CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Viral-mediated Delivery of Morphogenic Regulators Enables Leaf Transformation in *Sorghum bicolor* (L.)

Background/Objective

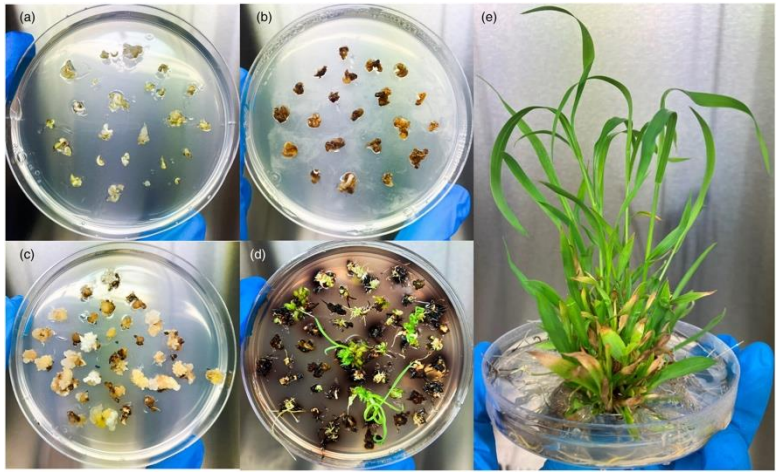
Recent advancements in monocot transformation, using leaf tissue as explant material, have expanded the number of grass species capable of transgenesis. However, the complexity of vectors and reliance on inducible excision of essential morphogenic regulators have limited widespread application. Plant RNA viruses, such as Foxtail Mosaic Virus (FoMV), present a unique opportunity to express morphogenic regulator genes. Furthermore, altruistic delivery of conventional and viral vectors could provide opportunities to simplify vectors used for leaf transformation, facilitating vector optimization and reducing reliance on morphogenic regulator gene integration.

Approach

In this study, both viral and conventional T-DNA vectors were tested for their ability to promote the formation of embryonic calli, a critical step in leaf transformation protocols, using *Sorghum bicolor* leaf explants. Morphogenic regulators - maize *Bbm (Bbm), Wus2 (Wus2), Wox2a (Wox2a)* and wheat GRF4-GIF1 (GRF4-GIF1) - were cloned in FoMV and tested as viral altruistic vectors using the Wang *et al.*, 2023 leaf-base transformation protocol as a proof-of-concept in sorghum.

Results

Although conventional leaf transformation vectors yielded viable embryonic calli (43.2 ± 2.9%: GRF4-GIF1, 50.2 ± 3%: *Bbm/Wus2*), altruistic conventional vectors employing the GRF4-GIF1 morphogenic regulator resulted in improved efficiencies (61.3 ± 4.7%). Altruistic delivery was further enhanced with the use of viral vectors employing both GRF4-GIF1 and *Bbm/Wus2* regulators, resulting in 75.1 ± 2.3% and 79.2 ± 2.5% embryonic calli formation, respectively.



Sorghum regeneration from leaf explants using conventional and altruistic T-DNA vectors and methods adapted from Wang *et al.*, 2023.

Significance/Impacts

This work provides an important proof-of-concept for the use of both altruistic vectors and viral-expressed morphogenic regulators for improving plant transformation. This study demonstrated that these technologies can be combined to increase efficiencies of embryonic calli formation, a limiting step in monocot leaf transformation, to improve protocol efficiencies and reduce experimental complexity. The continued improvement of leaf transformation protocols in monocot species is promising and has the potential to have important impacts on crop production worldwide.

Butler et al. 2025. "Viral-mediated Delivery of Morphogenic Regulators Enables Leaf Transformation in *Sorghum bicolor* (L.)." *Plant Biotechnology Journal*. DOI: 10.1111/pbi.70250.