

# Technical Module 2.2

## Improving Transformation of Farmer-preferred Cassava Genotypes

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Christian Lamm



Friedrich-Alexander-Universität  
Erlangen-Nürnberg





## **FEC production of CMD-resistant genotypes**

- › 98/0505 and 91/02324 successful



## **Chemical Boosters**

- › Addition of Hyperphyllin (HP) or Sodium Butyrate (SB) to medium
- › FEC induction medium: No effects observed
- › Regeneration medium: Higher regeneration efficiency in WT FEC
- › Effect on transformed FEC?



## **Genetic Boosters**

- › Several genes cloned and transformed
- › No obvious effects observed with first promoter-gene combinations  
→ *Discontinued to focus on transformation experiments*



## **Transformation of CMD-resistant genotypes**

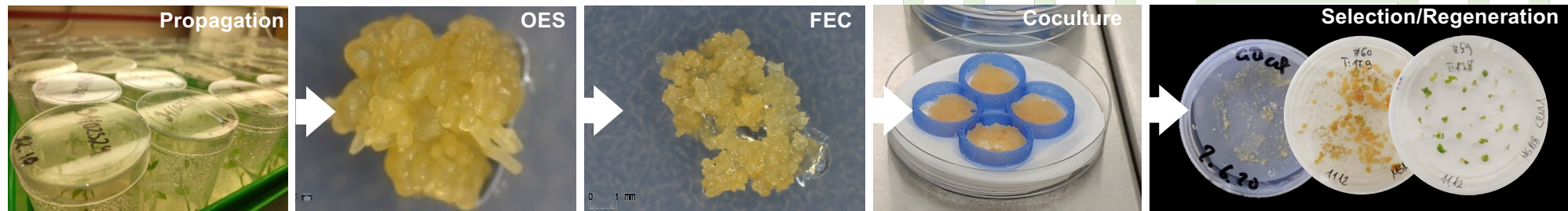
- › First transformation experiments initiated

## Relevant Genotypes in our adapted FEC Pipeline

### Producing FEC for booster efficiency testing:

- Protocol based on Bull et al. 2009 and Segatto et al. 2022
- Starting material: Immature leaflobes
- FEC induction medium containing Tyrosine HCl and Moxalactam

### → Five genotypes tested so far:

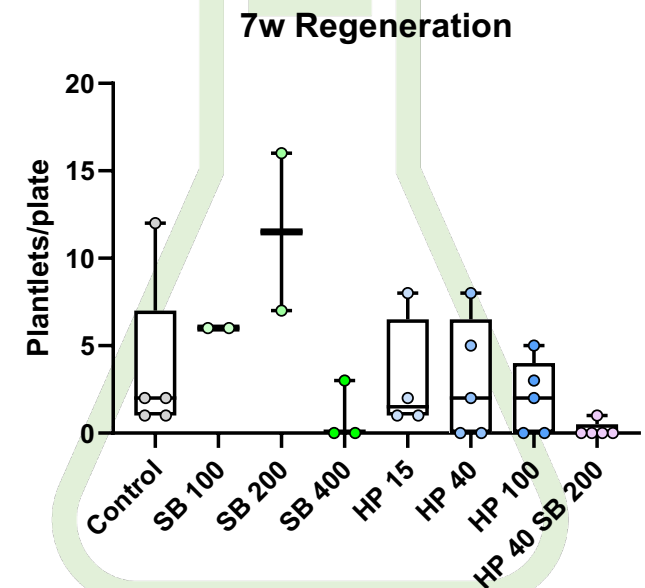
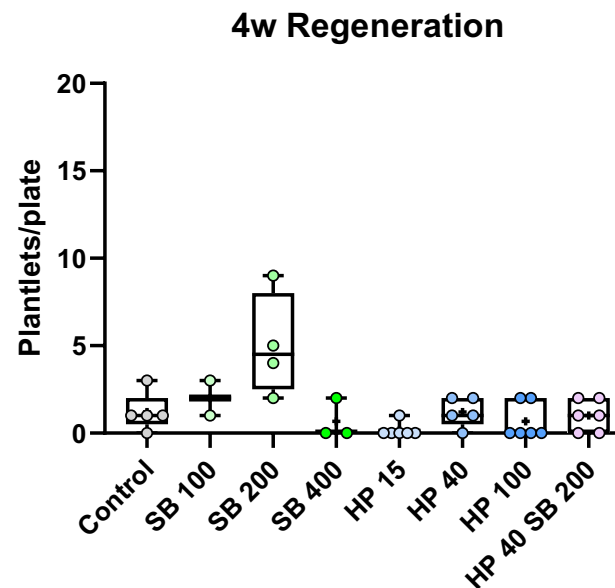
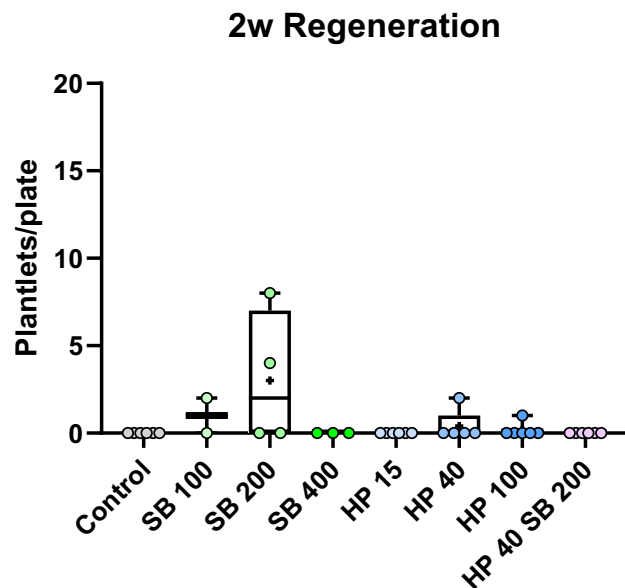


- FEC produced from three genotypes to study effects of boosters on regeneration
- Transformation experiments initiated in parallel

## Regeneration Experiment TMS60444 #1:

- Genotype TMS60444
- pNOS::GFP
- Regeneration medium supplemented with Hyperphyllin and/or sodium butyrate
- Quantification of emerging shoots/plate

- Contamination with *Agrobacterium* reduced number of plates/treatment
- Tendency to higher efficiency with 200  $\mu$ M SB

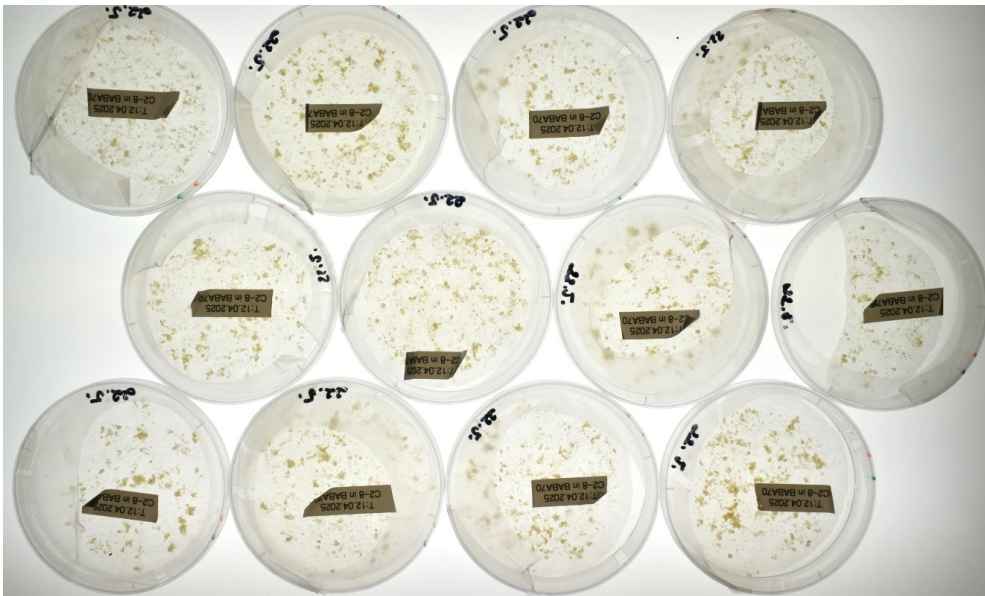


## Regeneration Experiment Baba-70

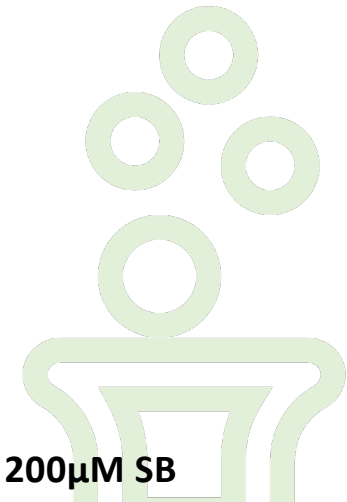
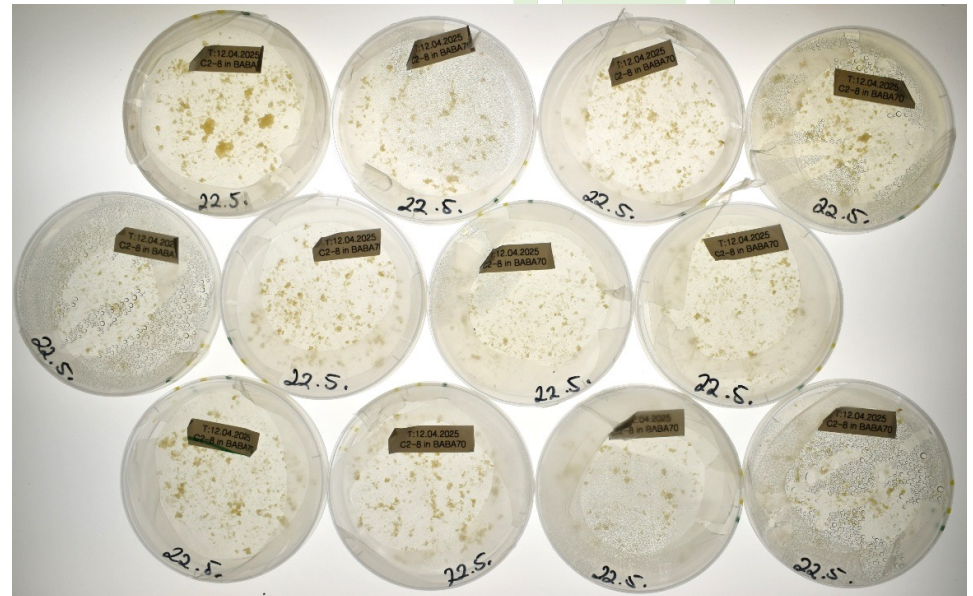
- Genotype Baba-70
- C2-8 / AKT2<sub>mut</sub> and empty vector
- Regeneration medium supplemented with sodium butyrate
- Quantification of emerging shoots/plate

→ Only few plates with regenerating plantlets

Regular regeneration medium



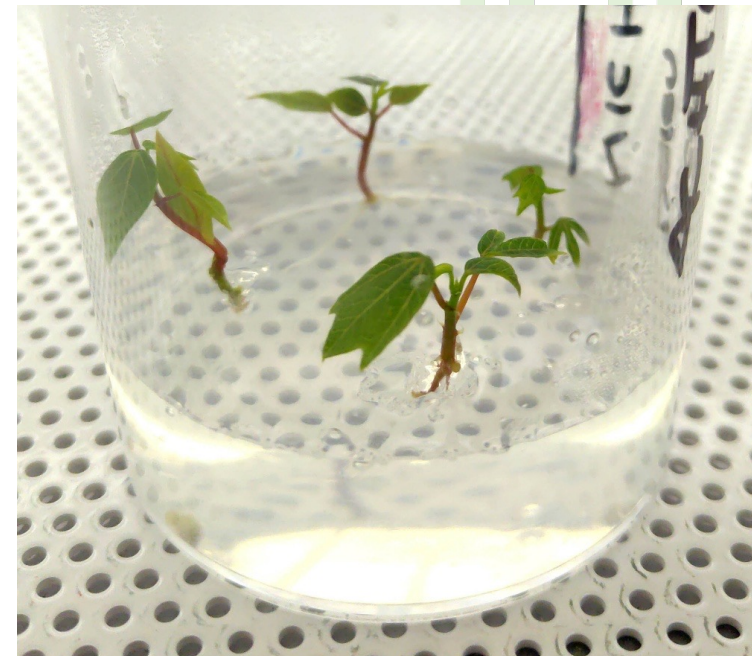
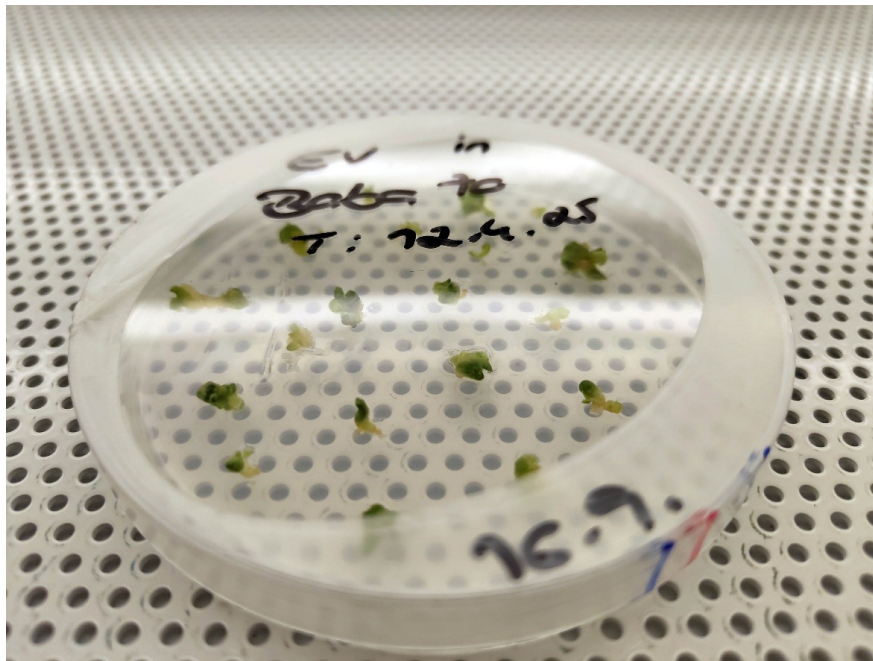
Regeneration medium + 200μM SB



## Regeneration Experiment Baba-70

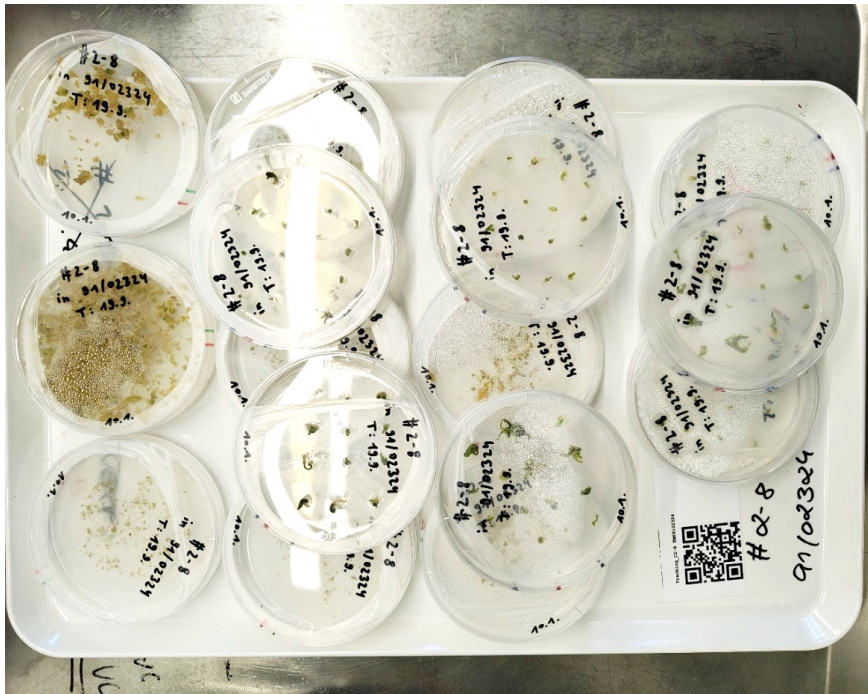
- Genotype Baba-70
- C2-8 / AKT2<sub>mut</sub> and empty vector
- Regeneration medium supplemented with sodium butyrate
- Quantification of emerging shoots/plate

→ Regenerated plantlets all die in Hygromycin-containing medium indicating failed T-DNA integration  
→ Repetition of experiment using adapted conditions



## C2-8 in TMS91/02324

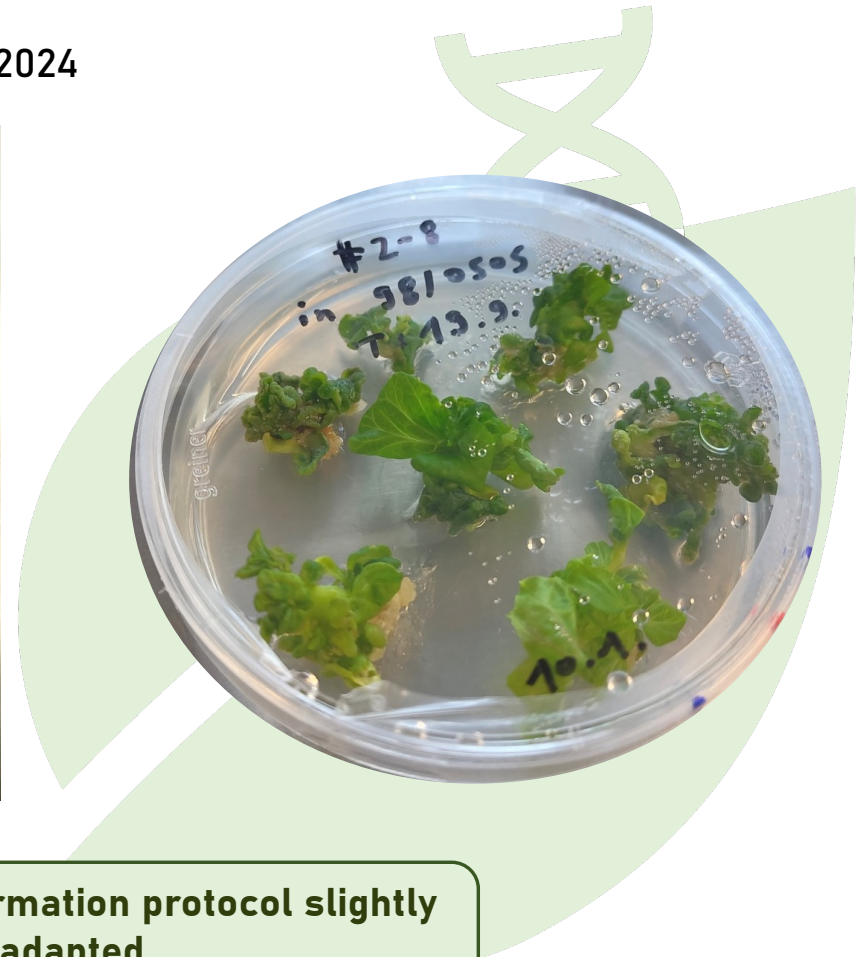
→ Transformation initiated with first FEC batch Sept 2024



- Routine transformation protocol slightly adapted (media composition, incubation times)
- Experiment produced many cotyledons
- Transformation fails during maturation/shoot regeneration

## C2-8 in TMS98/0505 „Fine face“

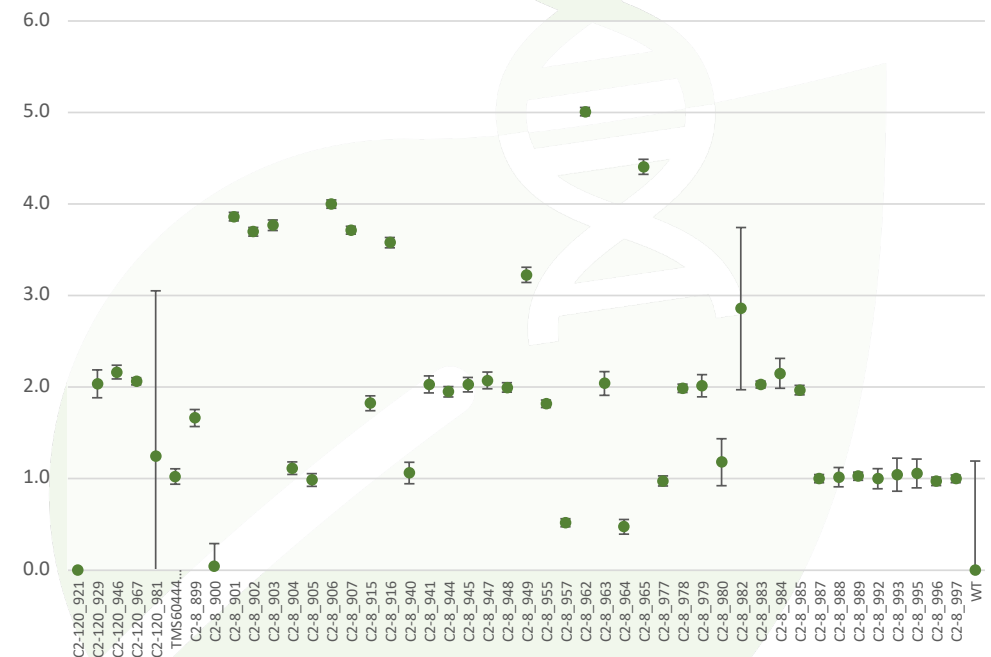
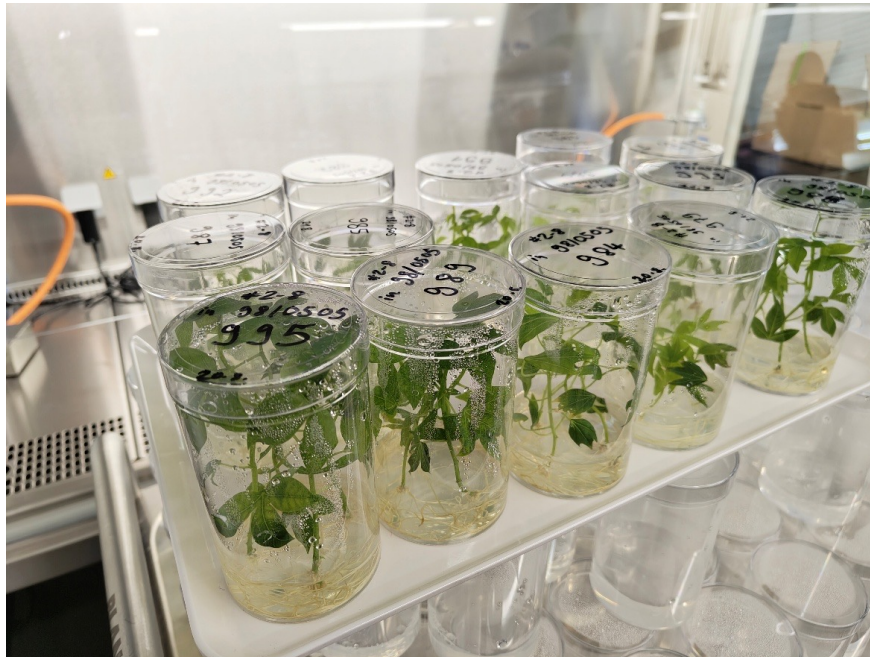
→ Transformation initiated with first FEC batch Sept 2024



→ Routine transformation protocol slightly adapted  
(media composition, incubation times)  
→ Regeneration occurred readily

## C2-8 in TMS98/0505 „Fine face“

→ Transformation initiated with first FEC batch Sept 2024



- Regenerated 51 transgenic lines
- Preliminary observation: Many lines show defects (irregular leaves, elongated internodes/petioles, ...)
- Whole genome sequencing confirmed general genomic integrity
- Digital PCR shows single- and multi insertion events as well as escape lines

## C2-8 in TMS98/0505 „Fine face“ – Initial greenhouse screening

→ Early cultivation with first 10 lines – 8 weeks after planting



WT\_TMS98/0505



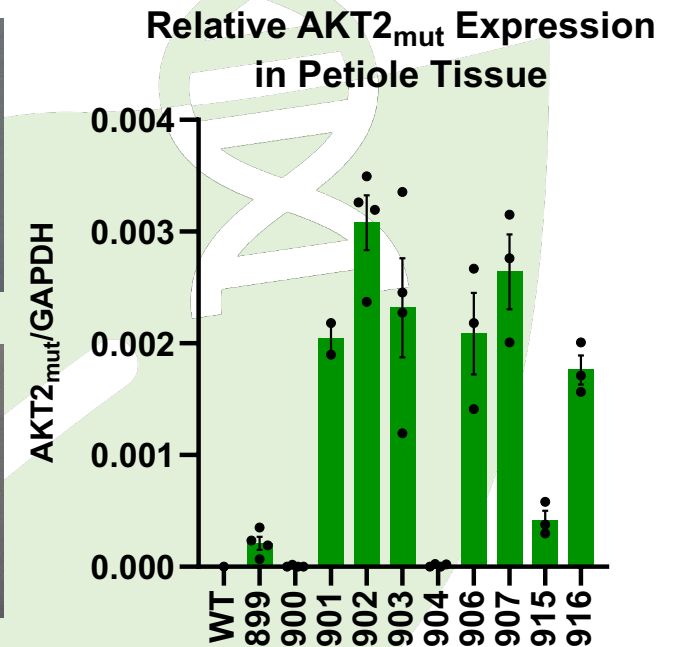
AKT2<sub>var\_904</sub>



AKT2<sub>var\_900</sub> (T-DNA negative)



AKT2<sub>var\_902</sub>



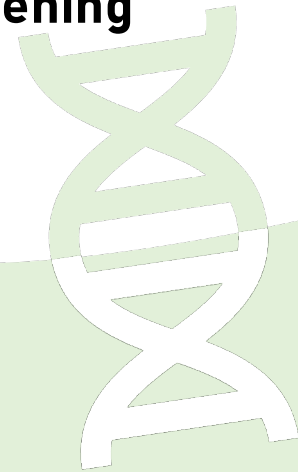
- Defects observable in all positive lines (to varying degree)
- Escape line 900 shows wildtype-like growth
- Positive line 904 without transgene expression in petioles with mildest defects

## C2-8 in TMS98/0505 „Fine face“ – Initial greenhouse screening

→ Early cultivation with first 10 lines – 15 weeks after planting

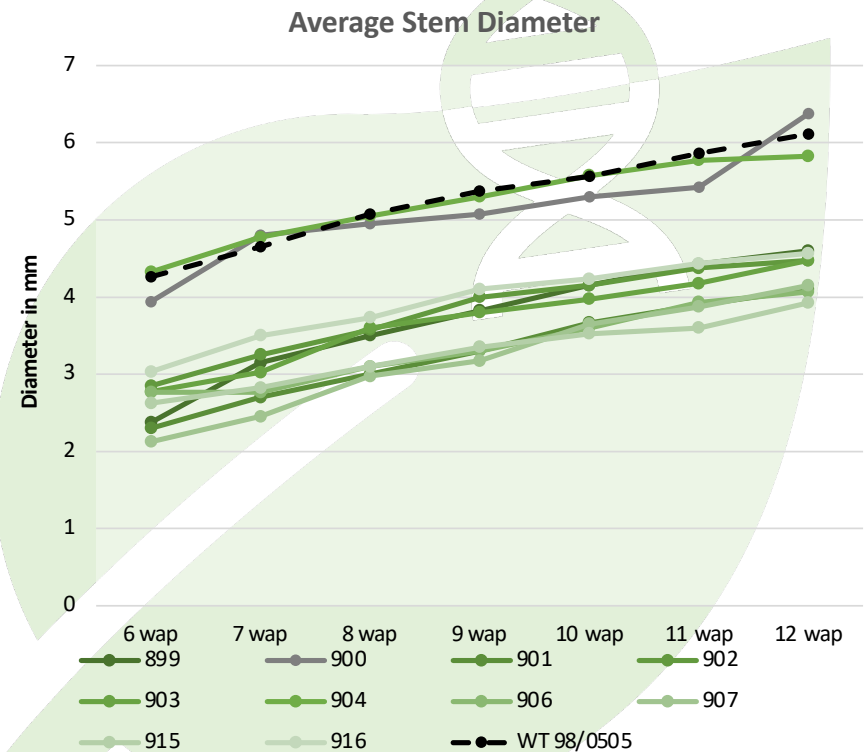
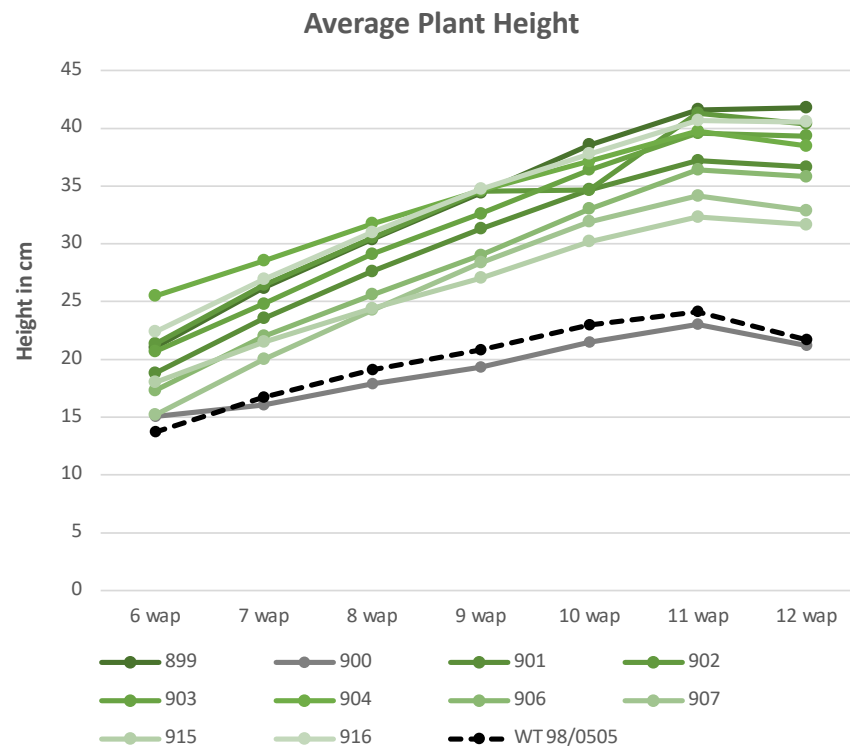


→ Defects remained after prolonged cultivation



## C2-8 in TMS98/0505 „Fine face“ – Initial greenhouse screening

→ Early cultivation with first 10 lines – 8 weeks after planting

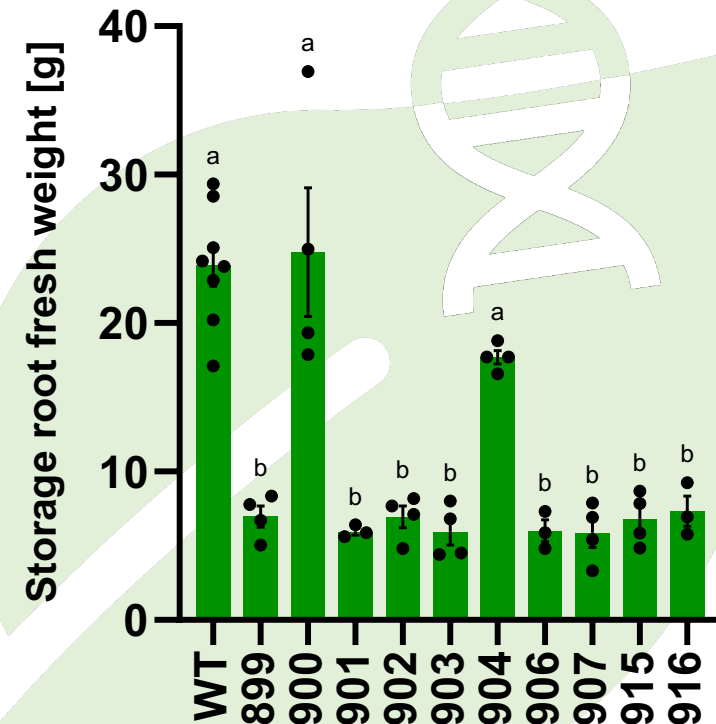
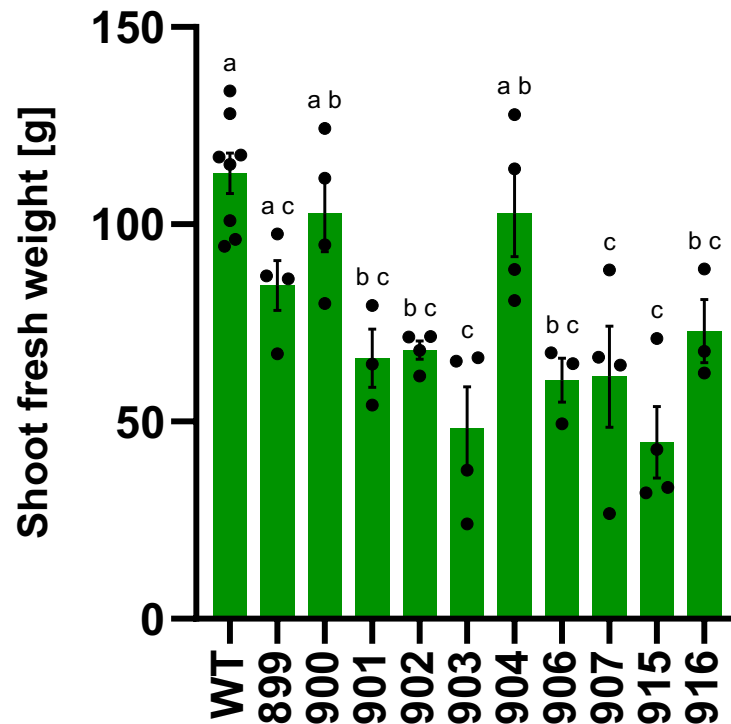


→ Transgene-expressing lines with increased shoot height,  
but lower stem diameter

→ Line 904 with stem diameter similar to WT and escape line 900

## C2-8 in TMS98/0505 „Fine face“ – Initial greenhouse screening

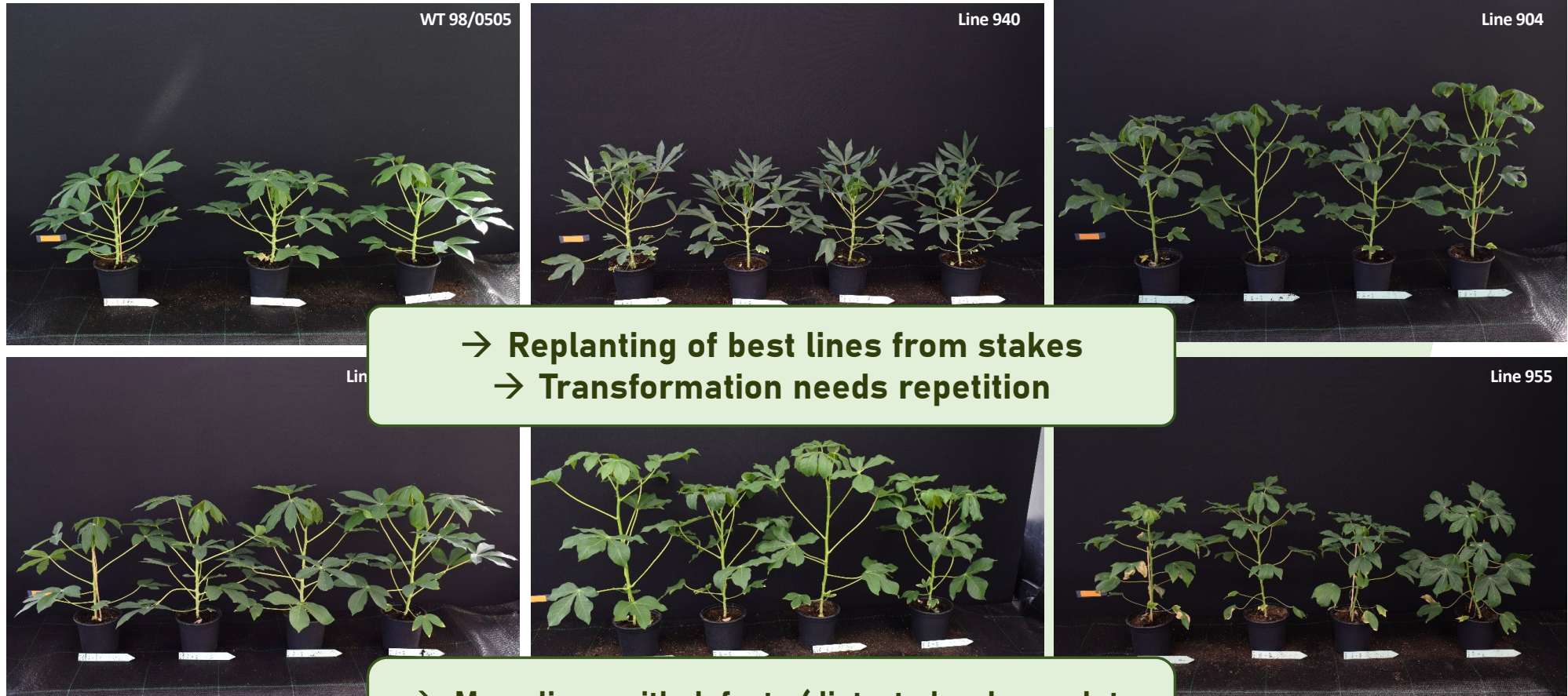
→ Early cultivation with first 10 lines – Harvest 16 weeks after planting



→ Shoot and root weight significantly decreased for all lines except escape line 900 and line 904

## C2-8 in TMS98/0505 „Fine face“ – Initial greenhouse screening

→ Second cultivation, 41 lines – 6 weeks after planting



→ Replanting of best lines from stakes  
→ Transformation needs repetition

→ Many lines with defects (distorted or lanceolate leaves, distorted stems)  
→ Several lines with less severe defects than others

## Converting a greenhouse climate chamber to a tissue culture light room



- Storage capacity for thousands of plants
- Micropropagation for Taiwan field trial 2026 initiated and on track

**FEC production of CMD-resistant genotypes**

- › 98/0505, 91/02324 and Baba-70 reproducibly successful

**Chemical Boosters**

- › Tendency to higher regeneration efficiency in TMS60444
- › Baba70 transformation failed irrespective of media composition  
→ Repetition ongoing

**Transformation of CMD-resistant genotypes**

- › TMS91/02324 failed during shoot regeneration
- › Transgenic TMS98/0505 lines generated,  
but plants show defects to varying degree,  
greenhouse screening ongoing
- › Regeneration itself not problematic, as escape lines are unaffected  
→ Repetition of transformation initiated (eV, C2-8, C56)

**Micropropagation for Taiwan confined field trial 2026**

- › Infrastructure established to enable propagation
- › Propagation ongoing and on track

# Thanks for listening!

Aaron Engelman, Eva Düll, Birgit Petersen, Julius Schulz,  
Michaela Reiser, Otilia Ciobotea, Sanika Lakshman





# Transformation of relevant Genotypes

21



