

Vitis hairy root cultures: A versatile biotechnological tool for the analysis of specialized metabolite production and functional genomics

Presenter: Sasha Marco Tietz
Co-authors: Tumelo Moyo, Dr Philip Young & Prof Melane Vivier

Photo by Stefan Els



Hmmmmm...and what
may these hairy roots
be?

Background

Hairy Roots?

- 1930-1960: Sign of plant disease.
- 1970-1980: *Agrobacterium rhizogenes* identified as cause.
- 1980-present: Establishment, development and optimization of hairy root cultures for over 100 plant species.

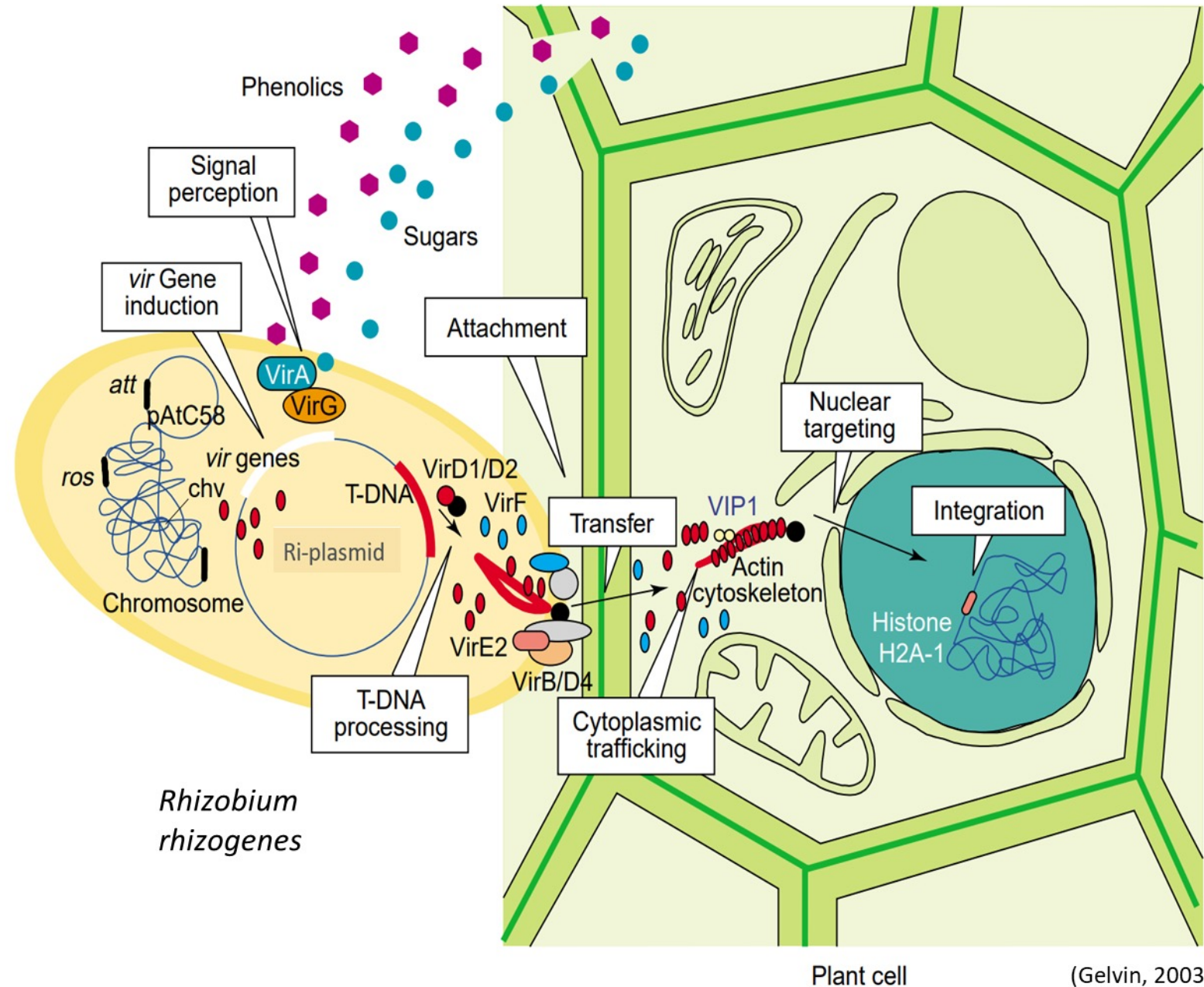


(plantdiseases.org)

Background

Hairy Roots in Nature

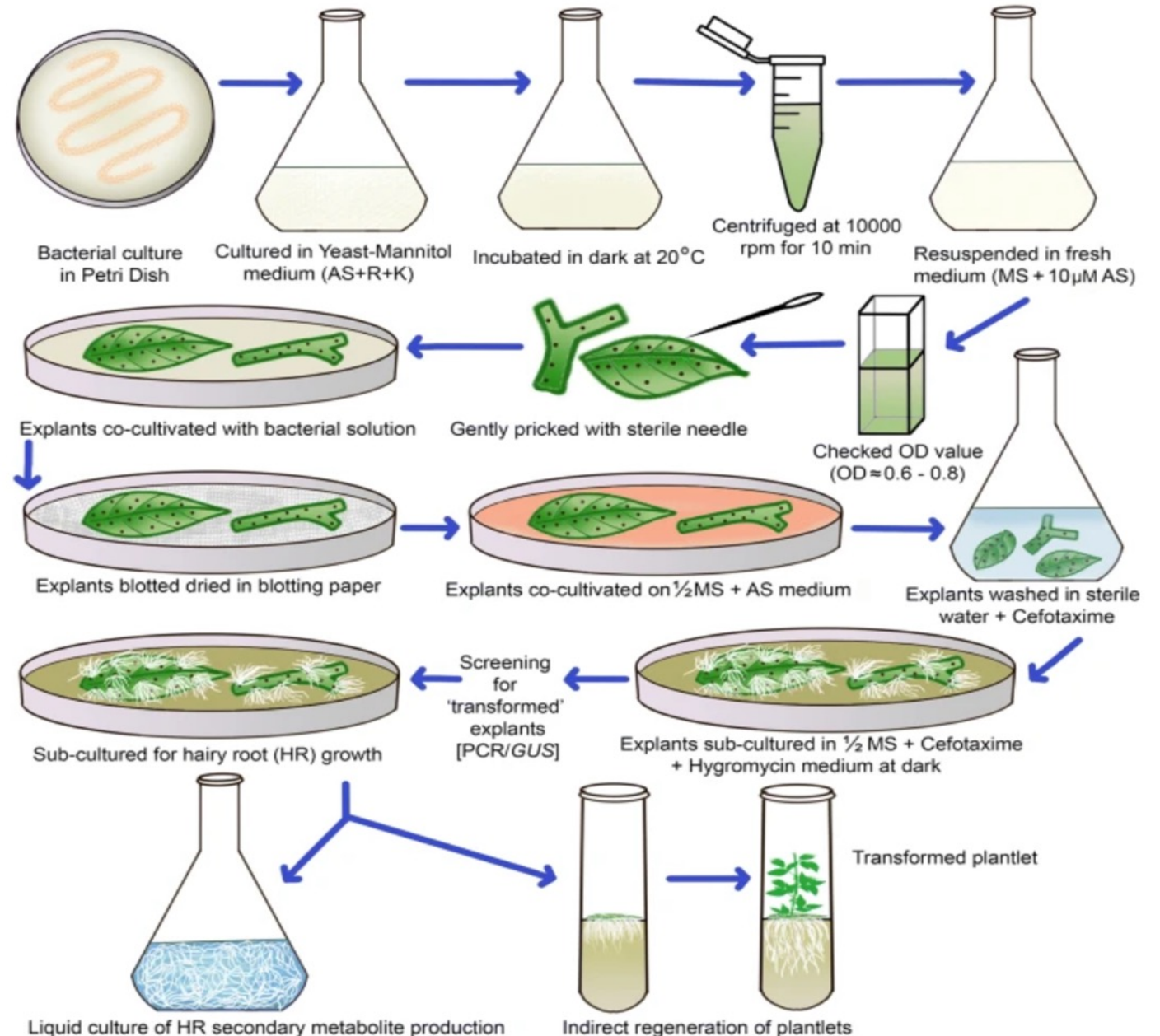
- *R. rhizogenes* infects plant via the integration of T-DNA into plant genome.
- *rol* genes modulate cell differentiation and plant growth – leads to formation of hairy roots at wounded site.
- Hairy roots produce high volume of opines – catabolized by *Rhizobium*



Background

Hairy Roots *in vitro*

- Enhanced growth rate on plant growth regulator free medium.
- Simple method to generate genetically and phenotypically stable transgenic plants/organ.
- Extracellular excretion of certain metabolites – simple purification

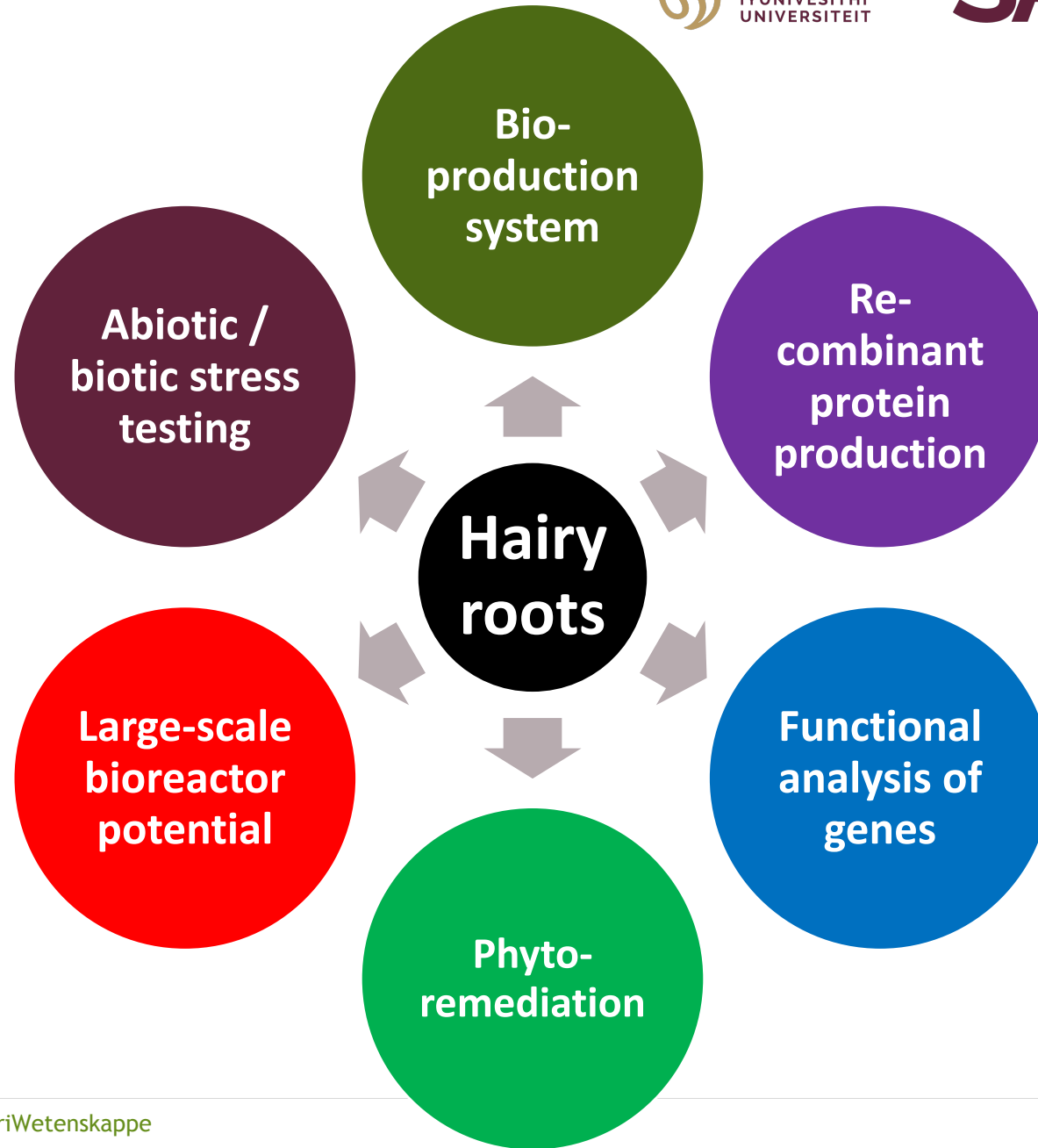




What's the point of
these weird roots?

Background

Applications of Hairy Roots



Generation of *Vitis* Hairy Root Cultures



Vitis vinifera scion cultivars:

- Redglobe
- Sultana
- Chardonnay

Rootstocks cultivars:

- Ramsey (*Vitis champinii*)
- Gravesac (*Vitis berlandieri* × *Vitis riparia* × *Vitis rupestris*)
- 143B (*Vitis riparia* × *Vitis vinifera*)
- SO4 (*Vitis berlandieri* × *Vitis riparia*)

Generation of *Vitis* Hairy Root Cultures

Excised internode method:

Day -30

Generation
of plant
materials



Grapevine *In vitro* plantlet



Generation of *Vitis* Hairy Root Cultures

Excised internode method:

Day -30

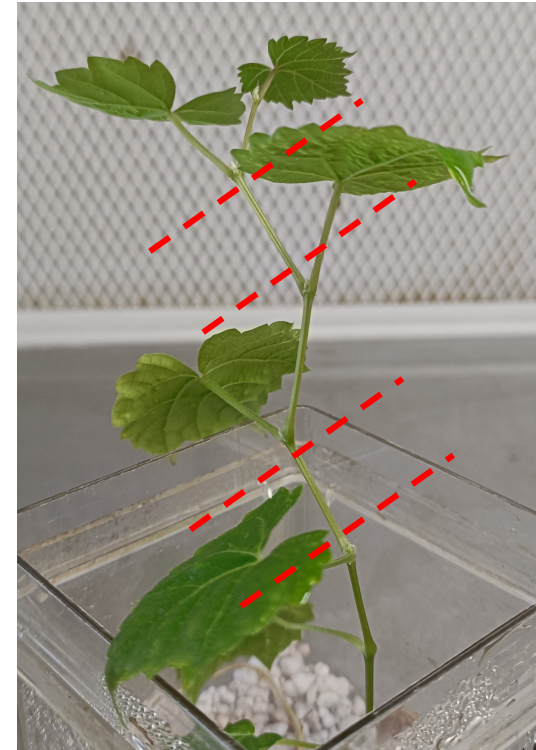
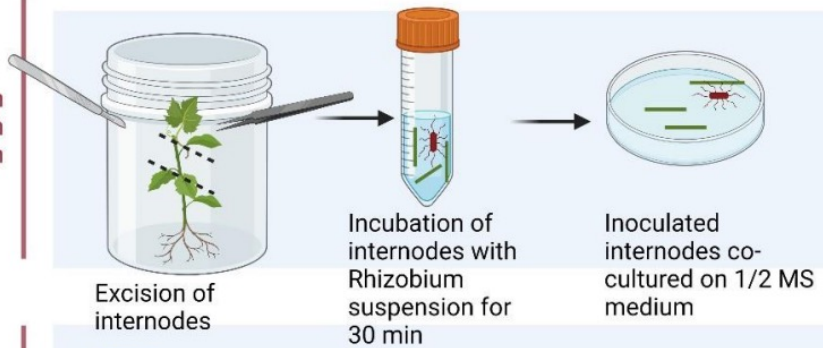
Generation
of plant
materials



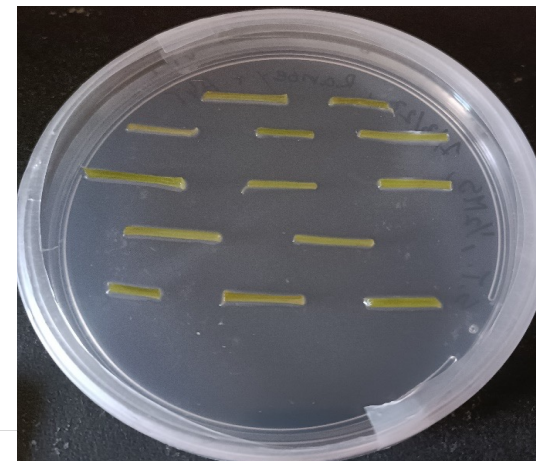
Grapevine *In vitro* plantlet

Day 1

Inoculation
with
Rhizobium



Rhizobium rhizogenes:
Strains: A4T, C58C1 and
LBA9402



Generation of *Vitis* Hairy Root Cultures

Excised internode method:

Day -30

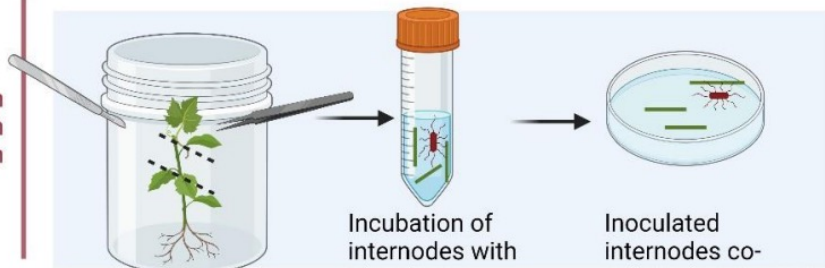
Generation
of plant
materials



Grapevine *In vitro* plantlet

Day 1

Inoculation
with
Rhizobium



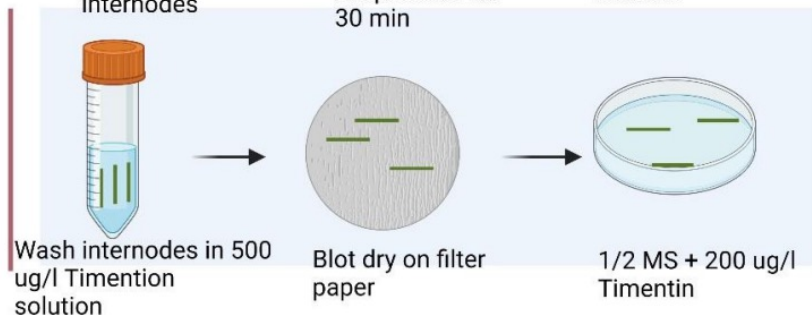
Excision of internodes

Incubation of internodes with Rhizobium suspension for 30 min

Inoculated internodes co-cultured on 1/2 MS medium

Day 3

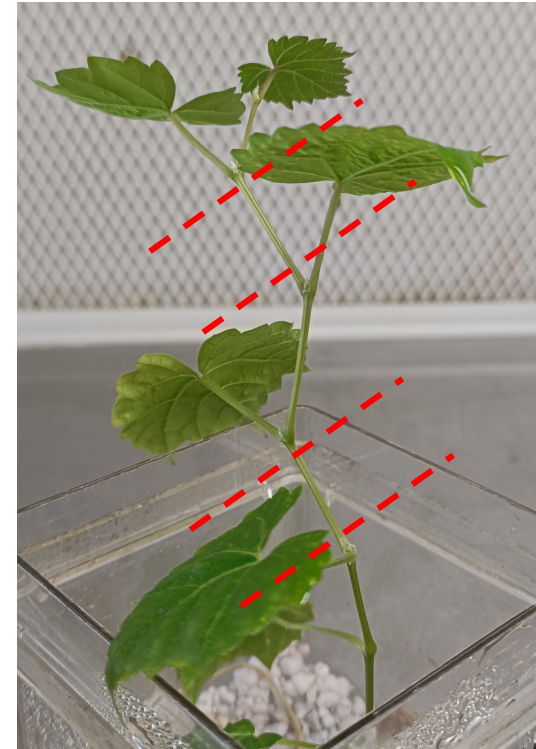
Rhizobium
elimination



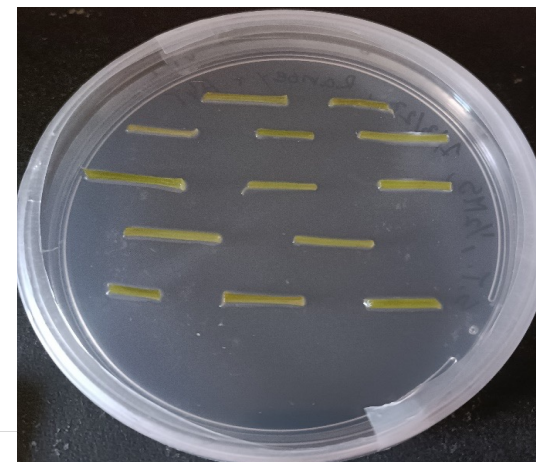
Wash internodes in 500 ug/l Timention solution

Blot dry on filter paper

1/2 MS + 200 ug/l Timentin



Rhizobium rhizogenes:
Strains: A4T, C58C1 and
LBA9402



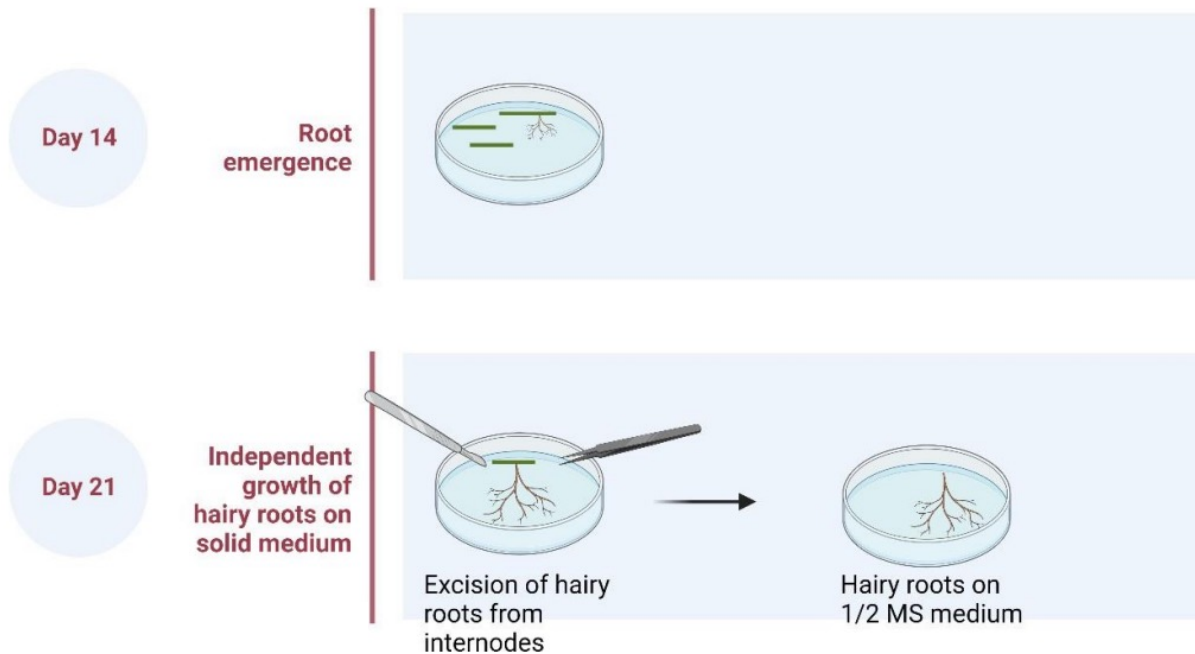
Generation of *Vitis* Hairy Root Cultures



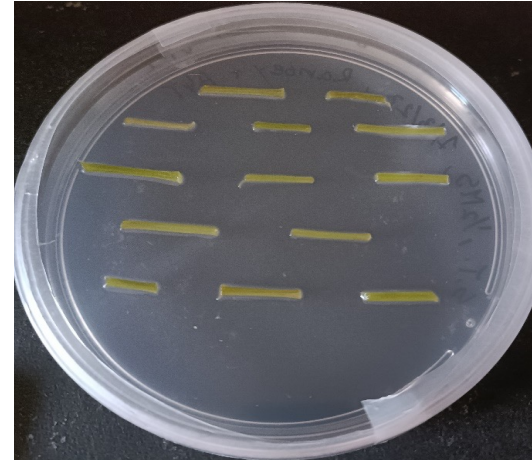
Stellenbosch
UNIVERSITY
IYUNIVESITHI
UNIVERSITEIT

forward together
sonke siya phambili
saam vorentoe

Excised internode method:



(created by Tumelo Moyo with BioRender.com)



2 days after infection



2 weeks after infection



3 weeks after infection



Day of excision

Generation of *Vitis* Hairy Root Cultures

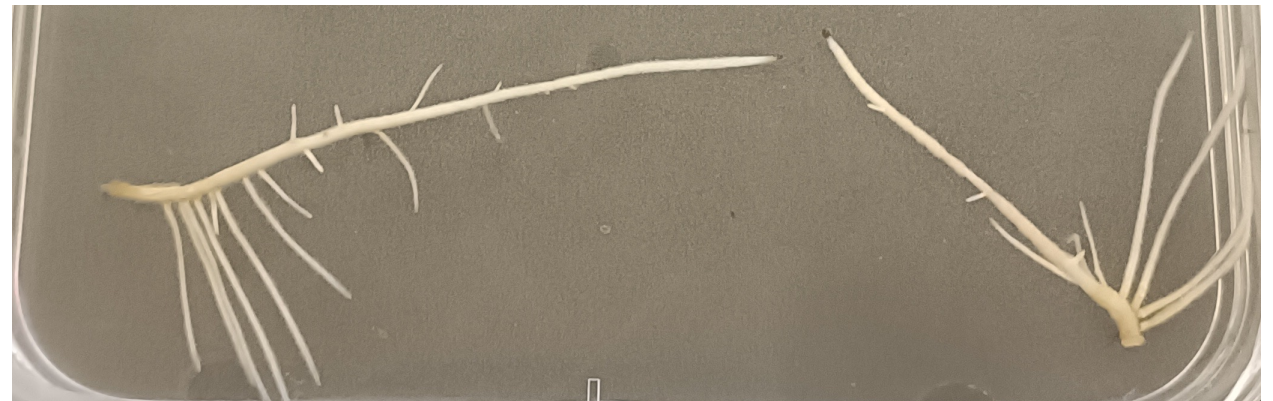
Hairy Root Development:



Day of excision from explant



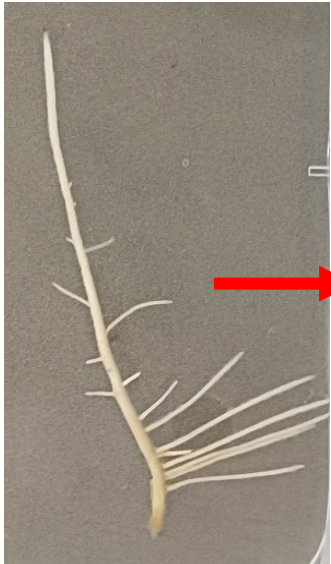
1 week after excision



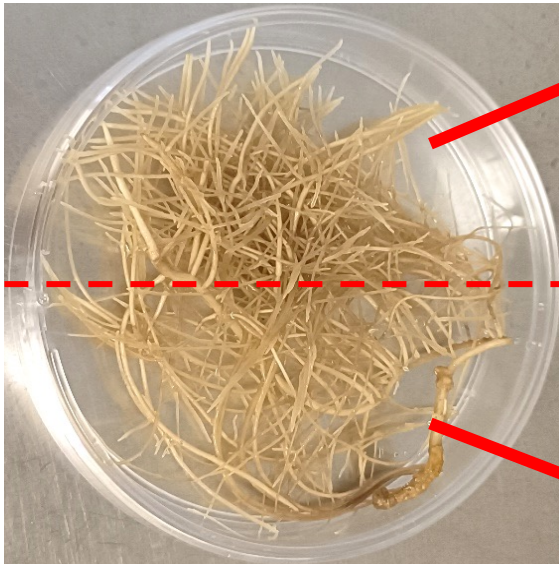
2 weeks after excision

Generation of *Vitis* Hairy Root Cultures

Hairy Root Development:



1: Initial weight 74 mg, final weight 3.841 g



1.1: initial weight 1.358 g, final weight 13.903 g



1.1: Weight 46.011 g



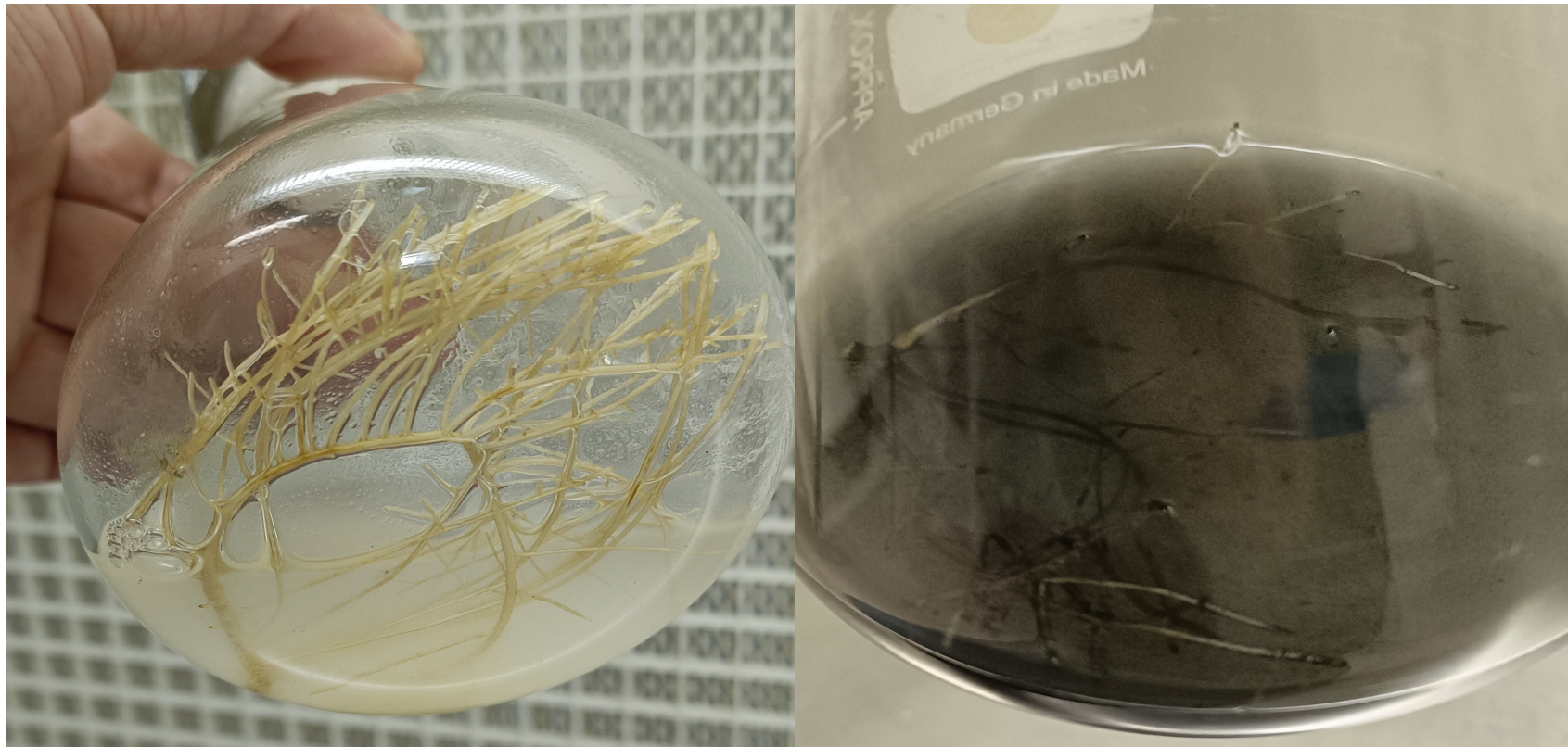
1.2: initial weight 2.158 g, final weight 18.258 g



1.2: Weight 38.174 g

Generation of *Vitis* Hairy Root Cultures

Anti-browning agent testing:



PVPP and L-glutamine

Activated charcoal

Generation of *Vitis* Hairy Root Cultures

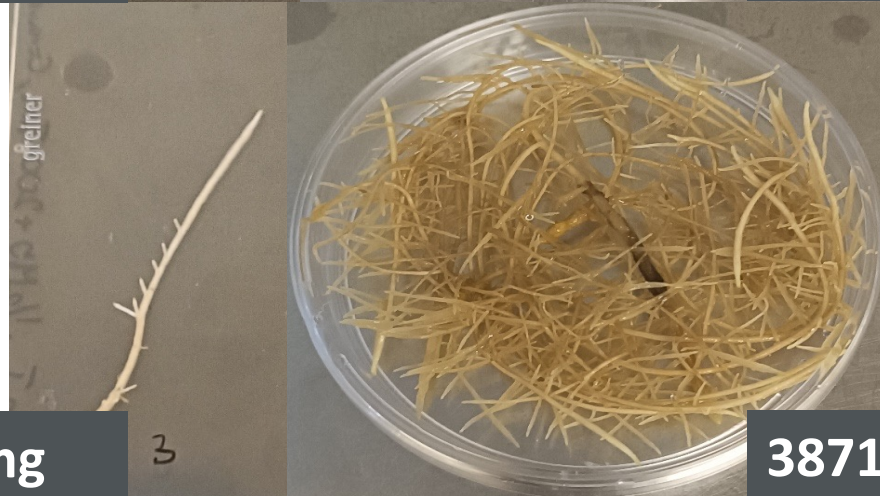
Anti-browning agent testing:

PVPP and L-glutamine



74 mg

3841 mg



41 mg

3871 mg

Activated charcoal



61 mg

1110 mg

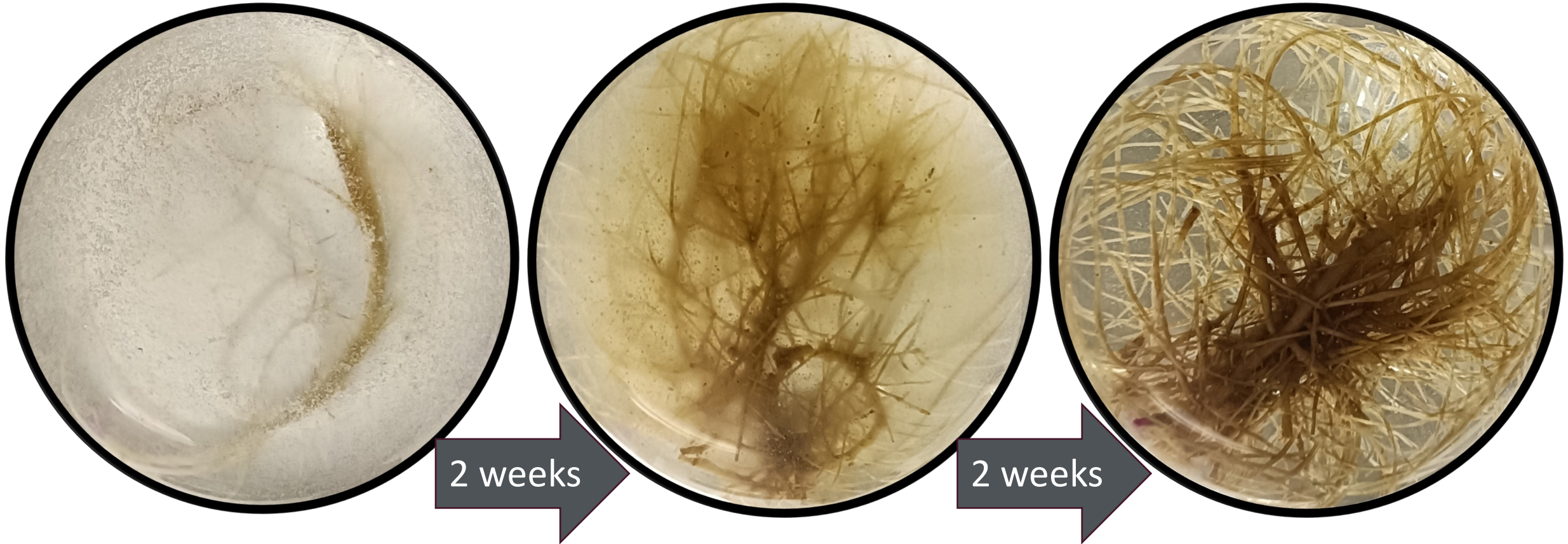


34 mg

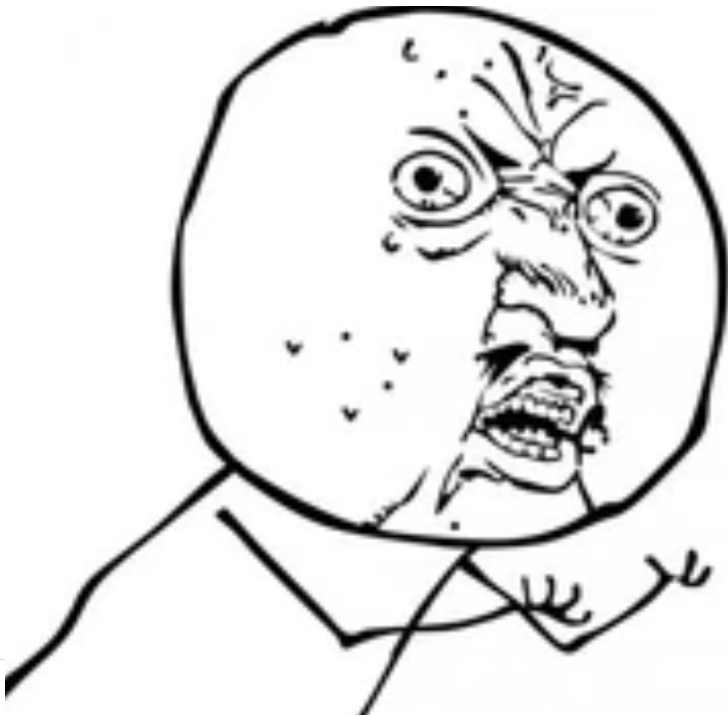
318 mg

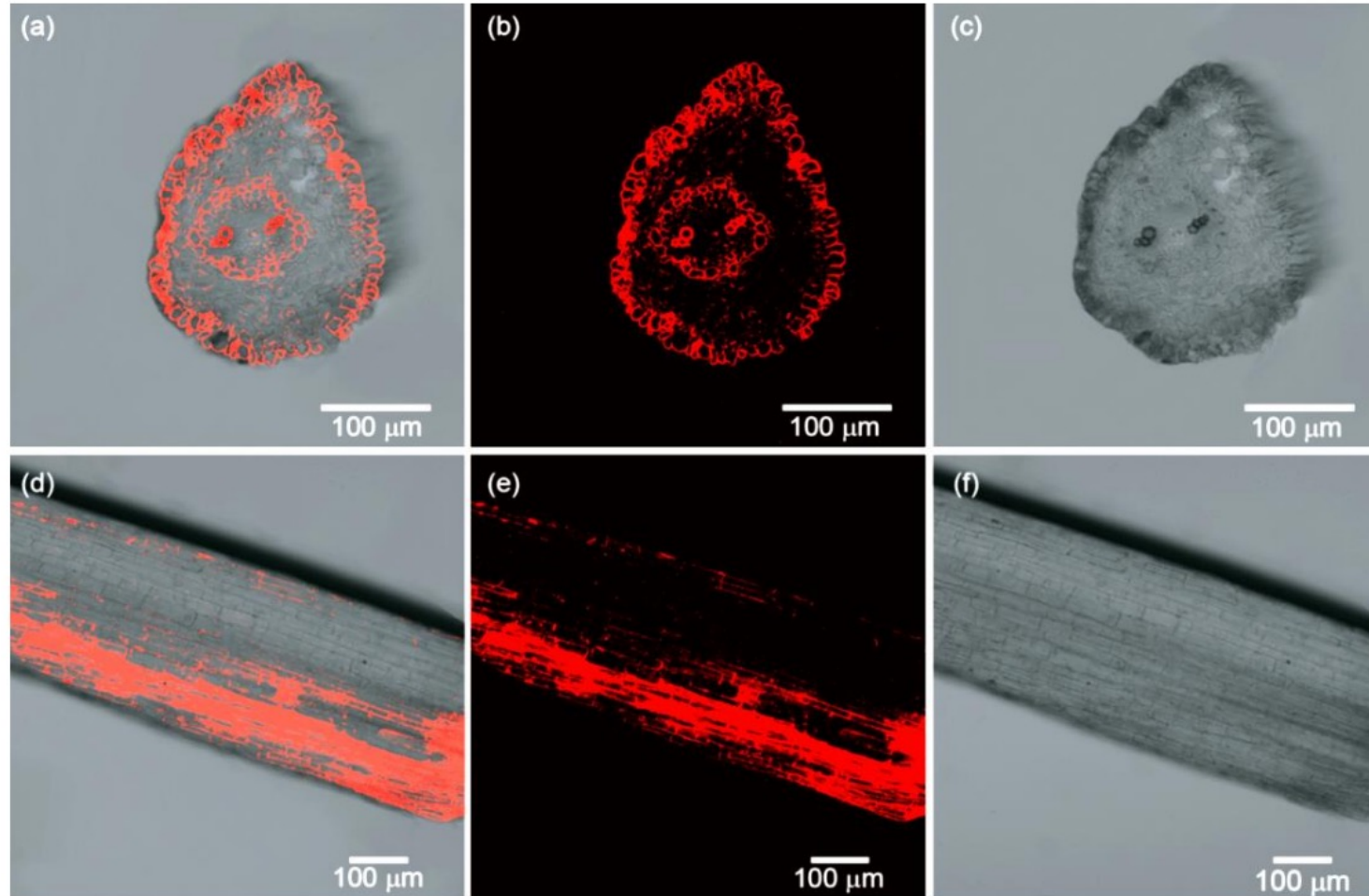
Generation of *Vitis* Hairy Root Cultures

Anti-browning agent testing:



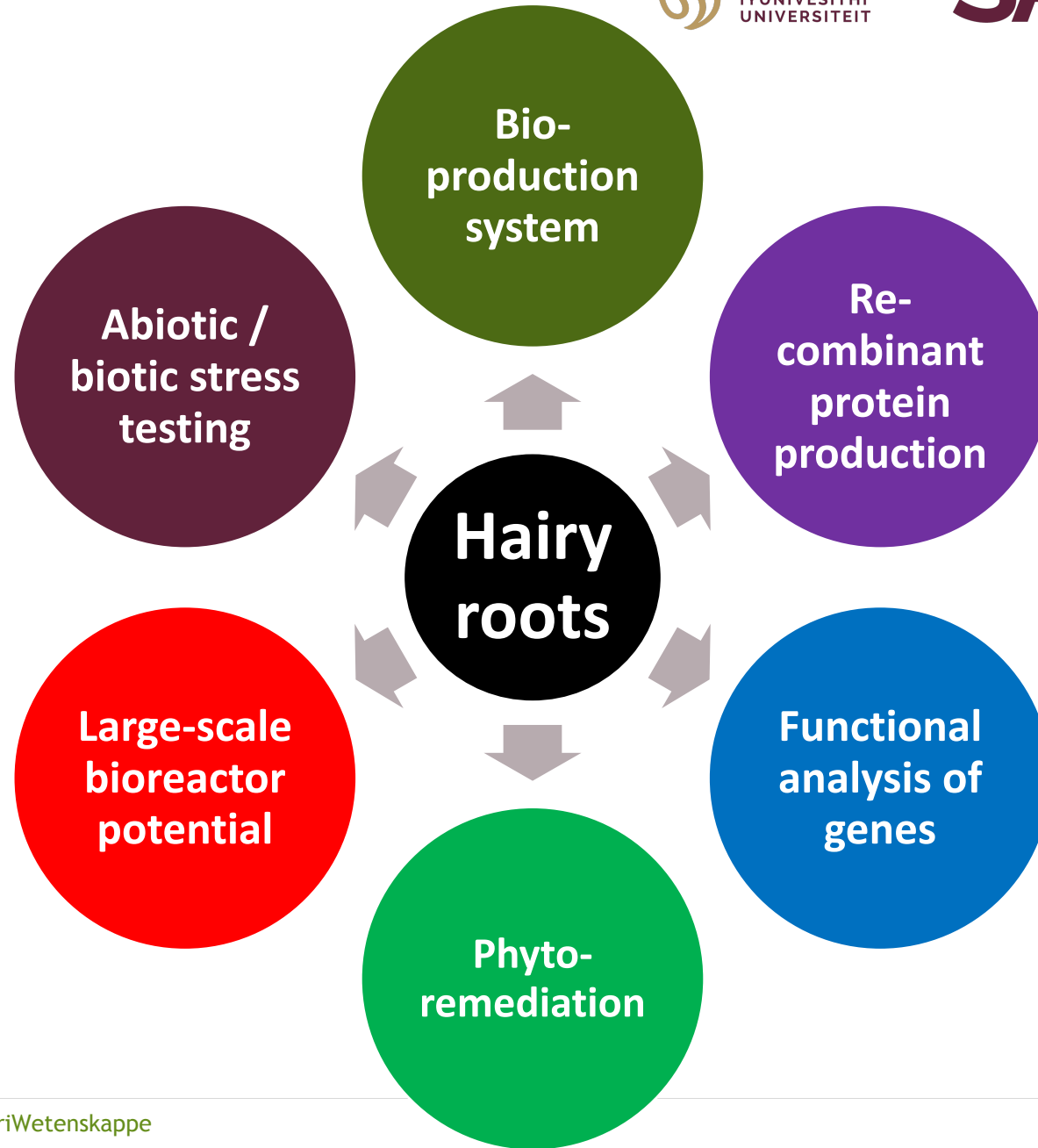
WHERE YOU GO
PVPPP????!!!





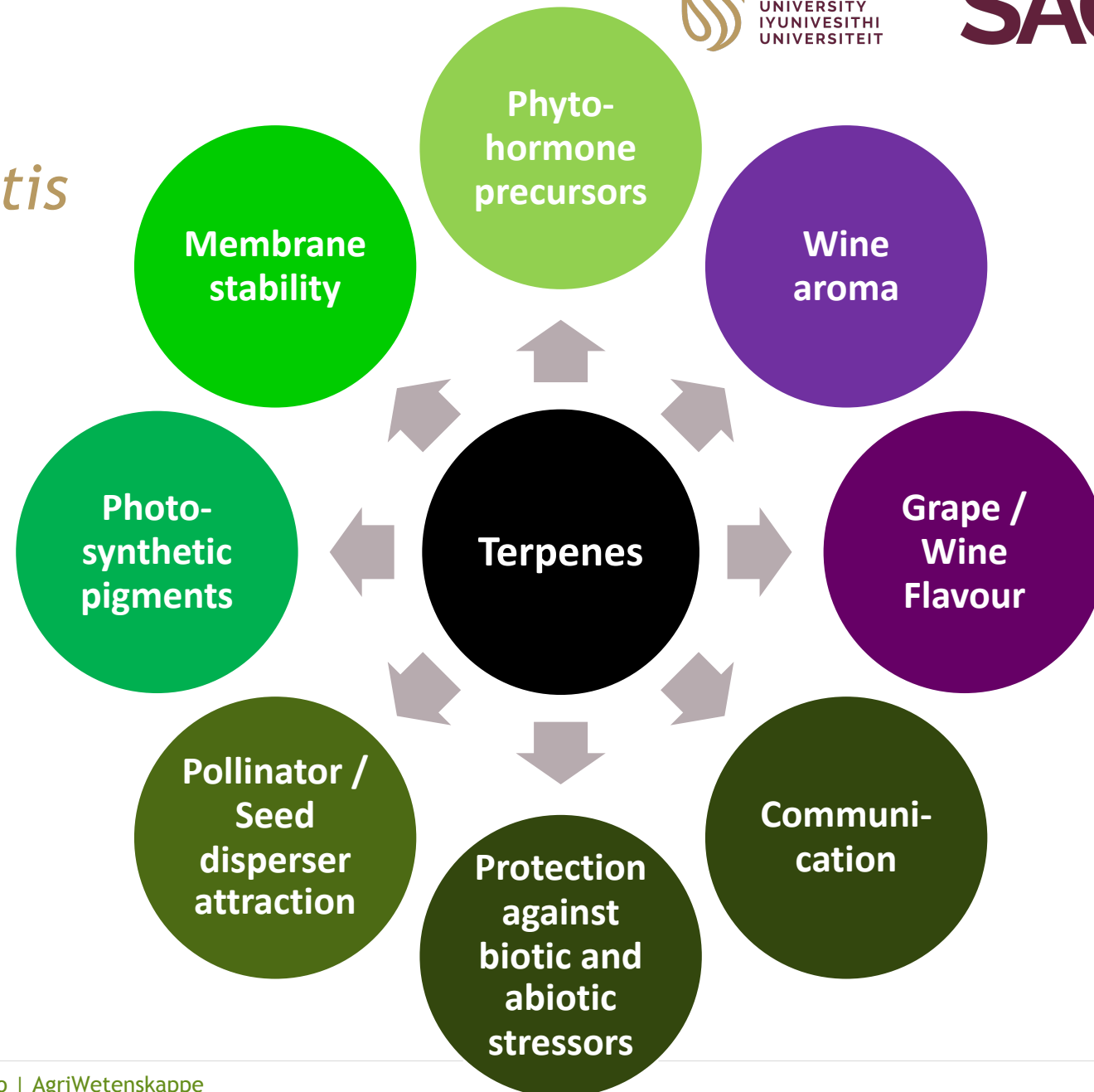
Background

Applications of Hairy Roots



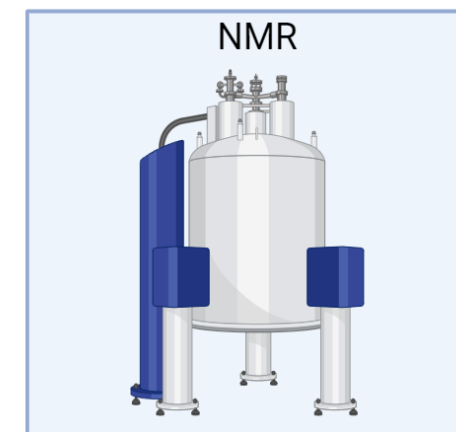
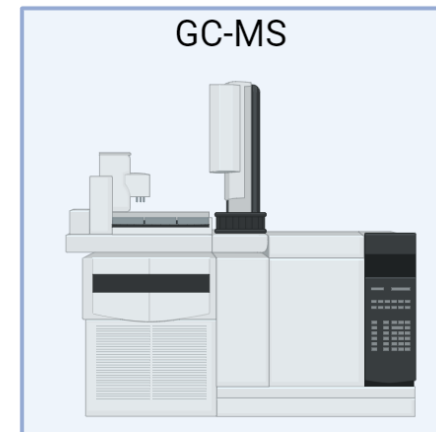
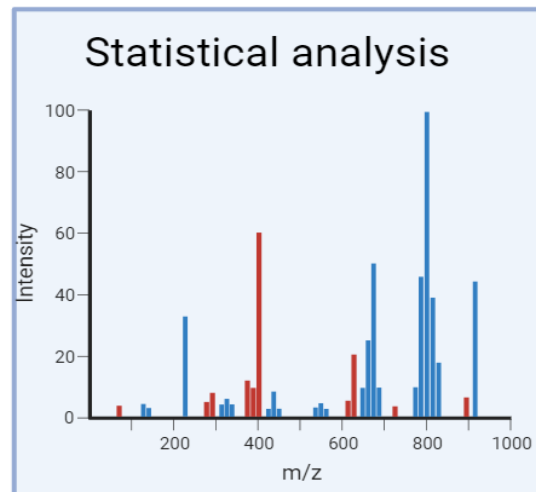
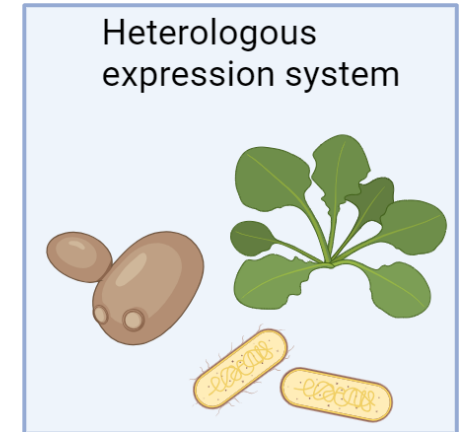
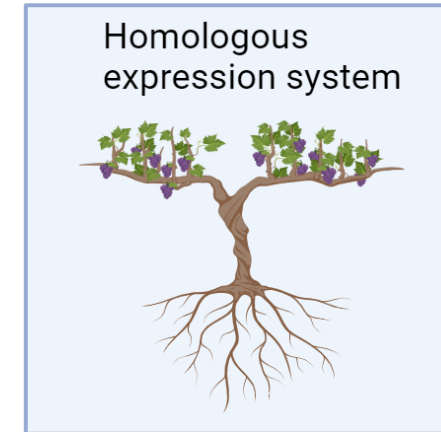
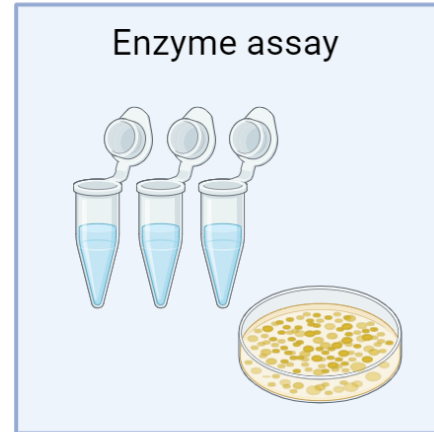
Background

Function of Terpenes in *Vitis*



Background

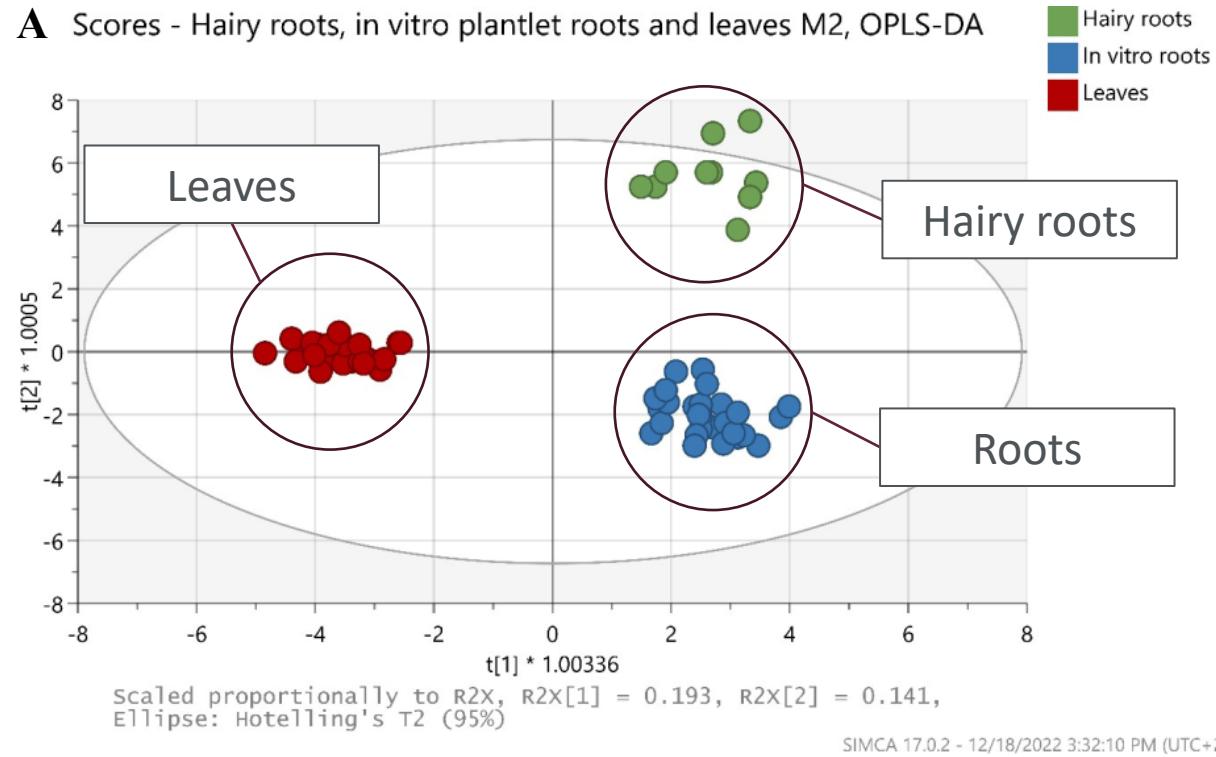
Functional characterization of Terpene synthases



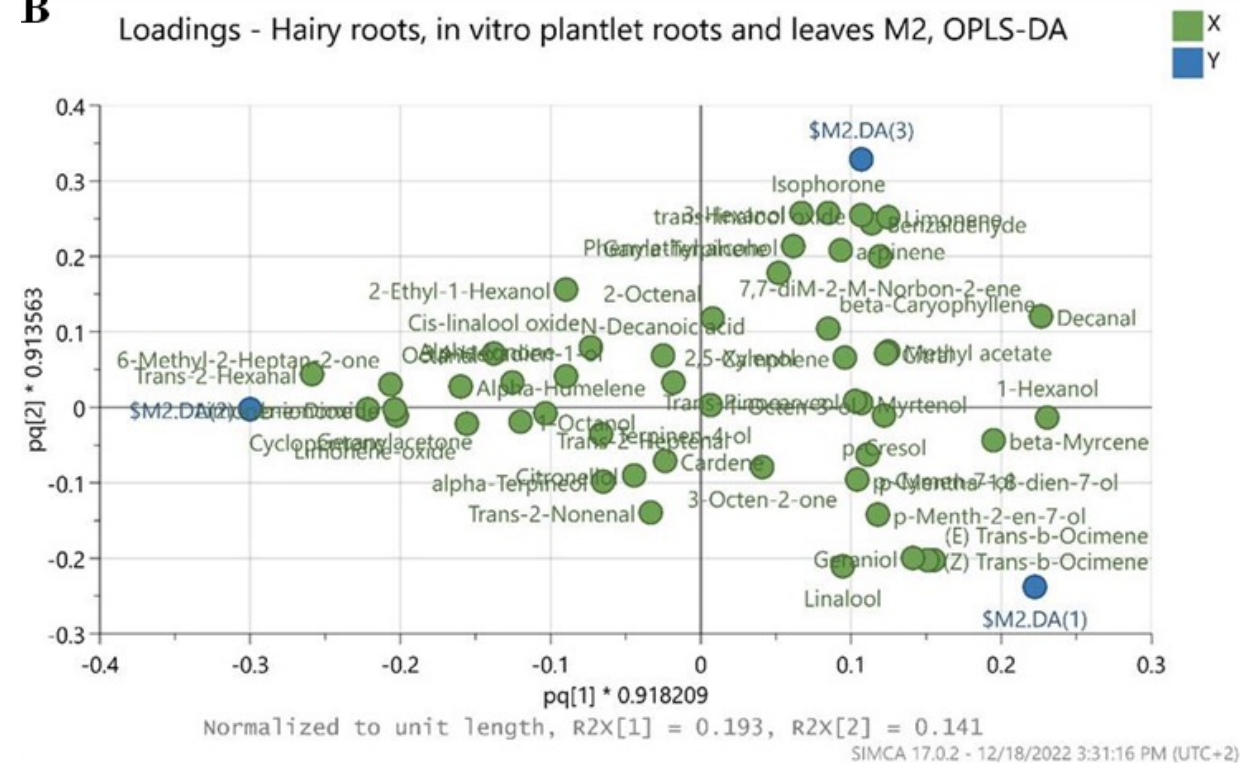
Baseline Terpene Profiles

Spatiotemporal Terpene synthase gene expression

A Scores - Hairy roots, in vitro plantlet roots and leaves M2, OPLS-DA



B Loadings - Hairy roots, in vitro plantlet roots and leaves M2, OPLS-DA

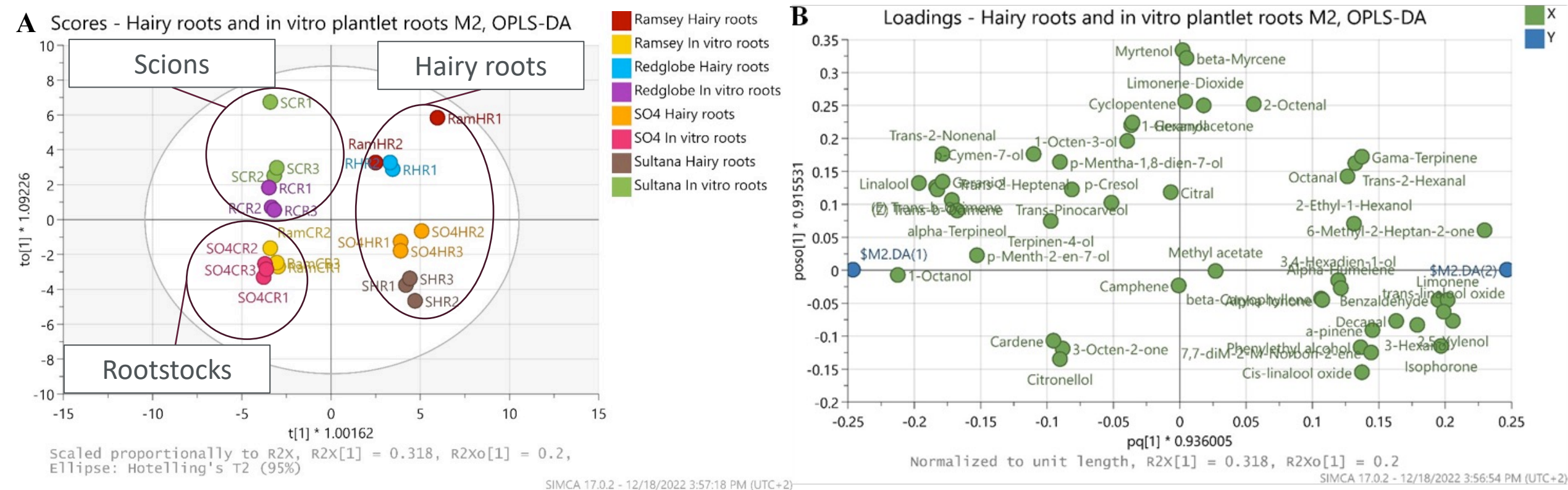


Hairy roots distinct from *in vitro* plantlet roots

- Plant-microbe interactions trigger defense responses in plants (Kiselev et al, 2007)
- The *rol* genes activate the transcription of defense genes even in the absence of plant defense hormones (Bulgakov, 2008)

Baseline Terpene Profiles

Spatiotemporal Terpene synthase gene expression



In vitro plantlet roots

- Scion/rootstock clustering observed

Hairy roots

- Scion/rootstock clustering not observed

Hairy Roots vs Wild Type Roots



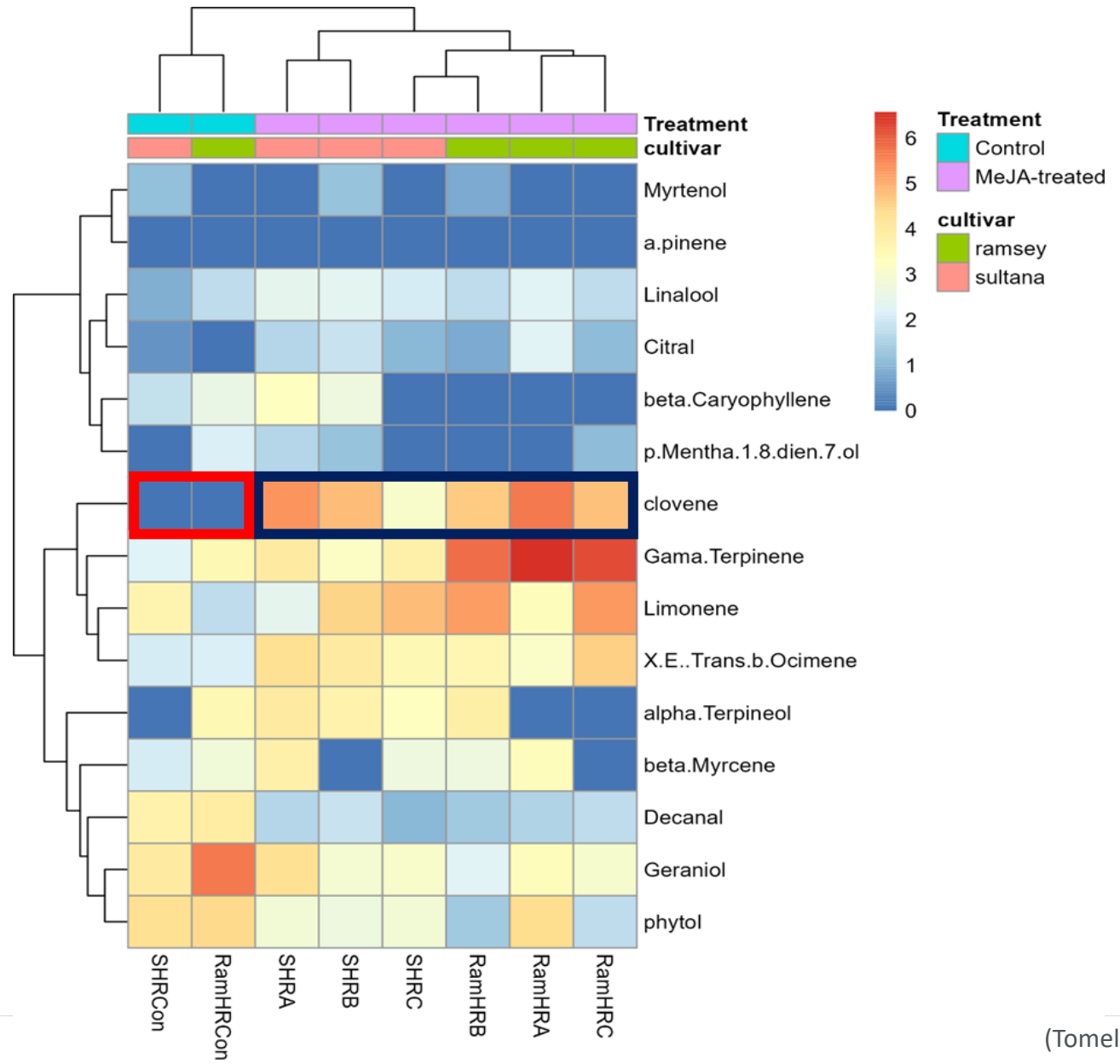
Hairy Roots vs Wild Type Roots



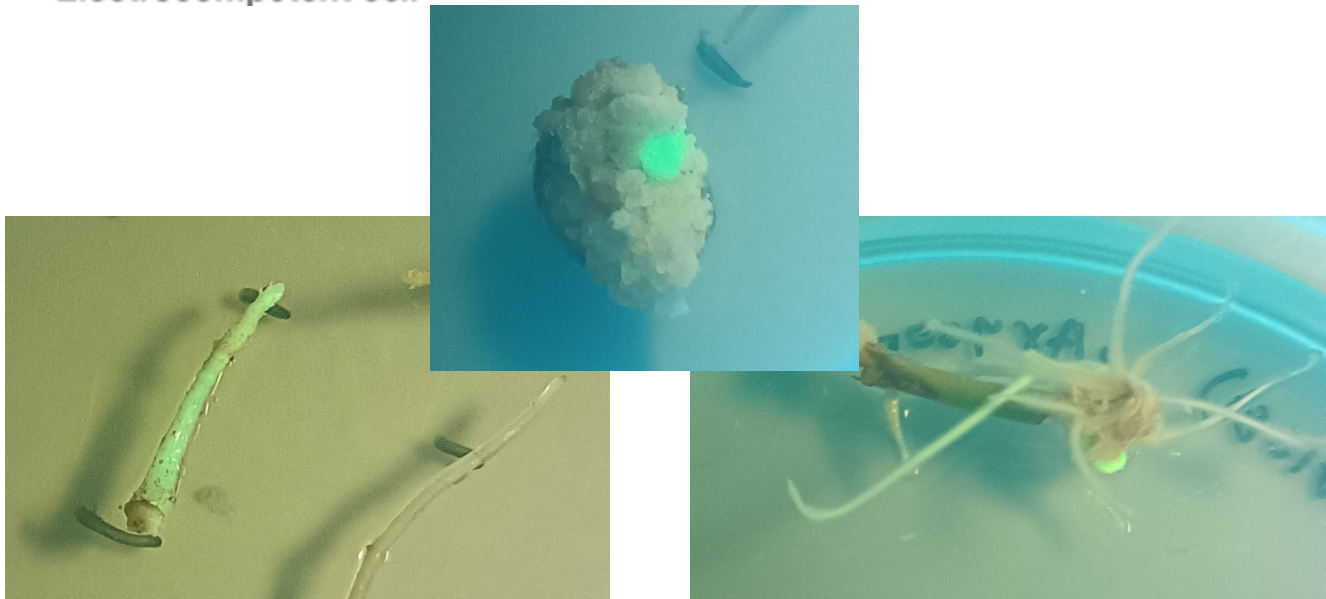
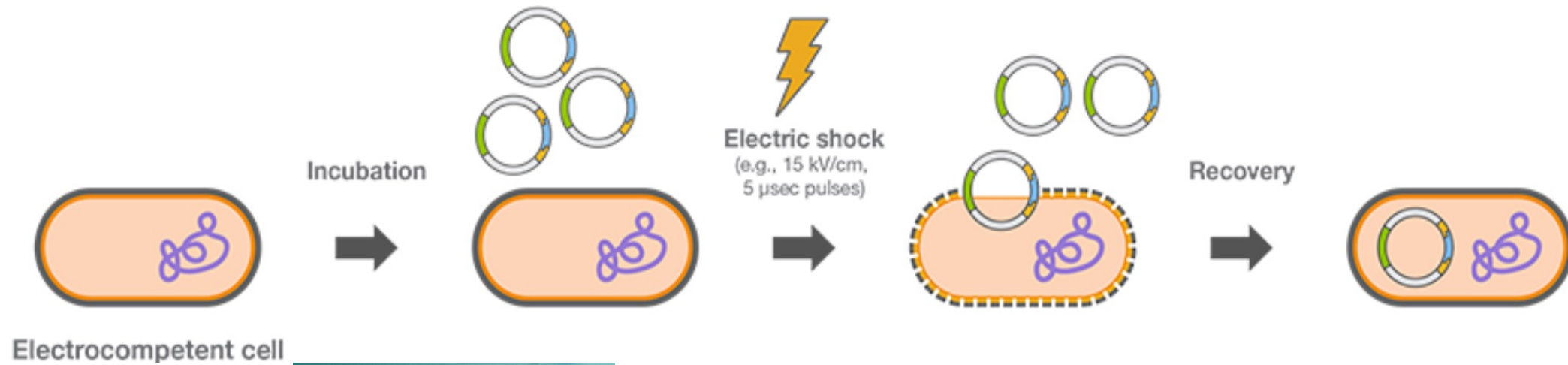
Induced Terpene Production

Untreated Hairy Roots vs MeJA treated Hairy Roots

- Methyl jasmonate (MeJA) treatment induced the accumulation of terpenes in hairy root cultures.



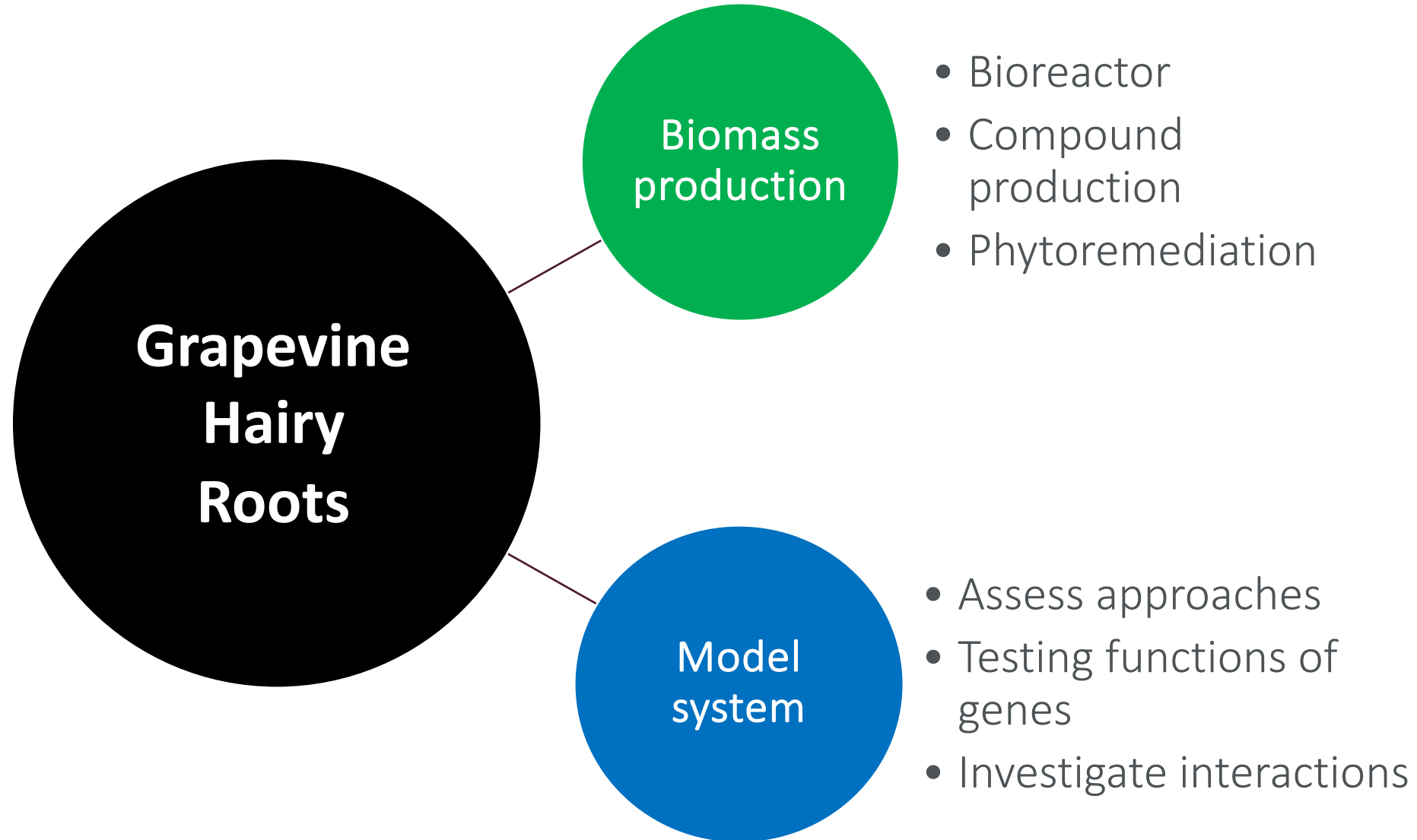
Establishing Transgenic *Vitis* Hairy Root Cultures



Conclusion

- Established a *Rhizobium*-mediated hairy root transformation protocol and generated hairy root cultures of various grapevine genotypes (Sultana, Redglobe, SO4, Ramsey, 143B and Gravesac).
- Tested analytical methods on grapevine tissue and obtained the hairy root baseline terpene profile for Sultana, Redglobe, SO4 and Ramsey grapevine genotypes.
- Showed that growth elicitor MeJA induced the production of selected terpenes in established hairy root cultures.
- Successful transformation of hairy roots provides scope to use the system for testing the functions of genes and constructs before whole plant transformation is implemented.

Future work



Thank you
Enkosi
Dankie



Photo by Stefan Els