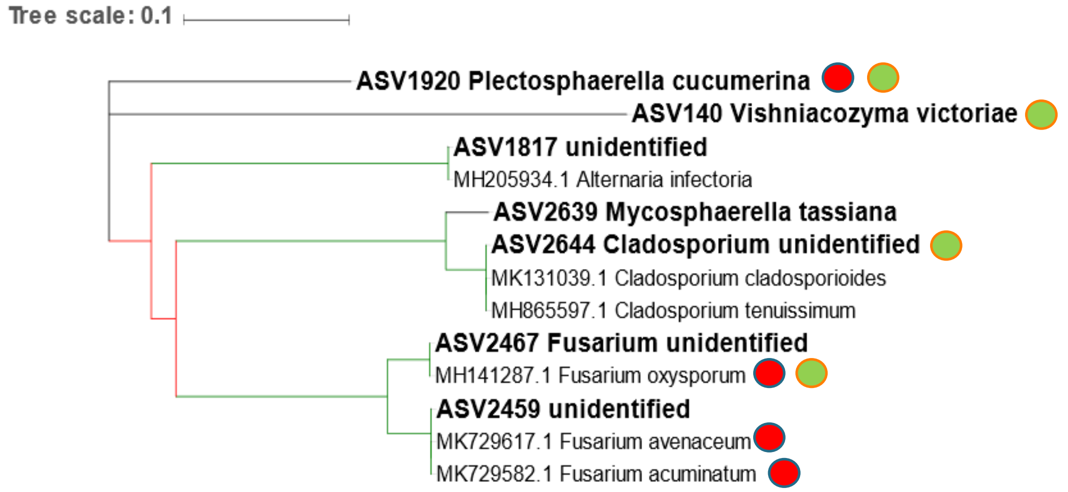
Taxonomic composition – Fungal ASV (ITS)

Total ASV	359
In all samples	7 = 2%
In one sample	180 = 50%



Core microbiome





	Bacteria (16S)	Fungi (ITS)
Core ASV	10	7
Total ASV	2%	2%
Total abundance	26%	60%

Neighbor Joining Trees based sequence alignment (Muscle) and bootstrapped with 100 iterations for bacterial (left) and fungal (right) core microbiomes. Branch colors refer to bootstrap values (ranged from 0.3 in red to 1 in green)

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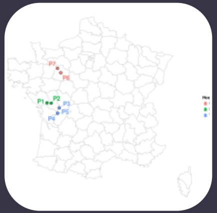
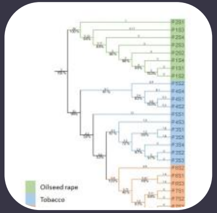
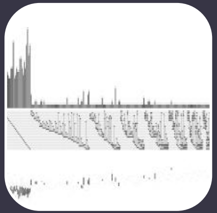


Sampling campaign



Genotype

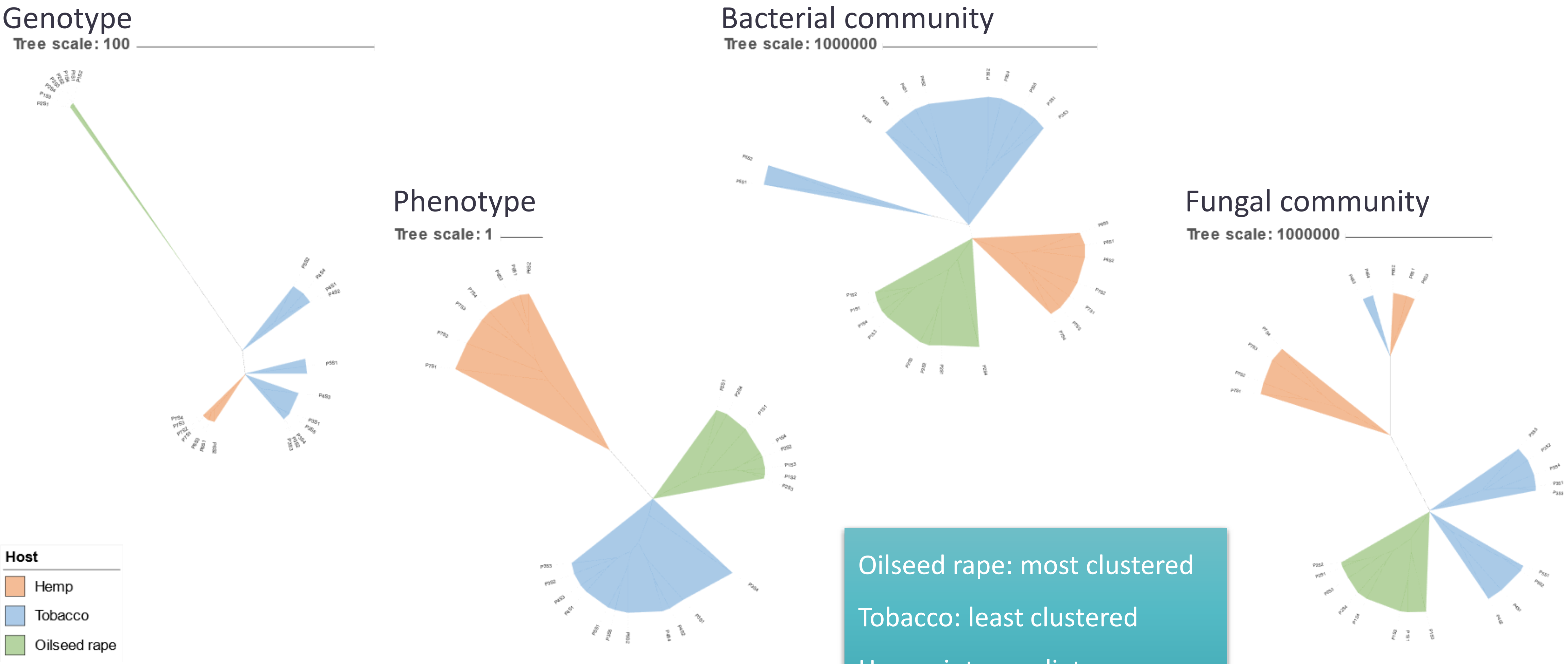
Phenotype



Microbiota

Comparison

Dendrogram comparison



Oilseed rape: most clustered

Tobacco: least clustered

Hemp: intermediate

UPGMA trees based on Euclidean distance and bootstrapped with 100 iterations

Conclusion

Seed microbial community

Composition

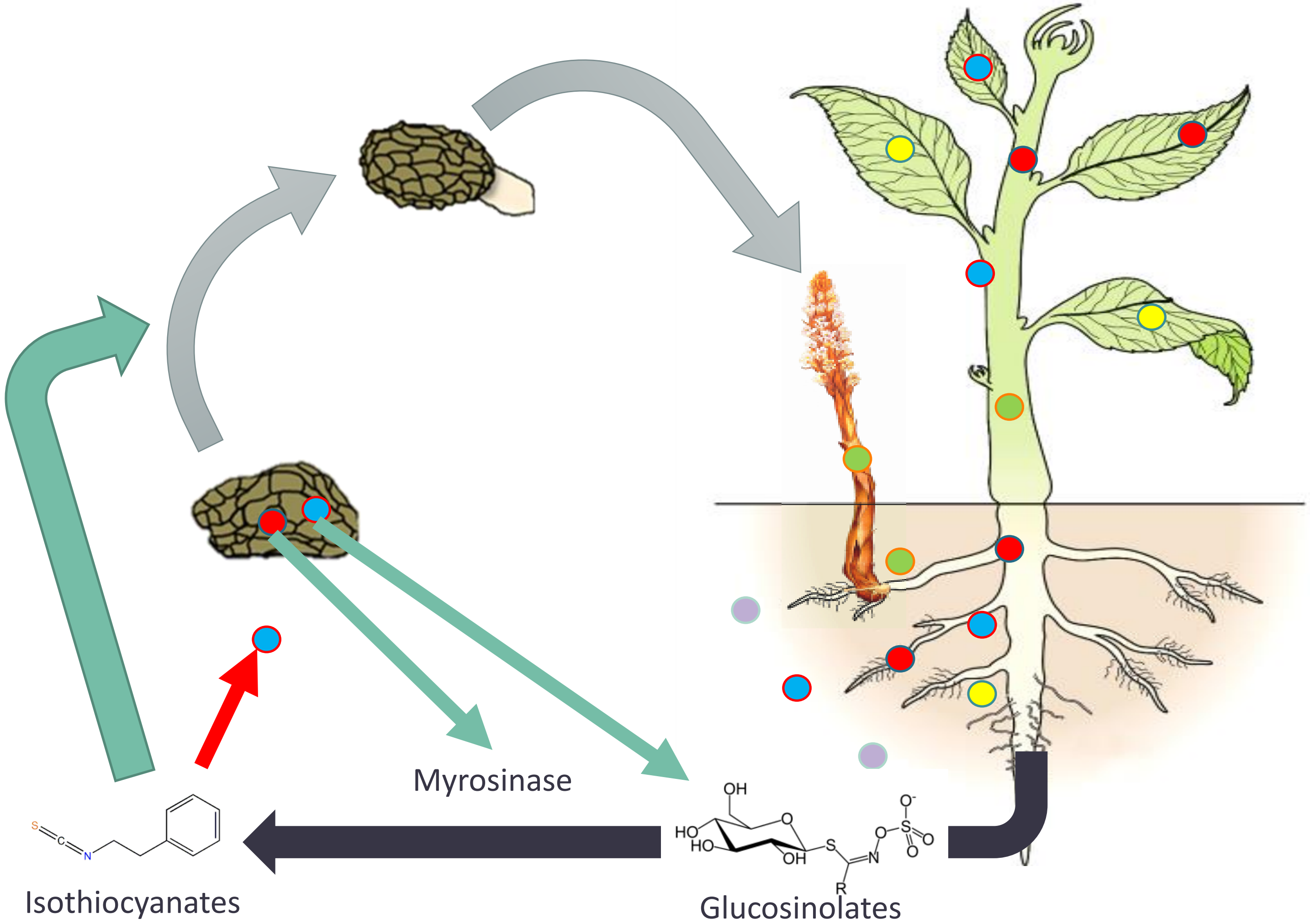
- Overview
  - 4 bacterial phyla
  - 2 fungal phyla
  - 7 bacterial genera
  - 6 fungal genera
- Core microbiota
  - 10 bacterial ASV
  - 7 fungal ASV

Influencing factors

- Originating host
  - + Bacteria
  - - Fungi
- Originating field
  - - Bacteria
  - + Fungi
- Link with Genotype & Phenotype
  - Similar clustering
  - Oilseed rape specialization

A tripartite dialogue

- Oilseed rape → glucosinolates
- *P. ramosa* G1 → oilseed rape
- Seed lots from Oilseed rape
  - More sensitive to 2PEITC
  - 4 specific ASV (2 bacterial & 2 fungal)
    - 🔴 Sphingobacterium
      - Myrosinase activity (Meulenbeld & Hartmans *et al.*, 2001)
    - 🔵 Leptosphaeria maculans
      - Sensitive to ITC (Andreasson *et al.*, 2001)
      - Induce glucosinolate production in *Brassica* sp. (Robin *et al.*, 2017)



Thanks to

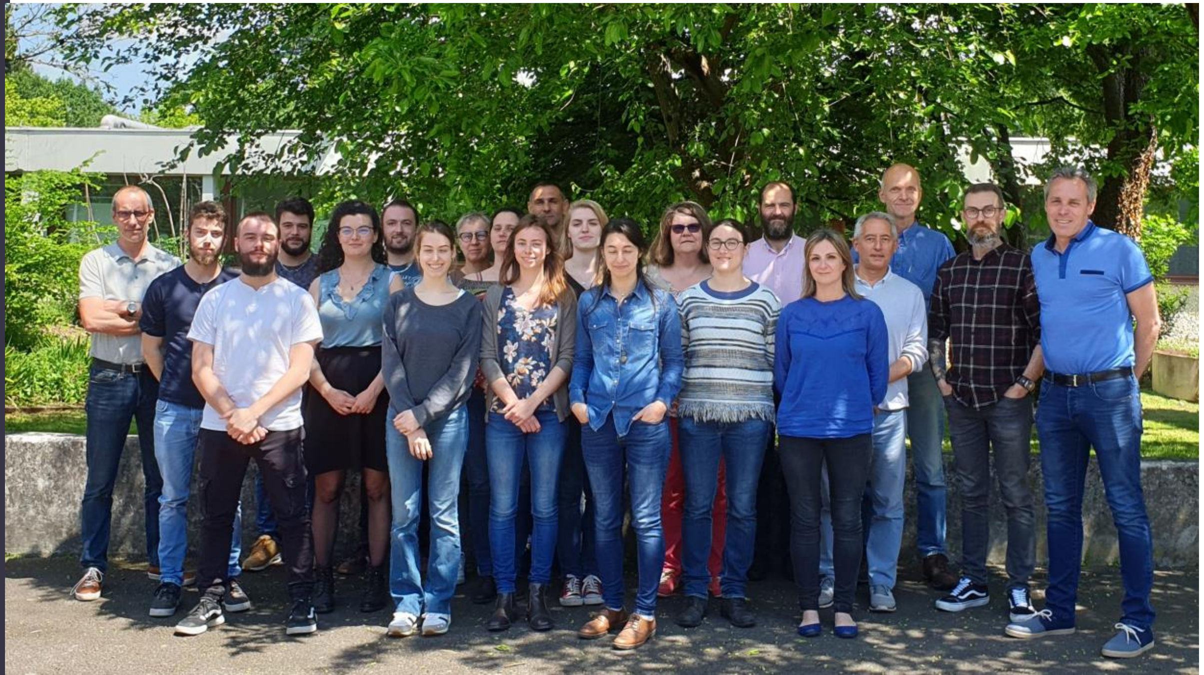
Lucie Poulin
Jean-Bernard Pouvreau

Sabine Delgrange

Alexandre Degraves

Coralie Marais
Muriel Bahut

Erwan Delage



Thanks for your attention !

Sources

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PICTURES

- https://news.nationalgeographic.com/news/2014/07/140724-gut-microbes-bacteria-bugs-science-health-food-weird/
- https://www.science-et-vie.com/archives/code-du-vivant-l-adn-a-en-fait-6-bases-32072

Development

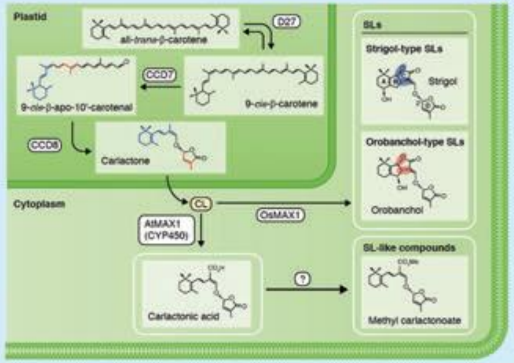
Strigolactone biosynthesis and signaling in plant development

Mauricio Lopez-Obando, Yasmine Ligerot, Sandrine Bonhomme, François-Didier Boyer and Catherine Rameau

The Company of Biologists

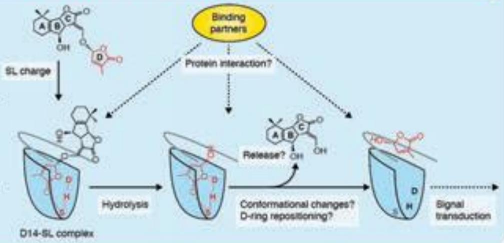
SL biosynthesis

The biosynthesis of strigolactones (SLs) and SL-like compounds involves an isomerase (D27) and two dioxygenases (CCD7 and CCD8) that successively convert all-trans-β-carotene into carlactone (CL), a key intermediate in SL biosynthesis; these enzymes are localized in the plastid. CL is then exported into the cytosol where it is converted into SLs and SL-like compounds by MAX1 and likely other as yet unidentified enzymes. To date, at least 20 naturally occurring SLs and five SL-like compounds have been characterized. These compounds may be transported inside the plant and in the rhizosphere. Only one transporter (PhPDR1) of the ABC family has yet been reported.



SL perception: coordinating enzymatic activity and reception

D14, a member of the α/β hydrolase superfamily, has been identified as an SL receptor in vascular plants. D14 can hydrolyze SLs but the importance of this enzymatic reaction is not clear as the hydrolysis products are thought to be inactive. *In vitro* experiments suggest that, while the SL ABC rings are released after hydrolysis, the trapping and repositioning of the D-ring in the receptor may cause changes in D14 affecting subsequent protein-protein interactions and signal transduction.



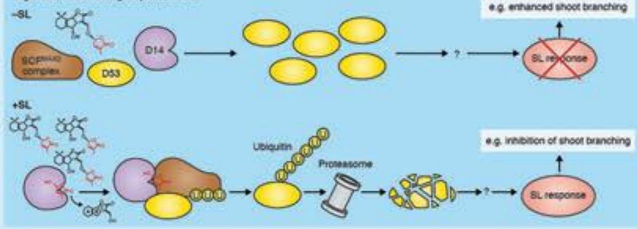
Models of SL signaling

Proteins involved

SL receptor (α/β hydrolase)	F-box protein (part of SCF complex)	Clp protease	Key
D14/AtD14/DAD2/RMS3	D3/MAX2/PhMAX2/RMS4	D53/SMXL	Rice/Arabidopsis/Petunia/Pea

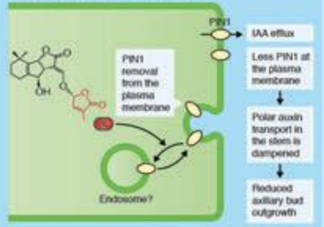
SLs trigger the degradation of proteins via the UPS

The perception and hydrolysis of SLs by D14 lead to the ubiquitylation-dependent degradation of D53 by a SCF^{MAX2} complex. D53 acts as a suppressor of SL signaling in the control of shoot branching and is proposed to interact with transcriptional co-repressors. These findings have led to a model in which low levels of SL allow D53 proteins to accumulate and repress the activity of putative target TFs by unknown mechanisms, thereby inhibiting the SL response. By contrast, when SL levels are high D53 becomes degraded, thus allowing response to SL.



SLs dampen polar auxin transport in the stem

SLs can also influence polar auxin transport, and hence shoot branching, by triggering the removal of PIN1 auxin exporter proteins from the plasma membrane. Whether this response requires D53 degradation is still not known.



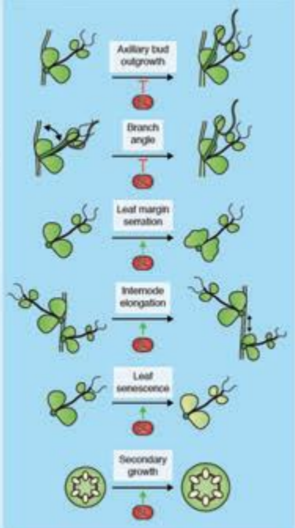
Multiple roles of SLs in plant life

SLs and SL-like compounds are ancient signaling molecules that control several aspects of plant development. They also have an important function in the rhizosphere for signaling the presence of a host root to symbiotic and parasitic organisms. More recently, SLs have been implicated in abiotic stress tolerance.

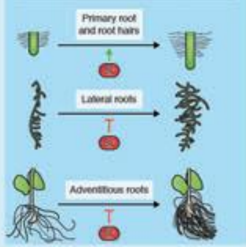
Key

	Several species	Single species
Activation/ stimulation	——→	┈┈→
Repression/ inhibition	——┤	┈┈┤
Controversial data	┈┈┈┈┈┈	

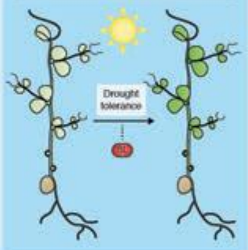
Shoot development



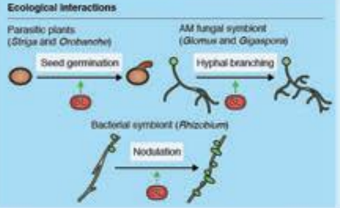
Root development



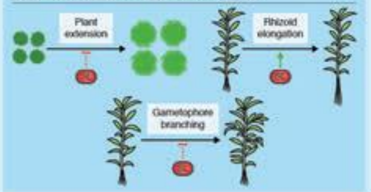
Tolerance to abiotic stress



Ecological interactions



Role in non-vascular plants (*Physcomitrella patens*)

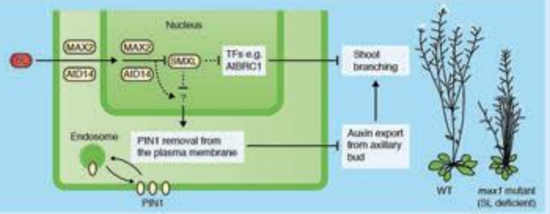


SL signaling and crosstalks for the control of shoot branching in different species

The role of SL signaling, and its interaction with other pathways, in the control of shoot branching has been studied in different species. It is still not clear whether models described in one species apply to other species.

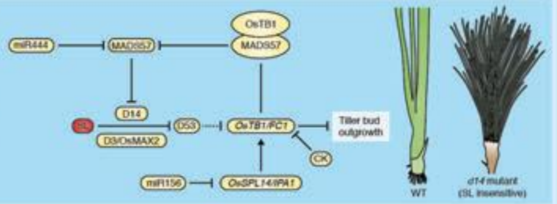
Arabidopsis

In *Arabidopsis*, SL signaling acts via MAX2 and AtD14 to promote the removal of PIN1 proteins from the plasma membrane and hence regulate polar auxin transport. SLs may also function via triggering the degradation of D53/SMXL, which would lead to consecutive effects on transcription factors (TFs) such as BRC1, although direct targets of SMXL proteins are still unknown.

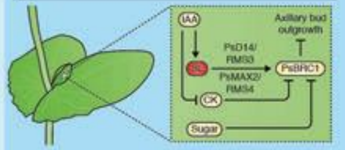


Rice

In rice, SL signaling acts via the degradation of D53 and via the TF *OsTB1* to inhibit tillering. *OsTB1* is activated by OsSPL14/IPA1, which directly binds to its promoter, and is transcriptionally repressed by CK. OsTB1 protein can bind to and inhibit the activity of MADS57, a TF that represses the *D14* gene encoding the SL receptor.

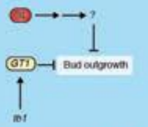


Pea



In Pea, *PsBRC1*, which encodes a TCP TF, integrates SL signaling at the transcriptional level with CK and sugar signaling pathways within the bud. Auxin (IAA) upregulates SL biosynthesis and represses CK biosynthesis. The link between SL perception and BRC1 is still unknown but SLs need RMS3 and RMS4 to upregulate *PsBRC1* transcript levels.

Domesticated Maize

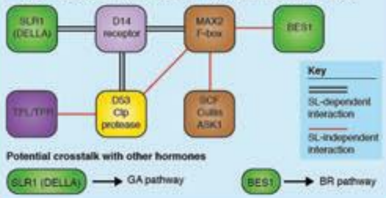


The major domestication gene *TB1* from maize appears to function in an SL-independent subnetwork with *GT1*, which encodes a class I homeodomain leucine zipper (HD-Zip) protein that acts downstream of *TB1*.

Protein interaction networks during SL signaling

SLs trigger the degradation of proteins via the UPS

The protein interaction network in SL signaling is poorly understood. Several protein-protein interactions between components of SL signaling have been shown to be SL independent or dependent. Interactions with key proteins in other hormone signaling pathways (e.g. SLR1, BES1) have also been observed, although the biological meaning of these interactions is not well understood.



SL signaling pathway evolution in the green lineage

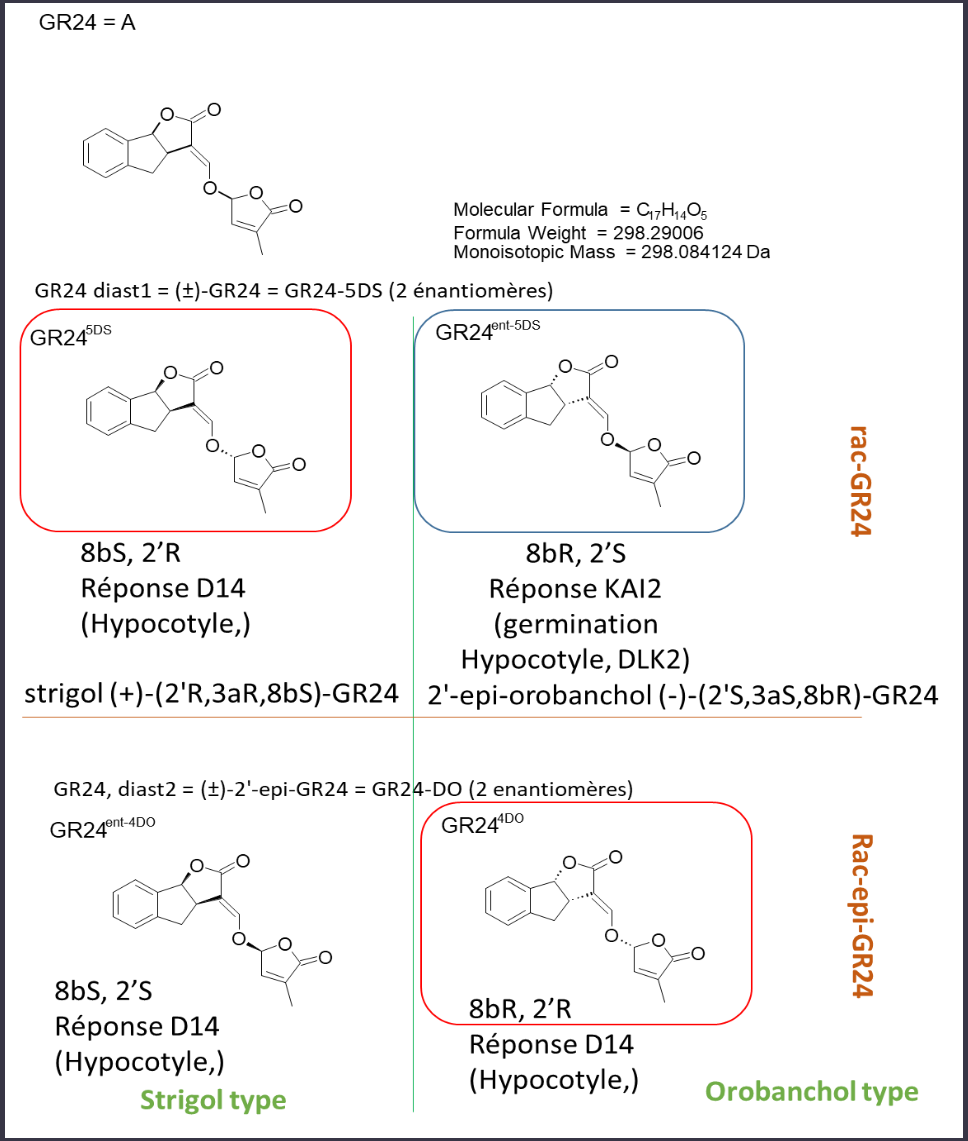
Reports on the presence of SLs are missing for gymnosperms and for *Selaginella* (denoted '?'). However, as known synthesis genes are present, these species are likely to produce SLs. When known, the number of homologous SL signaling genes is indicated. Question marks indicate that the occurrence of homologous genes is still debated, or that the number of genes is uncertain. Numbers of true homologous genes for D14 and KAI2 are indicated in separate columns. For clarity, the number of genes belonging to related α/β hydrolase clades (D14-like or KAI2-like) is not presented, since their role in SL signaling has not been characterized. Species from mosses and liverworts groups are mycotrophic, but as this is not reported for *P. patens* and *M. polymorpha* the occurence of AM symbiosis is noted in brackets.

			SLs	D14	KAI2	MAX2	D53/D53L-SMXL	AM symbiosis
Vascular plants	Angiosperms	*Pisum sativum*	✓	1	2	1	9	✓
		Arabidopsis thaliana	✓	1	1	1	8	✗
		Oryza sativa	✓	1	1	1	9	✓
	Gymnosperms	*Picea sitchensis*	?	0	1	1	2	✓
	Lycophytes	*Selaginella moellendorffii*	?	0	1	?	2	✓
Bryophytes	Mosses	*Physcomitrella patens*	✓	0	5	1	3	(✓)
	Liverworts	*Marchantia polymorpha*	✓	0	2	1	?	(✓)
Charophytes	Zygnematales	*Spirogyra pratensis*	✗	0	1	?	?	✗
	Charales	*Nitella hyalina*	✓	0	1	?	?	✗
Chlorophytes	Volvocales	*Chlamydomonas reinhardtii*	✗	0	0	0	0	✗

Abbreviations: AM, Arbuscular mycorrhizal; ASK1, ARABIDOPSIS SKP1 HOMOLOGUE1; BES1, BRI1-EMS-SUPRESSOR1; BR, Brassinosteroid; BRC1, BRANCHED1; CCD7, CAROTENOID CLEAVAGE DIOXYGENASE7; CCD8, CAROTENOID CLEAVAGE DIOXYGENASE8; CK, Cytokinin; CL, Carlactone; CYP450, CYTOCHROME P450; DAD2, DECREASED APICAL DOMINANCE2; D3, DWARF3; D14, DWARF14; D27, DWARF27; D53, DWARF53; FC1, FINE CULM1; GA, Gibberellin; GT1, GRASSY TILLERS1; IAA, Auxin (indole-3-acetic acid); IPA1, IDEAL PLANT ARCHITECTURE1; KAI2, KARRIKIN INSENSITIVE2; MADS57, MADS-BOX TRANSCRIPTION FACTOR57; MAX1, MORE AXILLARY GROWTH1; MAX2, MORE AXILLARY GROWTH2; miR156, microRNA156; miR444, microRNA444; PhPDR1, *Petunia hybrida* PLEIOTROPIC DRUG TRANSPORTER1; PIN1, PIN-FORMED1; RMS3, RAMOSUS3; RMS4, RAMOSUS4; SL, Strigolactone; SLR1, SLENDER RICE1; SCF, SKP-CULLIN-F-BOX; SMXL, SUPPRESSOR OF MAX2 1 (SMAX1)-LIKE; SPL14 , SQUAMOSA PROMOTER BINDING-LIKE PROTEIN14; TB1, TEOSINTE BRANCHED1; TCP, TEOSINTE BRANCHED1-CYCLOIDEA and PCF PROTEIN; TF, Transcription factor; TPL, TOPLESS; TPR, TOPLESS-related; UPS, Ubiquitin-proteasome system; WT, wild type.

doi: 10.1242/dev.120006

Four GR24 enantiomers



GR24 = A

Molecular Formula = $C_{17}H_{14}O_5$
Formula Weight = 298.29006
Monoisotopic Mass = 298.084124 Da

GR24 diast1 = (±)-GR24 = GR24-5DS (2 énantiomères)

GR24^{5DS}
8bS, 2’R
Réponse D14
(Hypocotyle,)
strigol (+)-(2'R,3aR,8bS)-GR24

GR24$^{ent\text{-}5DS}$
8bR, 2’S
Réponse KAI2
(germination
Hypocotyle, DLK2)
2'-epi-orobanchol (-)-(2'S,3aS,8bR)-GR24

rac-GR24

GR24, diast2 = (±)-2'-epi-GR24 = GR24-DO (2 enantiomères)

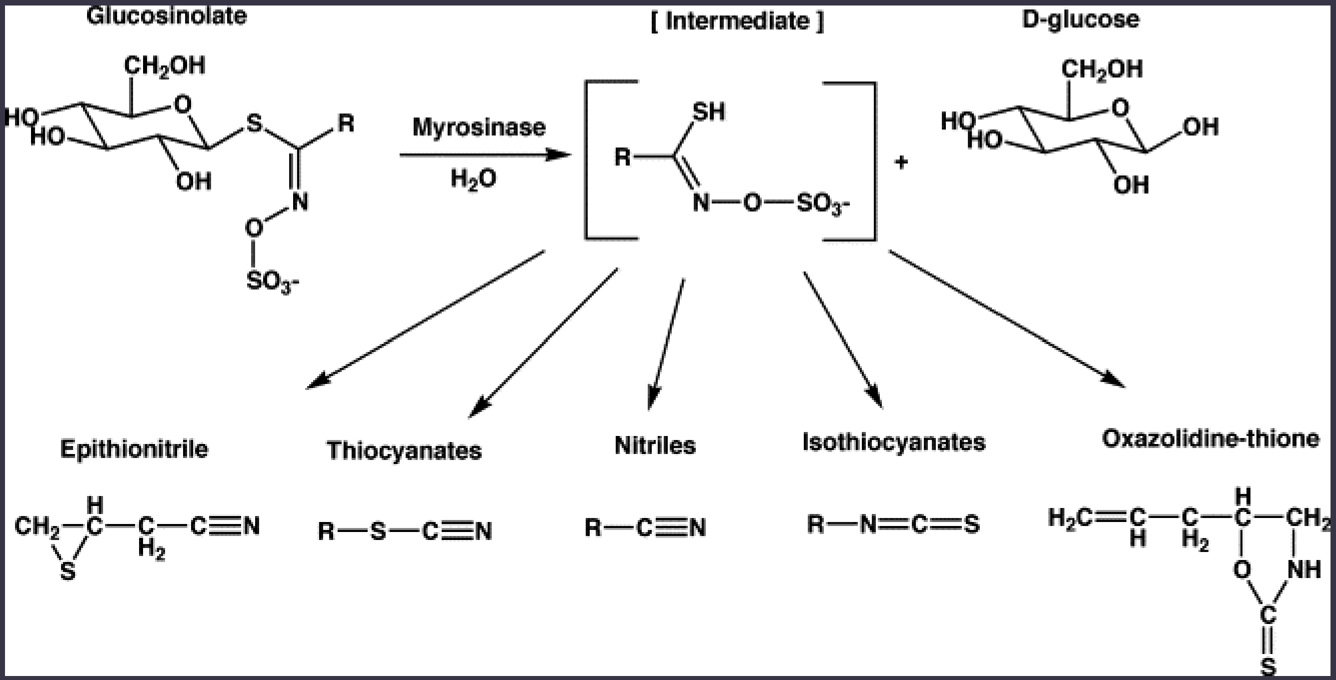
GR24$^{ent\text{-}4DO}$
8bS, 2’S
Réponse D14
(Hypocotyle,)
Strigol type

GR24^{4DO}
8bR, 2’R
Réponse D14
(Hypocotyle,)
Orobanchol type

Rac-epi-GR24

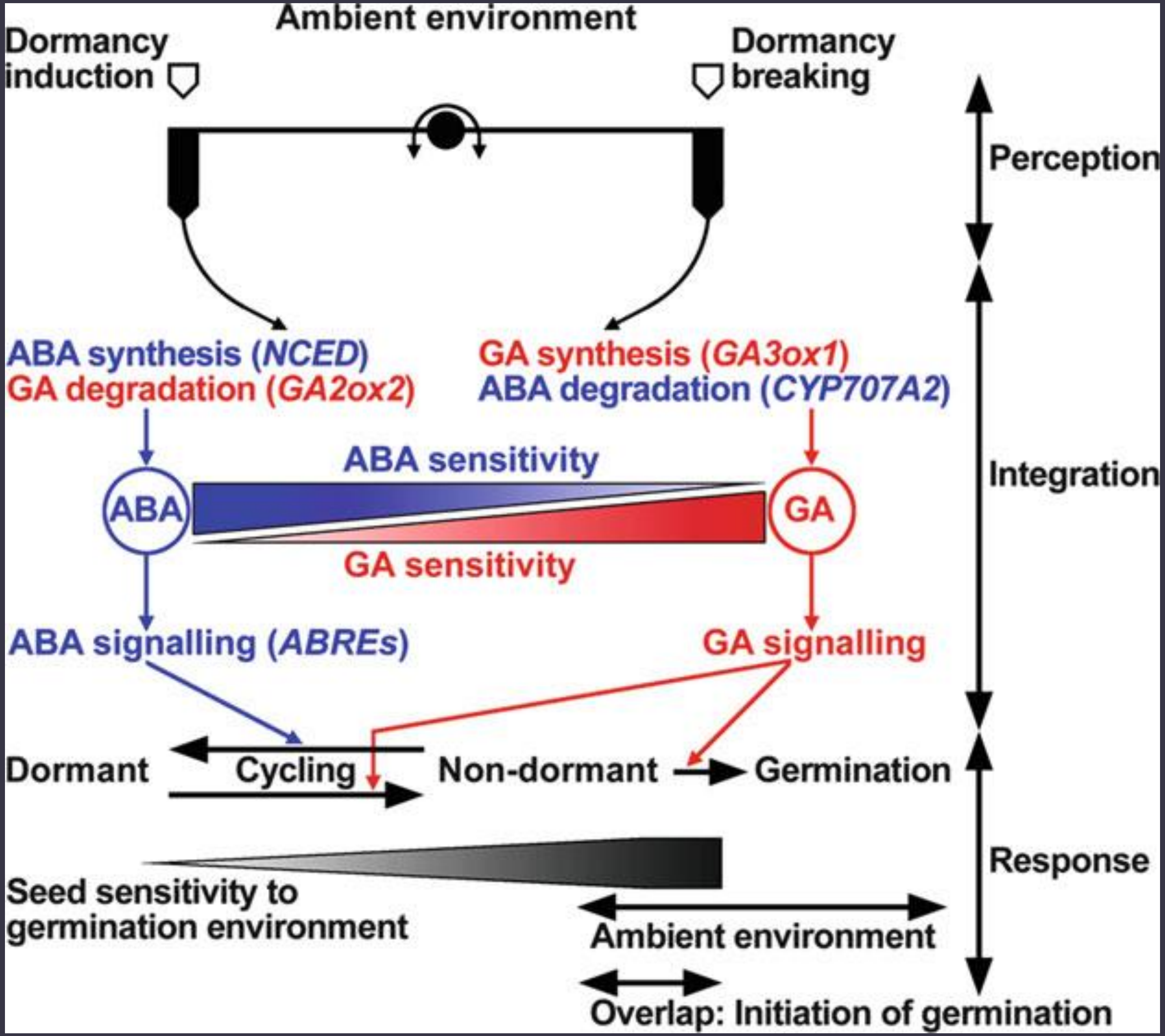
- Two response pathways
  - D14
  - KAI2
- Two SL families
  - Strigol type
  - Orobanchol type

ITC production



From Vaughn and Berhow, 2005

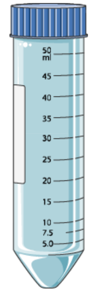
Germination



From Hilhorst *et al.*, 2010

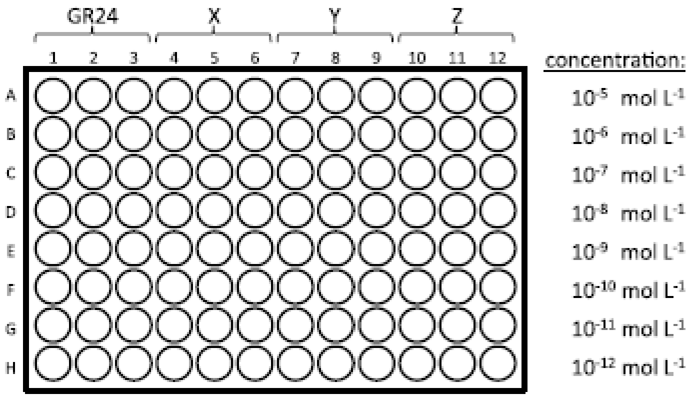
Seed germination assay (Pouvreau et al., 2013)

1



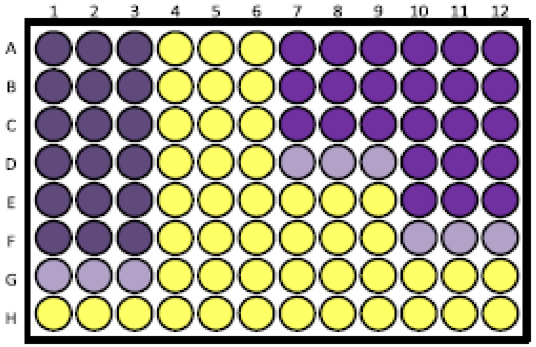
Seed disinfection and conditioning

2



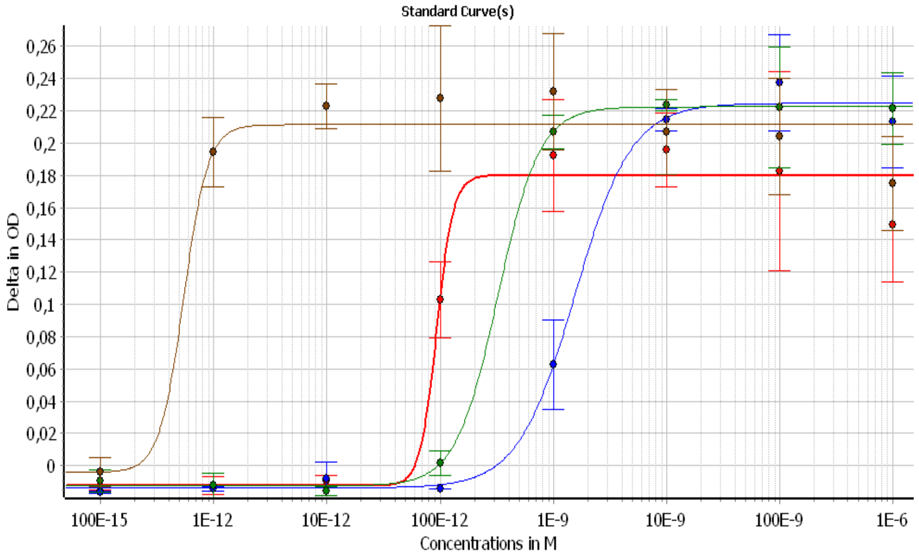
Seed distribution, stimulation, incubation and germination

3



MTT reduction and solubilization of formazan salts

4



Absorbance reading, extrapolation for dose-response analysis and EC50 determination

Marker genes

Bacteria (from Liu et al., 2012)

- 16S rRNA:
    - ribosomal subunit
- 23S rRNA:
    - component of the large prokaryotic ribosomal subunit (50S)
- 16S-23S rDNA ISR:
    - intergenic spacer region between the 16S rDNA and 23S rDNA loci in the rDNA operon
- rpoB:
    - subunit of DNA-dependent RNA polymerase
- gyrB:
    - β-subunit of DNA gyrase
- dnaK:
    - 70kDa heat shock protein (HSP70)
- dsrAB:
    - α and β subunits of an enzyme catalyzing the six-electron reduction of sulfite to sulfide
- amoA and amoB:
    - Ammonia monooxygenase subunits
    - oxidation of ammonium (NH_4) to nitrite (NO_2)

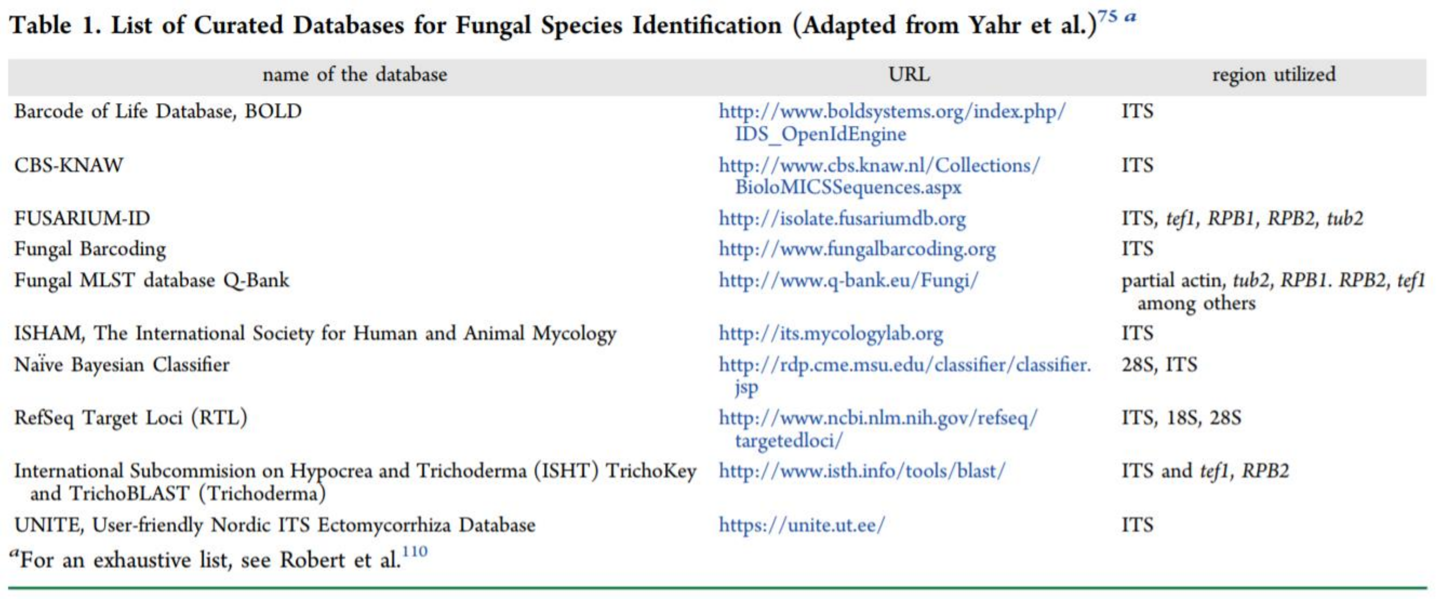


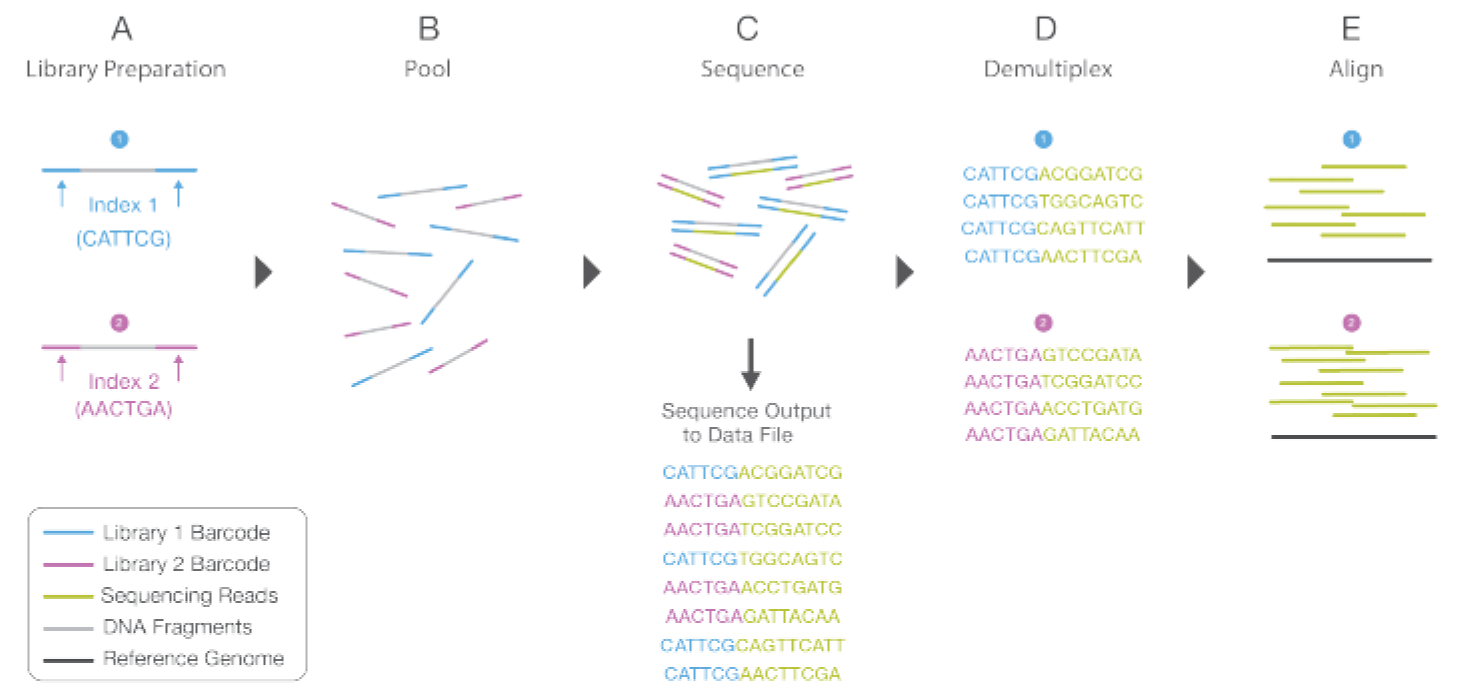
Table 1. List of Curated Databases for Fungal Species Identification (Adapted from Yahr et al.)[75] [a]

name of the database	URL	region utilized
Barcode of Life Database, BOLD	http://www.boldsystems.org/index.php/IDS_OpenIdEngine	ITS
CBS-KNAW	http://www.cbs.knaw.nl/Collections/BioloMICSSequences.aspx	ITS
FUSARIUM-ID	http://isolate.fusariumdb.org	ITS, *tef1*, *RPB1*, *RPB2*, *tub2*
Fungal Barcoding	http://www.fungalbarcoding.org	ITS
Fungal MLST database Q-Bank	http://www.q-bank.eu/Fungi/	partial actin, *tub2*, *RPB1*. *RPB2*, *tef1* among others
ISHAM, The International Society for Human and Animal Mycology	http://its.mycologylab.org	ITS
Naïve Bayesian Classifier	http://rdp.cme.msu.edu/classifier/classifier.jsp	28S, ITS
RefSeq Target Loci (RTL)	http://www.ncbi.nlm.nih.gov/refseq/targetedloci/	ITS, 18S, 28S
International Subcommision on Hypocrea and Trichoderma (ISHT) TrichoKey and TrichoBLAST (Trichoderma)	http://www.isth.info/tools/blast/	ITS and *tef1*, *RPB2*
UNITE, User-friendly Nordic ITS Ectomycorrhiza Database	https://unite.ut.ee/	ITS

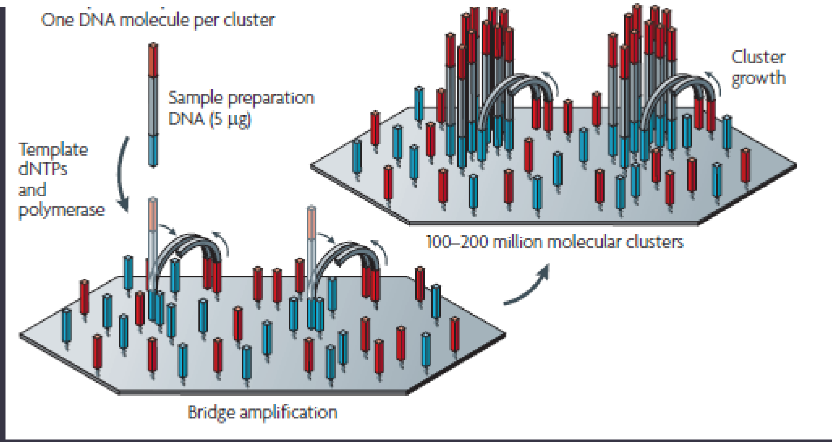
[a] For an exhaustive list, see Robert et al.[110]

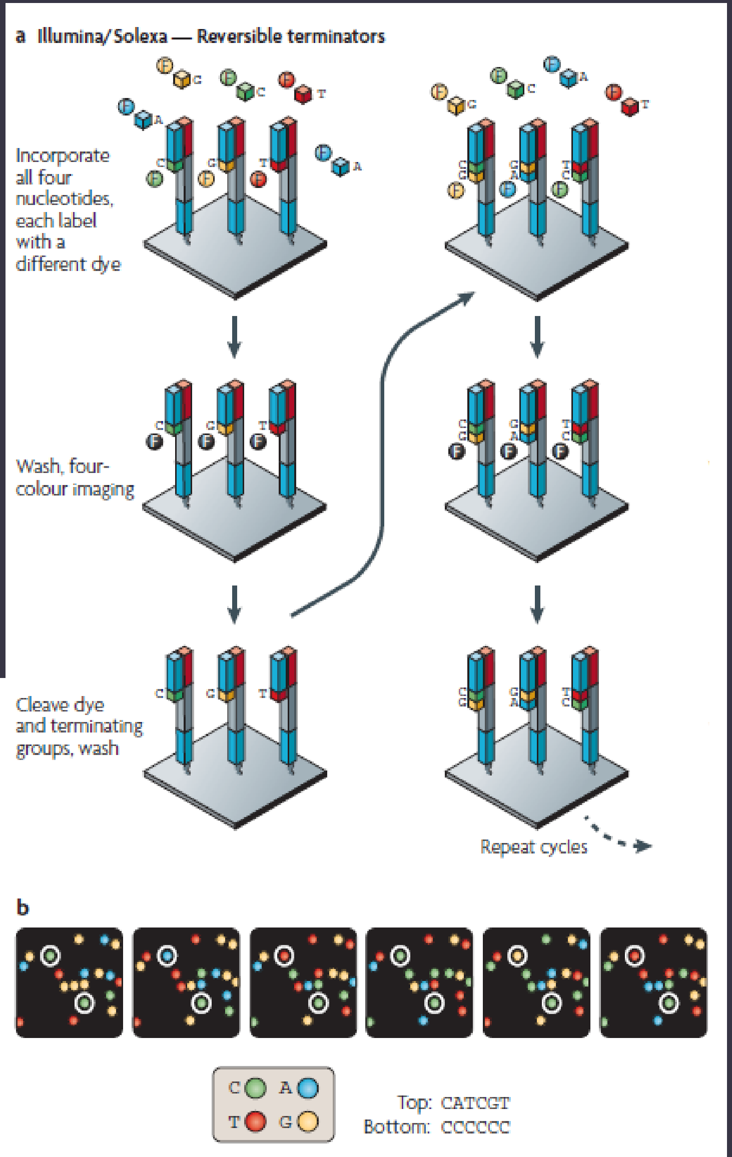
From Raja *et al.*, 2017

MiSeq sequencing



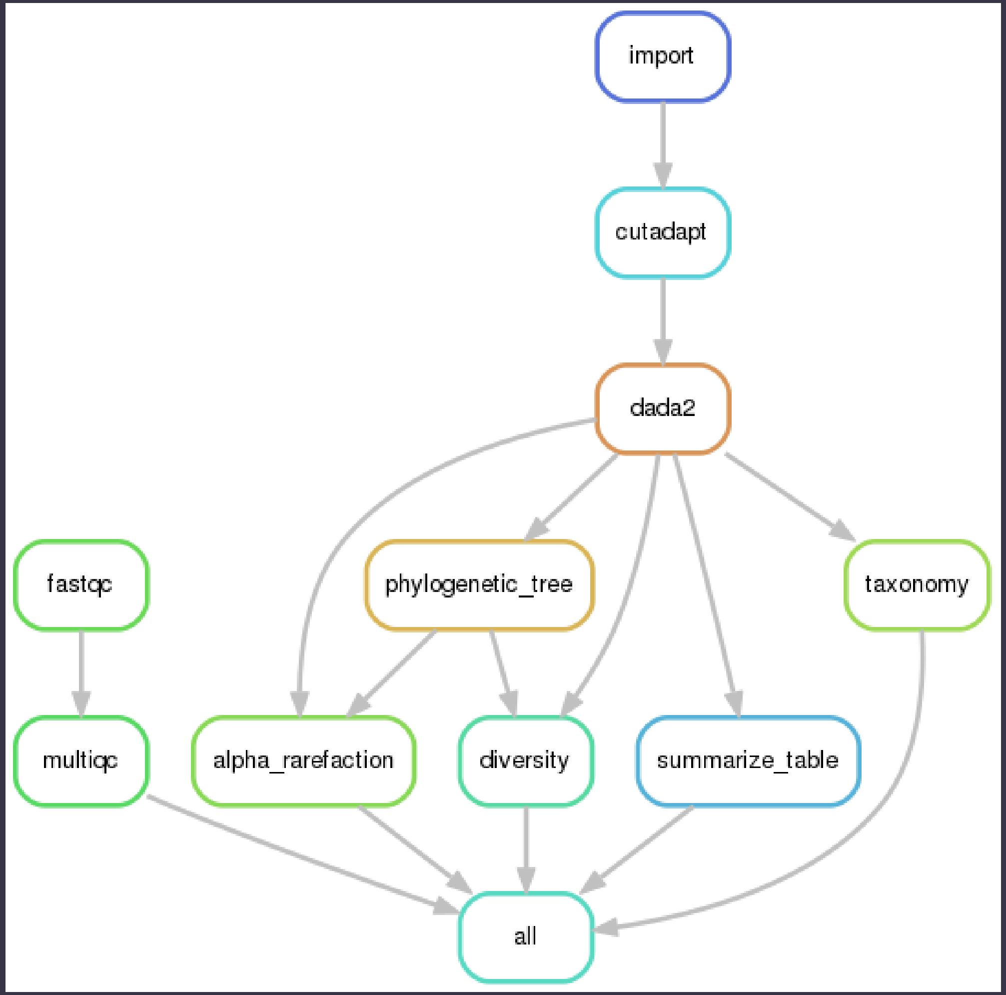
From Illumina



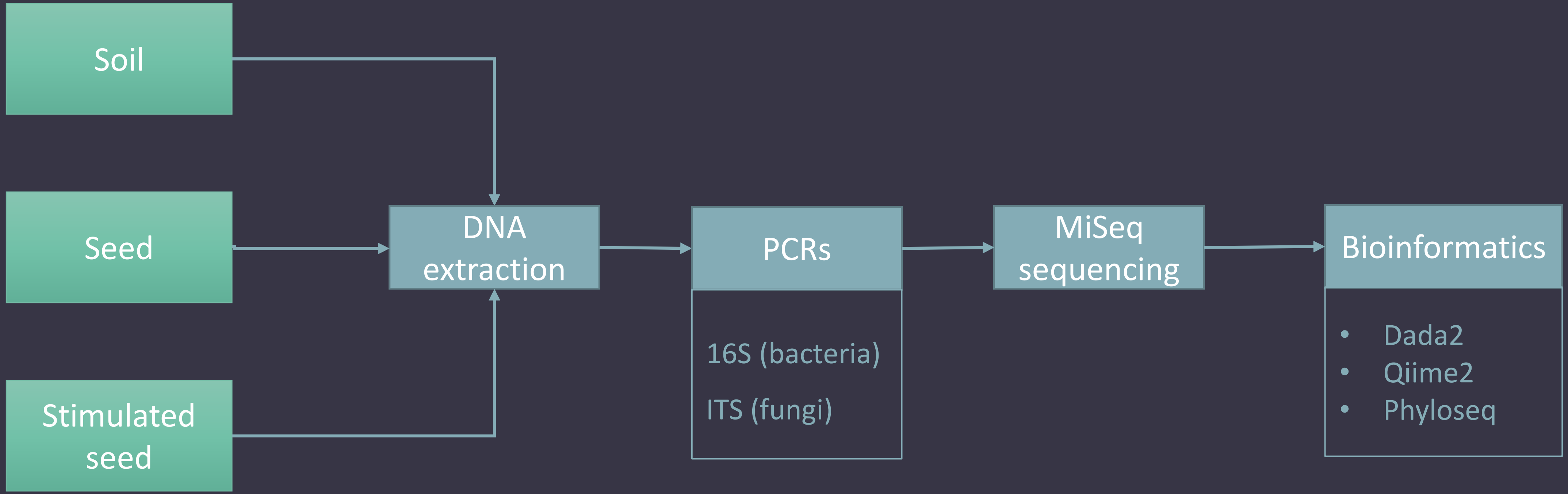


From Metzker, 2009

Qiime2 workflow



Microbiota profiling



Soil

Seed

Stimulated seed

DNA extraction

PCRs

16S (bacteria)

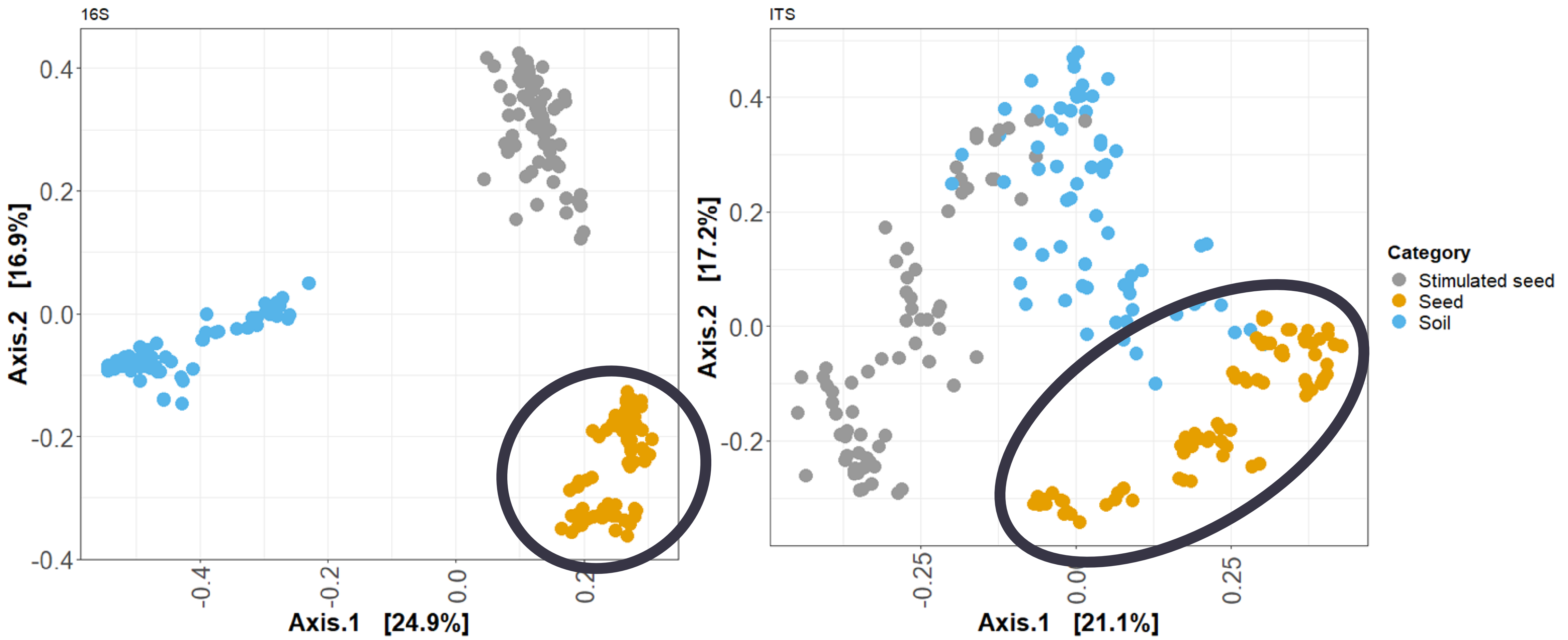
ITS (fungi)

MiSeq sequencing

Bioinformatics

- Dada2
- Qiime2
- Phyloseq

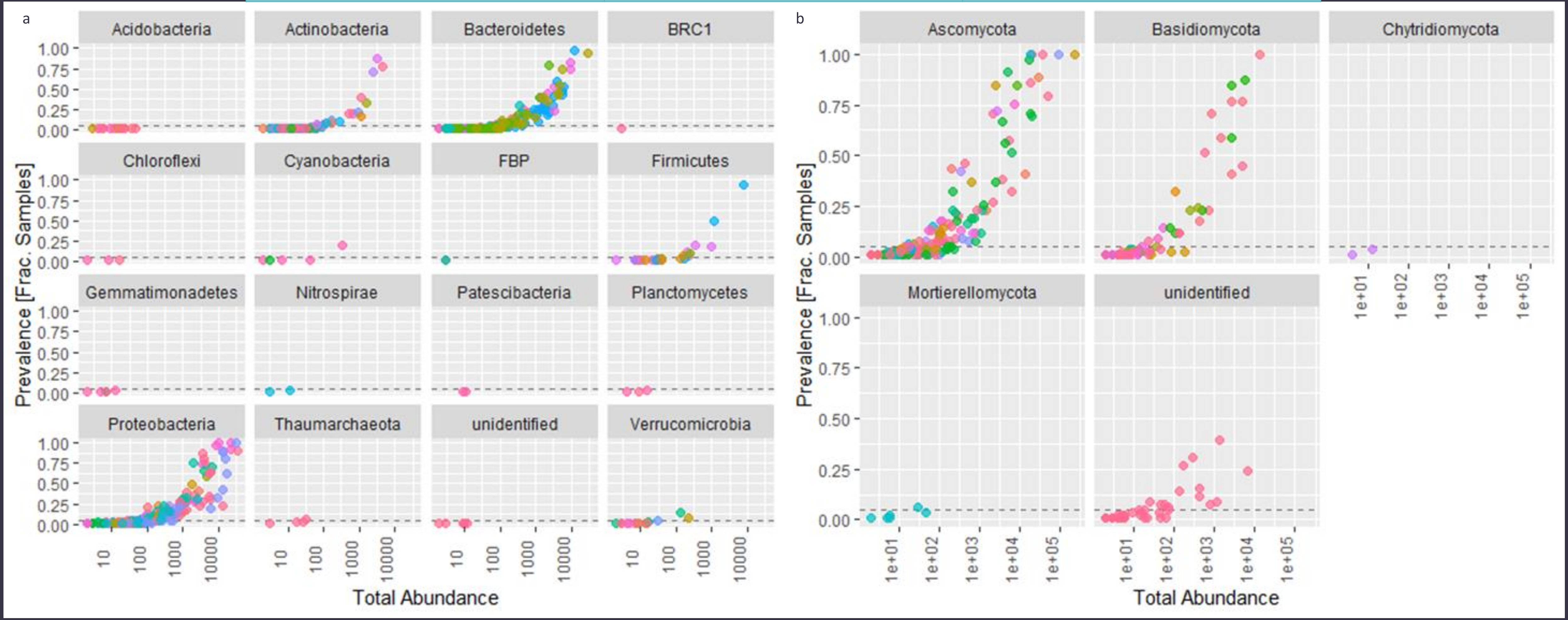
Global diversity



Samples diversity based on Bray-Curstis distances and depending on the sample categories (color)

Taxon

	Bacteria (16S)	Fungi (ITS)
Main phyla	4	2
Total ASV	90.50%	84.68%
Total abundance	99.76%	98.24%

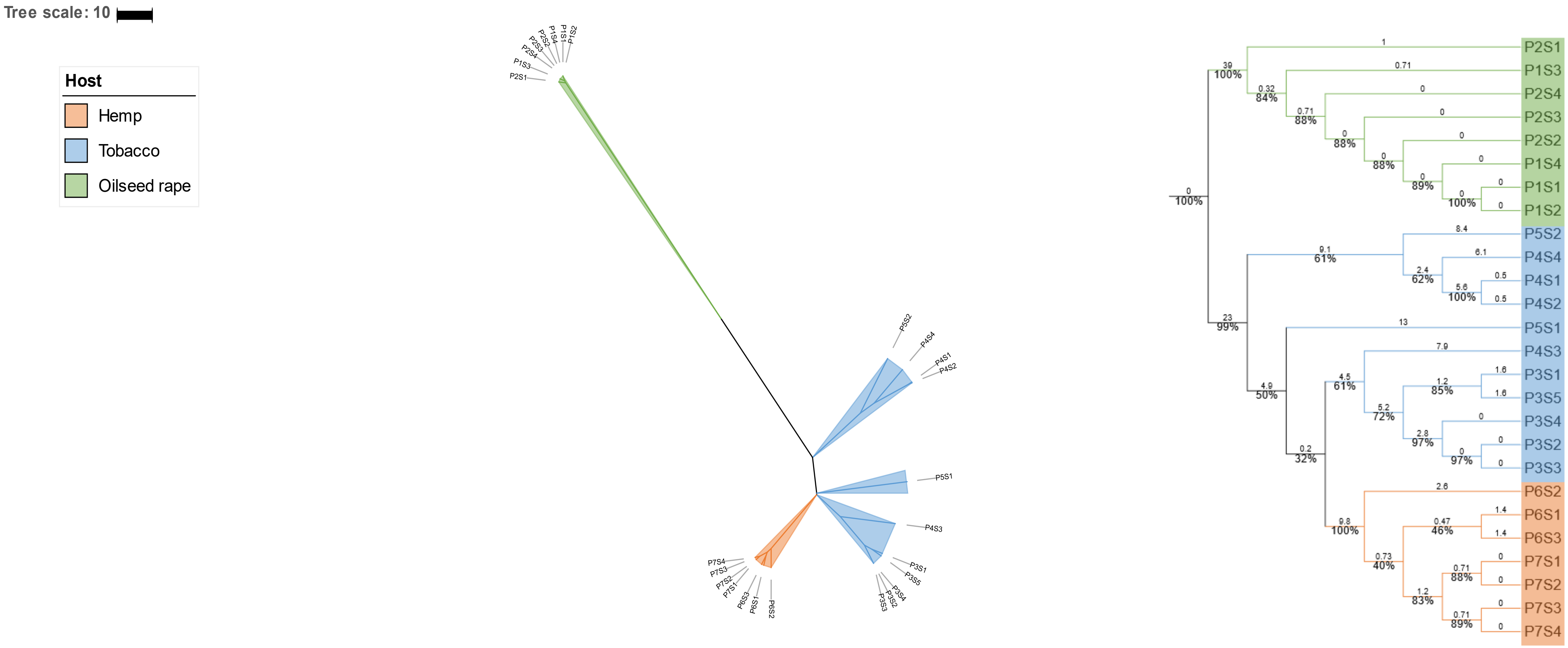


Taxonomic composition of the bacterial (left) and fungal (right) seed microbiota regarding the most abundant and prevalent phyla. Each dot represents a ASV and each color represents a genus.

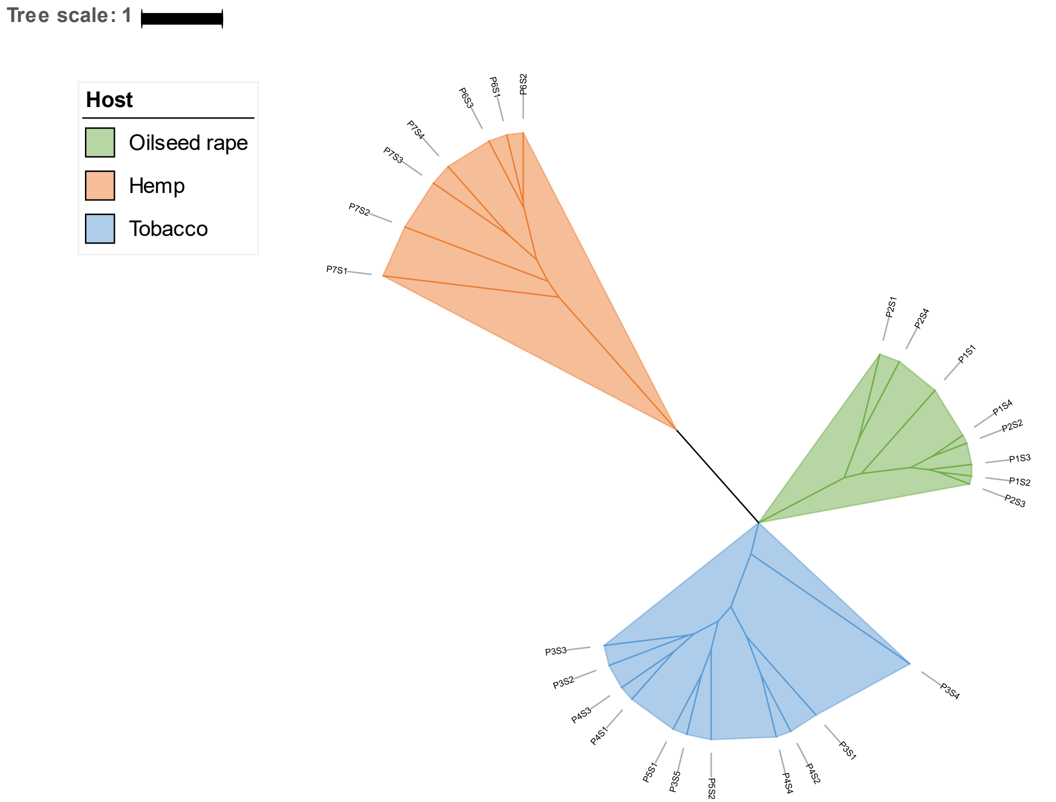
Host core

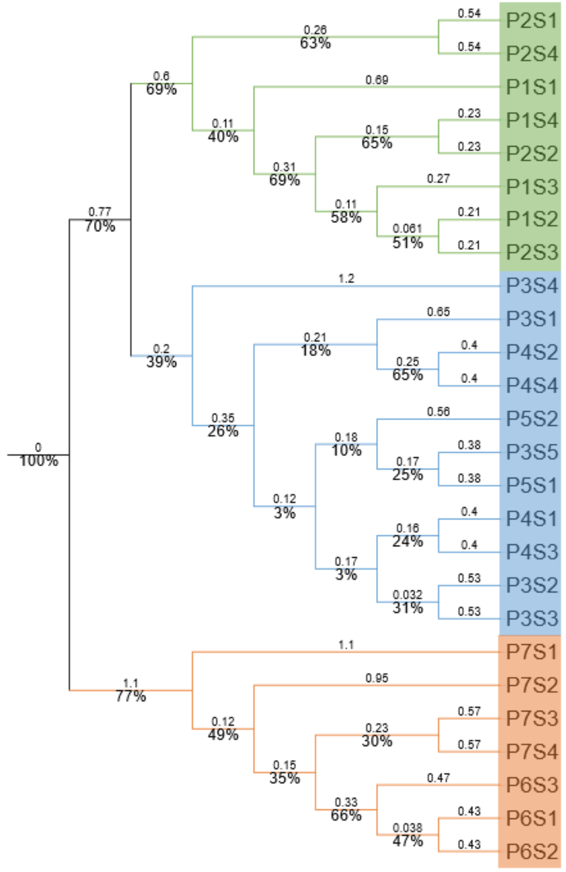
	Specific host	ASV id
16S	Oilseed rape	ASV668_Sphingobacterium sp. MIMdw12 ASV3317_Nannocystis_unidentified
	hemp	ASV718_Pedobacter_unidentified
	tobacco	ASV638_Sphingobacterium sp. 23D10-4-9 ASV2965_Pseudomonas_unidentified
ITS	Oilseed rape	ASV1153_unidentified ASV1708_Leptosphaeria_maculans

Genotyping

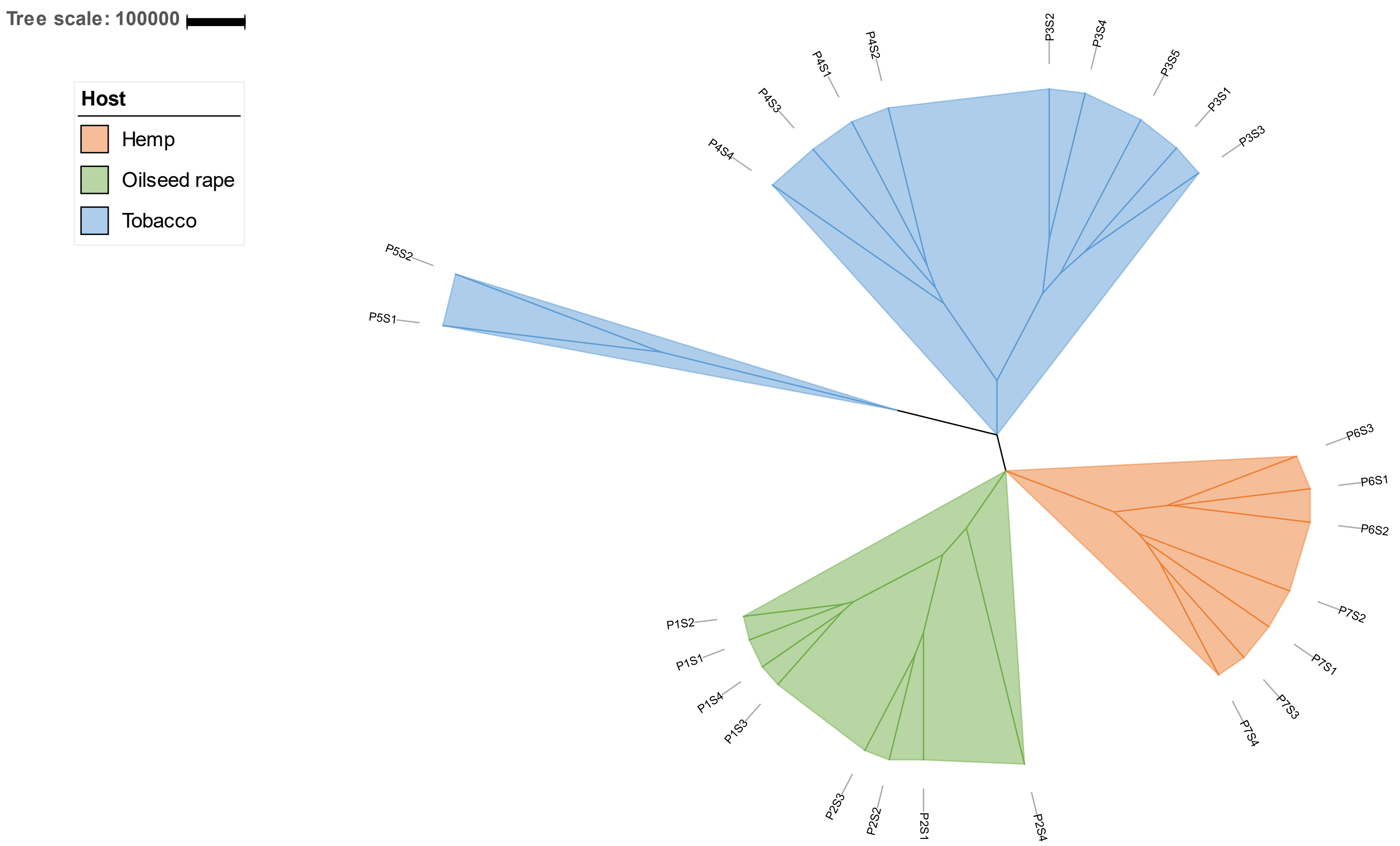

Tree scale: 10
Host
Hemp
Tobacco
Oilseed rape
P2S1
P1S3
P2S4
P2S3
P2S2
P1S4
P1S1
P1S2
P5S2
P4S4
P4S1
P4S2
P5S1
P4S3
P3S1
P3S5
P3S4
P3S2
P3S3
P6S2
P6S1
P6S3
P7S1
P7S2
P7S3
P7S4
100%
84%
88%
88%
88%
89%
100%
61%
62%
100%
99%
50%
61%
85%
72%
97%
97%
32%
100%
46%
40%
88%
83%
89%

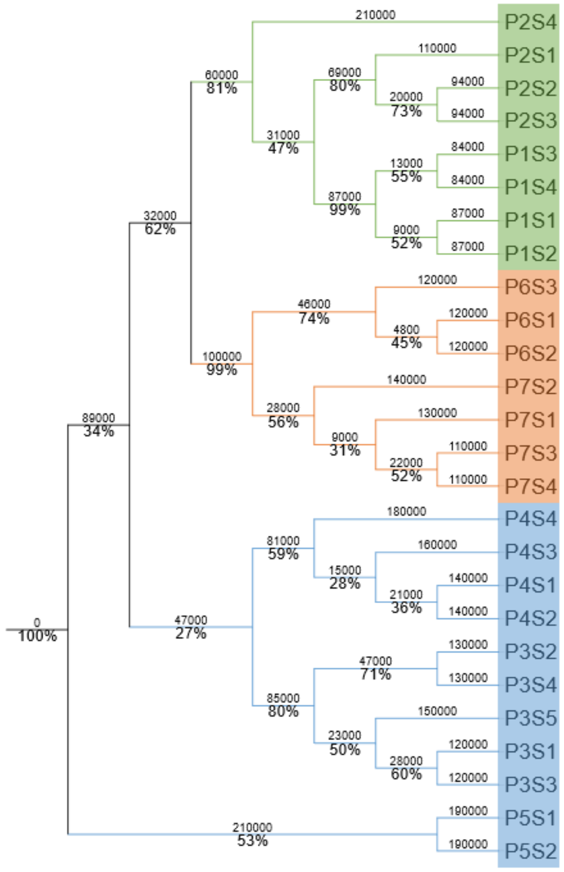
Phenotyping



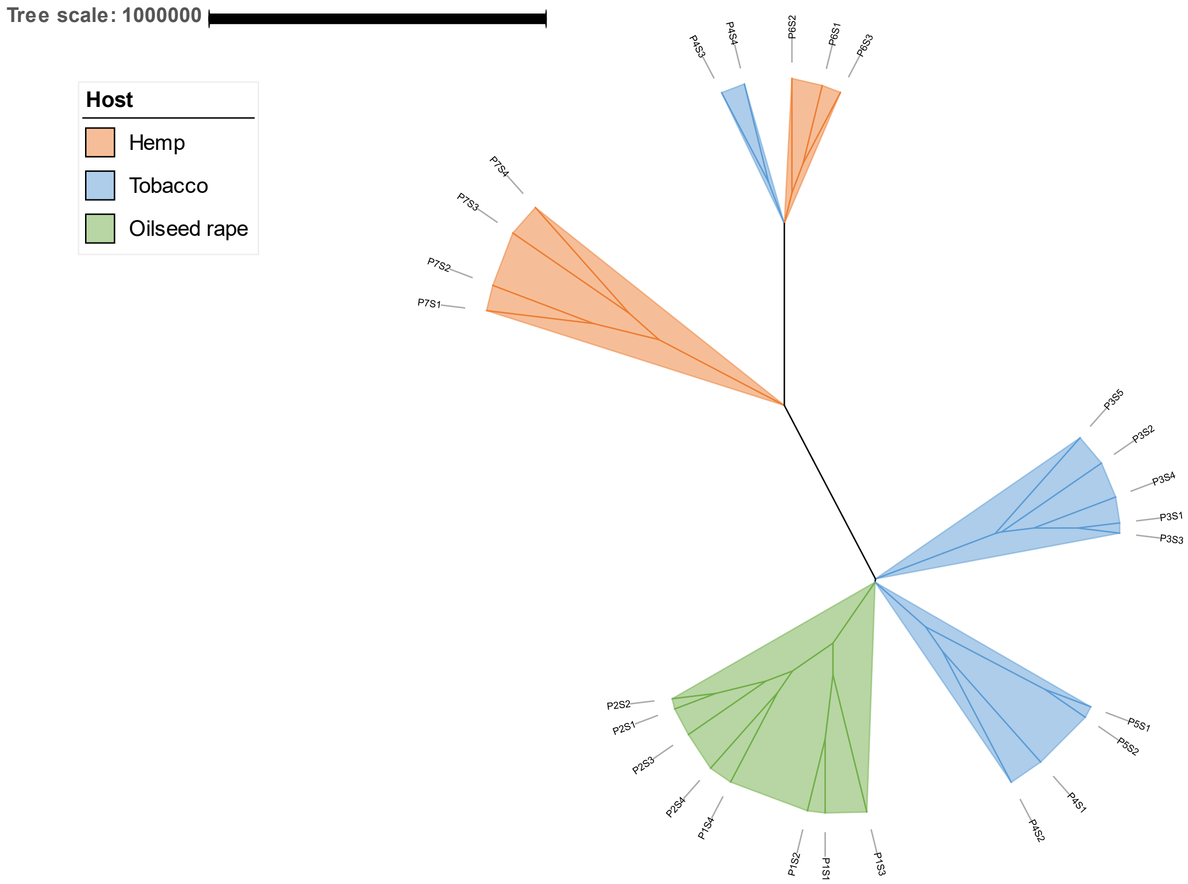


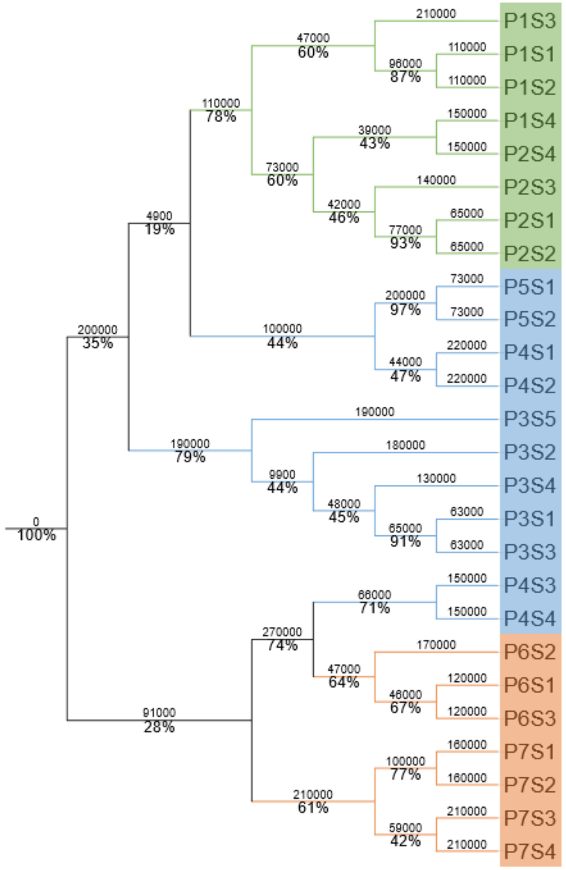
Bacterial community profiling



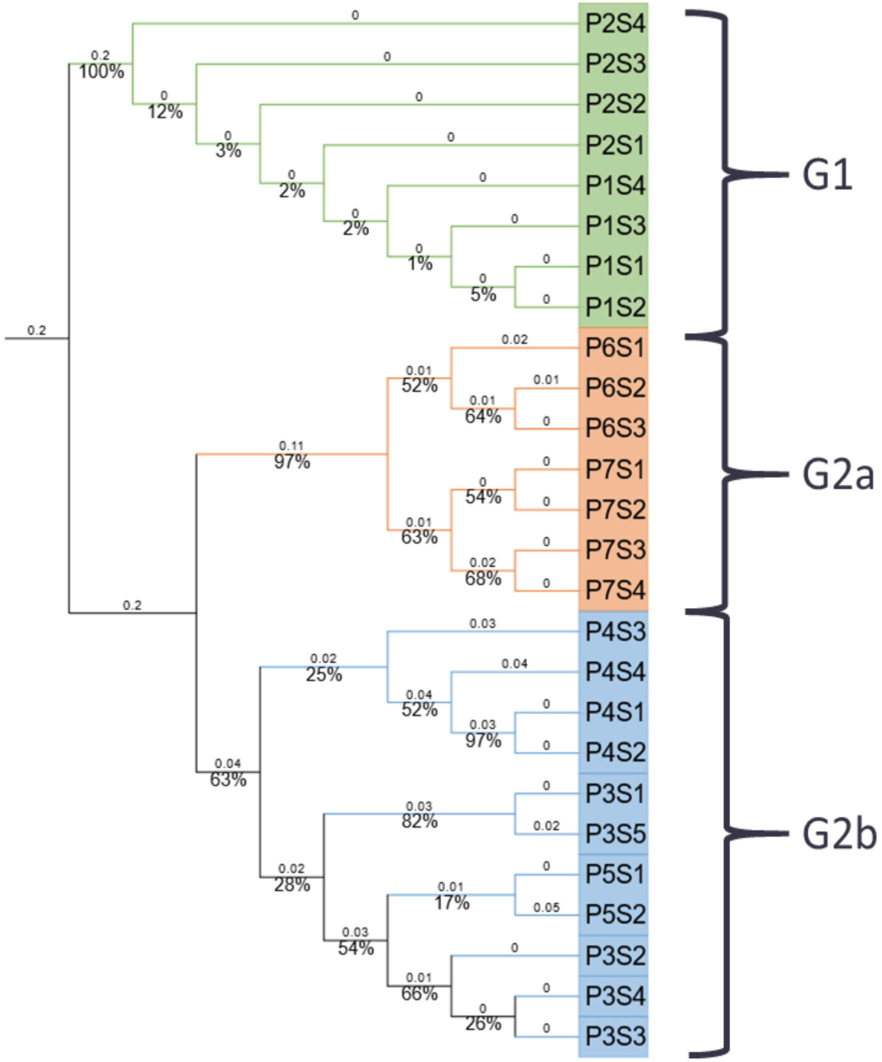


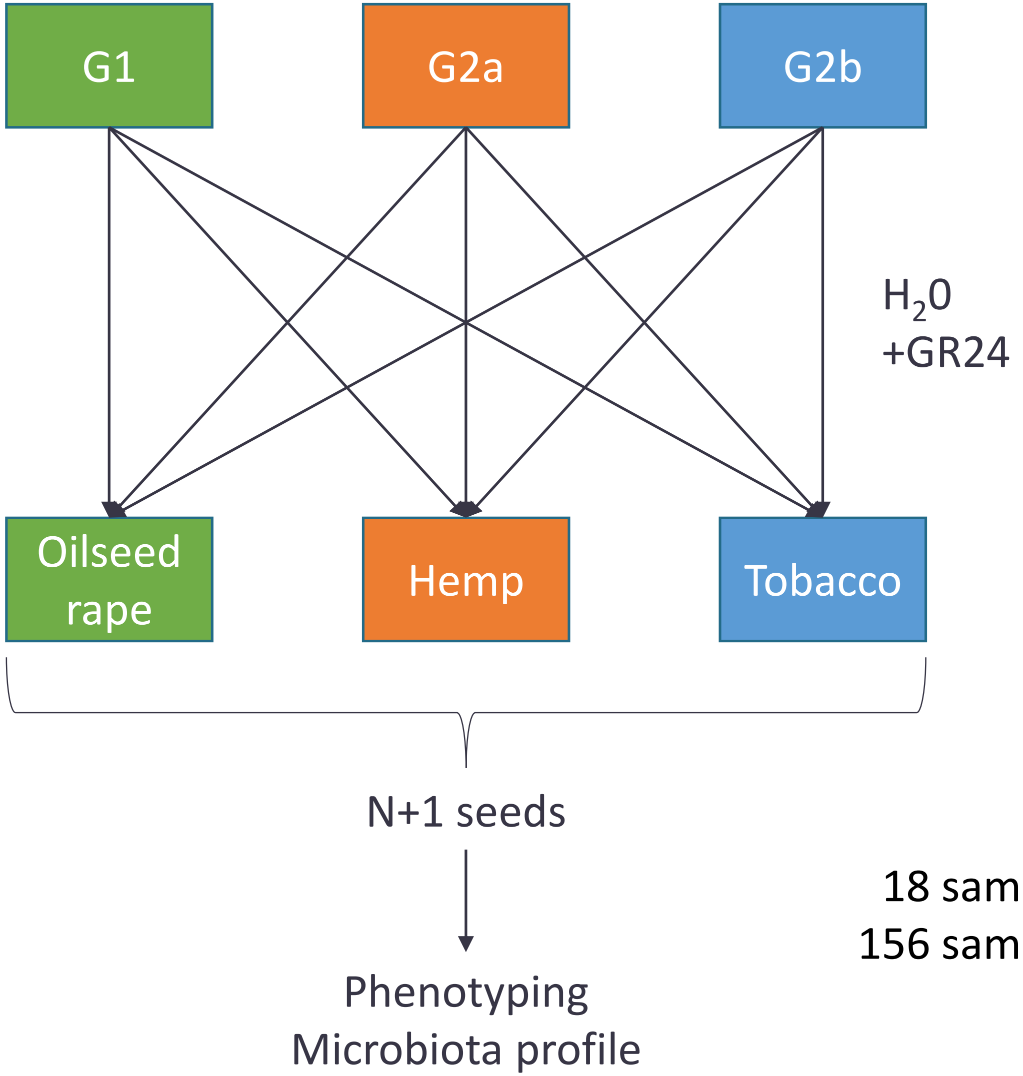
Fungal community profiling





Perspectives





G1
G2a
G2b

H_20
+GR24

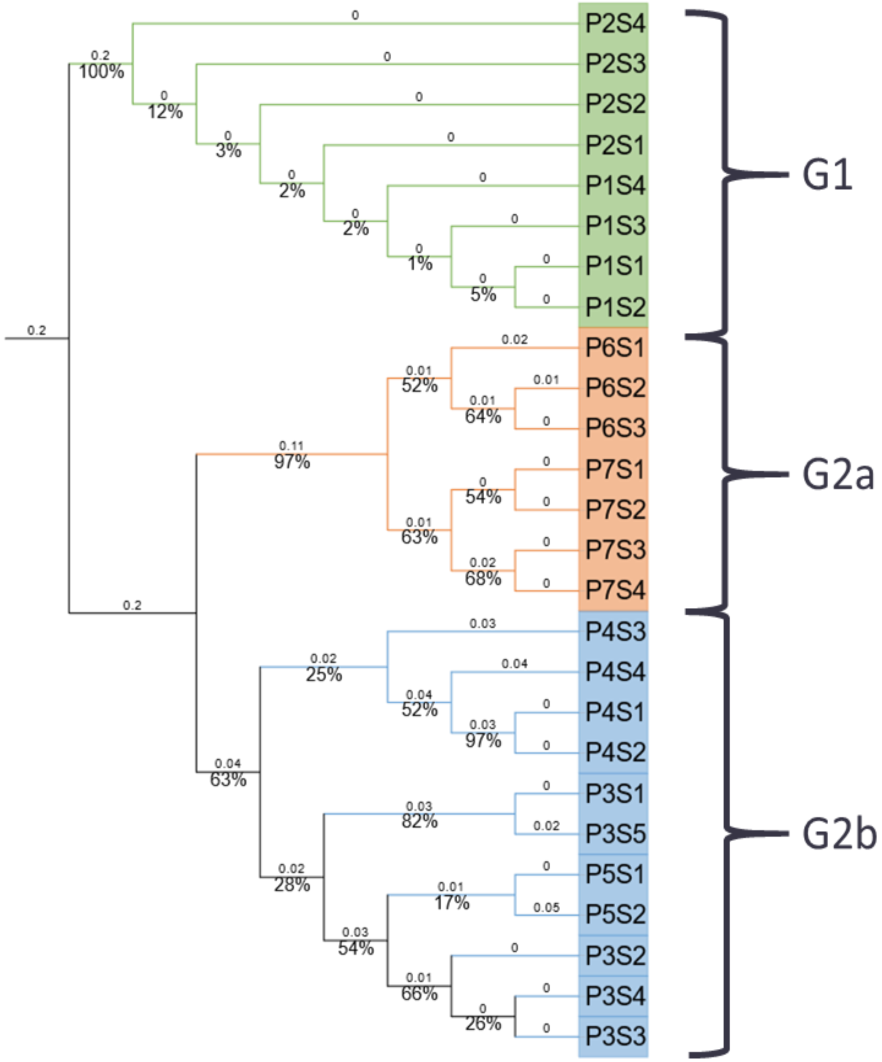
Oilseed rape
Hemp
Tobacco

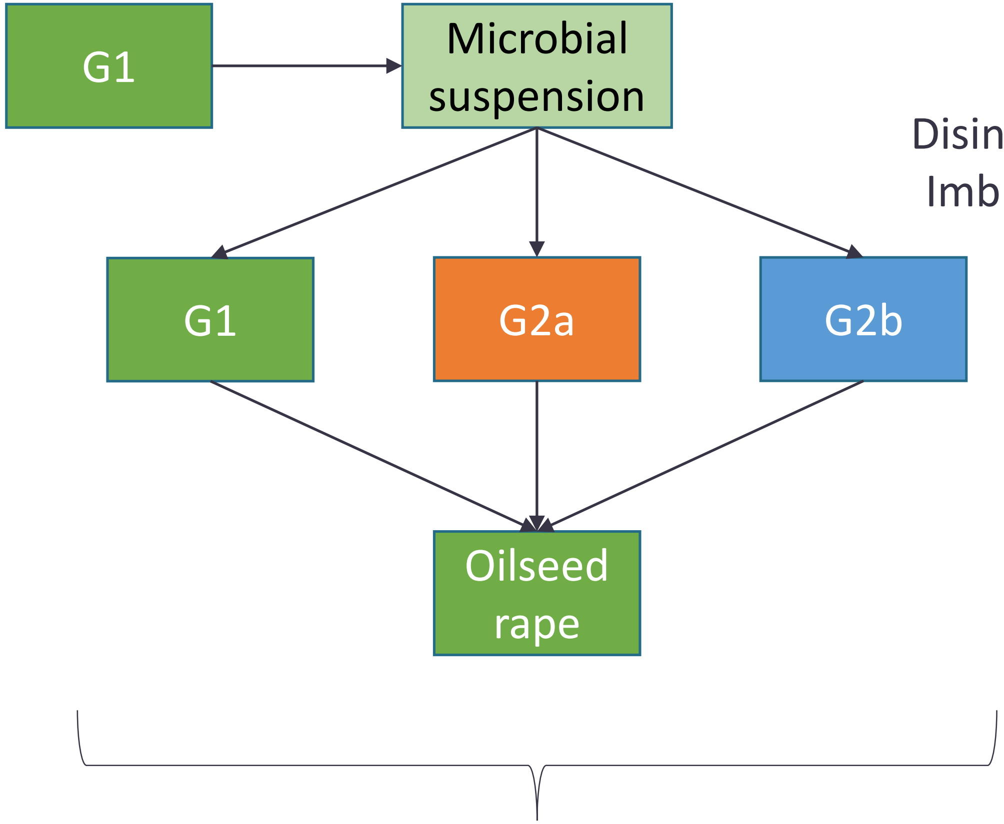
N+1 seeds

Phenotyping
Microbiota profile

18 samples
156 samples

Perspectives





G1

Microbial suspension

Disinfection
Imbibition

G1

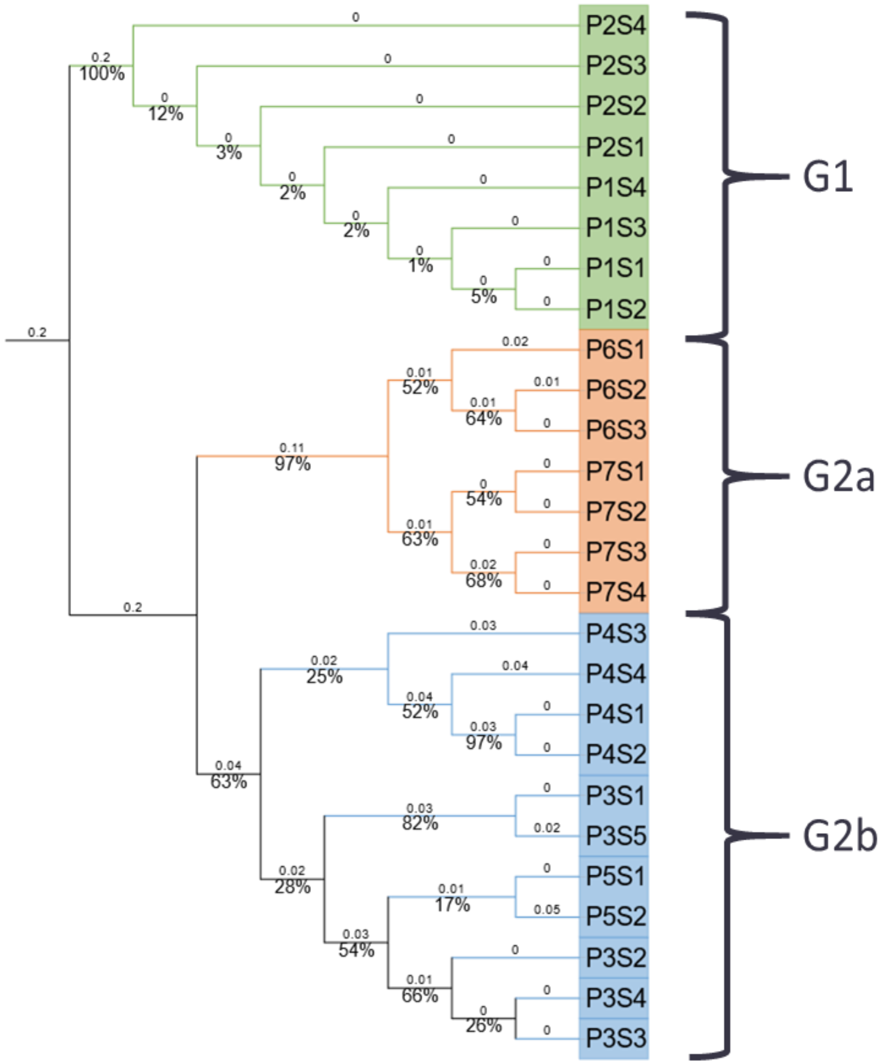
G2a

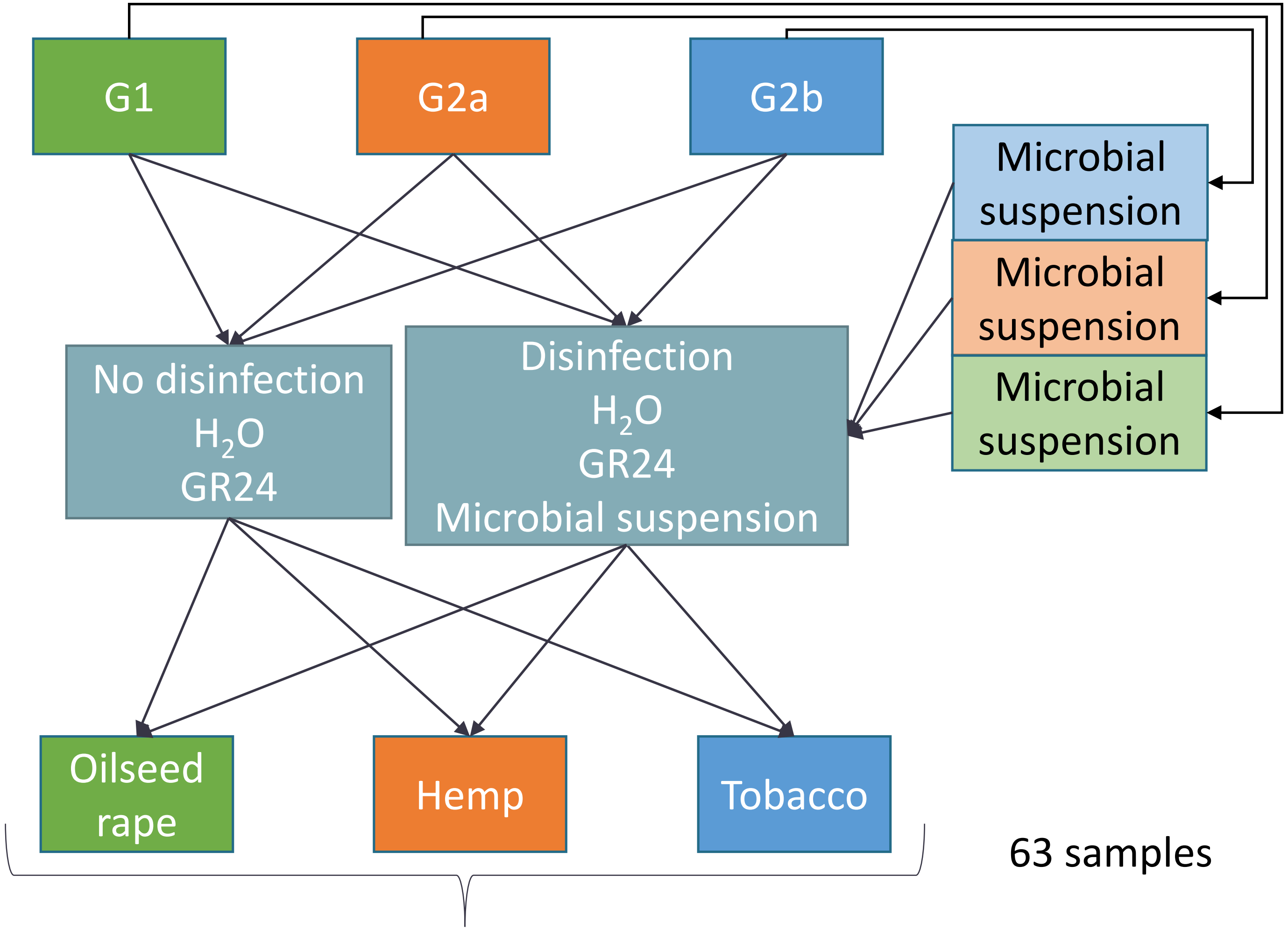
G2b

Oilseed rape

Phenotyping — 9 samples
Microbiota profile — 78 samples

Perspectives





G1

G2a

G2b

Microbial suspension

Microbial suspension

Microbial suspension

No disinfection
H_2O
GR24

Disinfection
H_2O
GR24
Microbial suspension

Oilseed rape

Hemp

Tobacco

63 samples

Phenotyping
Microbiota profile