



EM2.1:

Increasing sink strength by optimizing xylem parenchyma cell formation in storage roots

The Helariutta lab

University of Helsinki, Finland/

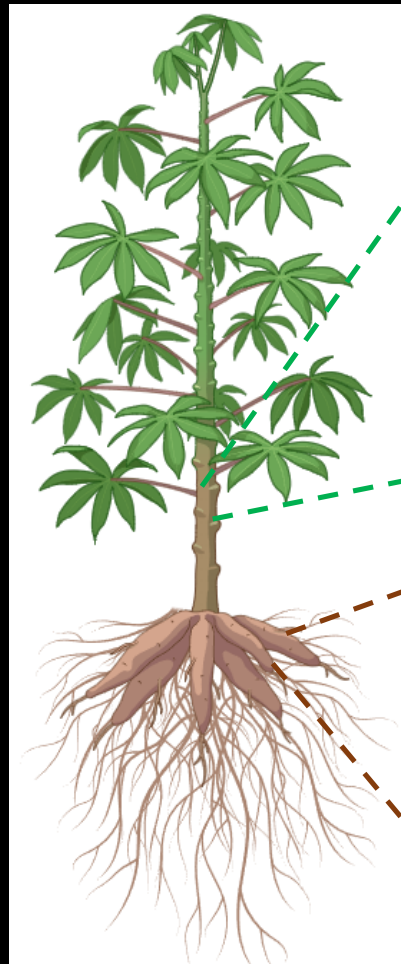
The Wolfgang team

The division of biochemistry, FAU, Germany

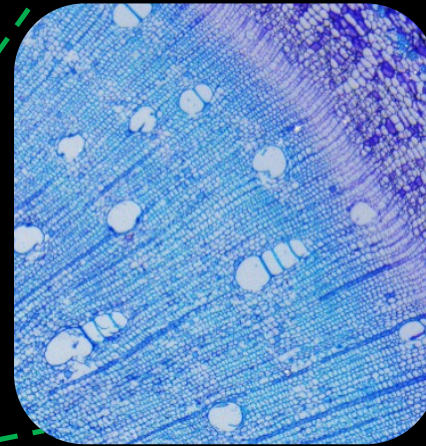


Arabidopsis with enhanced
secondary growth
pSAC51::n3GFP and Fuchsin staining

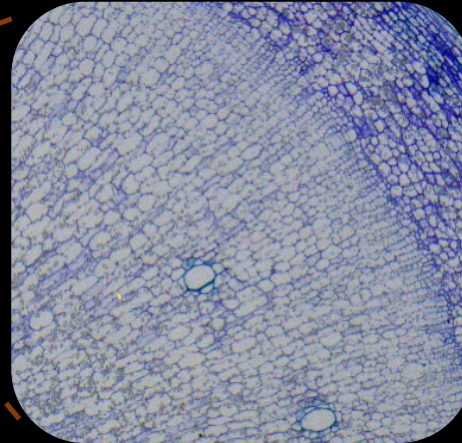
Plant Vascular Tissues - Connecting Tissue Comes in All Shapes



Stem

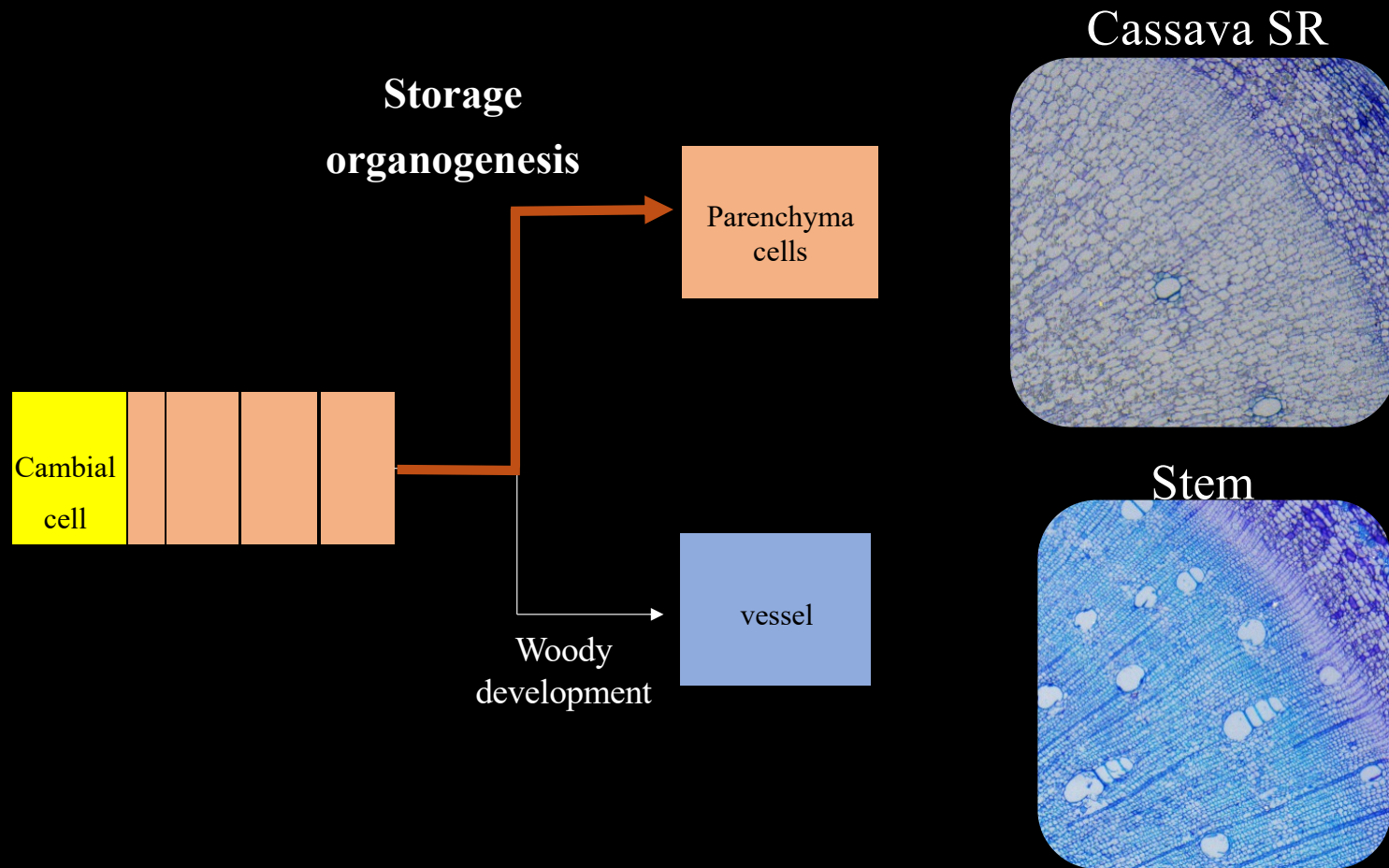


Cassava SR



Cassava images from the Wolfgang team, FAU

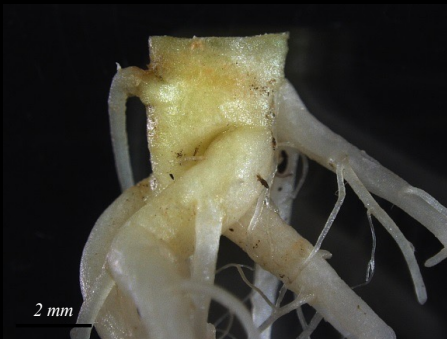
What are the key factors involved in the storage organogenesis?



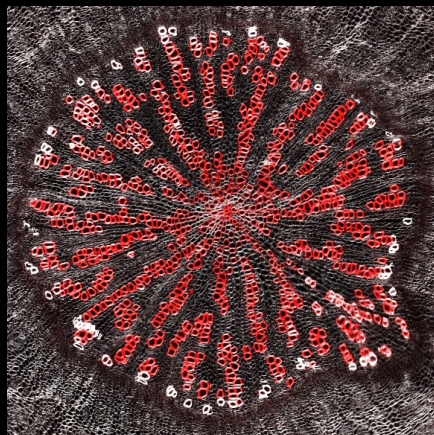
Cassava images from the Wolfgang team, FAU

Grafting between the *ovac* mutant and wild type generates a radish-like structure, Aradish

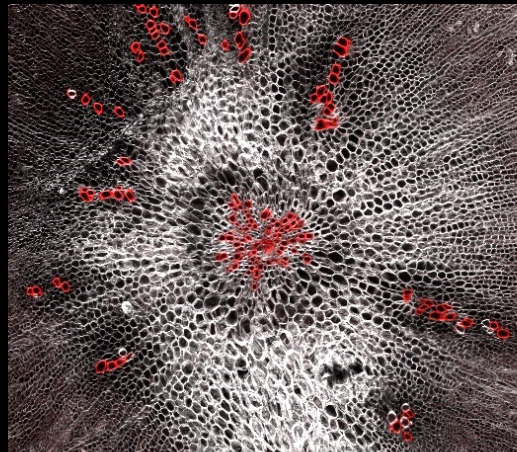
WT
WT



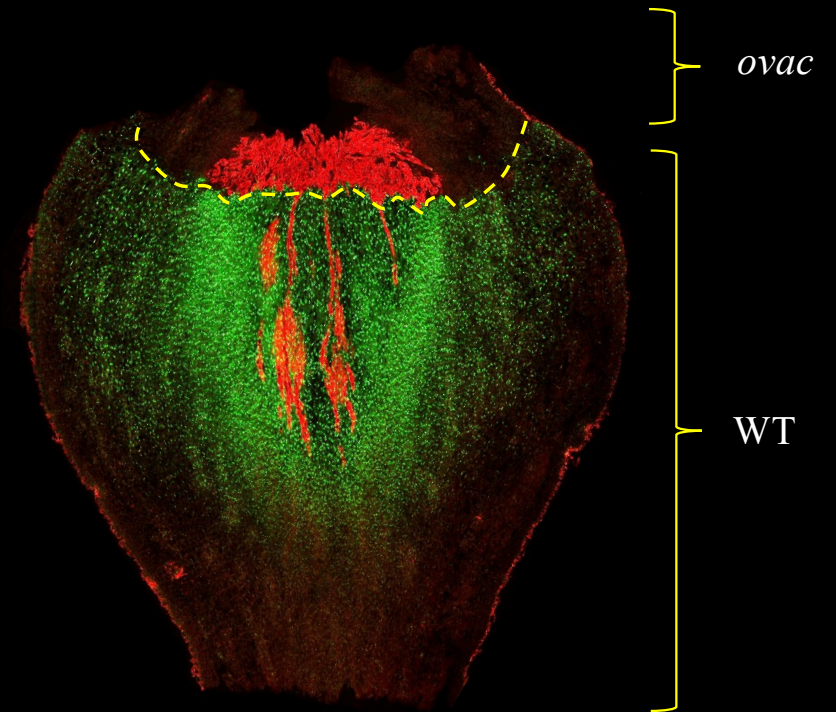
ovac
WT



Woody



Storage organ



Aradish
pSAC51::n3GFP and
Fuchsin staining

unpublished

The *ovac* scion induces the hypocotyl expansion in *Thellungiella* and *Cardamine*



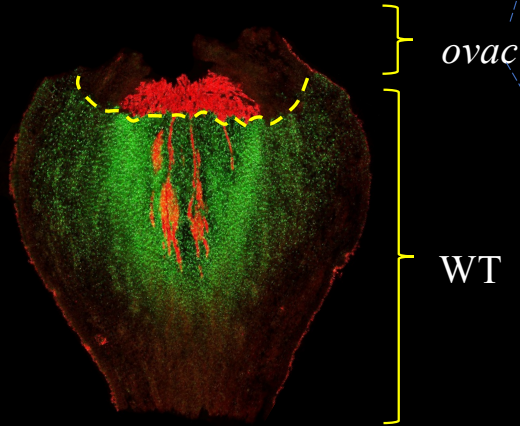
unpublished

12-week-after grafting

Tspm can act as a mobile signal that contributes to induction of translation of SACL genes

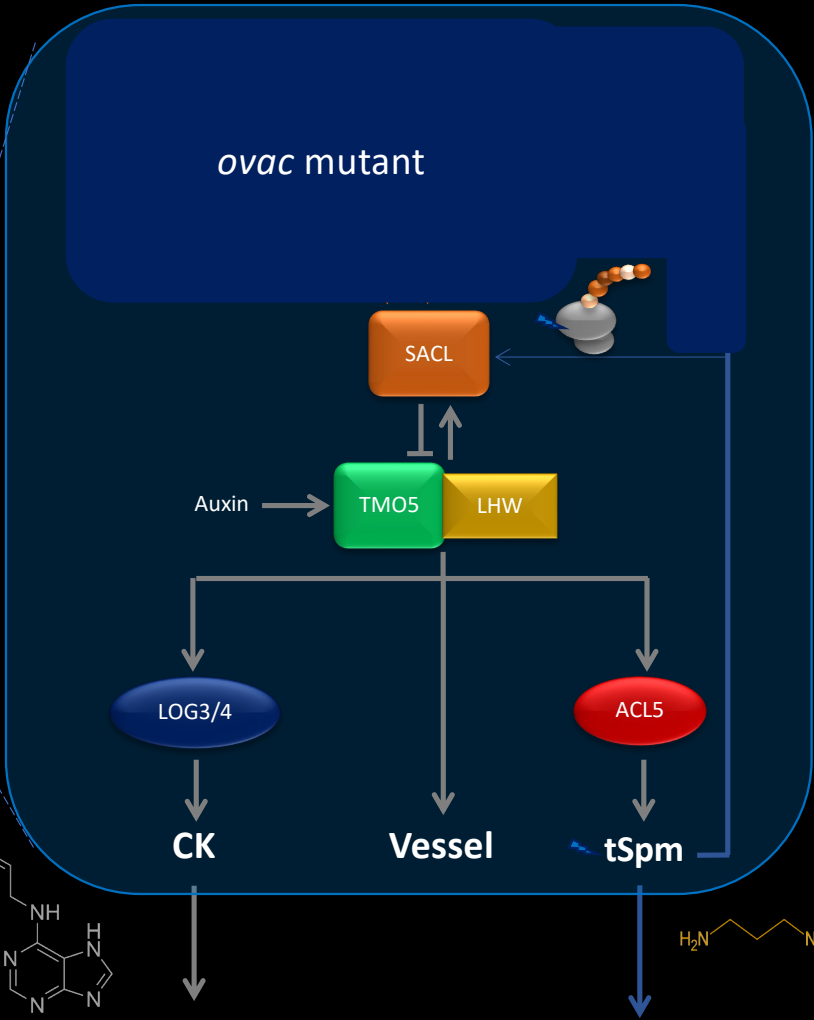
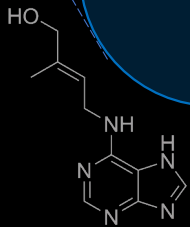
ovac overproduces cytokinins and **Tspm** to adequate level to induce storage organogenesis

Differentiating vessel

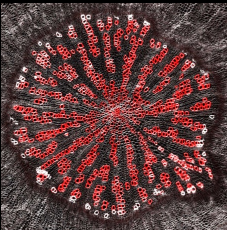


ovac

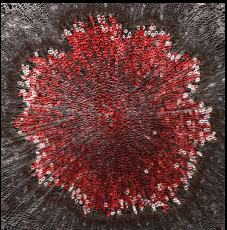
WT



WT



ovac mutant



tSpm can act as a mobile signal that contributes to induction of translation of SACL genes

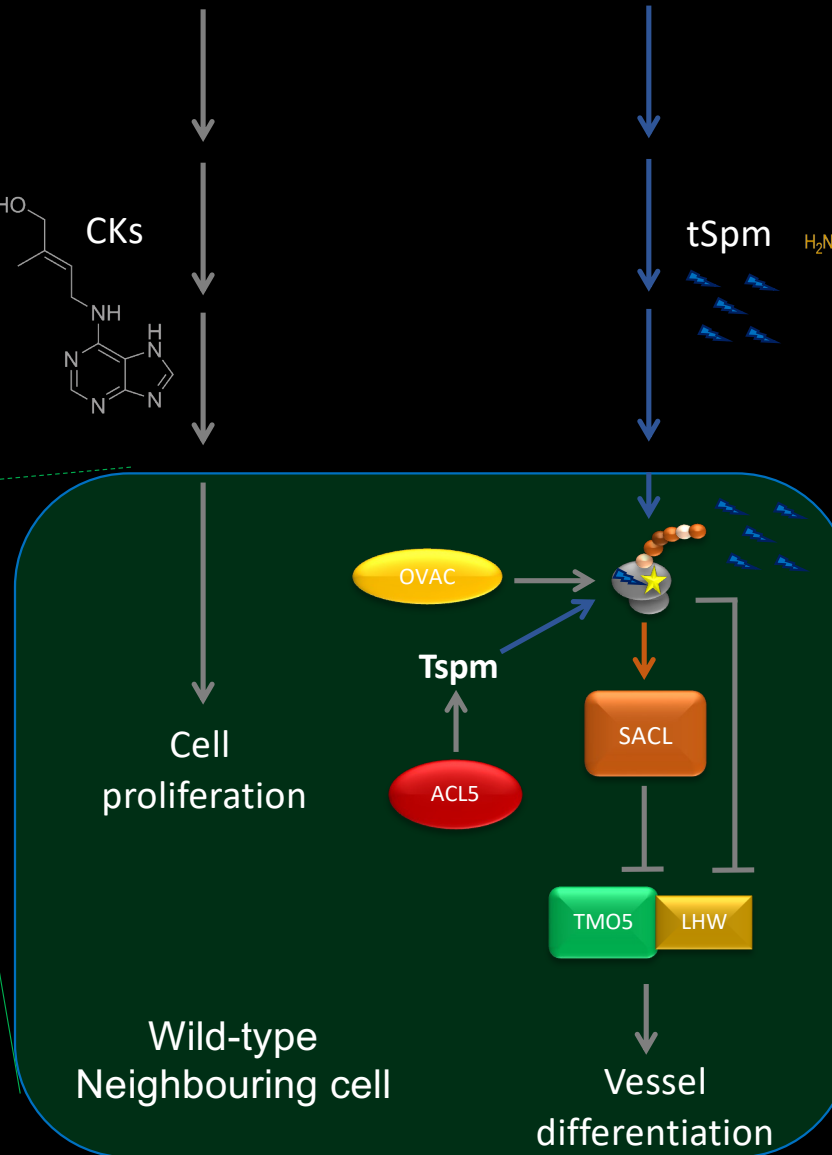
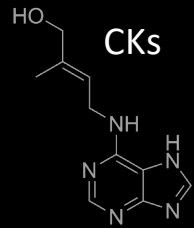


Arabidopsis
pSAC51::n3GFP and
Fuchsin staining

Enhanced cell proliferation

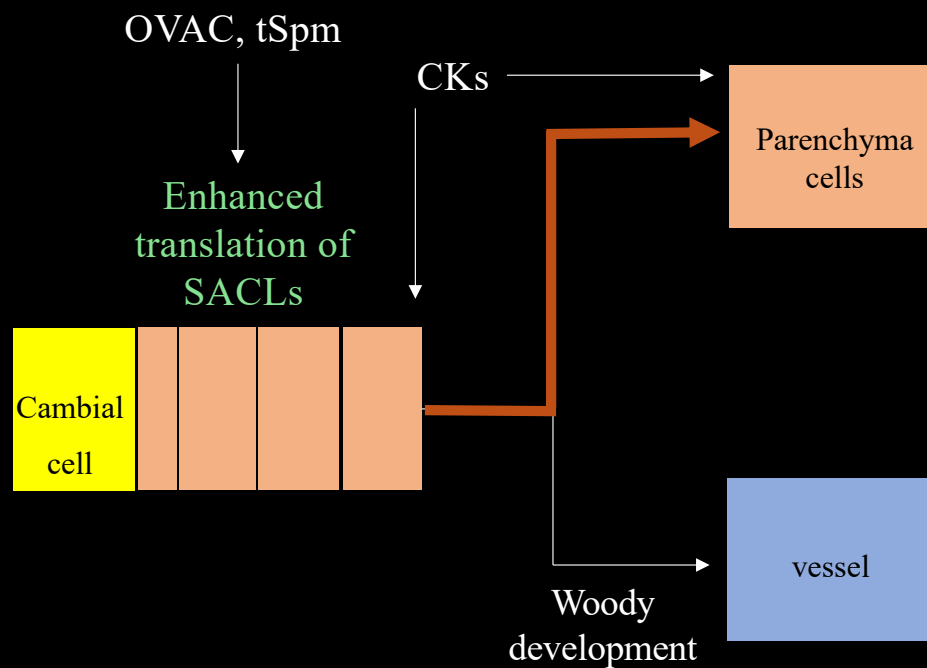
ovac

WT



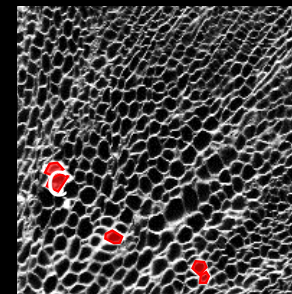
What are the key factors involved in the storage organogenesis?

OVAC-tSpm-SACL regulon and cytokinins

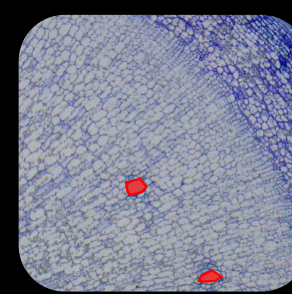


Storage organogenesis

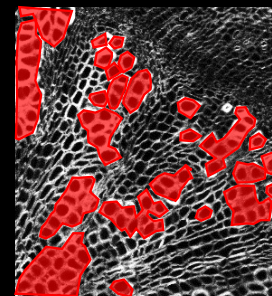
Aradish



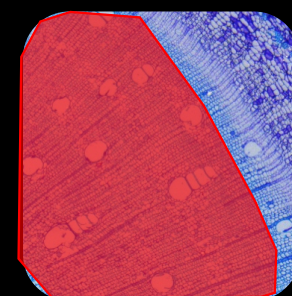
Cassava SR



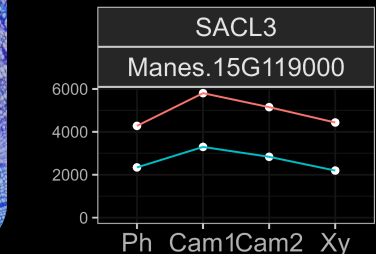
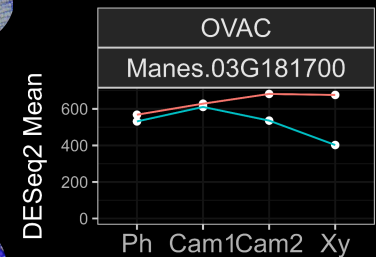
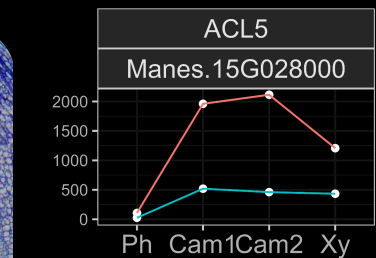
Arabidopsis



Stem



— Storage root — Stem



Cassava images and transcriptome data from

Wolfgang team, FAU

EM2.1 :

Increasing sink strength by optimizing xylem parenchyma cell formation in storage roots

Cytokinins

Thermospermine

EM2.1
} **Improving Hormonal signals**

1. Generating knockout of *meovac* mutant and grafting
2. Elevating biosynthesis of cytokinins and thermospermine in cassava in a storage tissue specific manner
3. Overexpression of OVAC in cassava

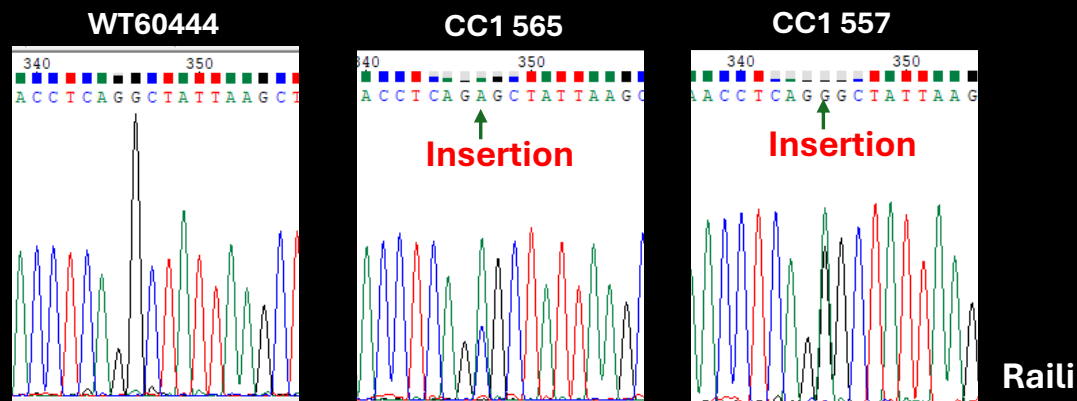
EM2.1_Manipulation of *OVAC* and cytokinin metabolism or signalling

1. Generating knockout of *meovac* mutant and grafting

24 lines of MeOVAC CRISPR/CAS9 transgenic cassava were generated.

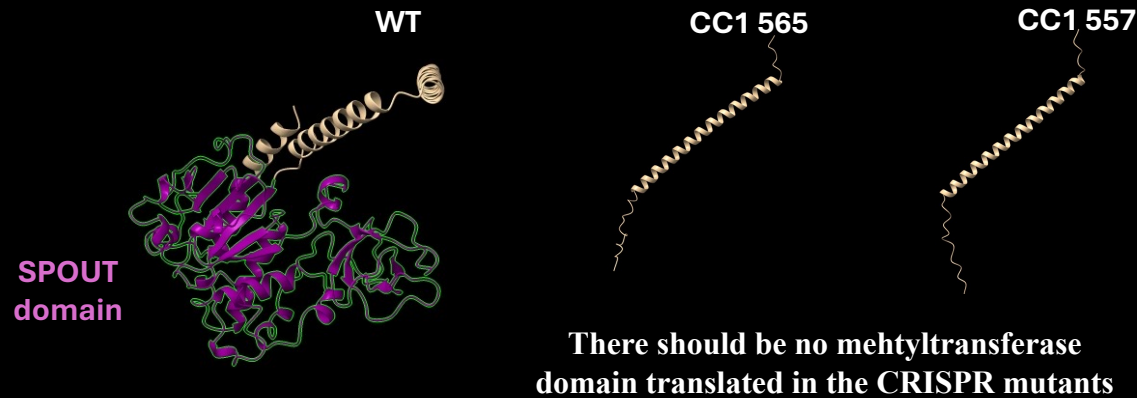


Genotyping isolated two independent lines with a nucleotide insertion in the MeOVAC



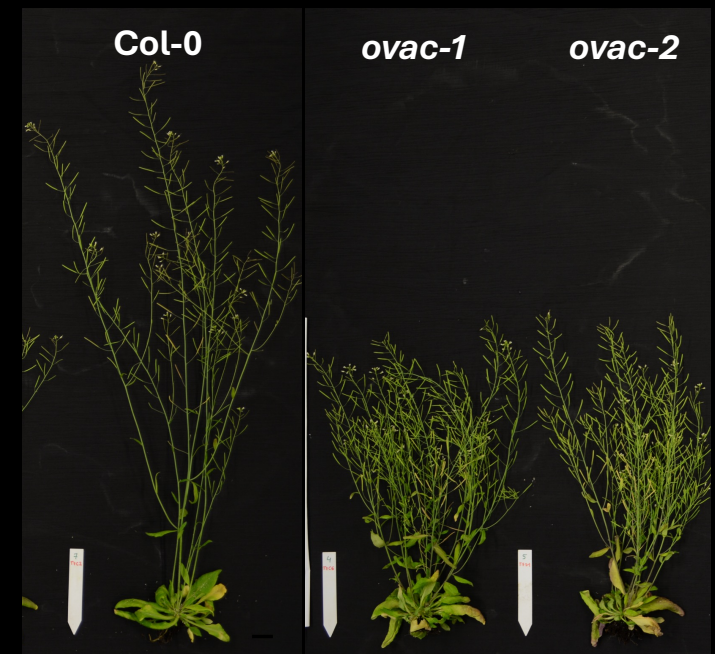
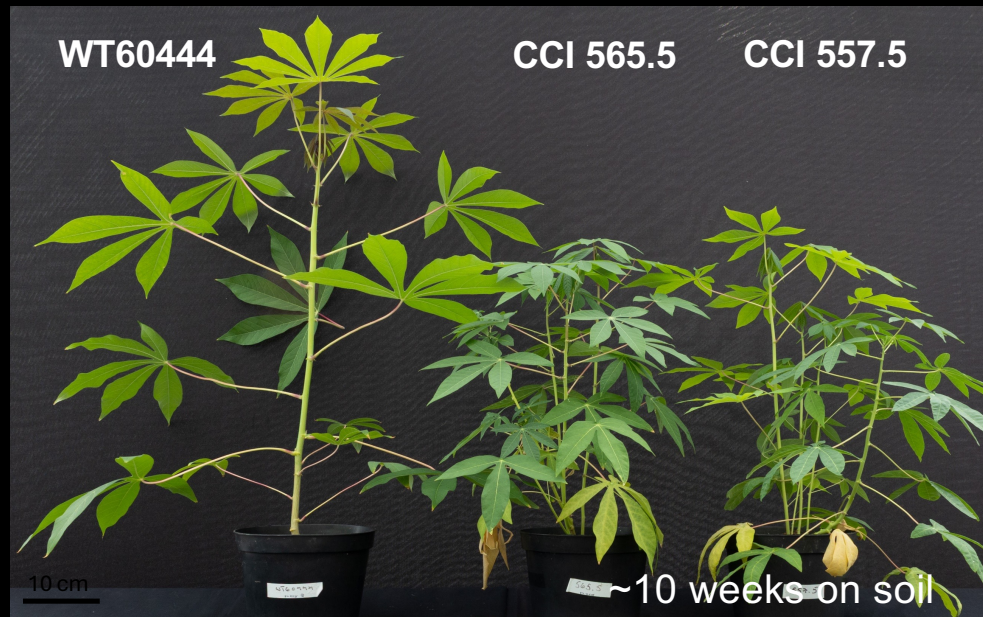
Frameshift results in premature stop
codon in following nucleotides

1. Generating knockout of *meovac* mutant and grafting



Loss-of-function strongly indicated; rRNA methylation measurement will confirm.

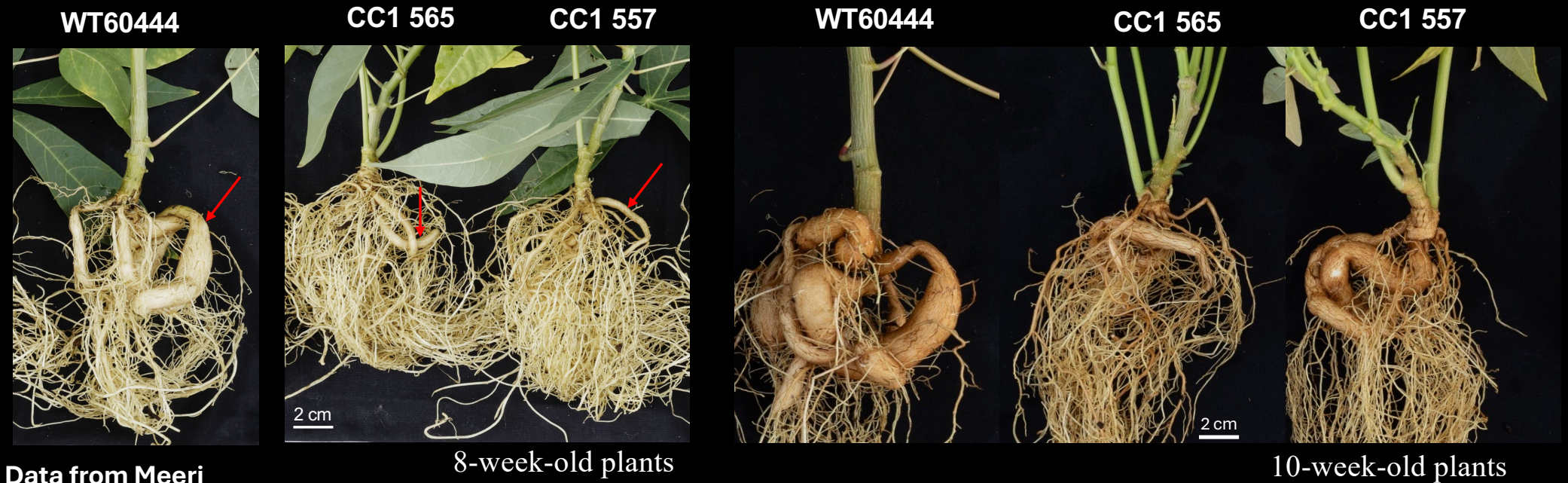
Data from Meeri



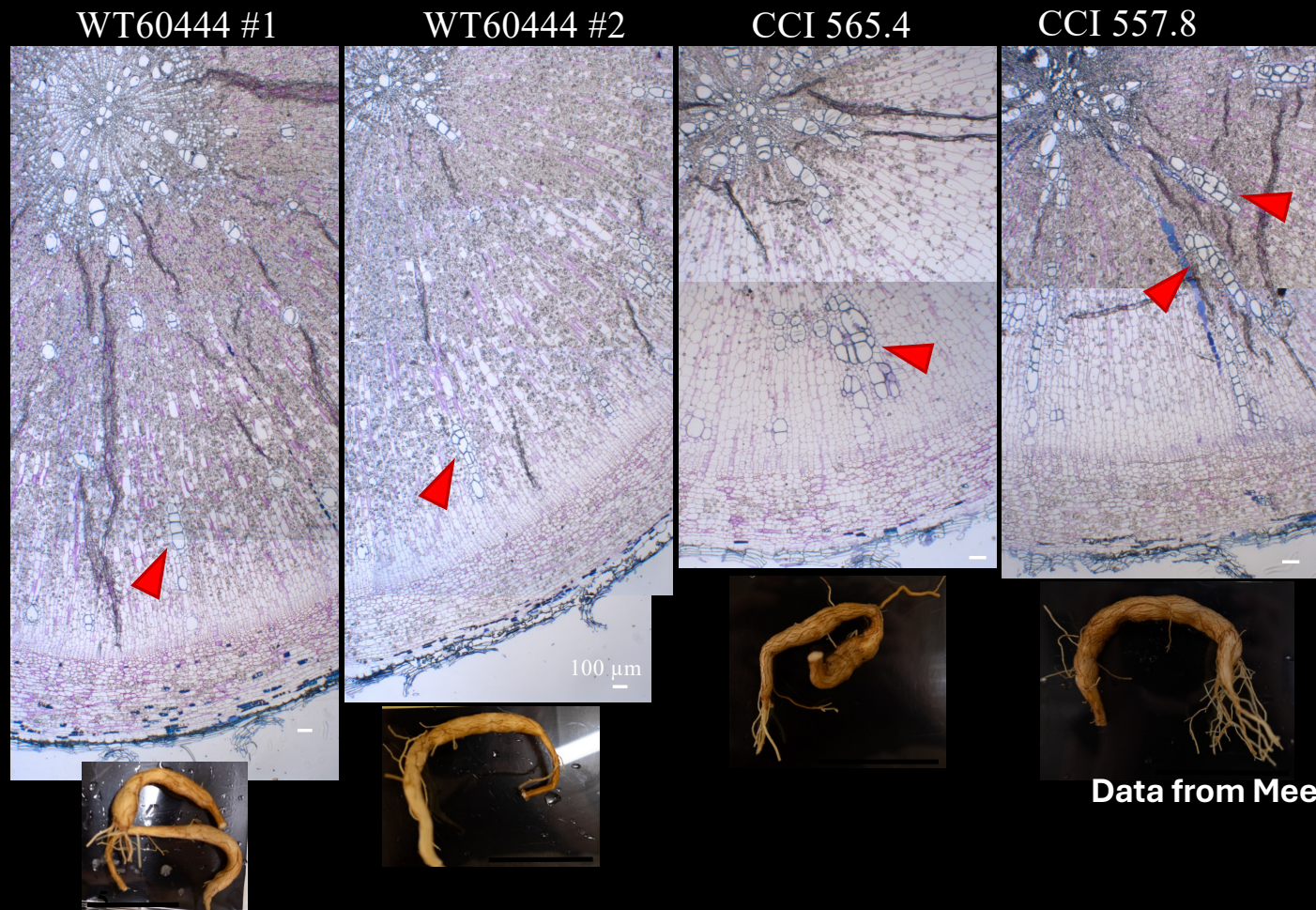
EM2.1_Manipulation of OVAC and cytokinin metabolism or signalling

1. Generating knockout of *meovac* mutant and grafting

The *meovac* CRISPR lines exhibit delayed and compromised storage organ development.



The *meovac* CRISPR lines exhibit enhanced vessel differentiation in storage roots.



OVAC-tSpm regulon is required for proper storage root development.

EM2.1_Manipulation of OVAC and cytokinin metabolism or signalling

1. Generating knockout of *meovac* mutant and grafting



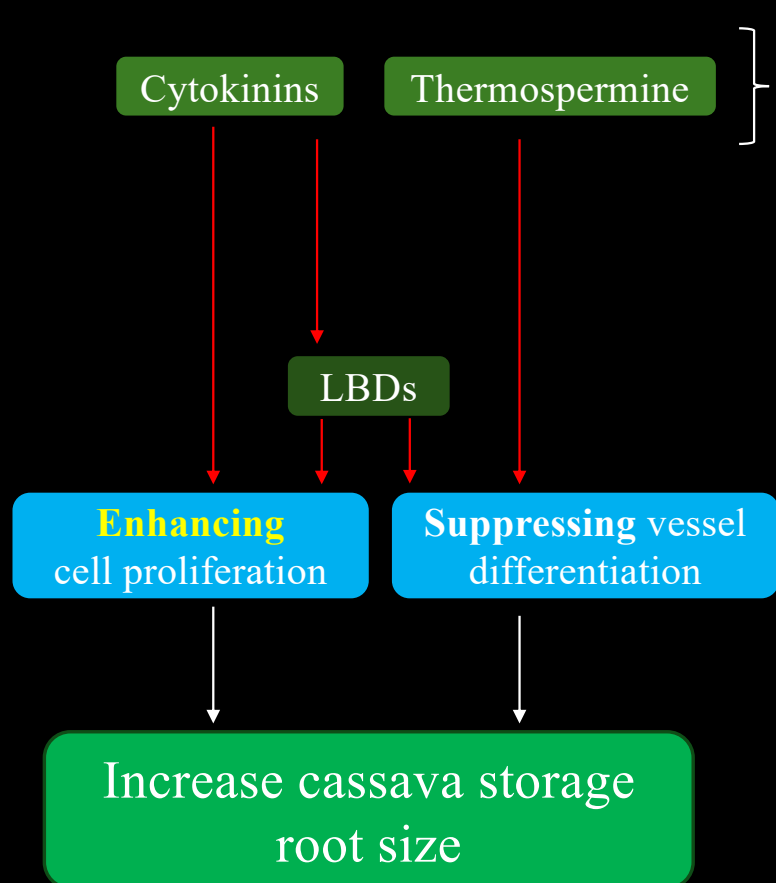
Data from Meeri



Grafting clips vs parafilm sealing of the (micro)graft junction

EM2.1 :

Increasing sink strength by optimizing xylem parenchyma cell formation in storage roots



EM2.1

Improving Hormonal signals

1. Generating knockout of *meovac* mutant and grafting
2. Elevating biosynthesis of cytokinins and tSpm in cassava in a storage tissue specific manner

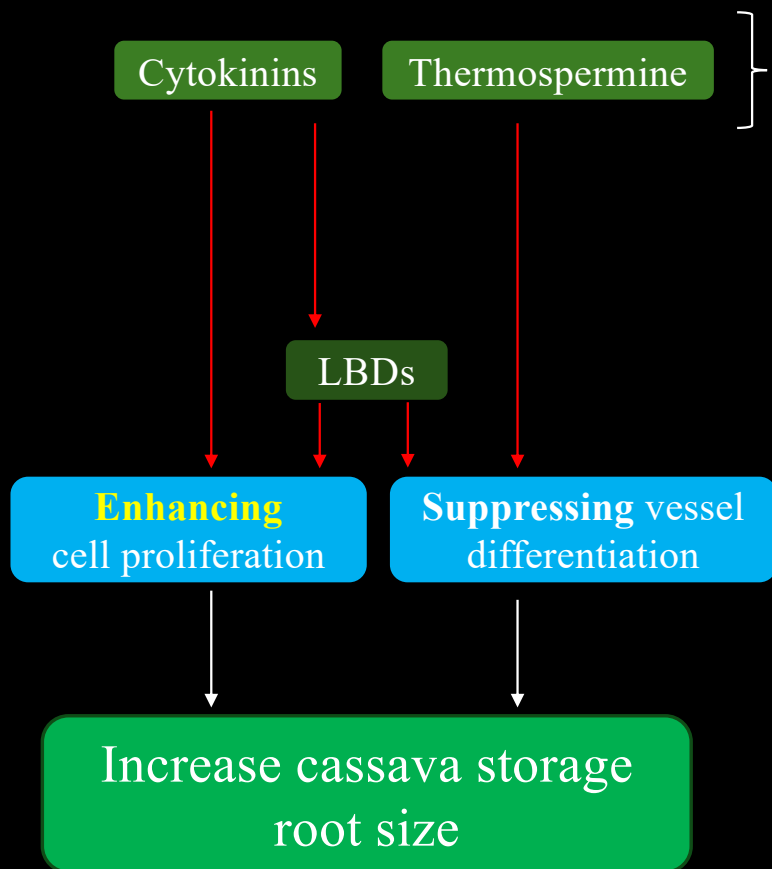
Cytokinins		tSpm	
<i>pStPat</i>	<i>AtIPT7</i>	<i>pMeACL5</i>	<i>AtBUD2</i>
<i>pStPat</i>	<i>AtLOG1</i>		
<i>pStPat</i>	<i>IPT7</i>		
<i>pStPat</i>	<i>AtLOG1</i>		

EM2.1 :

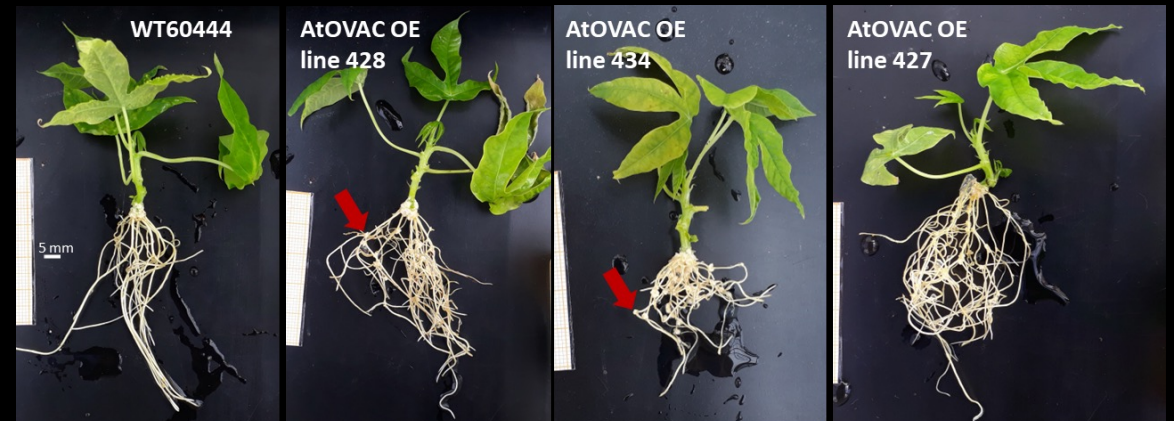
Increasing sink strength by optimizing xylem parenchyma cell formation in storage roots

EM2.1

Improving Hormonal signals



1. Generating knockout of *meovac* mutant and grafting
2. Elevating biosynthesis of cytokinins and thermospermine in cassava in a storage tissue specific manner
3. Overexpression of OVAC in cassava



Data from Raili

Acknowledgement

Dr. Christian Lamm

Dr. Wolfgang Zierer

Prof. Dr. Uwe Sonnewald

Meeri Makela

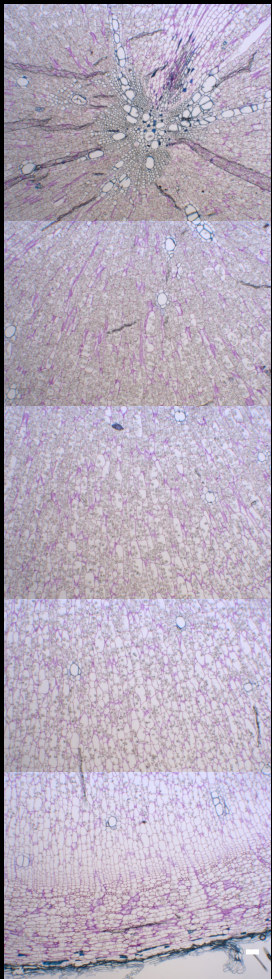
Dr. Raili Ruonala

Prof. Dr. Yrjo Helariutta

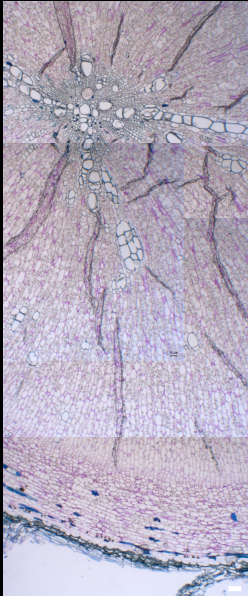


Thank you for your attention

WT60444 #2

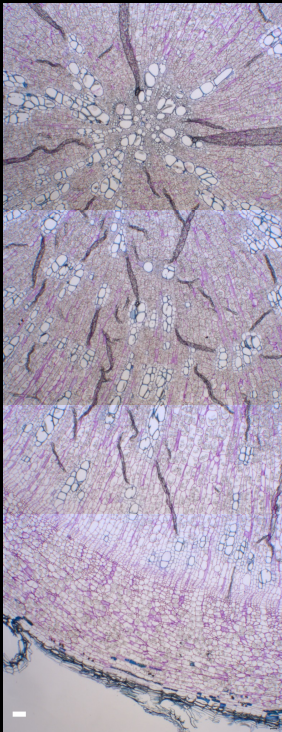


WT60444 #1

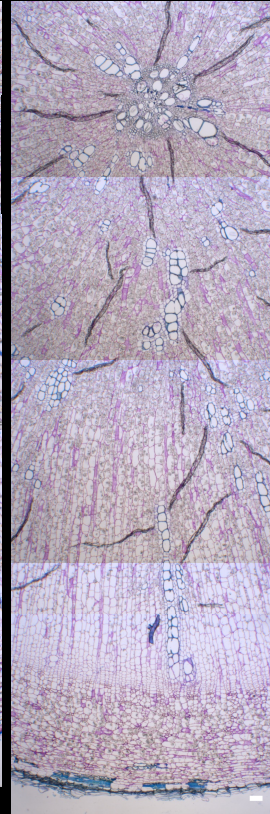
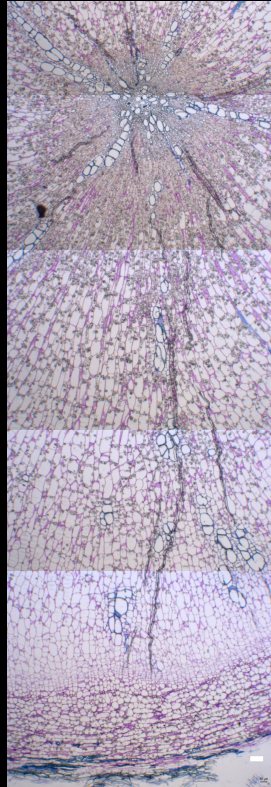


Scale bars:
100 μ m

Meovac 565.1



Meovac 557.1



Preliminary data repeat of cross-section of Meovac CRISPR loss-of-function at ~12 weeks in soil
Staining with Ruthenium Red and Toluidine Blue



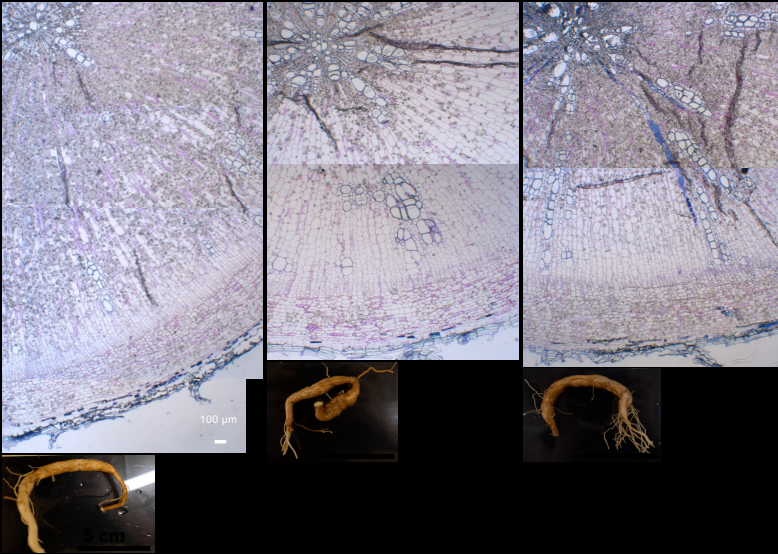
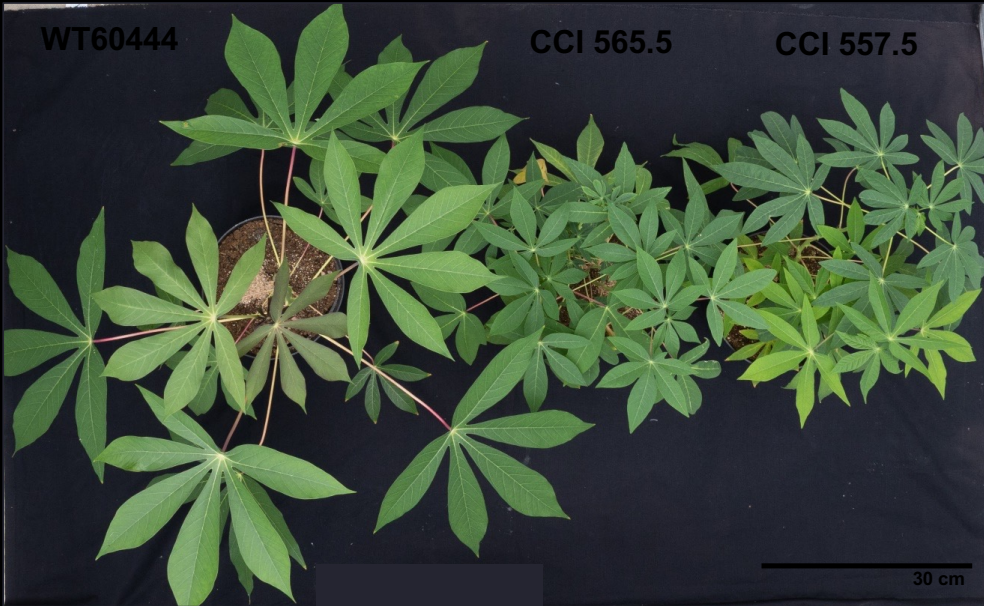
Cassava
(*Manihot esculenta*)

MeOVAC CRISPR
knockout

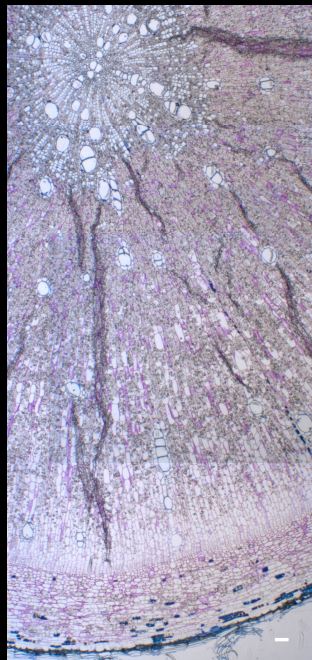
~10 weeks
in soil



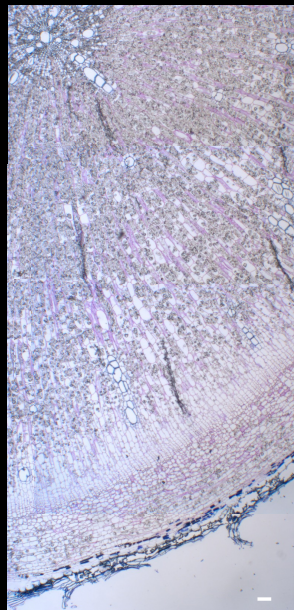
~9 weeks
in soil



WT60444 #1



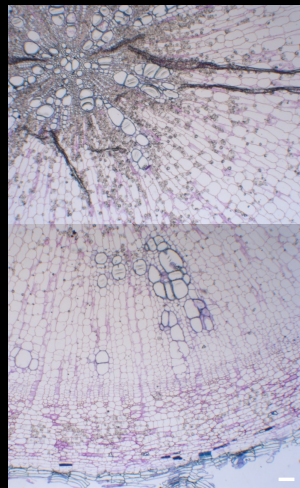
WT60444 #2



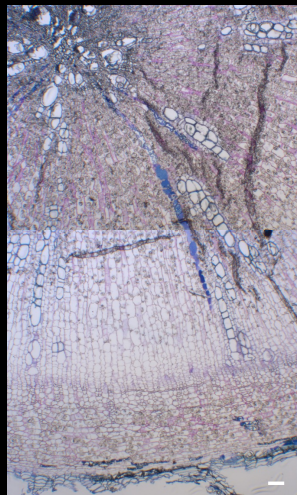
100 μ m



Meovac 565.4



Meovac 557.8



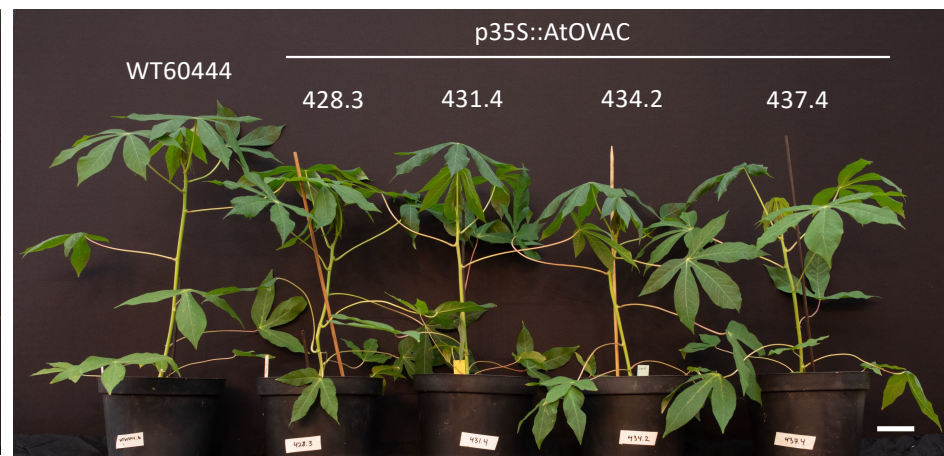
Preliminary data of fixed and plastic
resin embedded cassava storage
roots,
~10 weeks in soil

Ruthenium Red and Toluidine Blue
staining

WT60444



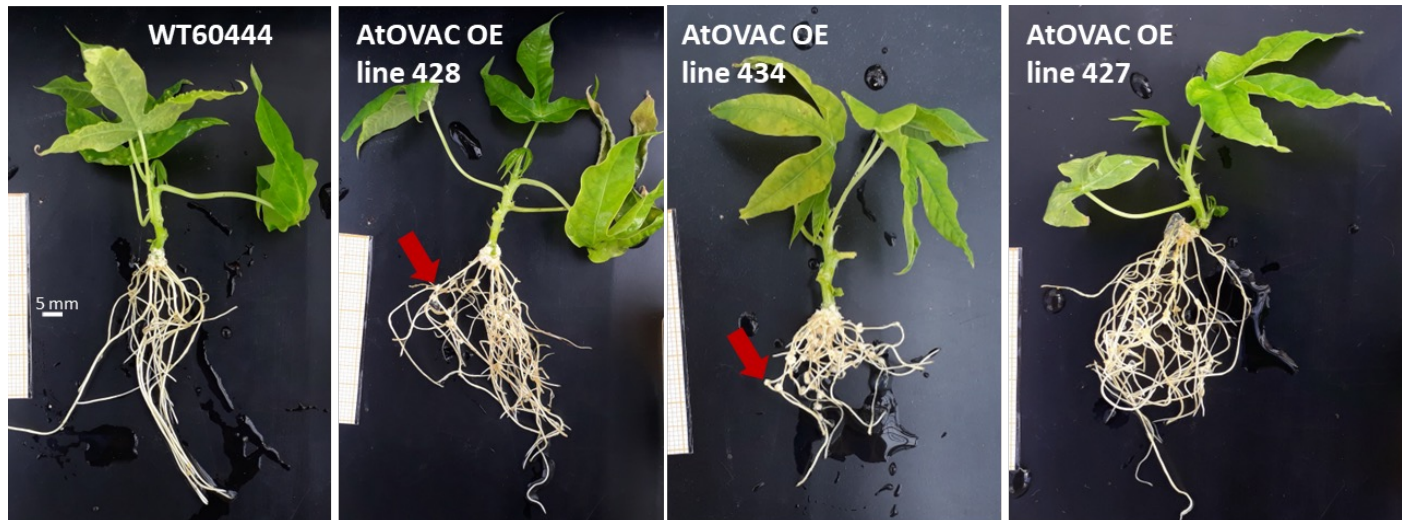
~4 weeks in soil



~5 weeks in soil



Current view on p35S::AtOVAC (OVAC over-expression, OE) lines, roughly 6 weeks in soil -> storage root phenotypes to be observed in due time (photo



Currently, the only root phenotype we have observed in the OVAC OE lines is from in vitro jars: there seems (callus-like?) thickenings in roots of many transgenic lines (photos 12.8.25)