

The role of secondary metabolites in the composition of root microbiome of Arabidopsis

Vivian Weng

Lab meeting presentation

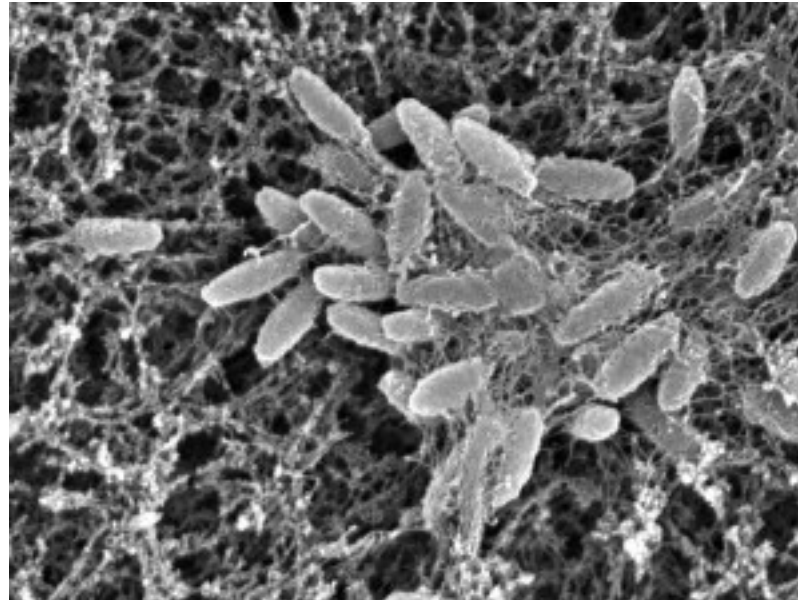
08/22/23



Microbiome



@ Boston
Children's



Plants are vital to human life



Grape



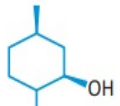
Maize



Coffee

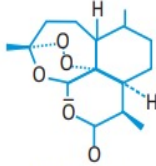
Plants produce secondary metabolites

Monoterpenes



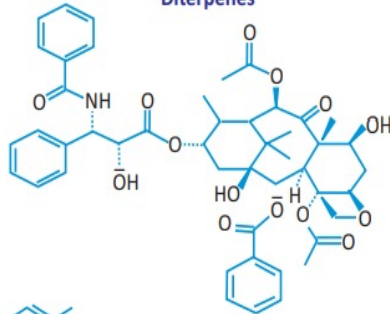
Menthol

Sesquiterpenes



Astemisinin

Diterpenes



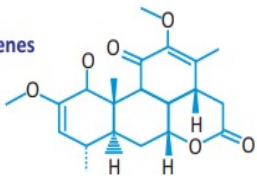
Paclitaxel

Tetraterpenes



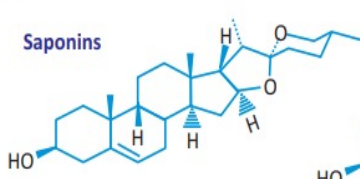
Lycopene

Triterpenes



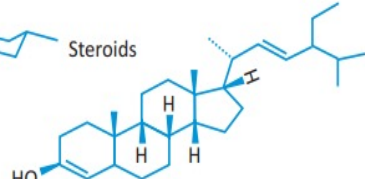
Quassin

Saponins



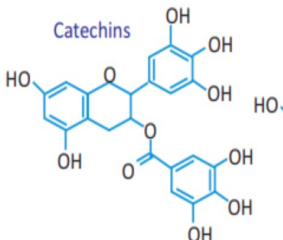
Dioegenin

Steroids



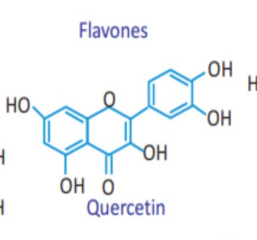
Sigmasterol

Catechins



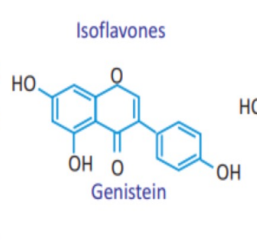
Epigallocatechin gallate

Flavones



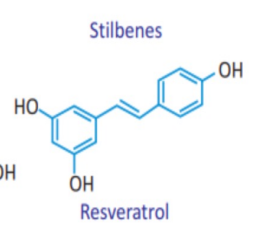
Quercetin

Isoflavones



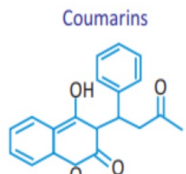
Genistein

Stilbenes



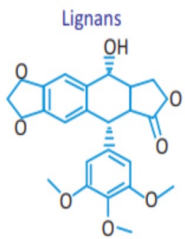
Resveratrol

Coumarins



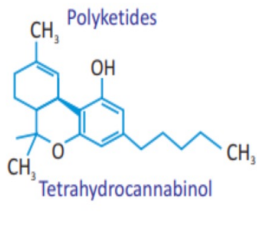
Warfarin

Lignans



Podophyllotoxin

Polyketides



Tetrahydrocannabinol

Volatiles

Eugenol
Linalool

Antioxidant
Antimicrobial
Antiseptic
Anti-Inflammatory
Analgesic

Saponins

Diosgenin

Anticancer
Anti-Inflammatory
Anti-Infectious
Antidiabetic
Antidyslipidemic
Antiobesity
Antioxidant

Alkaloids

Trigonelline
Glycine-betaine

Antidiabetic
Antidyslipidemic
Neuroprotective
Antimigraine
Antibacterial
Antiviral
Anti-Tumor

Flavonoids

Quercetin
Rutin
Vitexin
Isovitexin

Antioxidant
Anti-Inflammatory
Anticancer
Neuroprotective
Cardioprotective
Antihyperalgesic

Modified amino acids

4-hydroxyisoleucine

Antidiabetic
Antidyslipidemic
Antiobesity



Trigonella foenum-graecum

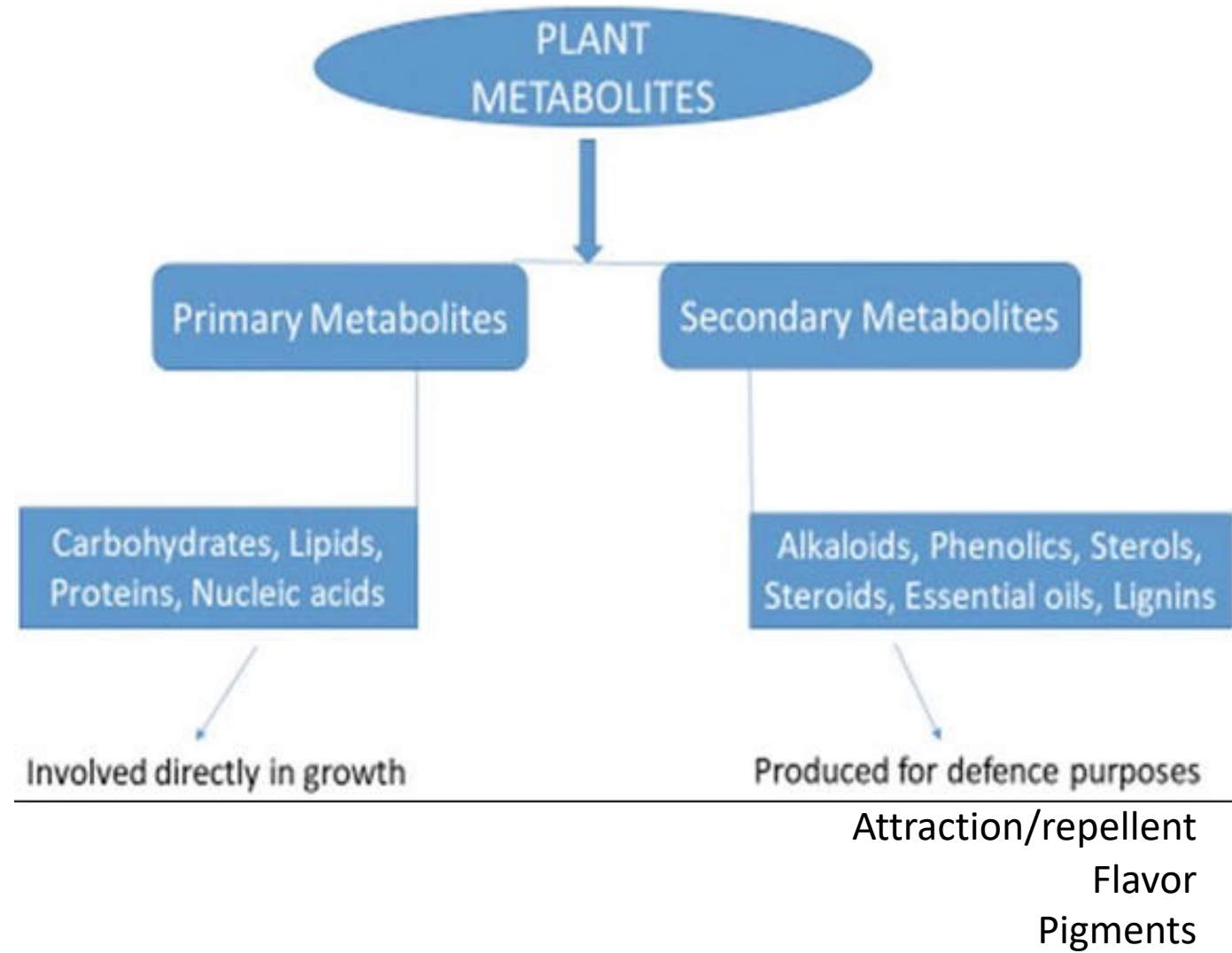
Fenugreek

Terpenoids and steroids

Phenolics, phenylpropanoids and polyketides

Secondary metabolites contribute to host fitness

- Primary metabolites are essential for growth and development
- Secondary metabolites are a byproduct of metabolism and are not essential for growth but contribute to the fitness of the host



Plants produce secondary metabolites



Grape



Polyphenol



Maize



DIMBOA



Coffee



Caffeine

Arabidopsis can be used to study how secondary metabolites are made and why they're important

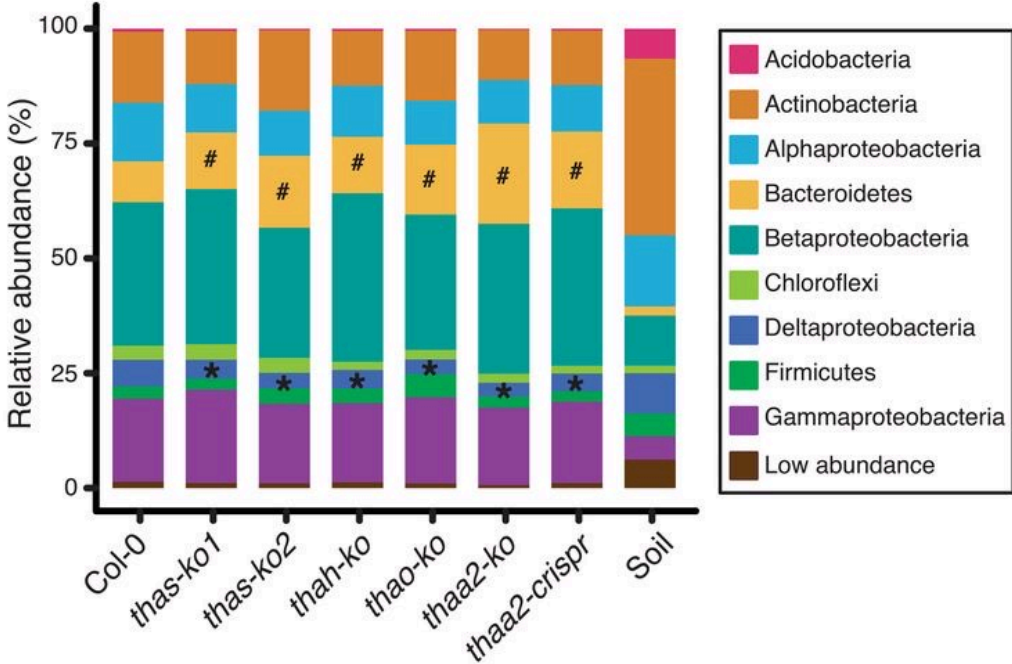
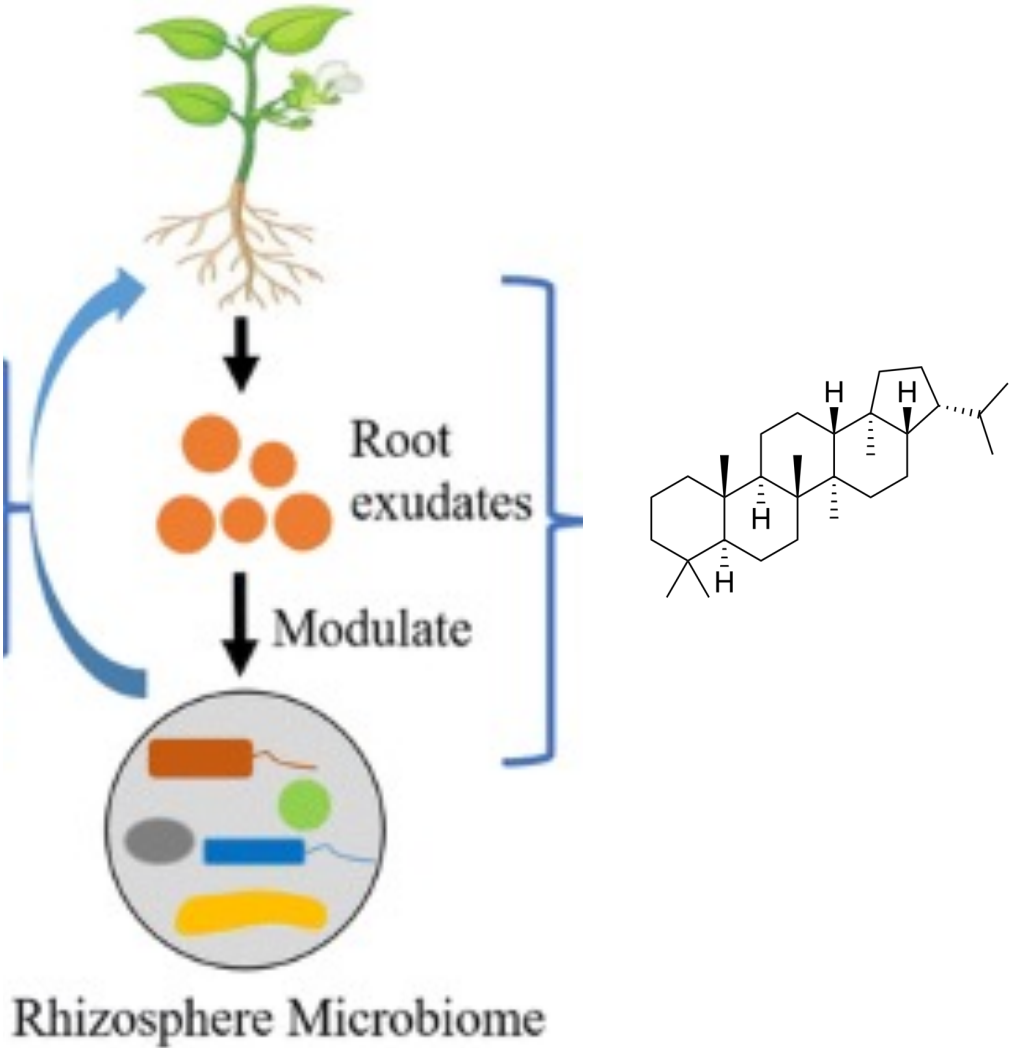
Arabidopsis is a “model organism” for studying plant genetics

- A member of the Brassicaceae family
- Easy to grow
- Genome is small (114.5 Mb) and already sequenced
- Genetically tractable
- Mutant libraries



Secondary metabolites modulate the root microbiome

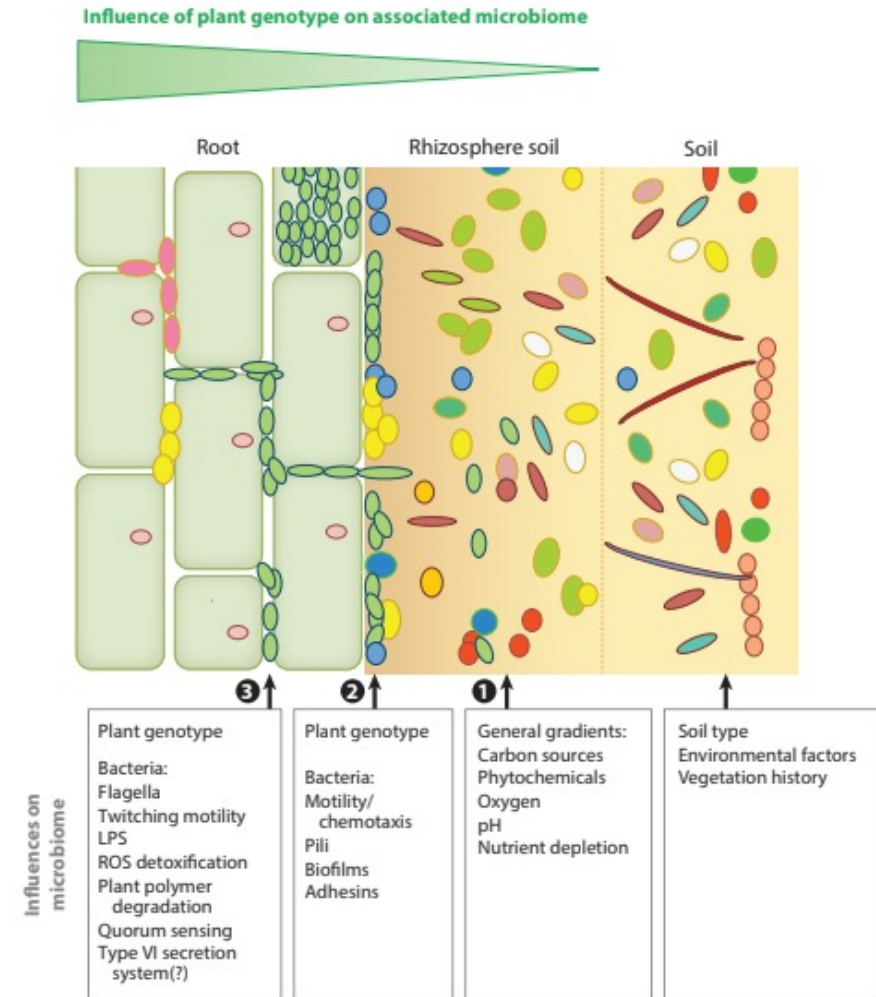
- Triterpenes (a secondary metabolite) are secreted by Arabidopsis roots



Root microbiome has a diverse composition

What is root microbiome?

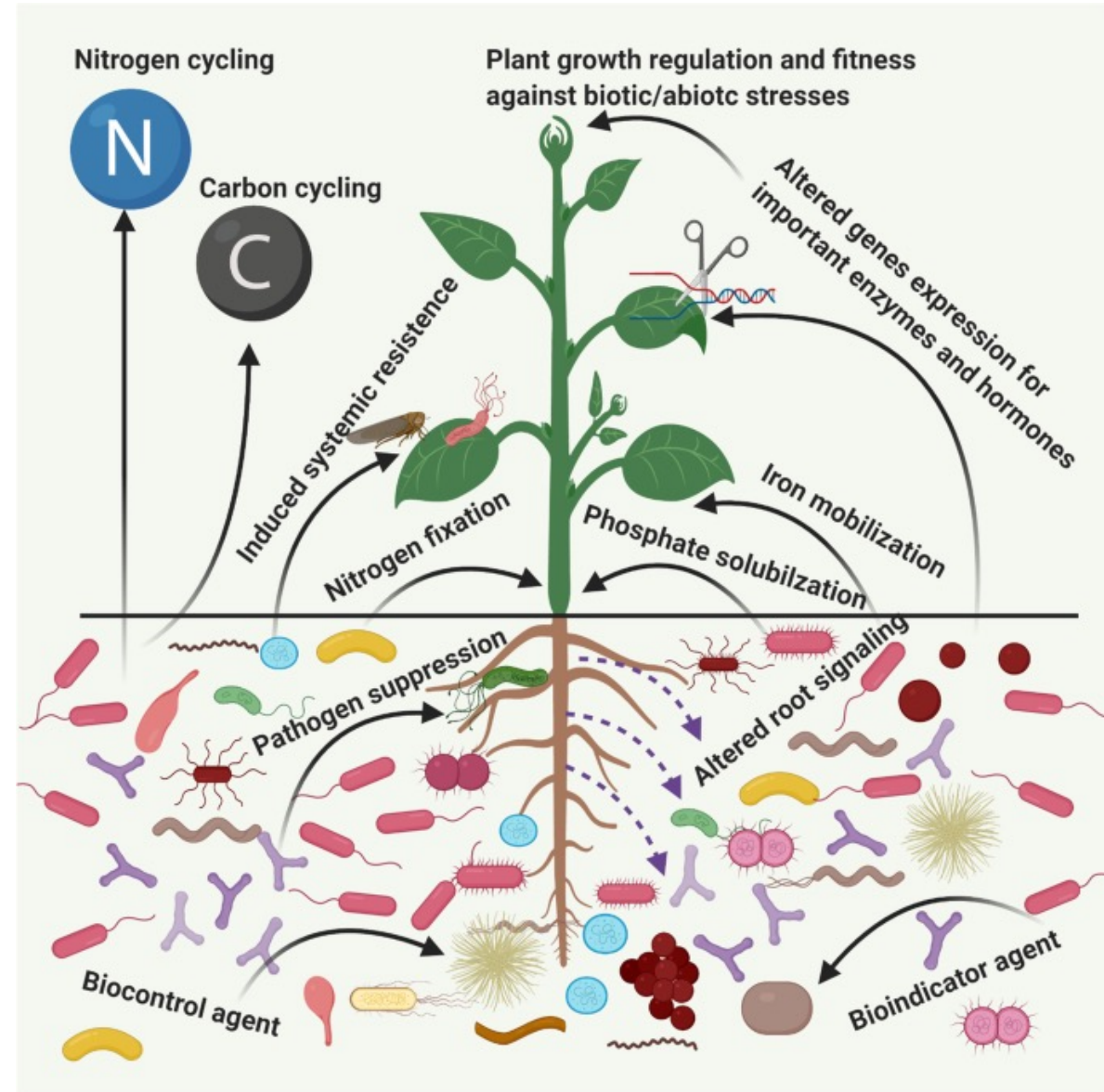
- The community of microorganisms that reside in and around plant roots
- Includes bacteria, fungi, archaea, and viruses
- Commonly consists of Proteobacteria, Actinobacteria, and Bacteroidetes



Healthy root microbiome is beneficial to the plant

How does it affect the plant?

- PGP (plant growth promoting) services
- Competes for resources against pathogens

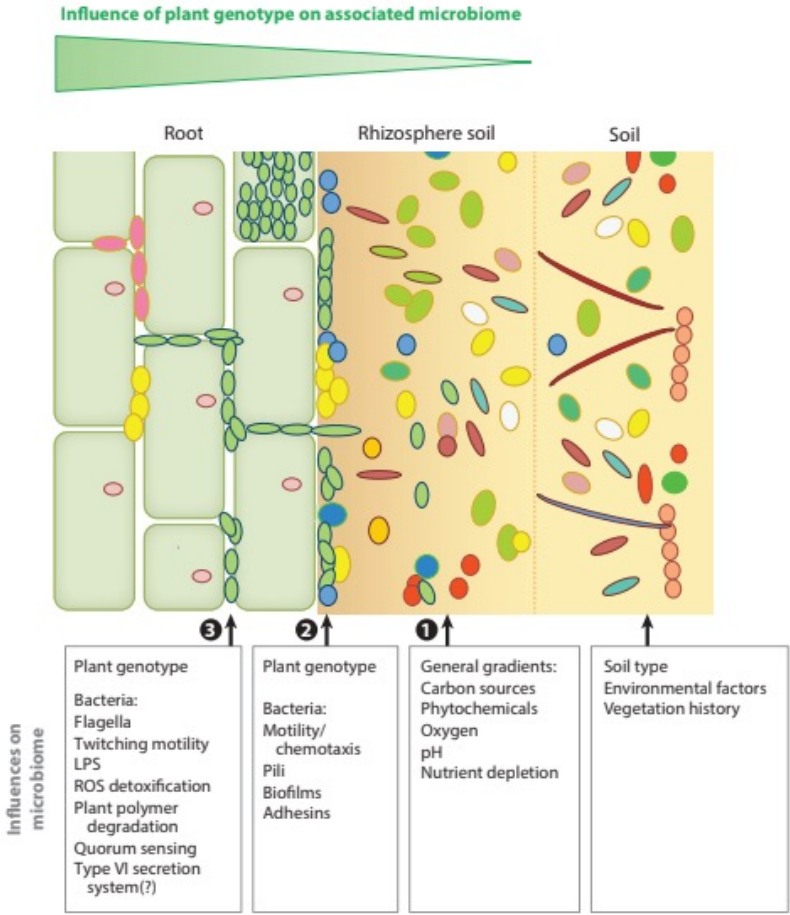


There are different compartments of root microbiome

Bulk soil

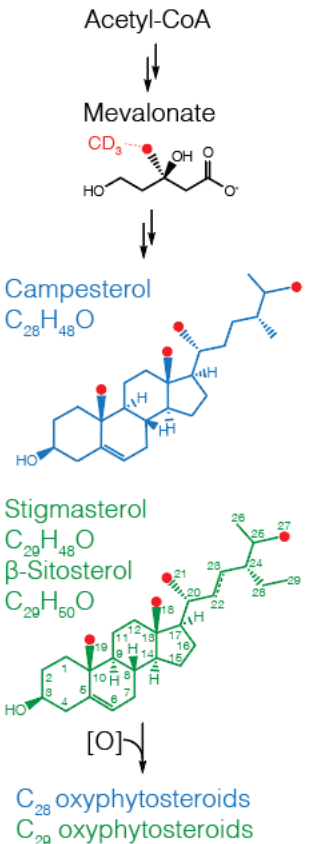
Rhizosphere (soil around the roots)

Endosphere (microbiome within the roots)

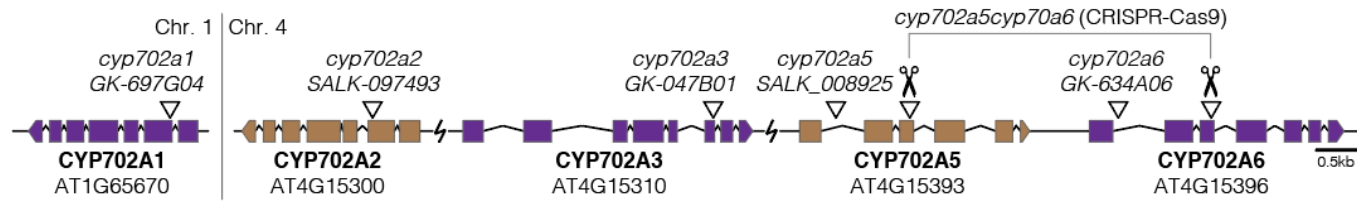


CYP702A genes are associated with phytosteroid production

phytosteroid

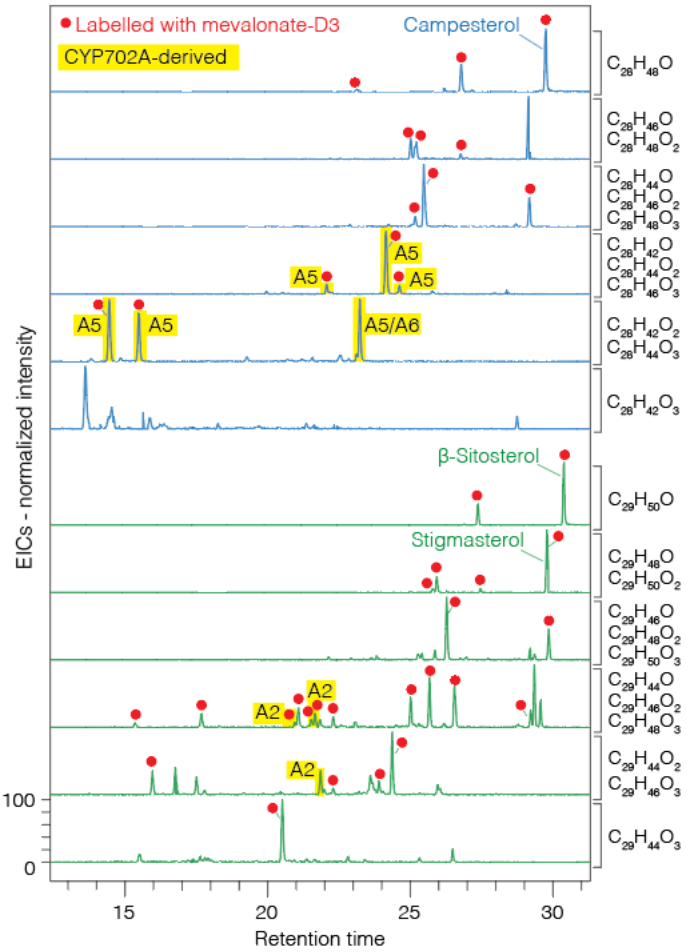
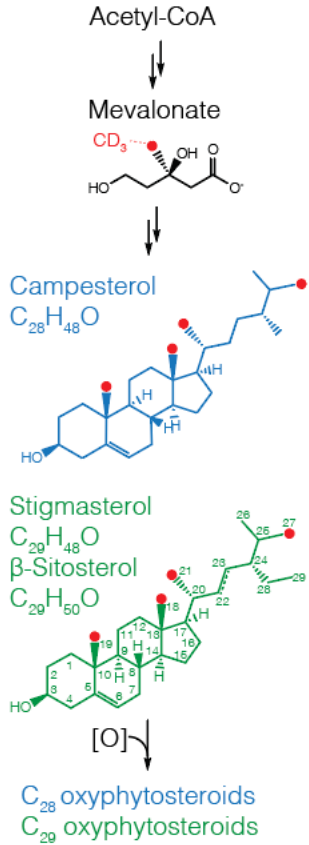


CYP702As (cytochromes P450)

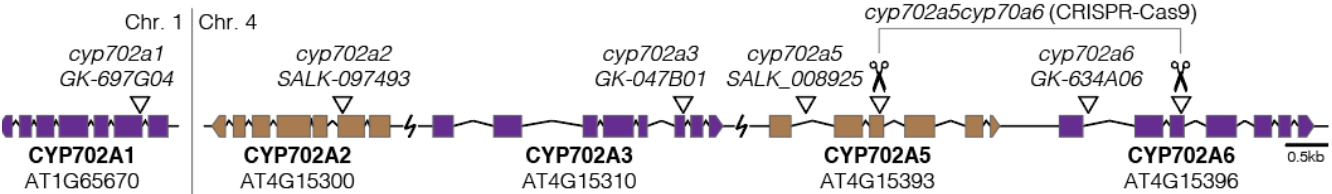


CYP702A genes are associated with phytosteroid production

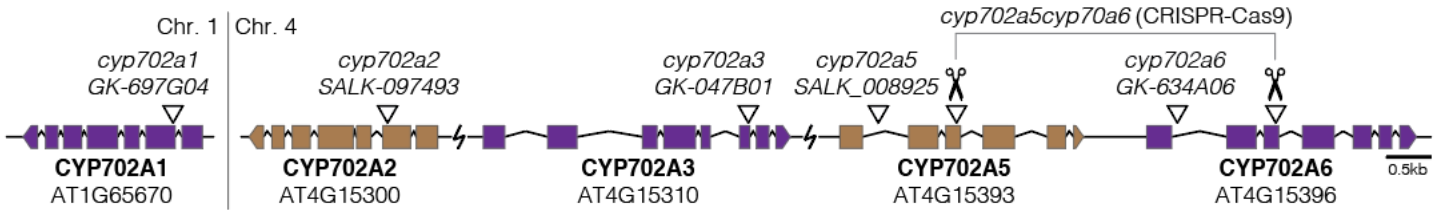
phytosteroid



CYP702As (cytochromes P450)



CYP702A genes are associated with phytosteroid production



T-DNA lines:

- cyp702a1*-TD-GK-697G04
- cyp702a2*-TD-SALK-097493
- cyp702a3*-TD-GK-047B01
- cyp702a5*-TD-SALK-008925
- cyp702a6*-TD-GK-634A06

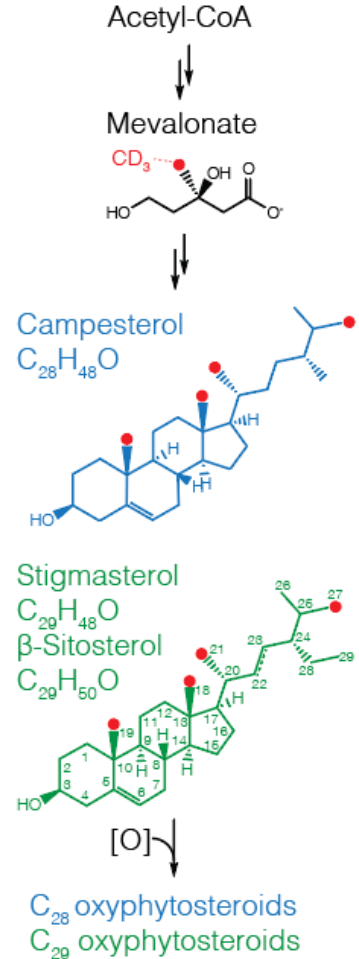
Knockout lines:

- cyp702a5cyp702a6*
- cyp702a1cyp702a5cyp702a6*
- cyp702a2cyp702a3cyp702a5cyp702a6*
- cyp702a1cyp702a2cyp702a3cyp702a5cyp702a6*
(Quintuple mutant)

Knockdown lines:

- CYP702A2 in *cyp702a5cyp702a6*
- CYP702A3 in *cyp702a5cyp702a6*
- CYP702A2CYP702A3 in *cyp702a5cyp702a6*
- CYP702A2 in *cyp702a1cyp702a5cyp702a6*
- CYP702A3 in *cyp702a1cyp702a5cyp702a6*
- CYP702A2CYP702A3 in *cyp702a1cyp702a5cyp702a6*

No consistent phenotype!



Do phytosteroids produced by the CYP702 enzymes affect the Arabidopsis root microbiome?

Experimental Design

2 types of soil



2 types of soil from Jingke's garden (A and B)

8 Arabidopsis genotypes



1. WT
2. CYP702 quintuple mutant
3. CYP702A5 overexpression
4. CYP702A6 overexpression
5. CYP87 mutant
6. CYP87 CDS2 overexpression
7. CYP87 CDS3 overexpression
8. DpCYP87 overexpression

4 replicates



64 pots total
- 32 with soil A
- 32 with soil B
8 pots per genotype
5 plants per pot

Pots labelled A1, A2, B1, B2, etc.

1 developmental stage



Harvest after 4-6 weeks

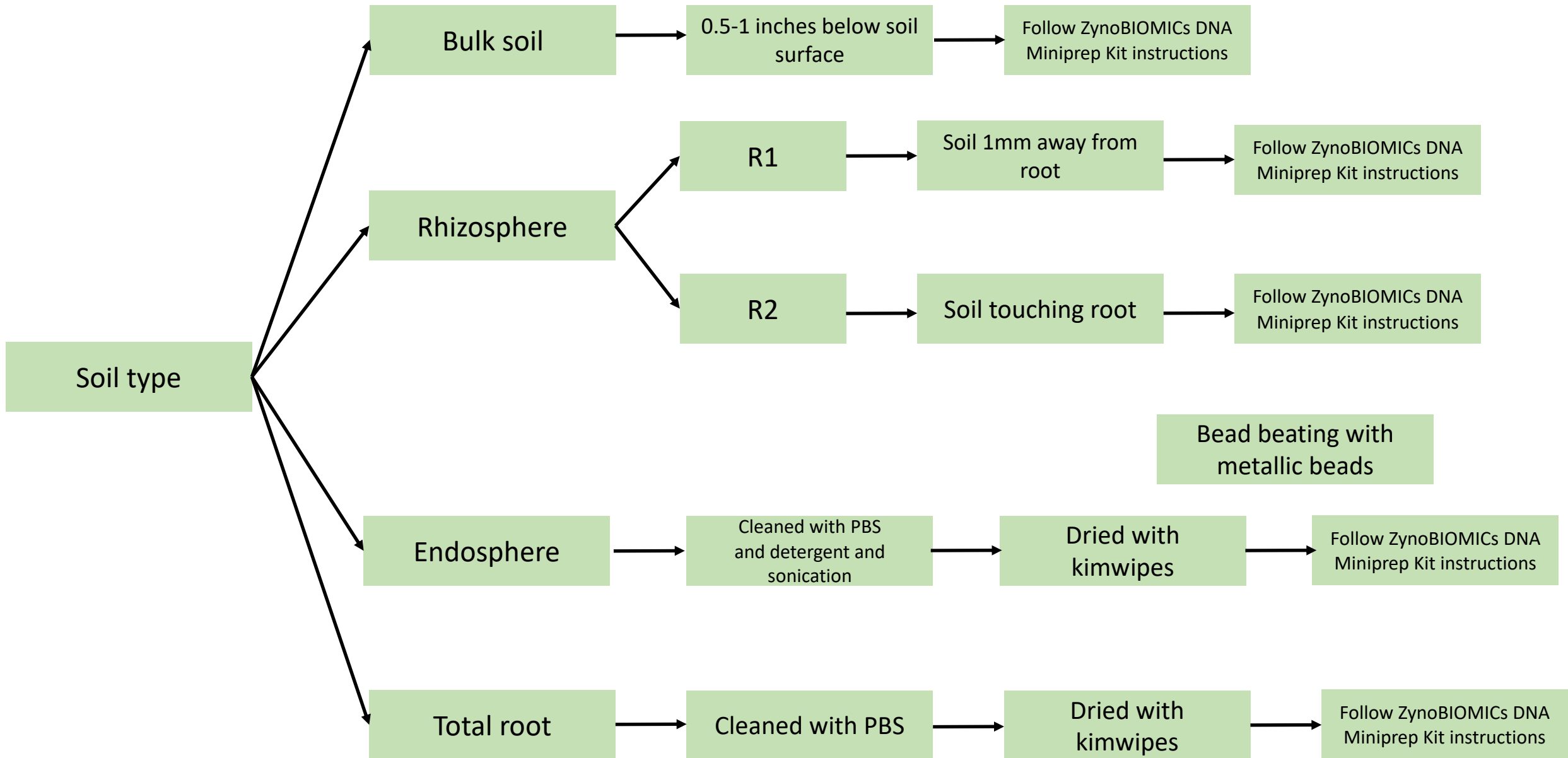
4 types of collection

1. Bulk soil
2. Rhizosphere
3. Endosphere
4. Total root

Microbiome sampling

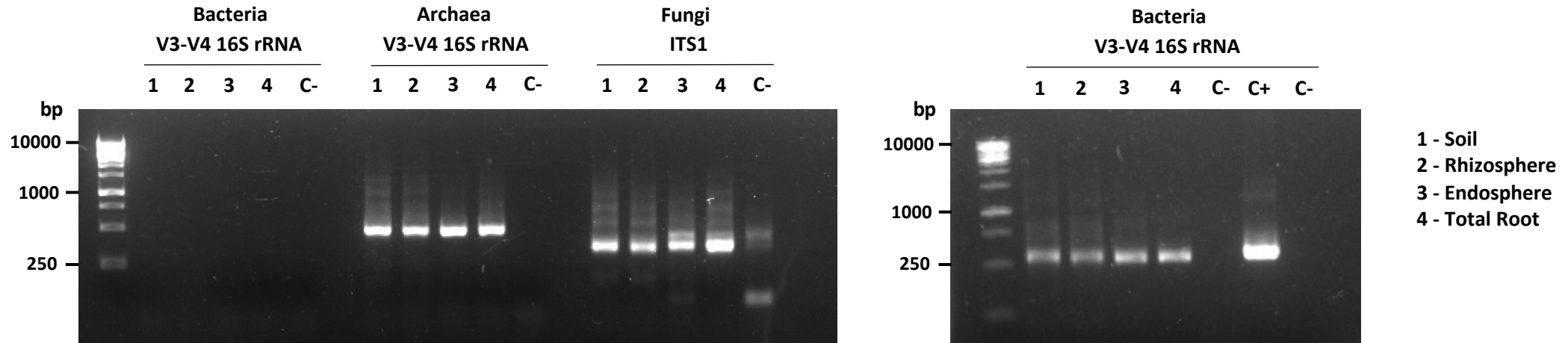


DNA collection



Validation of the DNA extraction protocol

Standardization protocol starting with ~100 mg of soil or ~100 mg of roots



Plant growth progress (3 weeks)



Soil type A



Soil type B

1. WT
2. CYP702 quintuple mutant
3. CYP702A5 overexpression
4. CYP702A6 overexpression
5. CYP87 mutant
6. CYP87 CDS2 overexpression
7. CYP87 CDS3 overexpression
8. DpCYP87 overexpression

Acknowledgements



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