Optimizing a Hairy Root Transformation Pipeline in Pumpkin Utilizing an Ultraviolet Fluorescent Reporter

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Introduction

- Plant transformation is the process of integrating foreign DNA into the genome of a host organism, so that the DNA will be permanently expressed in the organism.
- The plant pathogen *Rhizobium rhizogenes* inserts its own DNA into the host genome, leading to the formation of structures called hairy roots.
- Plant transformation using *R. rhizogenes is* cheaper and less labor intensive than other transformation methods [1]. However, only the roots are transgenic, so these changes are not inherited by the next generation.
- Previously, we modified existing hairy root transformation procedures [1,2] to work for our research system. However, we still needed to optimize several additional parameters.

Project Objective

- The goal of my project was to determine which *R. rhizogenes* strain and vector combinations have the best transformation efficiency.
- I tested 3 strains of *R. rhizogenes* in combination with 3 vectors and 3 promoter types to identify the largest transformation efficiency.
- Successful transformation was determined using visual observation of an ultraviolet-responsive fluorescent protein (eYGFPuv), which glows bright green under UV light [3].

Biological Materials

- Pumpkin seedlings: *Cucurbita pepo* variety 'Casperita'
- *R. rhizogenes* strains: K599, ArQual, and ATCC15834
- Vector types: pMIN-Ri (low-copy plasmid), pMIN-VS1 (high-copy plasmid), pCAMBIA1305 (high-copy plasmid)
- Promoters: 35S (high expression, from a virus), CmYLCV (moderately high expression, from a virus), and SlUbi (moderate expression, from a plant)

Hairy Root Transformation

1. Shelled seeds were sterilized with bleach, then rinsed with sterile water. Seeds were placed onto agar media, and plates were sealed with micropore tape. The plates were placed in a growth chamber at a 45° angle to germinate in darkness for seven days (**Figure 1**).

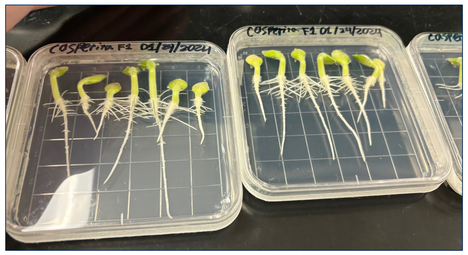

Figure 1. Pumpkin seedlings after 6 days growing on media plates in the dark.

2. Three days prior to inoculation, *R. rhizogenes* strains were taken from glycerol stocks and streaked onto LB media plates with kanamycin, then incubated for three days at 28°C.
3. Pumpkin seedlings roots were cut at the root-hypocotyl transition, then inoculated four times with a small needle dipped in the bacteria.
4. Inoculated seedlings were transferred to 0.5X MS media containing the antibiotic timentin to prevent bacterial overgrowth.
5. Seedlings were covered with filter paper to create a moist and low air-flow environment for the seedlings to regrow roots (**Figure 2**).

Figure 2. Pumpkin seedlings post bacterial inoculation plated on 0.5X MS media and covered with filter paper.

6. 2 weeks post inoculation, roots were cut off once more and seedlings were monitored for glowing green roots visible under UV light, which indicated expression of the eYGFPuv fluorescent protein (**Figure 4**).

Results

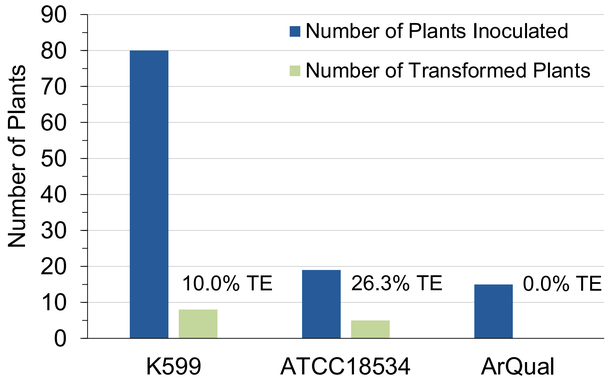

Figure 3. Number of plants inoculated compared to the number of plants transformed for each bacterial strain, with respective transformation efficiency (TE) indicated. A transformed plant is defined as a plant with at least one glowing root.

- The current transformation efficiency for K599 is 10.8%, while the rate for ATCC15834 is 26.3% (**Figure 3**). Plants inoculated with ArQual did not produce any glowing roots within the scope of this experiment.
- It appears that transformation efficiency is highest when using the pCAMBIA1305 vector containing the viral promoter 35S (data not shown).

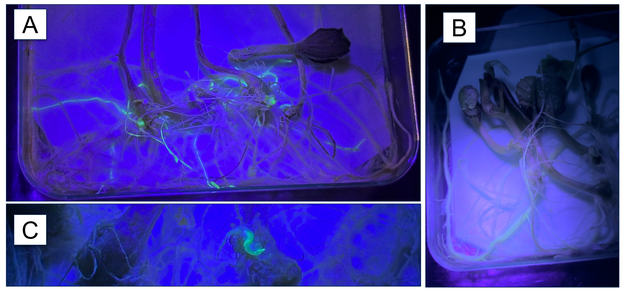

Figure 4. Visual differences in fluorescent protein expression observed under UV light. (**A**) Roots expressing 35S::eYFPuv. (**B**) Roots expressing SlUbi::eYGFPuv. (**C**) Roots expressing CmYLCV::eYGFPuv.

- Roots expressing SlUbi::eYGFPuv (**Figure 4B**) show less fluorescence than roots expressing the viral promoter 35S::eYGFPuv (**Figure 4A**) or CmYLCV::eYGFPuv (**Figure 4C**).
- These results suggest that fluorescence is an effective indicator of successful transformation and is easily visible under UV light.

Conclusions and Future Work

- The highest rates of transformation were observed using *R. rhizogenes* ATCC15834 in combination with the pCAMBIA1305 vector.
- Expression of eYGFPuv appeared greatest when the fluorescent protein was expressed under the viral promoters 35S and CmYLCV, although the glowing roots were still visible when the plant SlUbi promoter was used.
- Ongoing research through summer 2024 will focus on completing this analysis using additional vector and promoter combinations.

References

[1] Ilina, Elena L *et al*. (2012) *Annals of Botany* 110(2): 479-489. doi: 10.1093/aob/mcs086

[2] Ho-Plágaro, Tania *et al*. (2018) *Plant Methods* 14: 34. doi: 10.1186/s13007-018-0304-9

[3] Yuan, Guoliang *et al*. (2021) *Horticulture Research* 8: 234. doi: 10.1038/s41438-021-00663-3

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