# CASSIII: Editing starch synthesis and stability in Cassava

**Christina Joy Müller**
**Annual Meeting 2025, IITA, Nigeria (Zoom)**
25 Sept 2025

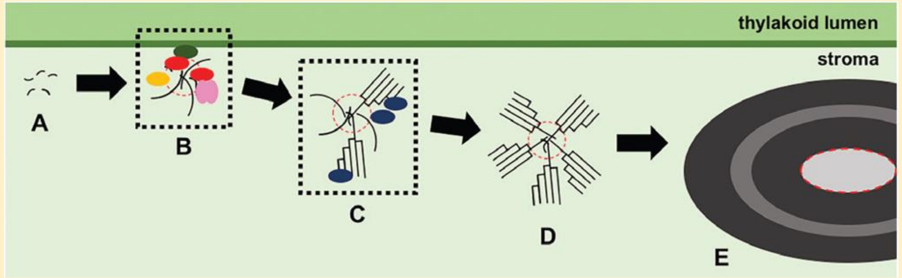
# Starch – our main focus

**Aim**
To *understand* and *improve* Cassava starch **biosynthesis** and **stability** by making targeted gene expression changes aimed at **yield improvement**.

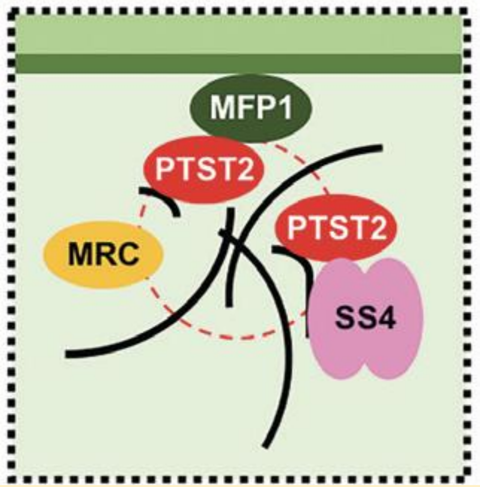
**Approach**
1) alter starch initiation
2) modify starch stability

# Two ongoing approaches

**I. Increase/alter starch initiation** by over-expressing starch initiation genes




Seung and Smith, 2018




*STARCH SYNTHASE 4 (SS4)*

*PROTEIN TARGETING TO STARCH2 (PTST2)*

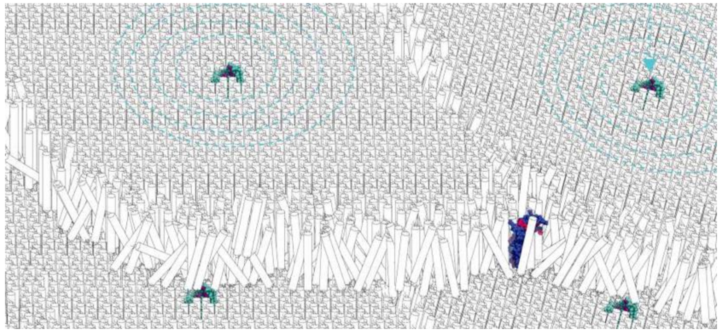
*MYOSIN-RESEMBLING CHLOROPLAST PROTEIN (MRC)*

*MAR-BINDING FILAMENT-LIKE PROTEIN 1 (MFP1)*

**II. Edit starch stability** with altered expression of starch stabilizing proteins

*EARLY STARVATION 1 (ESV1)*

*LIKE EARLY STARVATION (LESV)*

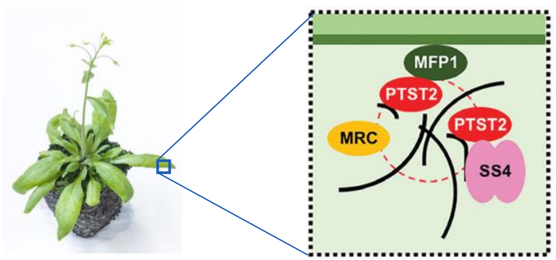


Liu et al. 2023

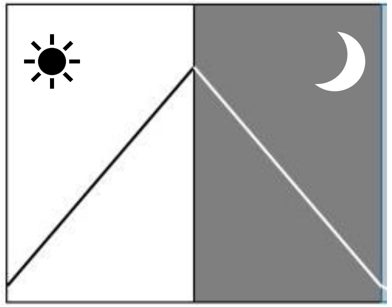
# Transitory starch vs storage starch

## Arabidopsis leaves

transitory starch

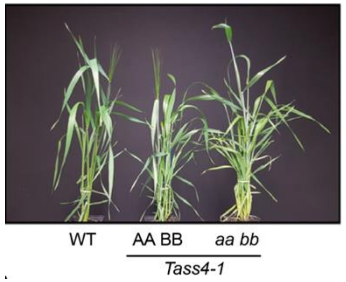



Seung and Smith, 2018

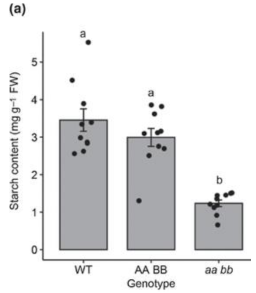


Graf and Smith, 2011, modified

## Wheat endosperm



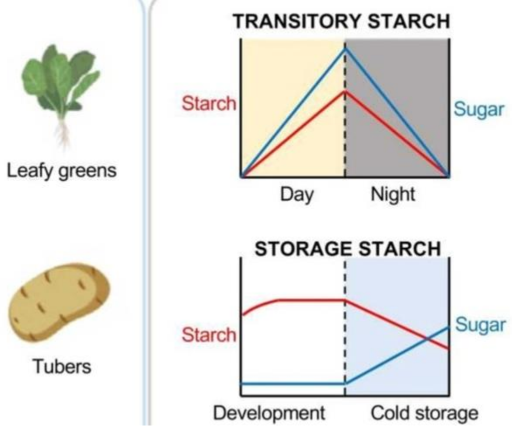




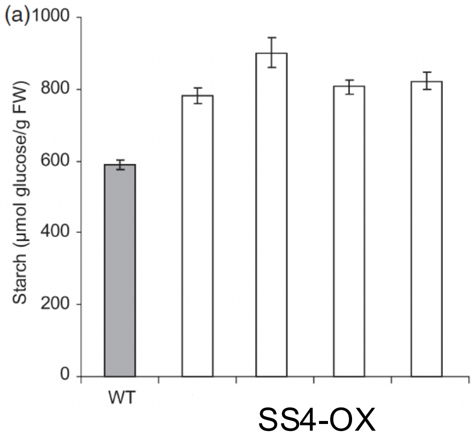

Hawkins et al 2021

SS4 and PTST2 initiate starch granules in endosperm amyloplasts

## Potato tubers

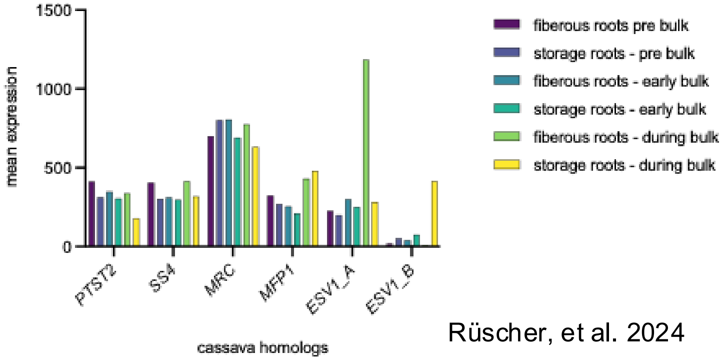



Yu et al. 2022




Gámez-Arjona et al. 2011

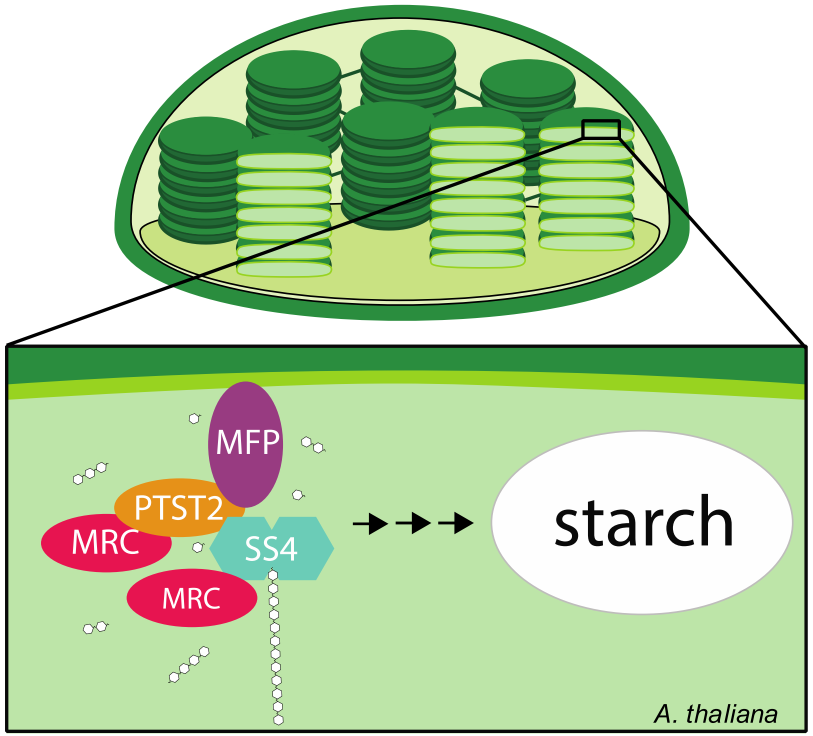
## Cassava storage roots

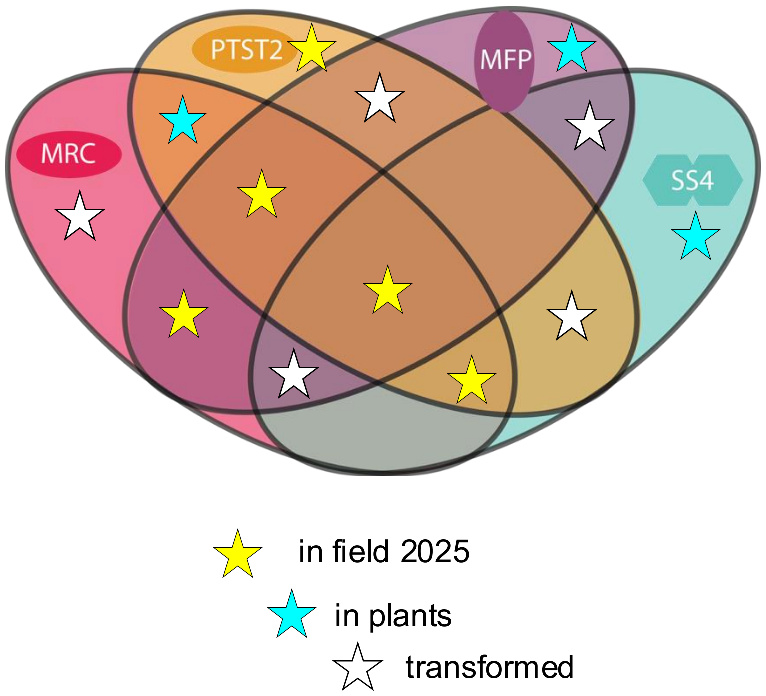



Rüscher, et al. 2024

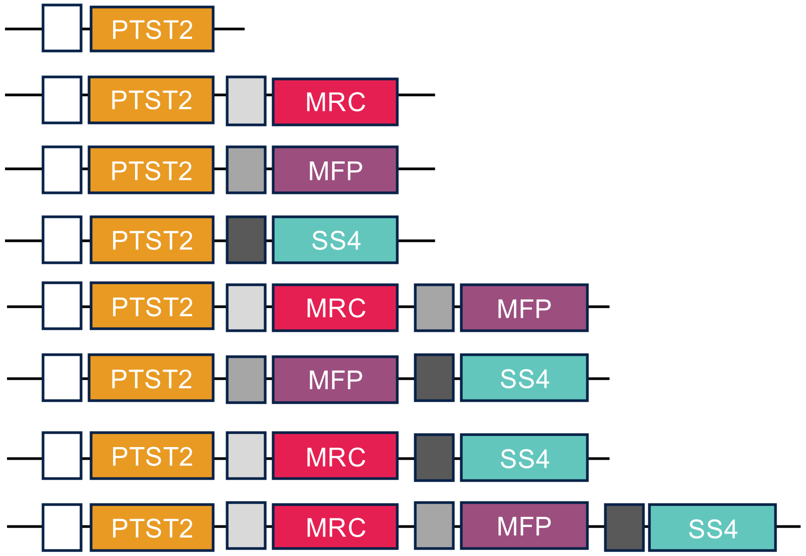
mechanisms of starch initiation largely unknown

# Single, double, triple and quadruple OX lines to find the best starch initiation combo in cassava

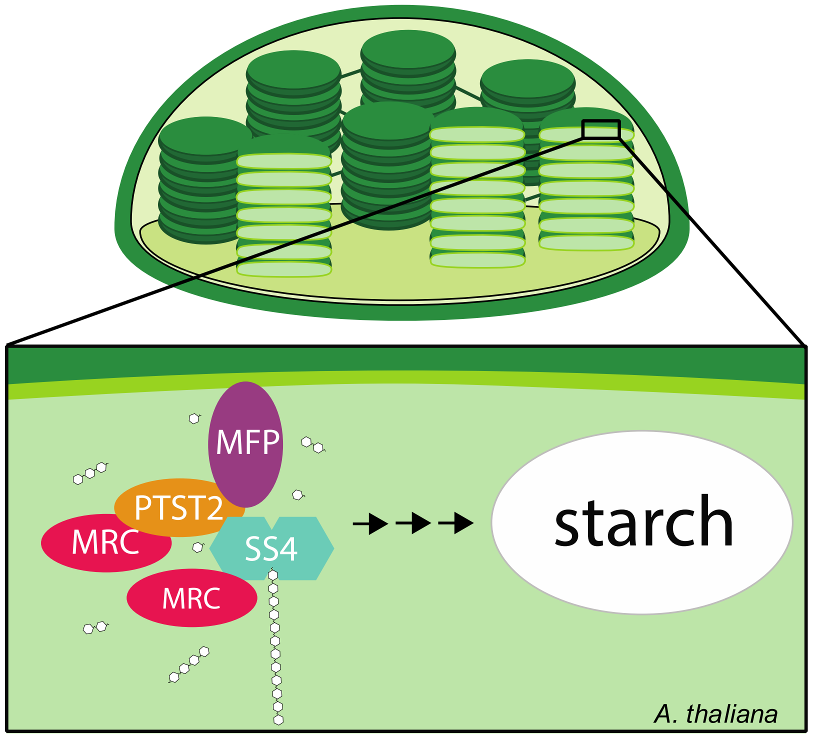


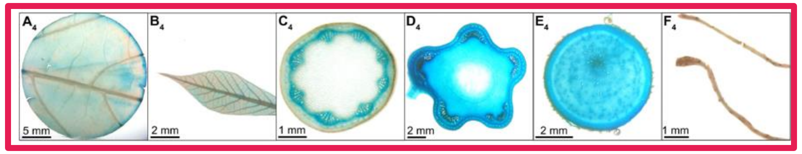

Example with PTST2:

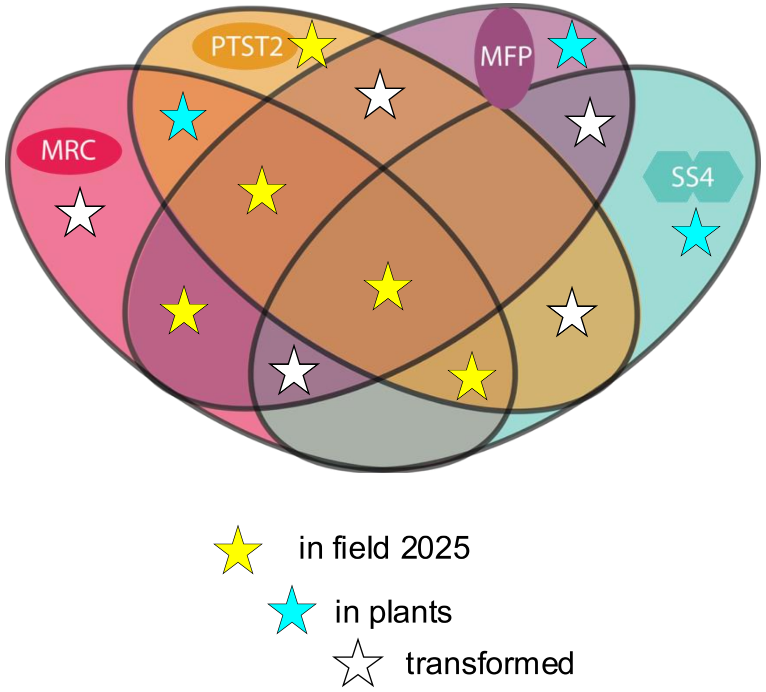


# All constructs designed with *rooty* promoters

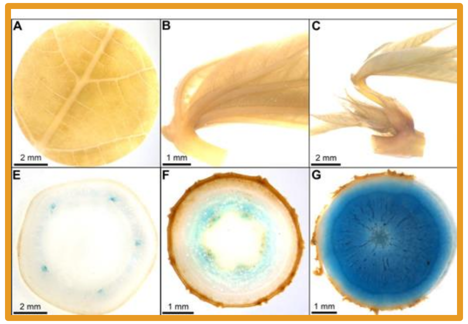



*pMeGBSS1:GUS*

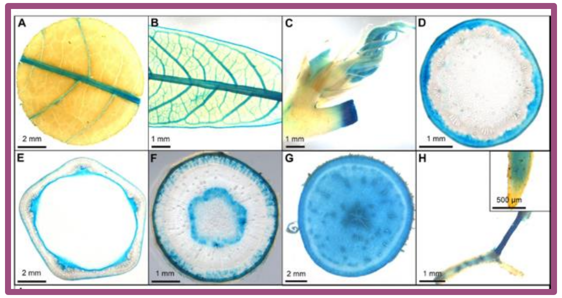




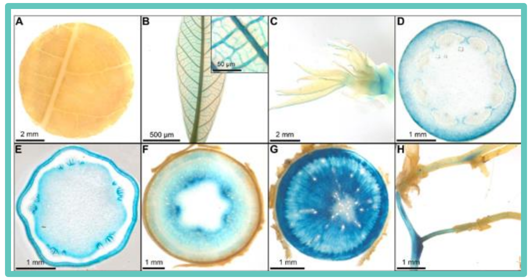

*pStPatatin:GUS*



*pStGBSS1:GUS*

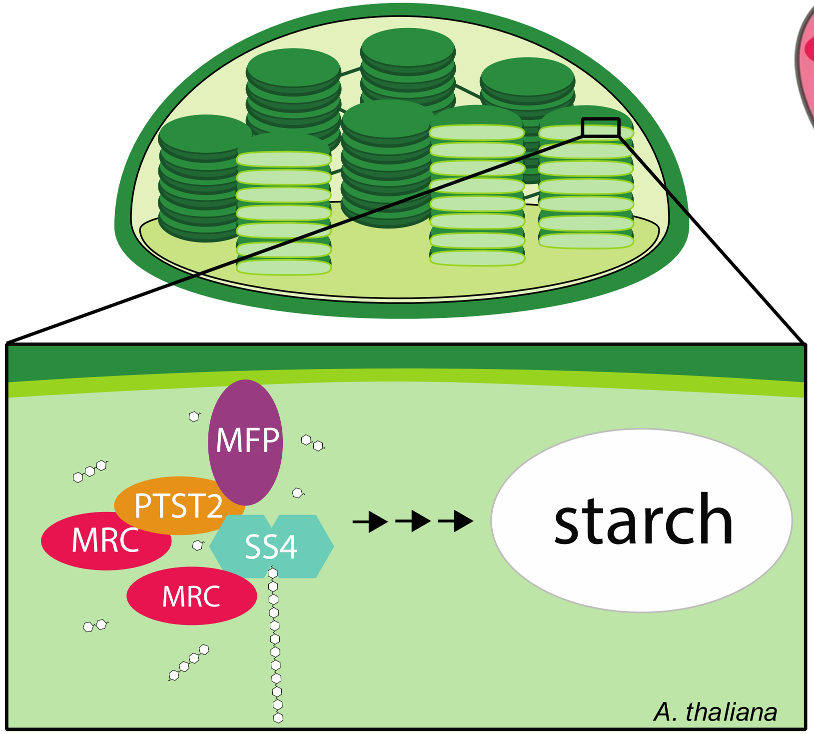


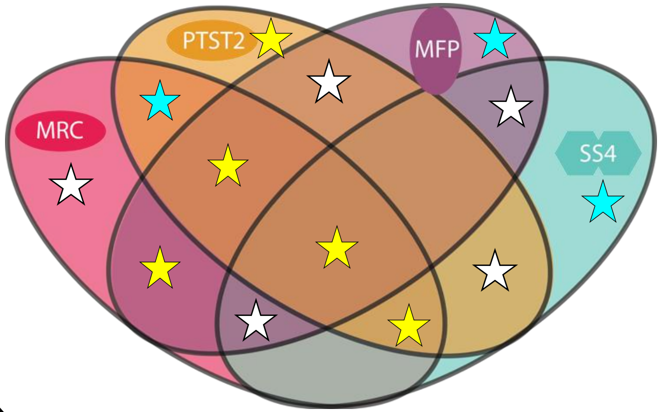
*pMeGPT2:GUS*



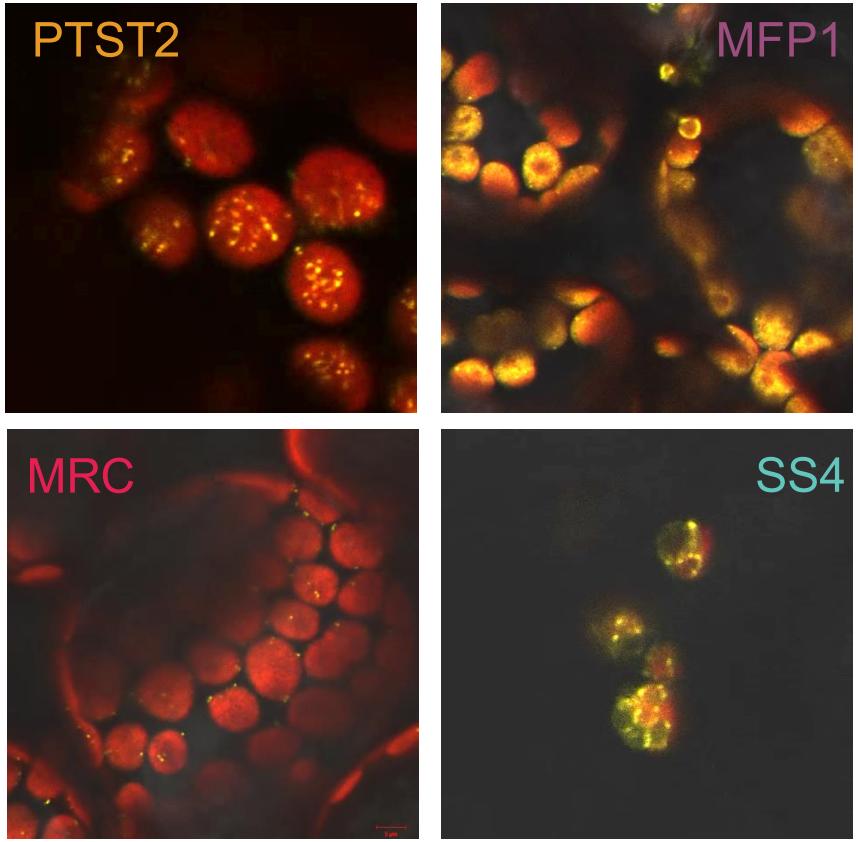
Zierer, Anjanappa, et. al 2022

# All constructs localize to sub-chloroplastic domain similar to in A.th.



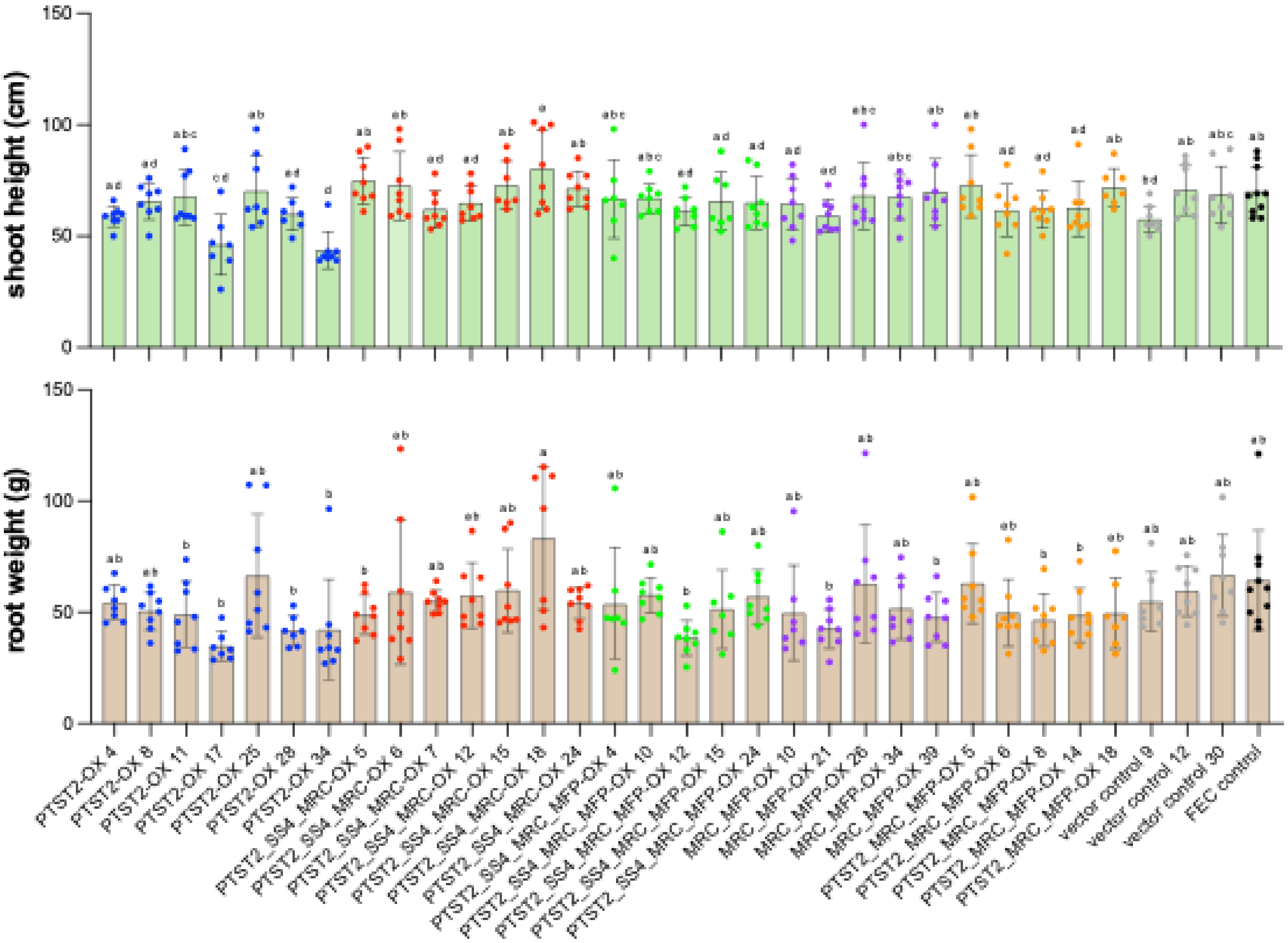

Transient expression in tobacco



# Growth metrics

Pre-screened

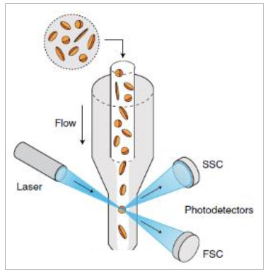
- transgene presence
- copy number
- southern blot
- western blot
- qPCR



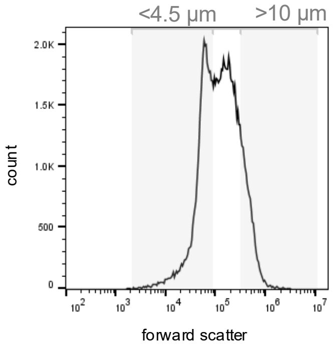

ETH zürich

# Flow cytometry and SEM help characterize starch size and shape

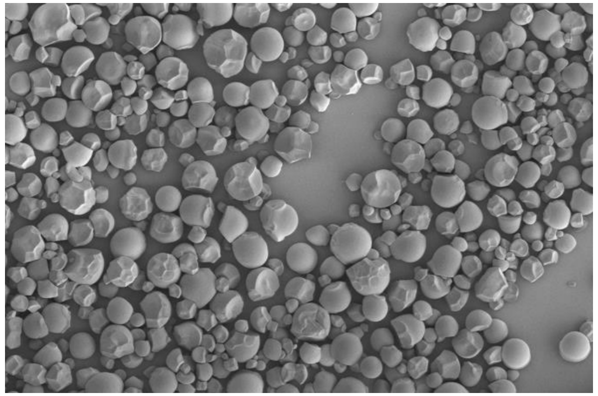
**Flow cytometry**
Between 4 – 20 µm




Mage et al, 2019

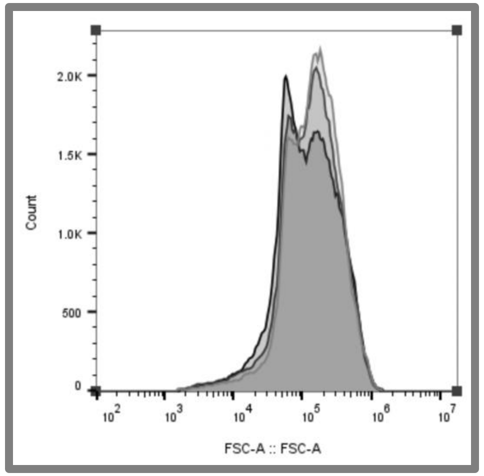


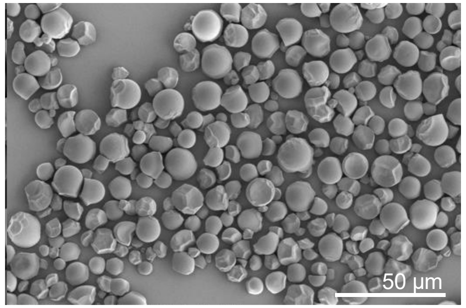

**SEM**



bimodal

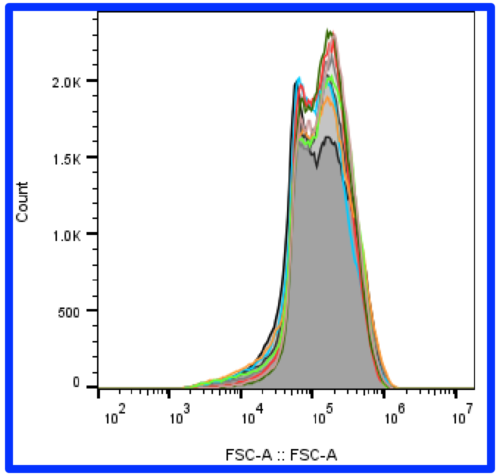
controls



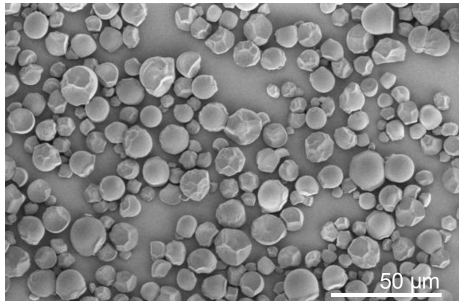




bimodal, no apparent difference

37488



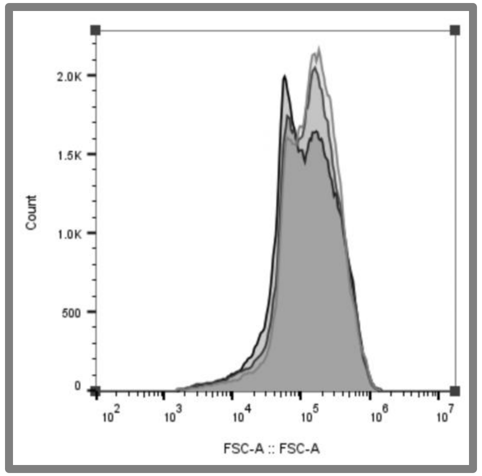

PTST2-OX
~~SS4-OX~~
~~MRC-OX~~
~~MFP1-OX~~

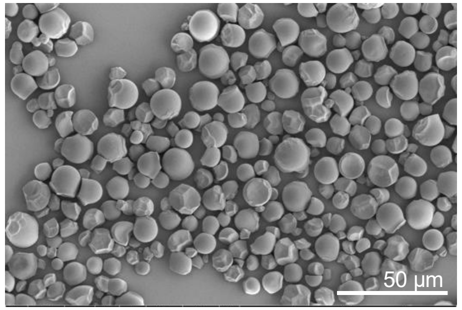



COLORED LINES → independent line representatives bimodal

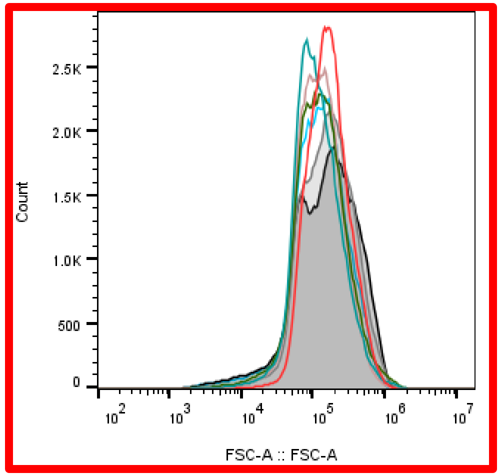
smaller granules, but also more complex shapes (angular / jagged)

controls




37493




PTST2-OX
SS4-OX
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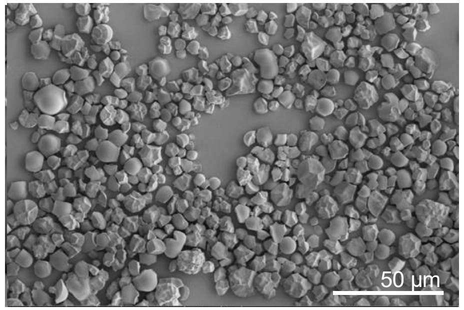
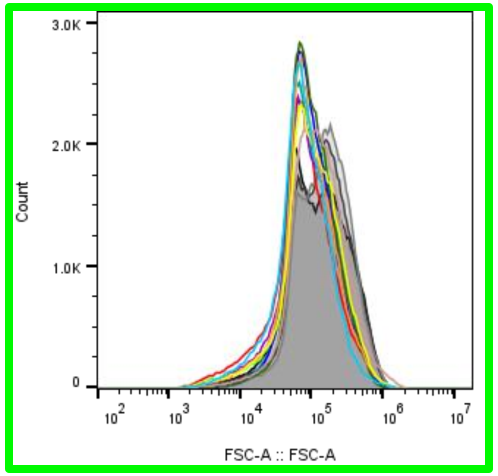
~~MFP1-OX~~




37499




PTST2-OX
SS4-OX
MRC-OX
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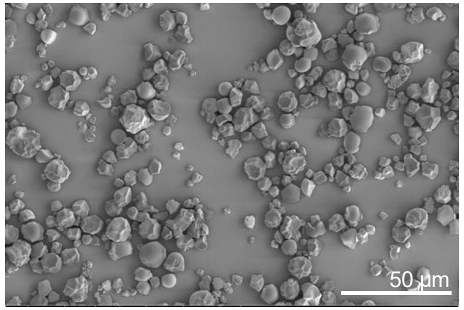
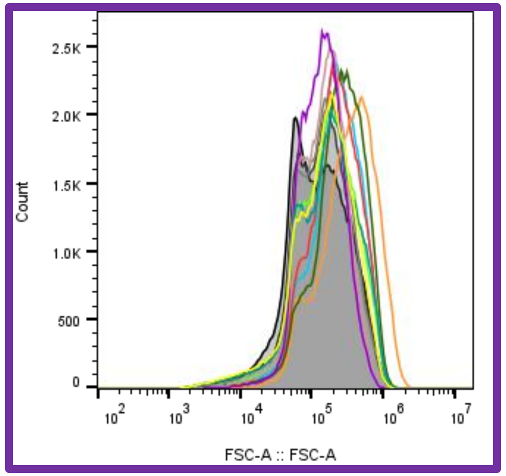



When MFP1 is increased, the granules appear smaller larger, rounded granules

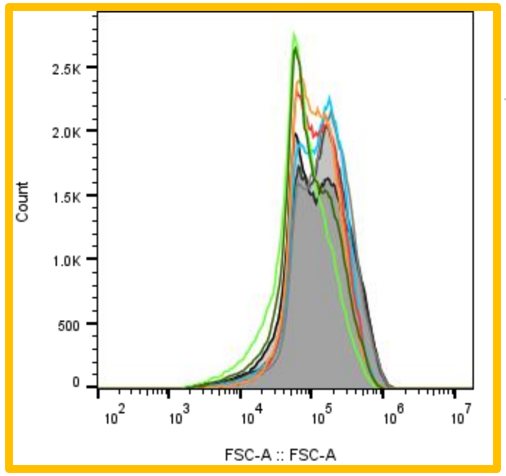
smaller size, more bimodal, jagged

37501




~~PTST2-OX~~
~~SS4-OX~~
MRC-OX
MFP1-OX

37502




PTST2-OX
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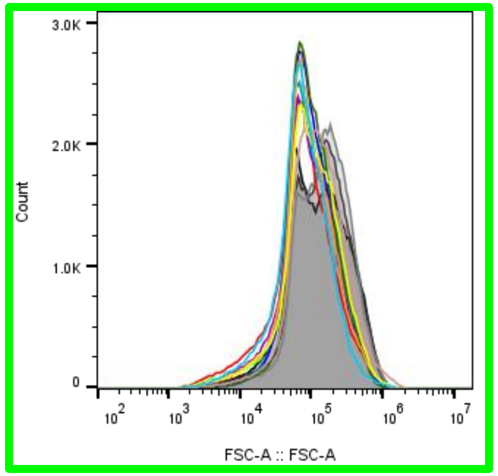
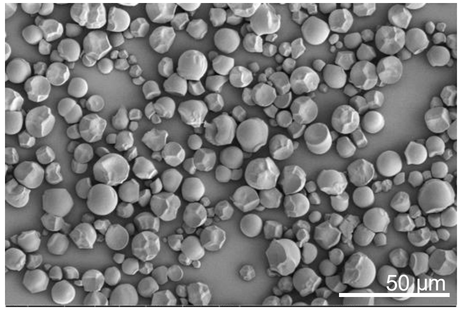
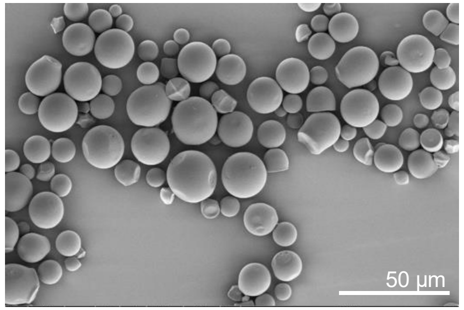
MFP1-OX

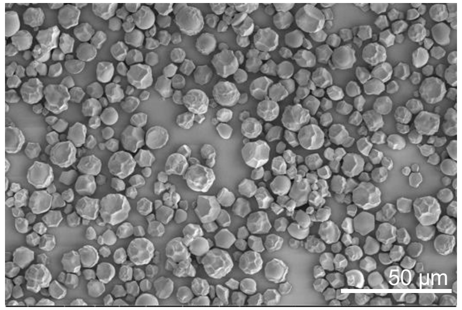
37499

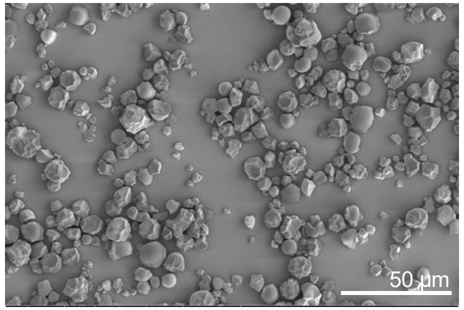



PTST2-OX
SS4-OX
MRC-OX
MFP1-OX



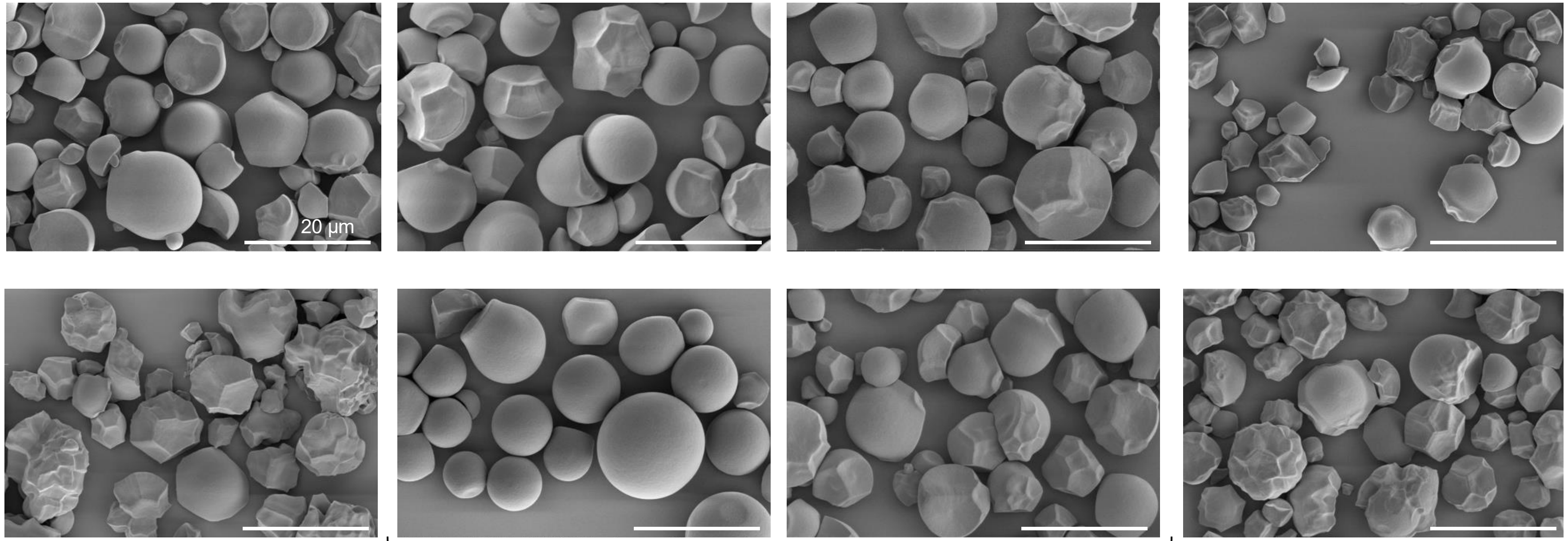
# Overexpression of starch initiation genes in cassava changes starch development

PTST2-OX
~~SS4-OX~~
~~MRC-OX~~
~~MFP1-OX~~

PTST2-OX
SS4-OX
MRC-OX
~~MFP1-OX~~

60444

vector control




PTST2-OX
SS4-OX
MRC-OX
MFP1-OX

~~PTST2-OX~~
~~SS4-OX~~
MRC-OX
MFP1-OX

PTST2-OX
~~SS4-OX~~
MRC-OX
MFP1-OX

changes may affect starch quality traits

# Outlook I.

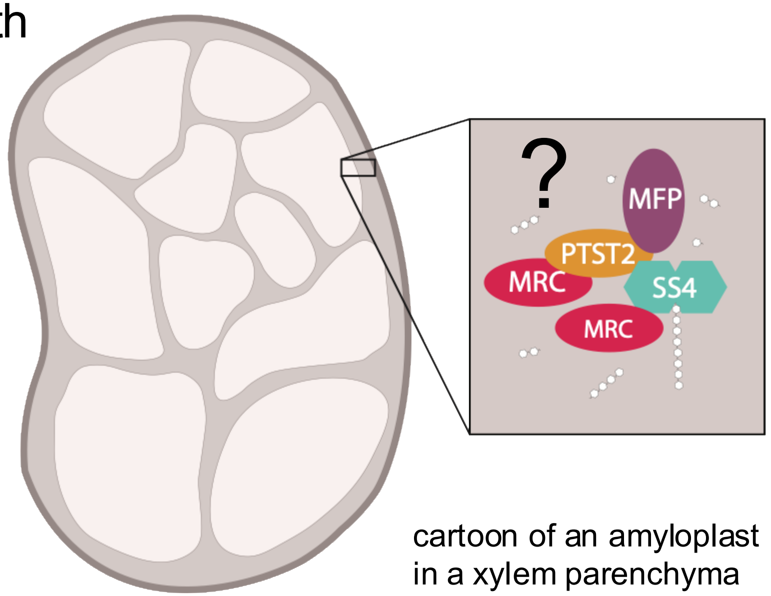
**Takeaway: We can modify root starch development using known leaf starch initiation gene homologs in various combinations – changing the shape and size of starch and hopefully quality traits.**

- The effects so far do not seem to be detrimental to shoot or root growth (greenhouse)

**Next:** Microscopy to characterize these granules at the amyloplast level

- Characterize upcoming lines that have been transformed
- Isabel says she is up for the challenge! 
- All constructs are represented in **CTF2025**

**Isabel Williams**
Doctoral Student




cartoon of an amyloplast in a xylem parenchyma

# Two ongoing approaches

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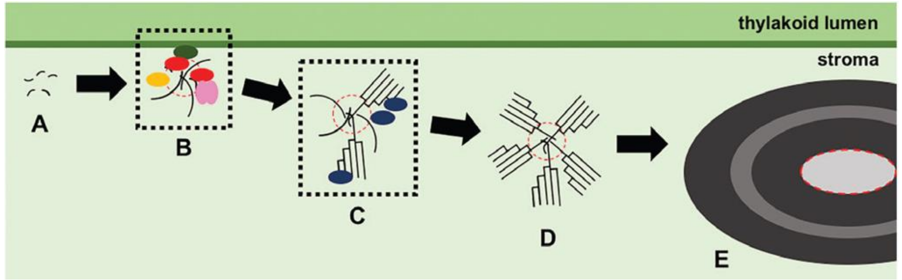
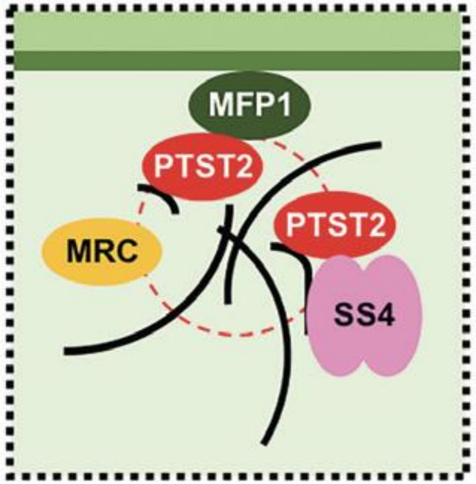



Seung and Smith, 2018

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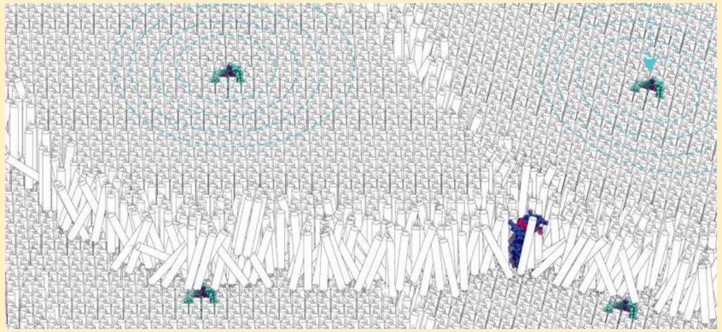
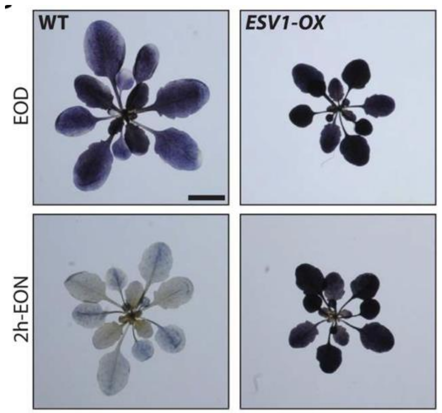


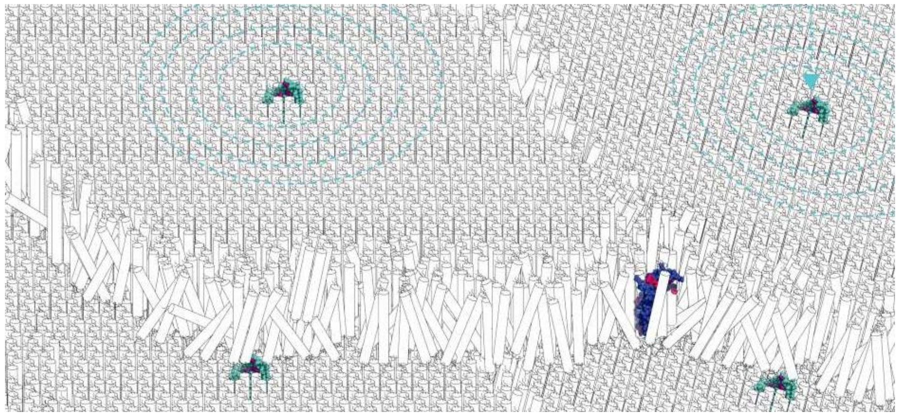
Liu et al. 2023

# ESV1 promotes starch stability



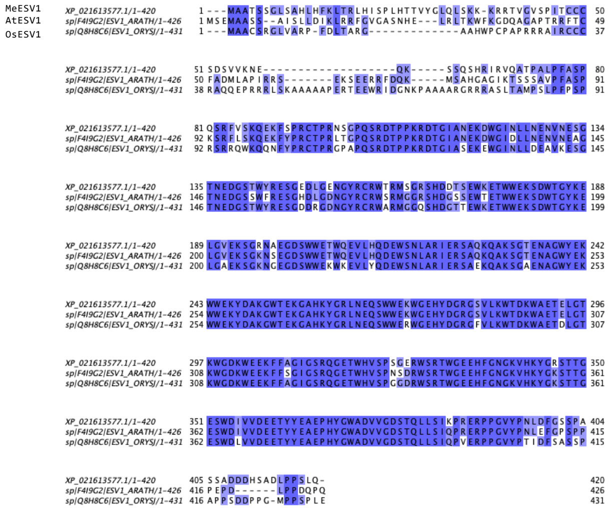

Liu et al. 2023


Feike et al. 2016

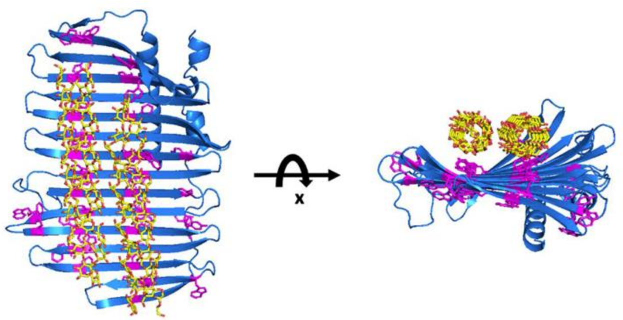


Liu et al. 2023

Cassava, Arabidopsis and Rice conservation:

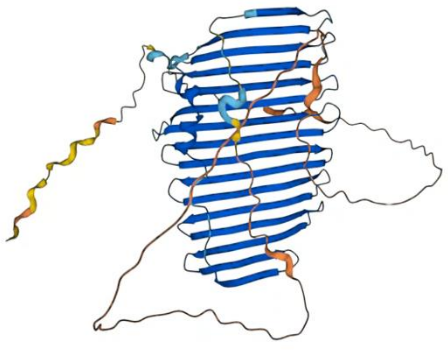



ClustalW

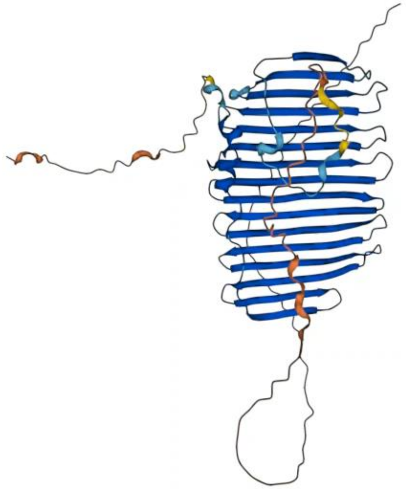



amylopectin double helices and LESV model, Osman, et al. 2024

AlphaFold prediction



*A. thaliana* ESV1

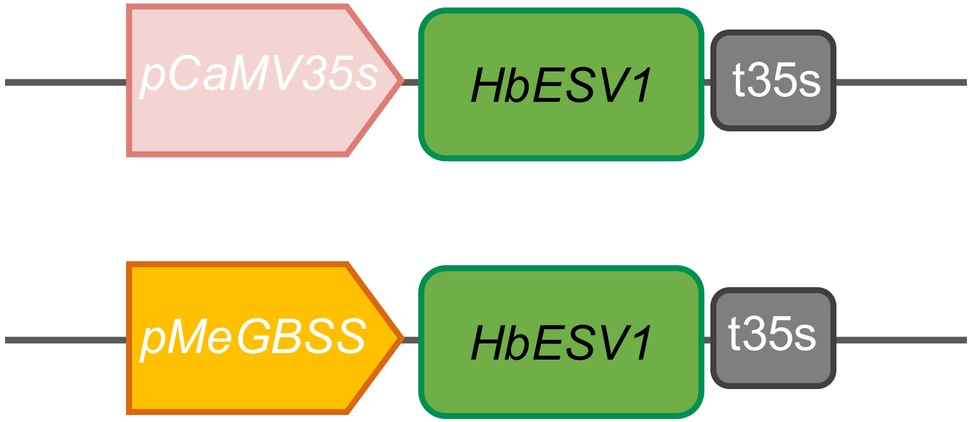


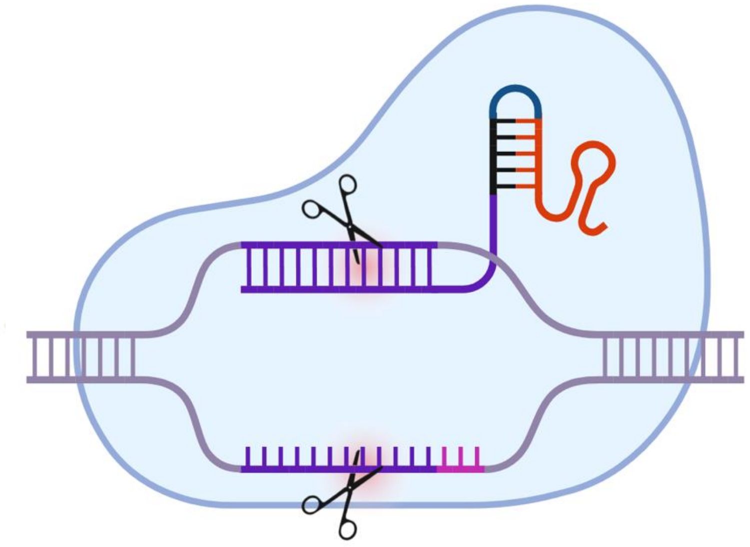
*M. esculenta* ESV1

# Editing ESV1 expression

ESV1
over-expression

ESV1
knock-down



*MeESV1*

image from BioRender

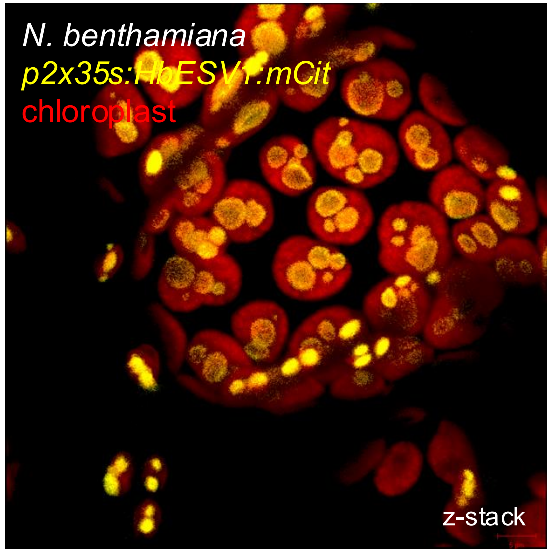
How is plant growth / starch altered?

---| STARCH DEGRADATION ?

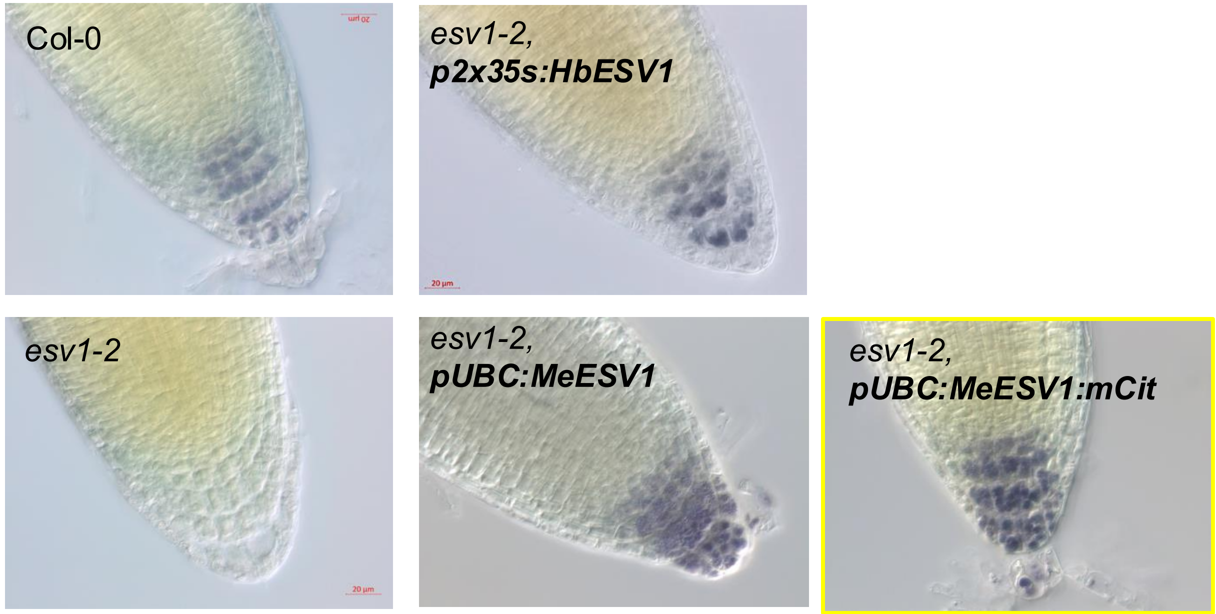
→ STARCH DEGRADATION ?

# MeESV1 complements AtESV1 function

Transient HbESV1 localizes at/near starch




Hb/MeESV1 rescues *esv1* phenotype (T1)

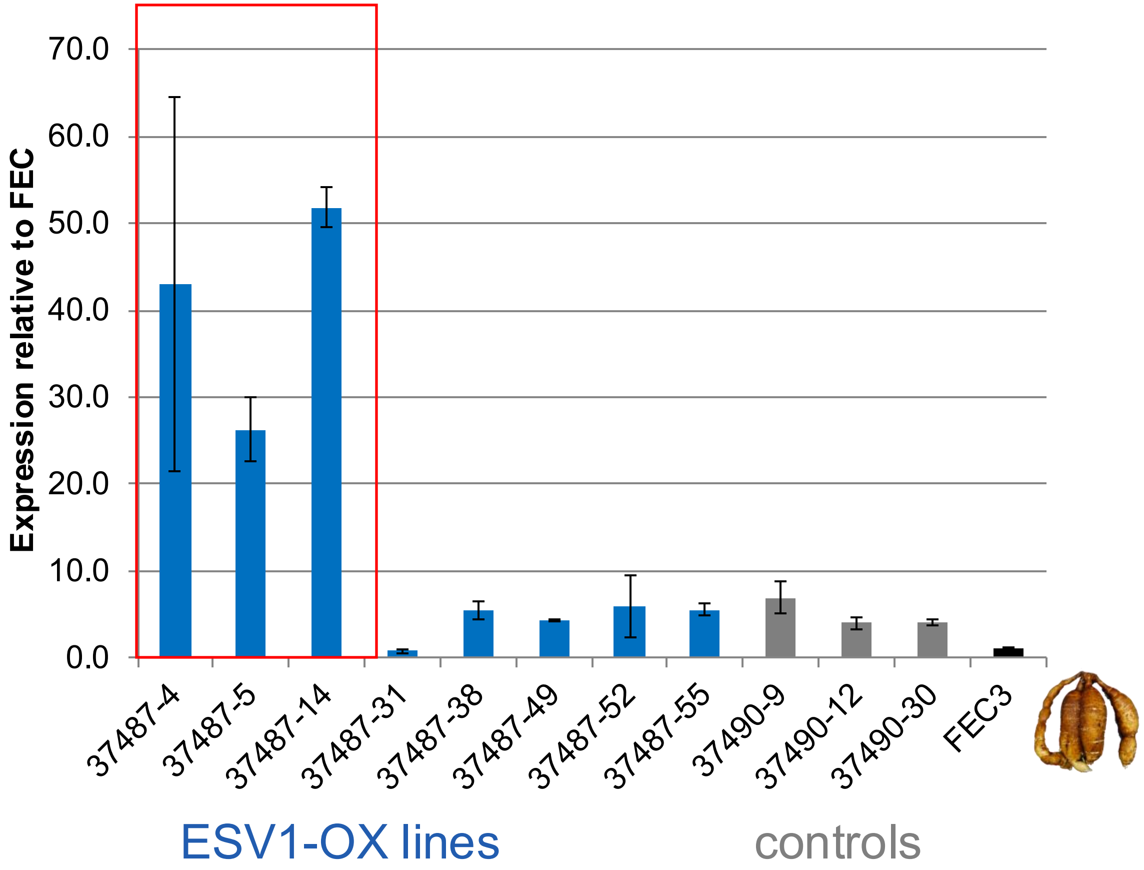


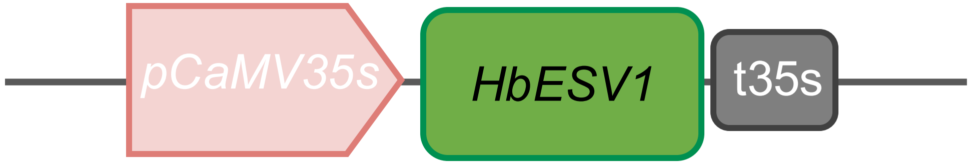


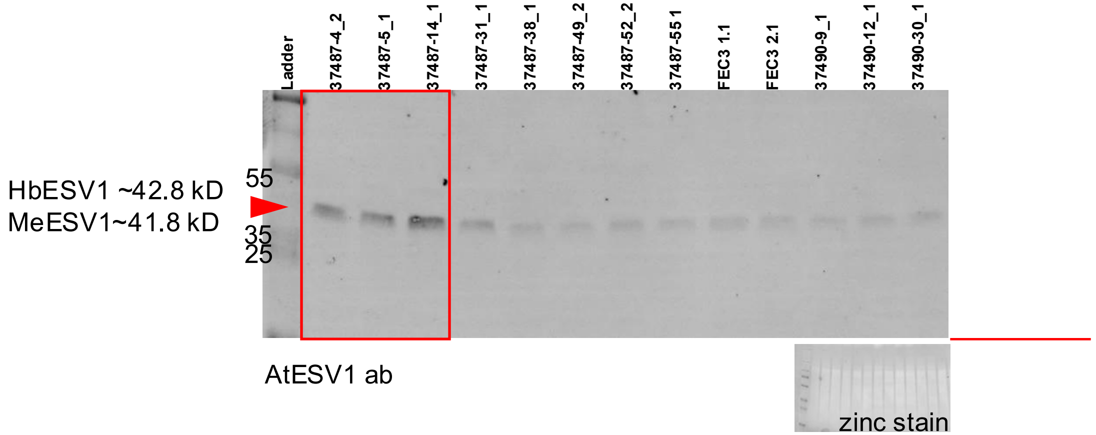
constructs: Vinaya Röhrl

☑ localization
☑ conserved function

# Three independent lines strongly overexpress ESV1



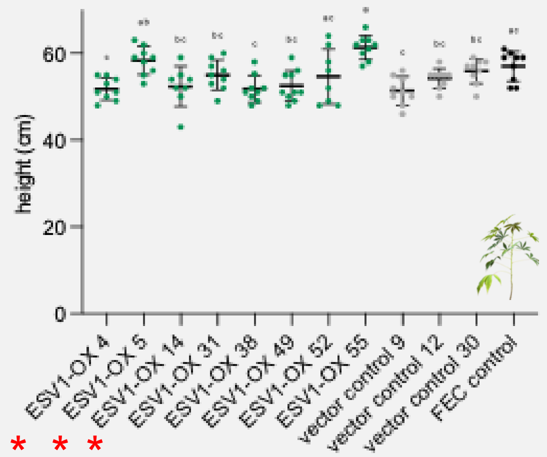



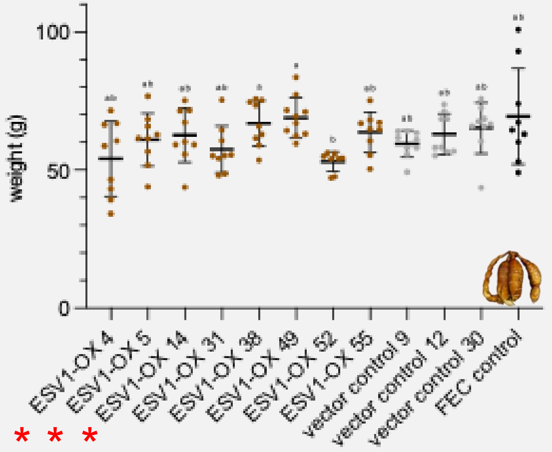




☑ overexpression

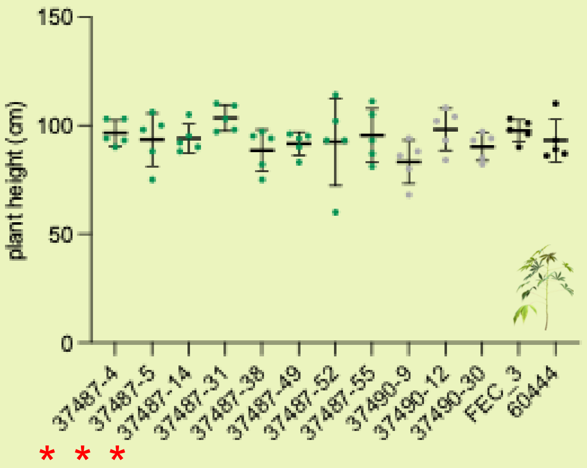
# Growth metrics of ESV1-OX lines

## GREENHOUSE

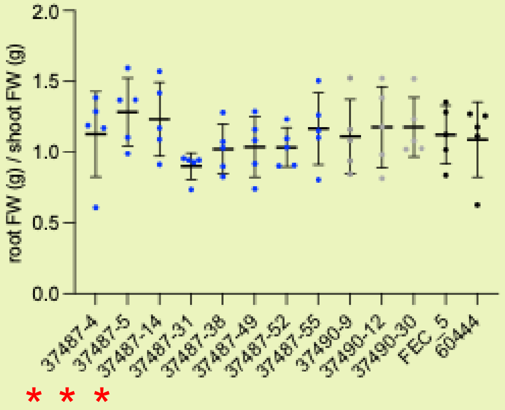


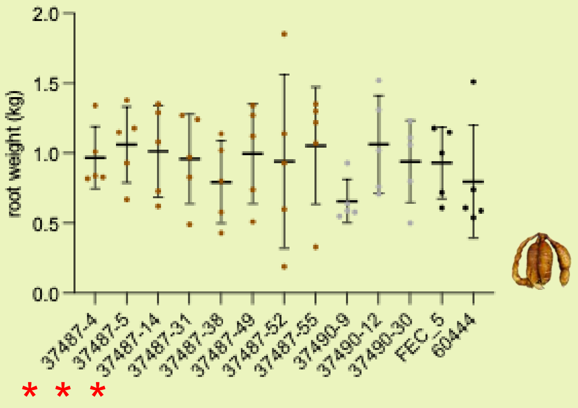




## FIELD - TAIWAN

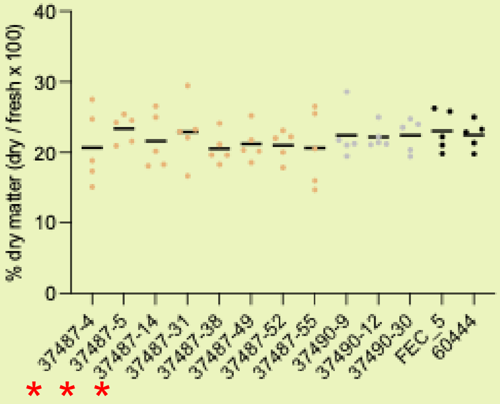



root:shoot ratio



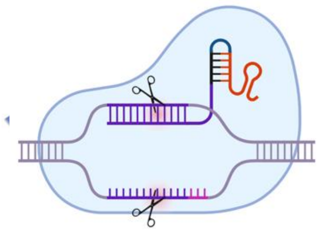

% dry matter




No growth penalty from ESV1-OX

* strong ESV1-OX

# Three independent CRISPR lines that strongly affect MeESV1



*MeESV1*

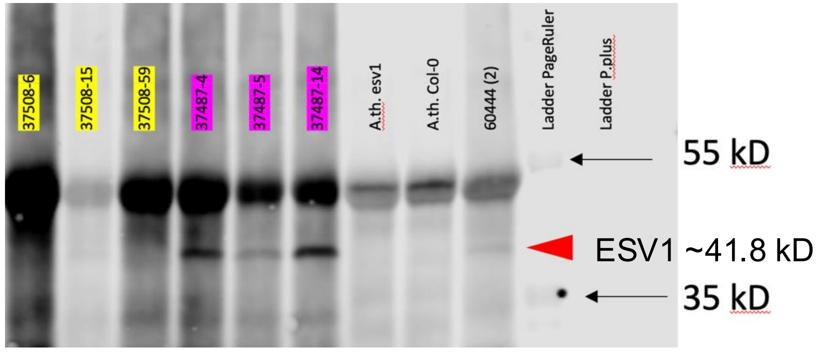
graphic from Biorender

> 6 million reads, Illumina

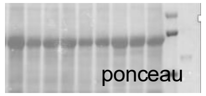
| Sample | position | REF | ALT | % total reads | Output |
|---|---|---|---|---|---|
| **37508_6** | 264 | GAC | GAAC | 92.1 | frameshift, premature stop |
| **37508_9** | 264 | GAC | GC | 76.0 | frameshift, premature stop |
| **37508_34** | 264 | GAC | GC | 72.9 | frameshift, premature stop |
| **37508_59** | 264 | GAC | GC | 98.7 | frameshift, premature stop |
| **37508_71** | 265 | ACTGG | AT | 63.0 | 3nt Δ, in frame |
| **37508_71** | 257 | TGAAAAGG | TG | 26.5 | 6nt Δ, in frame |
| **37508_15** | 262 | AGGAC | AGGGAC | 95.3 | frameshift, premature stop |
| **37508_54** | 254 | TAATGAAAAGGACTGGGCATCAACTTGTTGAATGAAA | TA | 35.8 | 36nt Δ, in frame |
| **37508_35** | 258 | GAAAAGGACT | GT | 78.3 | frameshift, premature stop |
| **37508_14** | 261 | AAGGACT | AT | 67.8 | frameshift, premature stop |
| **37508_50** | - | no change | - | - | - |

CRISPR

ESV1 OX

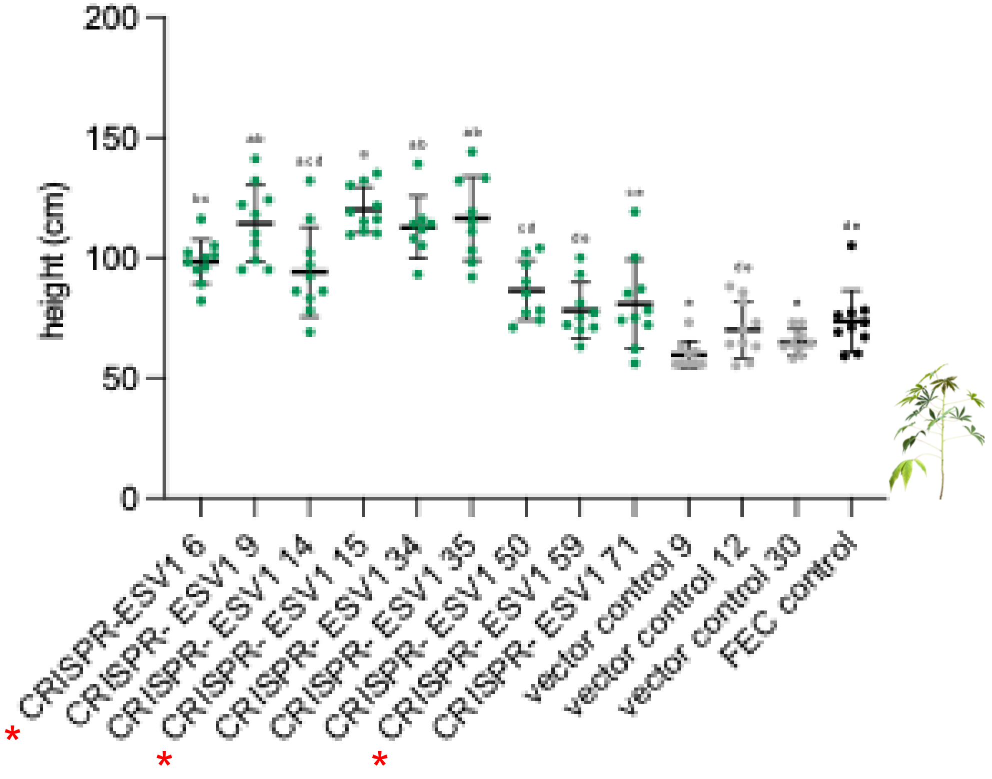


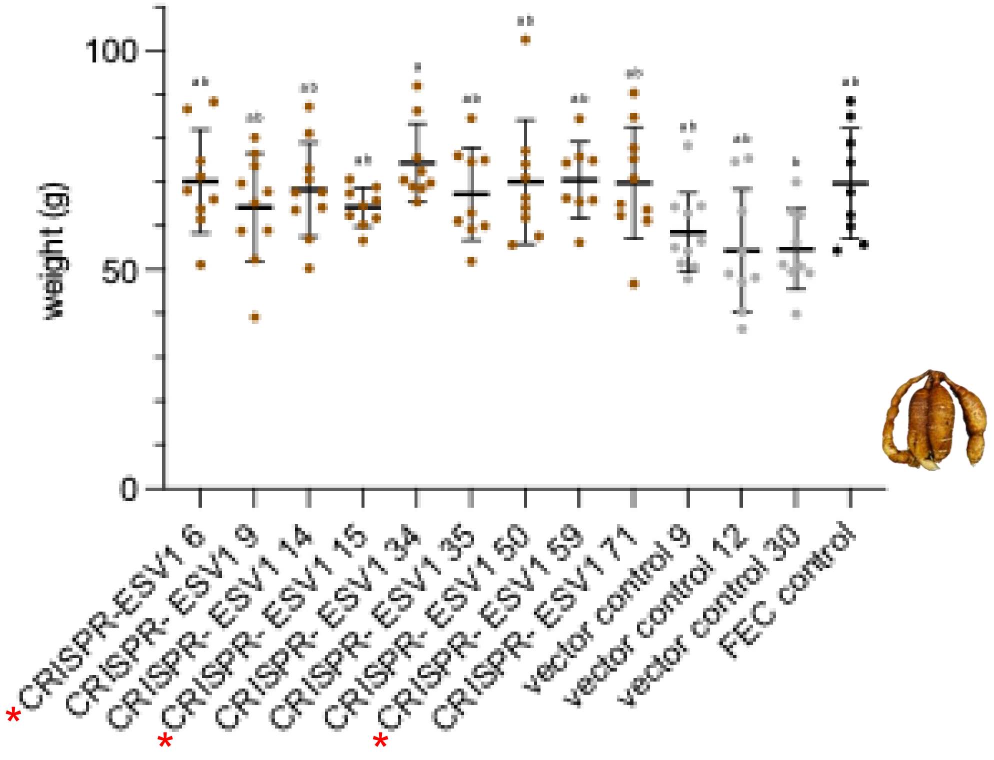

AtESV1 ab




☑ loss of expression

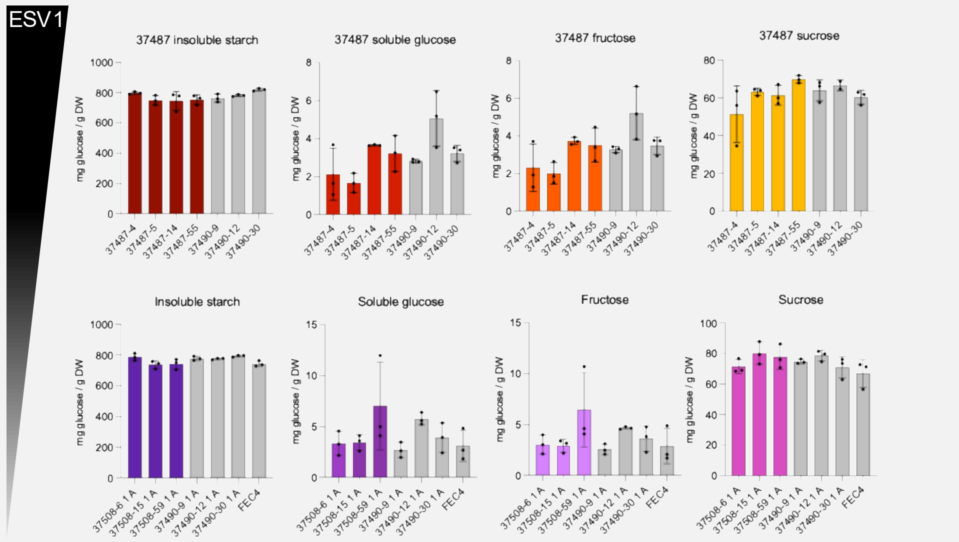
# Growth metrics of CRISPR-ESV1



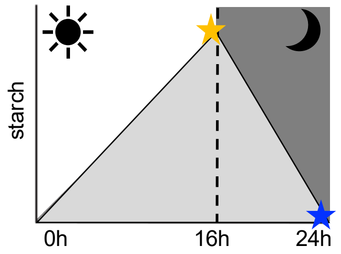



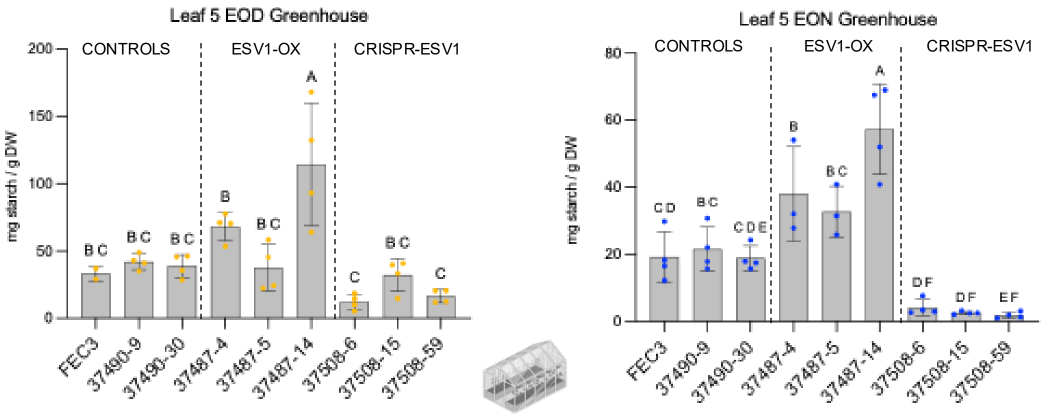

- CRISPR-ESV1 plants grow taller in greenhouse (more accessible sugar?)
- Loss of ESV1 does not significantly affect root weight (good conditions)

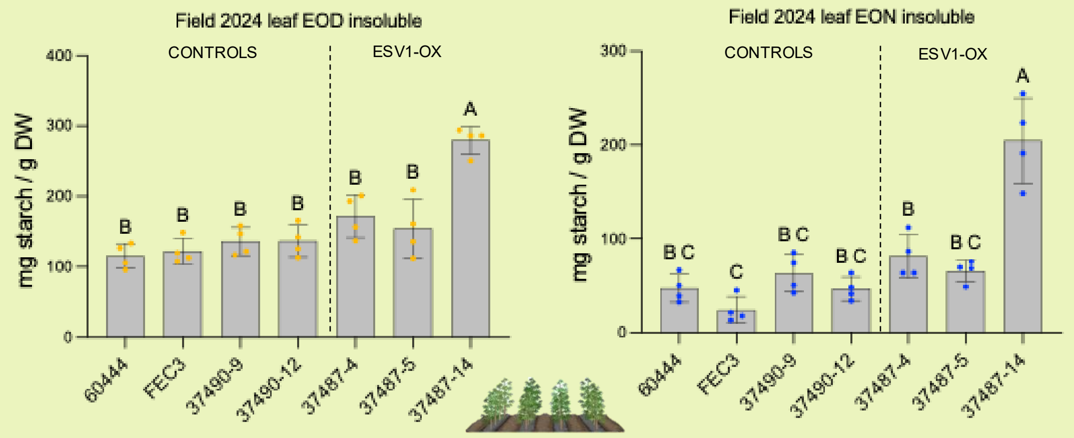
# Looking more closely at starch…




No differences in starch or soluble sugar quantity in greenhouse grown plants



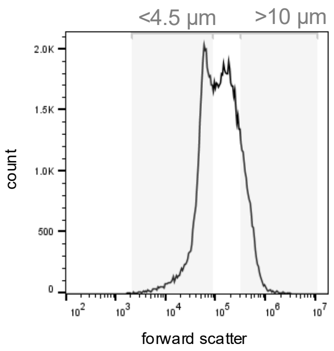



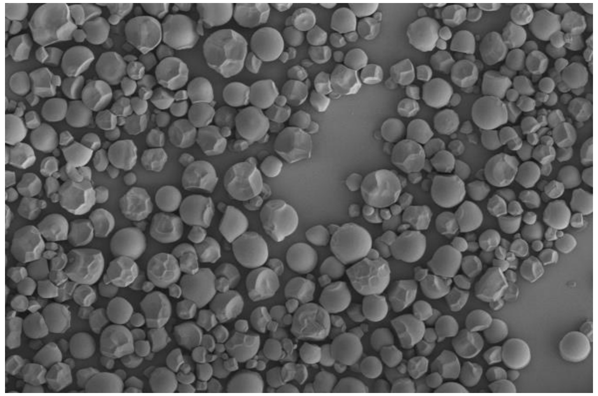

More leaf starch with increasing ESV1 and less leaf starch with loss of ESV1

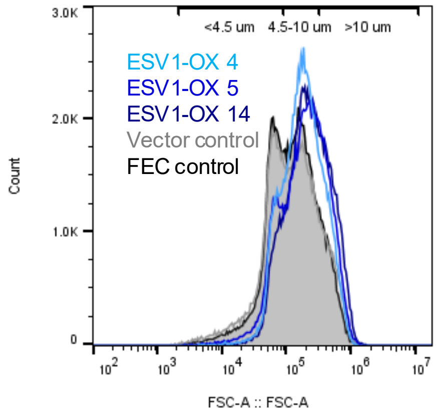
# Flow cytometry suggests ESV1-OX forms more large starch granules

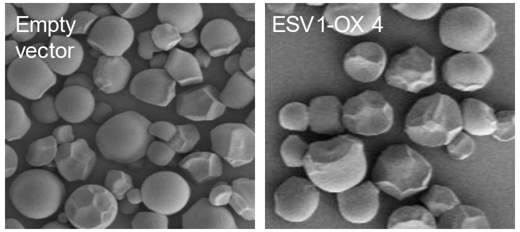
**Flow cytometry**
Between 4 – 20 µm



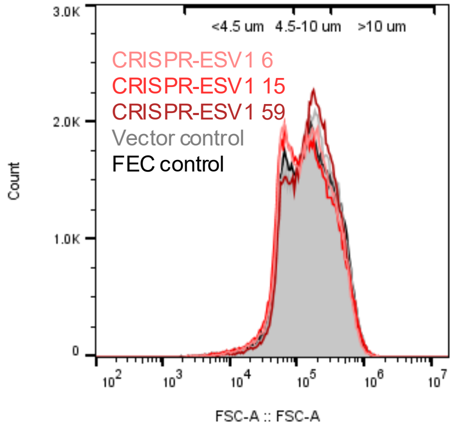

**SEM**

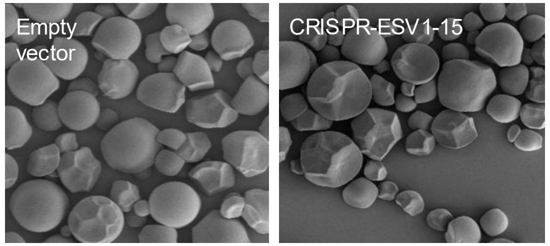





ESV1-OX leads to more larger granules

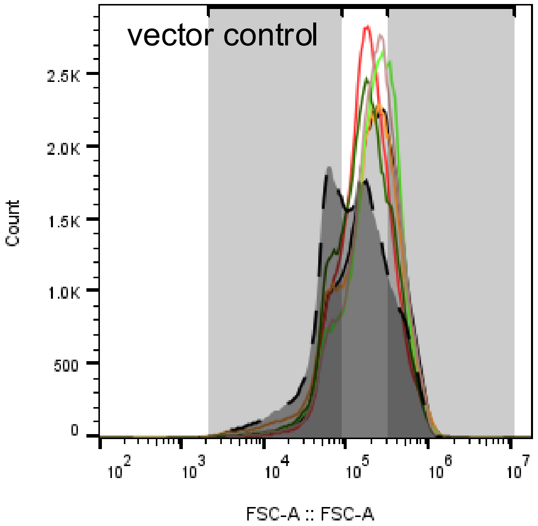


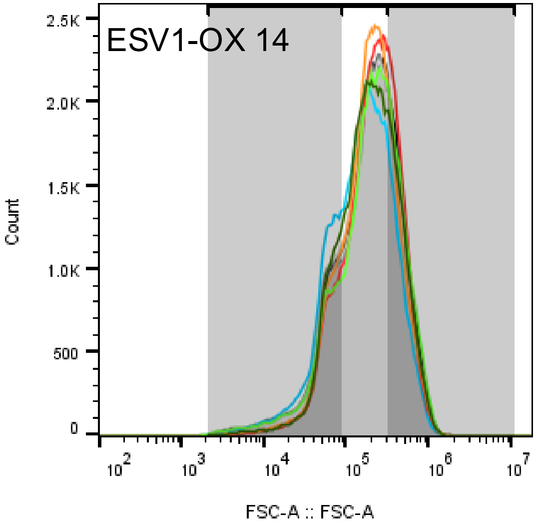




Loss of ESV1 has no obvious size/shape effect in root starch

# ESV1 may promote early buildup of starch in storage roots

Field-grown cassava contains more larger starch, almost monomodal

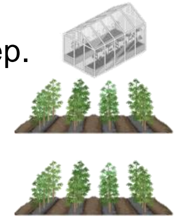


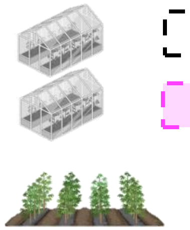

greenhouse control rep.

60444 field control

COLORED LINES
→ field reps.



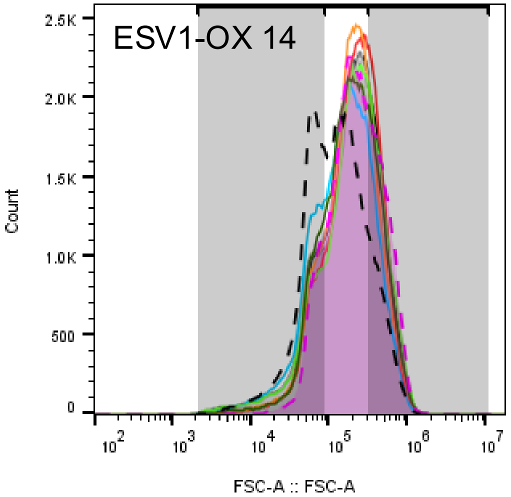
Size and shape of ESV1-OX starch same in greenhouse and field

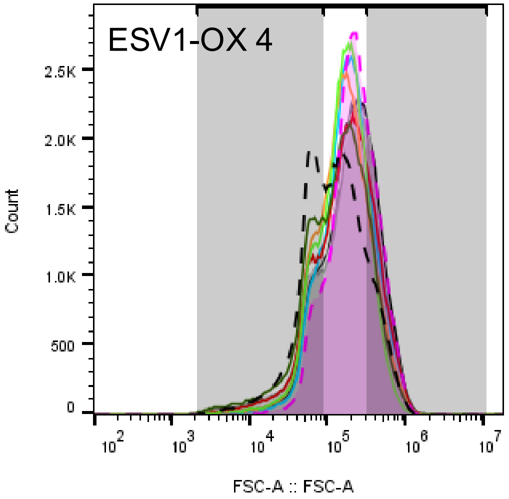


greenhouse control rep.

greenhouse 37487 rep.

COLORED LINES
→ field reps.



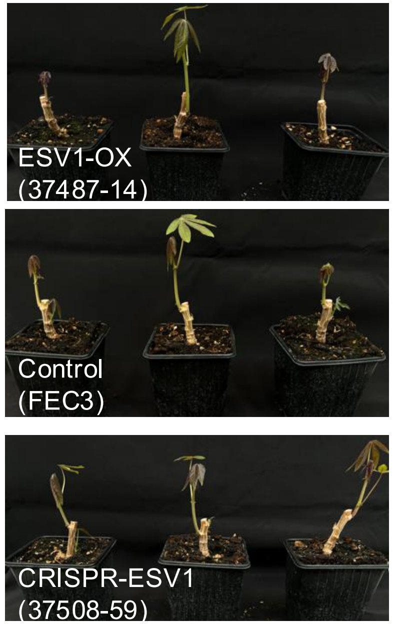




Higher ESV1 levels may allow granules to reach their size potential more quickly.

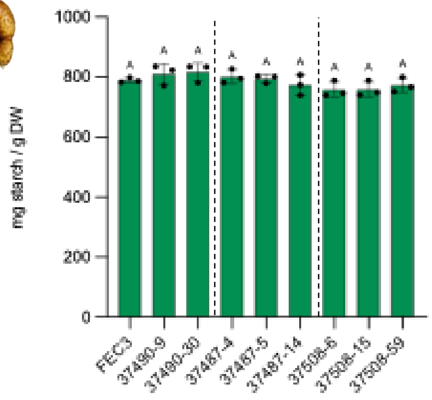
# ESV1 may stabilize storage starch during environmental stress (ratooning)

0 DAR

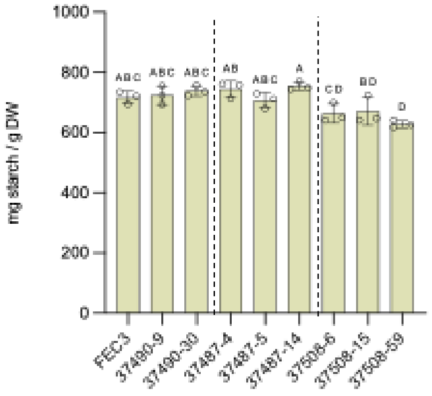
10 DAR




0 DAR

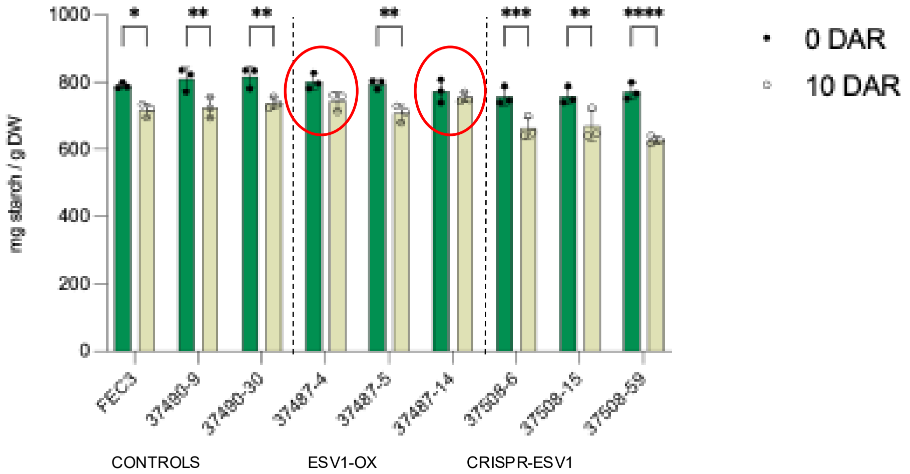



10 DAR




ESV1-CRISPR roots have significantly less starch 10 DAR.

0 and 10 DAR




Controls have significantly reduced starch after 10 DAR.

ESV1 OX lines 37487-4 and -14 show **NO** significant difference in starch after 10 DAR.

ESV1 CRISPR lines 37508-6 and -59 show **more significant reduction** in starch after 10 DAR.

Demonstrates the ability of ESV1 as a biotechnological tool to regulate the availability of storage starch

# Outlook II.

ESV1
over-expression

ESV1
knock-down

**Takeaway: High ESV1 levels increase leaf starch, maintain root starch under stress, and do not come at a cost of plant growth or yield.**

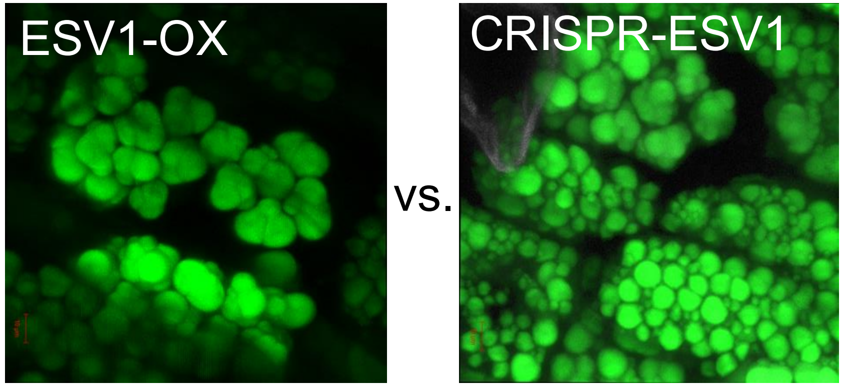
- May be important in developmentally young starch granules (further study required)
- The CRISPR-ESV1 line is valuable in understanding ESV1's endogenous role - generally inversely complements much of the ESV1-OX results

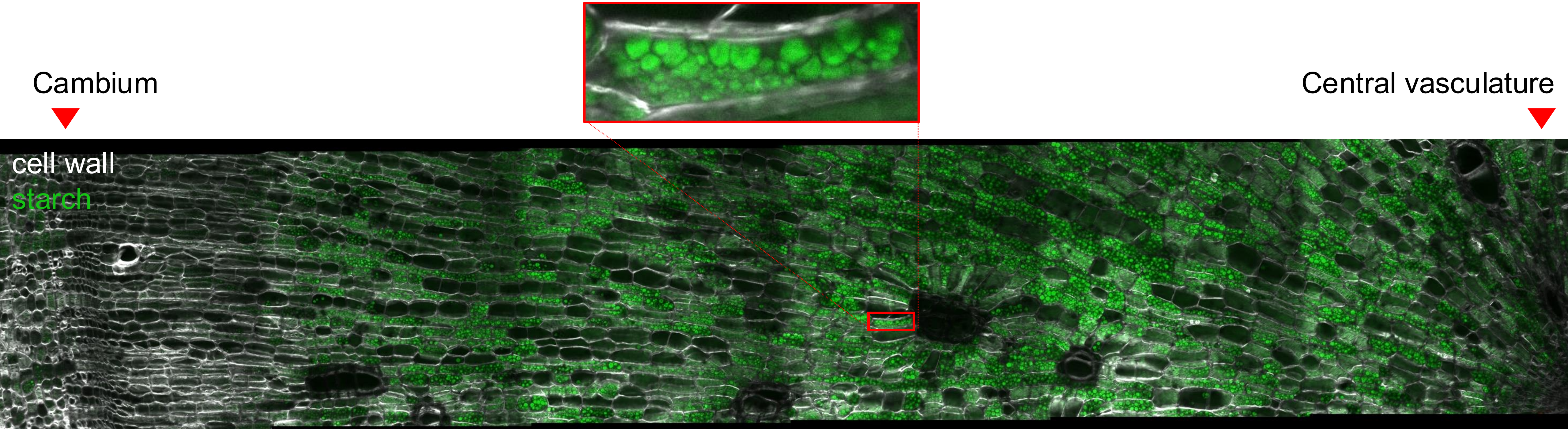
**Next:** We are exploring earlier developmental stages and the effect on starch stability using ESV1 tools

- Larger stress test
- Characterize further the more 'rooty' *pMeGBSS:HbESV1* line
- ESV1 OX lines in **Taiwan 2025 field – fewer lines, increasing replicates for CFT2026**

# In progress…

- Are there different sized starch granules in early vs. late bulking roots? *Flow cyt suggests yes…*
- Are there **spatial-temporal differences** in the number and size of starch in the xylem parenchyma?
- ESV1 and starch initiation lines will greatly contribute…



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Credit: Andrea Ruckle, in Eschikon, ETHZ

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