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

Andrew S. Fister, Lena Landherr, Melanie Perryman, Yufan Zhang, Adam Clemens, Mark J. Guiltinan, and Siela N. Maximova

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**
- Aclimated SE plants
  - Bentwood Stock Plant
  - Rooted Cutting

SE plants at
The Nestle Farm, Ecuador

Ave. yield of 2.6 t/ha from SE plants

Photos: Mark Guiltinan, PSU
Research Needed to Optimize the Cacao Somatic Embryogenesis Protocols (SE)

- 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 Guiltinan, PSU
Main Research Questions

• 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?

Photos: Mark Guiltinan, PSU
SE regulation by transcription factor (TF) proteins
TcLEC2 TF gene has higher expression in cacao SE tissue

Zhang et al., BMC Plant Biology 2014, 14:106
Study of the function of TcLec2 TF protein using genetic transformation

Agrobacterium transformation vector including TcLec2:GR

Agrobacterium transformation process

Flowers → Primary SE → Cotyledons from primary SE transformed with Agrobacterium → Transgenic secondary SE → Transgenic Plants
To test the function of the *TcLec2* gene in SE flowers and leaves from *TcLec2:GR* transgenic plants were cultured to produce new SEs.

Embryo development and maturation from floral and leaf explants.
SEs were regenerated at very high rates from floral and leaf explants
The overexpression of TcLec2 TF protein caused induction of other genes involved in SE embryo development.
Conclusions

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?