



# **Regulating transcription factors to alleviate plant tissue and genotype limitations of cacao somatic embryogenesis**

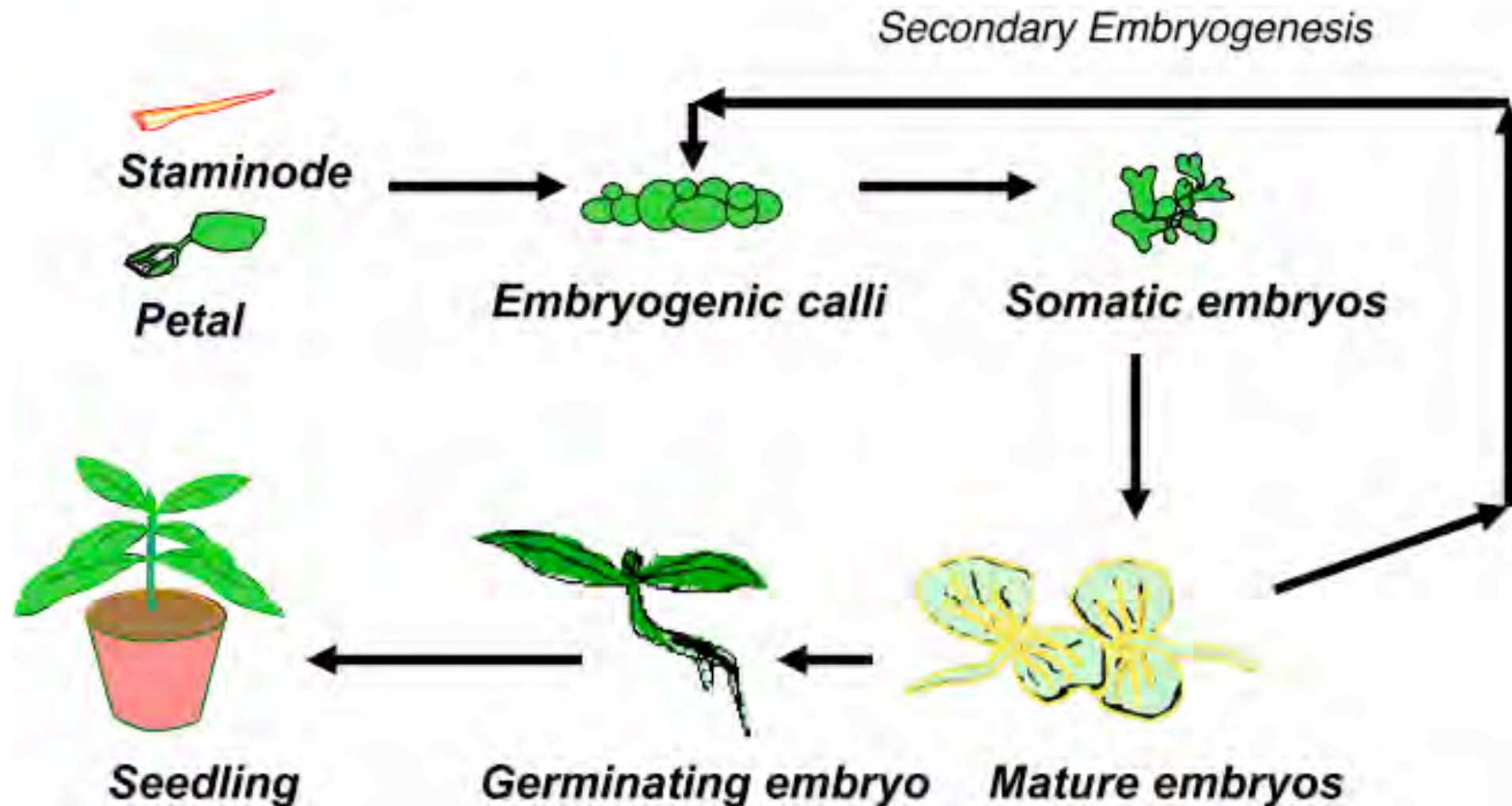
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*Lima, Peru*

**14 November 2017**



# Propagation by Cacao Somatic Embryogenesis





# Integrated Propagation System (NextGen Cacao Propagation Pipeline)

*In the laboratory*



Floral Parts



Somatic  
Embryos



Plantlets



*In the nursery*



Acclimated SE  
plants



Bentwood  
Stock Plant



Rooted Cutting



SE plants at  
The Nestle Farm, Ecuador

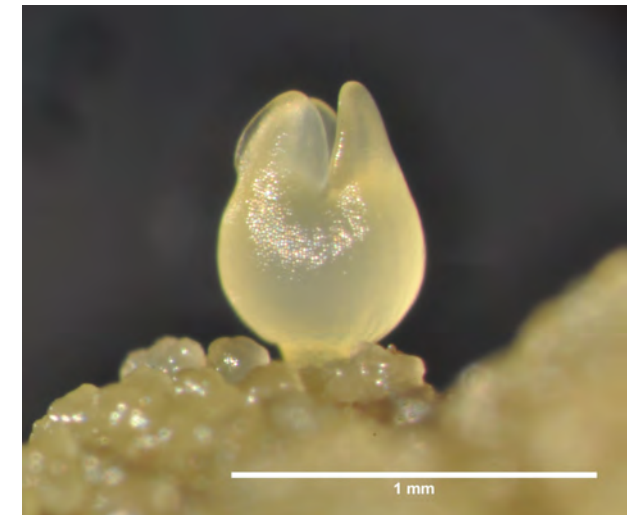


Ave. yield of 2.6 t/ha from SE plants

# Research Needed to Optimize the Cacao Somatic Embryogenesis Protocols (SE)

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- Optimization of the protocols for new genotypes
- Increasing the proportion of high quality embryos
- Improvement of the maturation process
- Increasing the rates of embryo to plant conversion



Photos: Mark Gultinan, PSU

# Main Research Questions

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- What biological mechanisms controlling cacao SE?
- What are the differences between genotypes in the response to SE?
- What metabolic pathways are the most important in SE initiation and maturation?
- What are the key molecules involved – DNA, RNA, proteins, metabolites?



Seed storage albumins, cupins, oleosins

Stem cell maintenance

Wus

PKL

LEC1

LEC2

AGL15/18

LBD40

FUS3

ABI3

ABI5

AtEM1, AtEM6

PEI1

EEL

MAE

SWN

CLF

SERK 1

CLV 1/3

bZIP67

L1L

CRC, SUS2

MIR156A, MIR156C

SPL10, SPL11

VAL1

BBM

WRI1

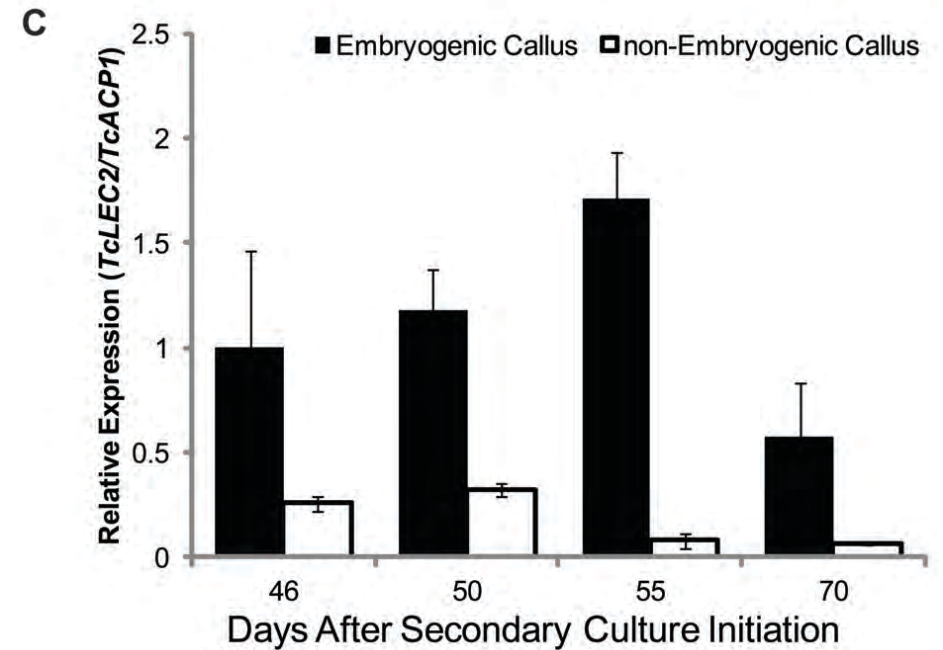
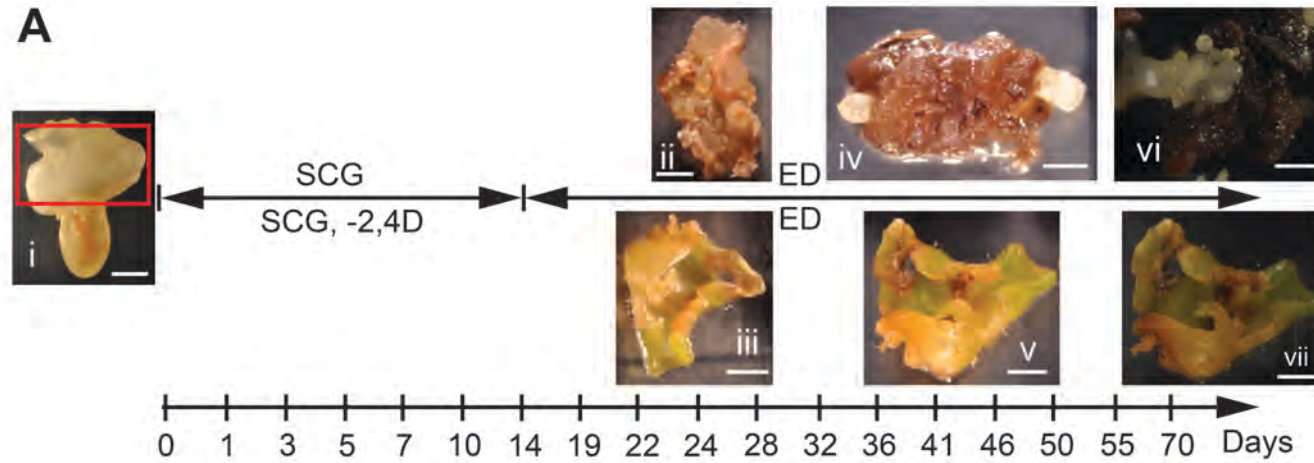
Fatty acid biosynthesis

Inducer/Enhancer of SE

Repressor of SE



# *TcLEC2* TF gene has higher expression in cacao SE tissue



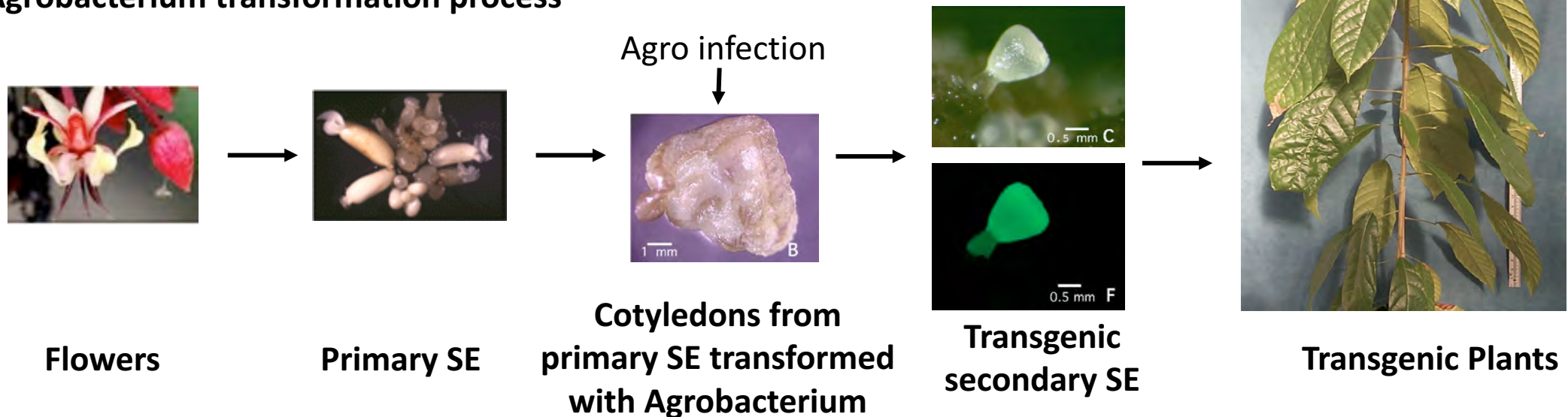


# Study of the function of TcLec2 TF protein using genetic transformation

## Agrobacterium transformation vector including *TcLec2:GR*



## Agrobacterium transformation process

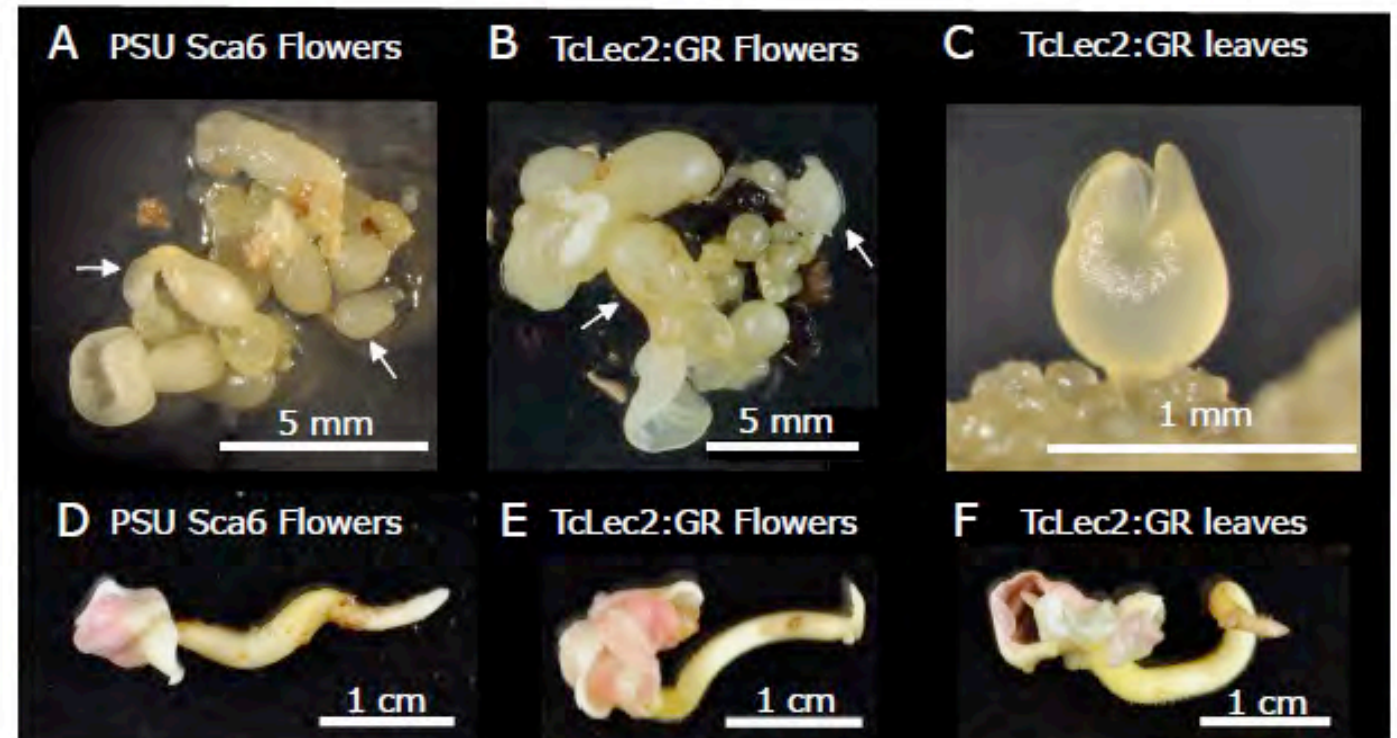
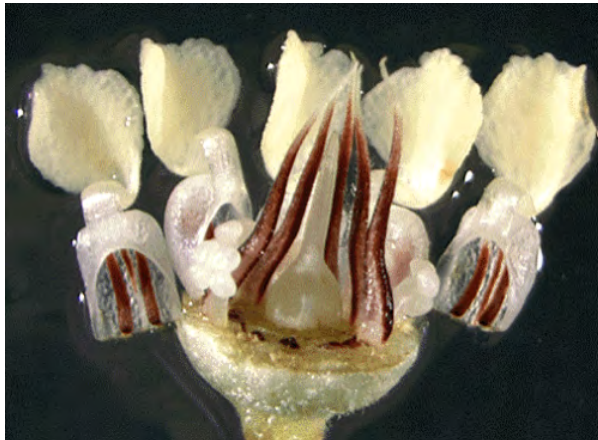






# To test the function of the *TcLec2* gene in SE flowers and leaves from *TcLec2:GR* transgenic plants were cultured to produce new SEs

## Explants

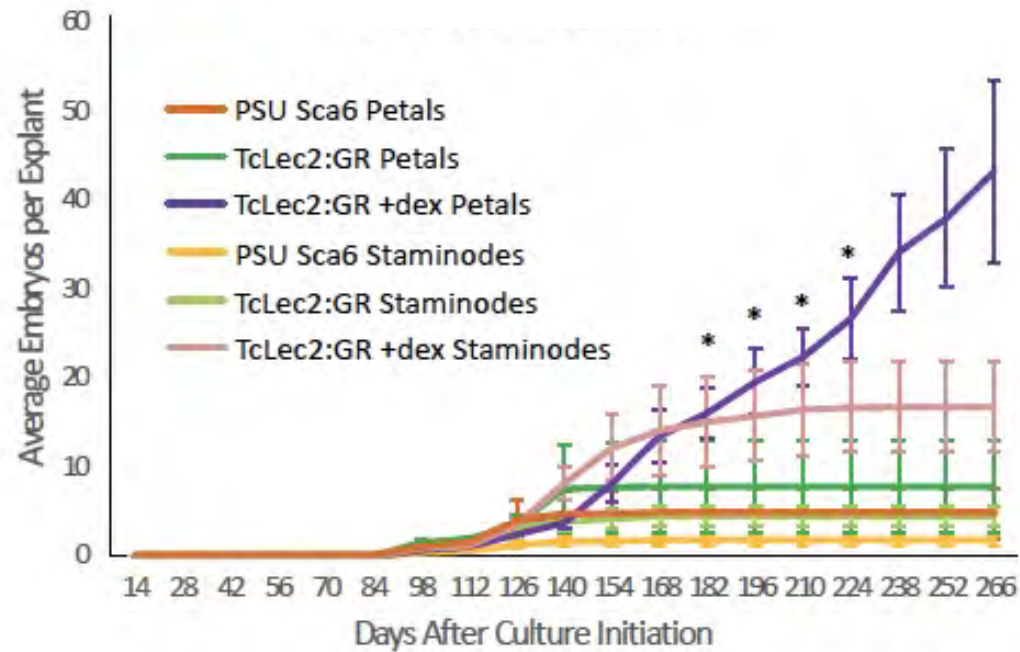


Embryo development and maturation from floral and leaf explants. .

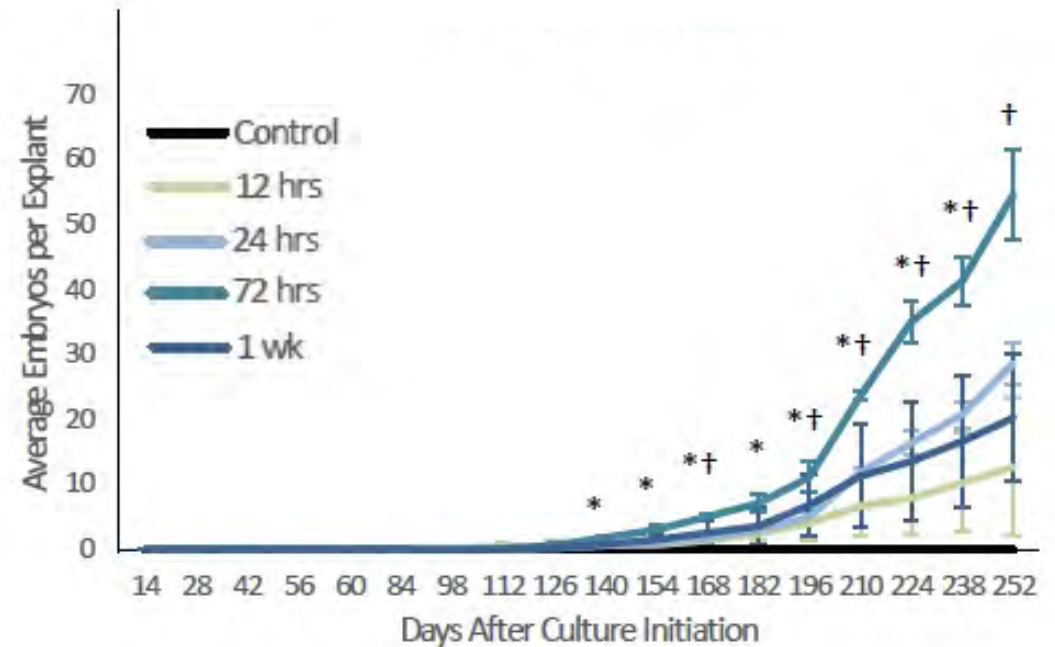


# SEs were regenerated at very high rates from floral and leaf explants

## SE from floral explants

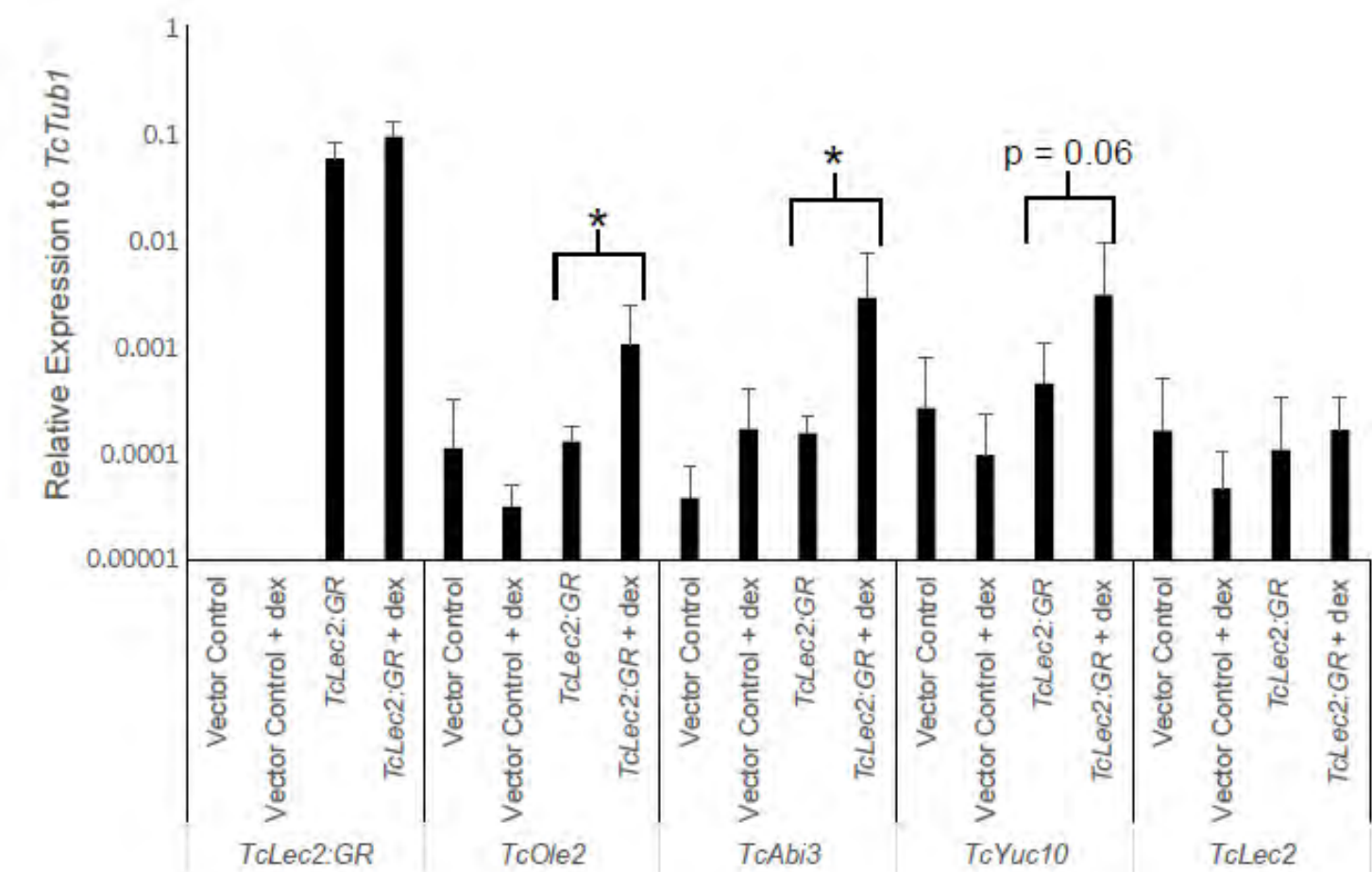


## SE from leaf explants





# The overexpression of TcLec2 TF protein caused induction of other genes involved in SE embryo development

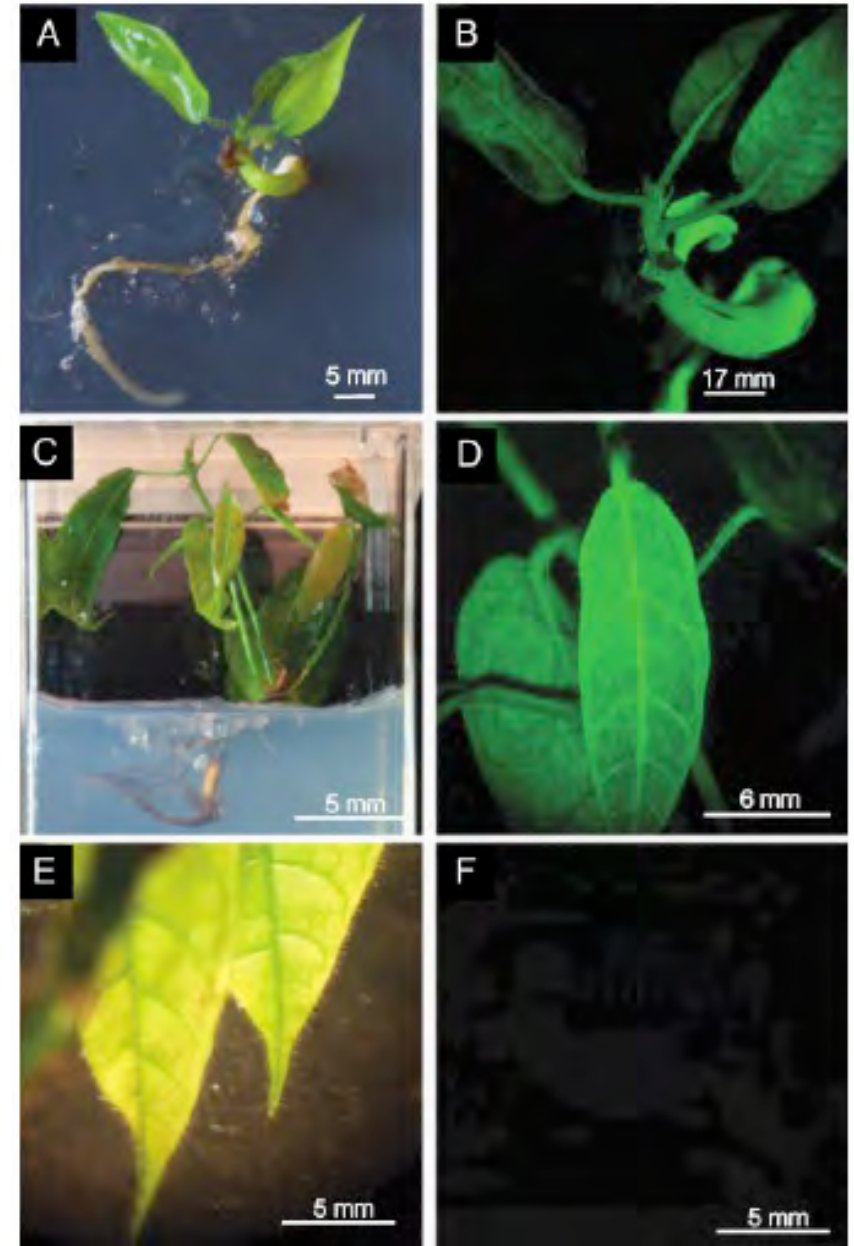





# Conclusions

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1. TcLec2 is an important TF regulator of SE in cacao.
2. Overexpression of TcLec2 contributes to high production of SEs from flowers and leaves.
3. The overexpression of TcLec2 needs to be controlled and restricted to specific stages of the culture development.
4. The TcLec2 expressing SE from leaves were successfully converted to healthy plants.







Thank You for Your Attention

Questions?