Sterilization of turf bermudagrass line FB1628 (*Cyondon. spp*) to induce calli for *Agrobacterium*-mediated transformation

Rebecca O. Arias*[1], Adina Y. Grossman[1], Kevin Begcy[2], Kevin E. Kenworthy[1], Esteban F. Rios[1]

[1]Agronomy Department, University of Florida, Gainesville, FL 32611, adinaygrossman@ufl.edu, kenworth@ufl.edu, estebanrios@ufl.edu
[2] Environmental Horticulture Department, University of Florida, Gainesville, Florida 32611, kbegcy.padilla@ufl.edu
*Corresponding Author: arias.rebecca@ufl.edu

UF IFAS UNIVERSITY of FLORIDA | PLANT BREEDING | AGRONOMY DEPARTMENT

Introduction

- Bermudagrass (*Cyondon. spp*) is a widely grown perennial warm-season turfgrass
- Tropical sod webworm (TSW) (*Herpetogramma phaeopteralis)* is a major pest of Bermudagrass, impacting various industries

Figure 1. TSW pupa and cocoon. Credit: Steven Arthurs, University of Florida

Objectives

Improve sterilization techniques to reduce rate of contamination of turf bermudagrass experimental line FB1628 for *Agrobacterium*-mediated transformation.

Figure 2. Hybrid bermudagrass used on a golf course. Credit: J. Bryan Unruh, UF/IFAS

Figure 3. Bermudagrass grown in a greenhouse at the University of Florida, Gainesville, FL

Sterilization Methods

1. Aseptic collection of stems from greenhouse
2. Cutting of stems to extract nodes
3. 10-minute rinse of nodes in DI-Water
4. Transfer to laminar flow hood
5. 1-minute bath in 70% ethanol
6. 20-minute bath in 1.2% NaOH (+ 2~3 drops of Tween 20)
7. Rinse in DI water for 5 minutes, twice
8. 15-minute bath in 0.1% PPM solution
9. Cut nodes to 5mm length, then place onto MS plates
10. Wrap plates while still in laminar flow hood

- Bermudagrass was harvested in Citra, FL and transported back to the lab
- Plants were then propagated and grown in a greenhouse at the University of Florida in Gainesville, FL
- Plants are kept on tables and off the floor to reduce contaminants
- PPM inclusion in callus induction media did not prevent microorganism contamination. Addition of PPM to a 3rd final DI-water rinse significantly reduced contamination.

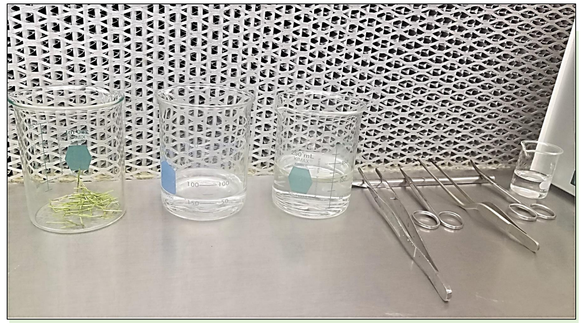

Figure 4. From left to right: Bermudagrass nodes, 70% ethanol, 1.2% NaOH solution, and sterilized tools

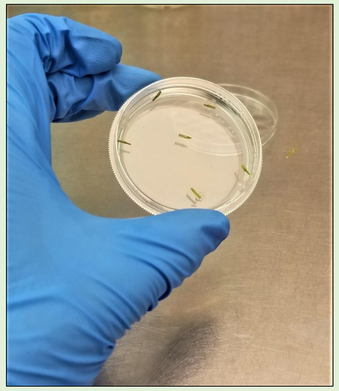

Figure 5. Nodes after the sterilization process.

Figure 6. Stack of plates ready to be wrapped and put away to incubate

Conclusion

With contamination under control, the project can move forward with calli induction and focus on *Agrobacterium*-mediated transformation

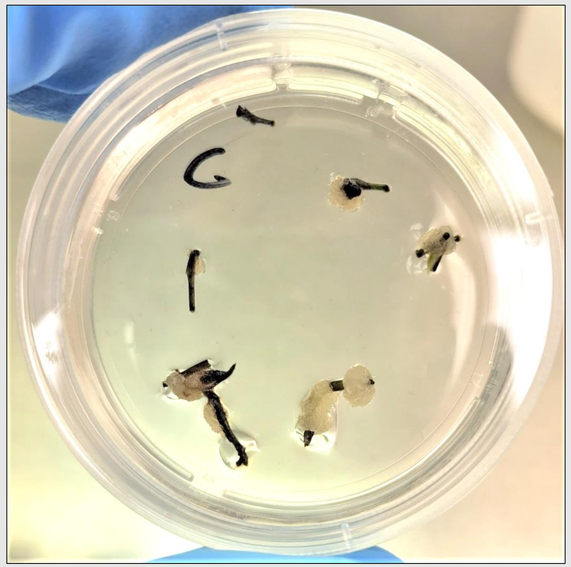

Figure 7. Successful callus formation after a 2-week period

References

Taliaferro, C. M., Rouquette, F. M., & Paul, M. (2004). Bermudagrass and Stargrass. *Agronomy Monographs*, 417-475. doi: https://doi.org/10.2134/agronmonogr45.c12

Tofangsazi, N., University of California, R., & Arthurs, S. P. (2012, October). Tropical Sod Webworm. Gainesville, Florida, United States: University of Florida. Retrieved from http://entnemdept.ufl.edu/creatures/ORN/TURF/Tropical_sod_webworm.htm

Trenholm, L. E., Unruh, J. B., & Shaddox, T. W. (1991). *Bermudagrass for Florida Lawns.* Retrieved from UF IFAS Extension: https://edis.ifas.ufl.edu/lh007