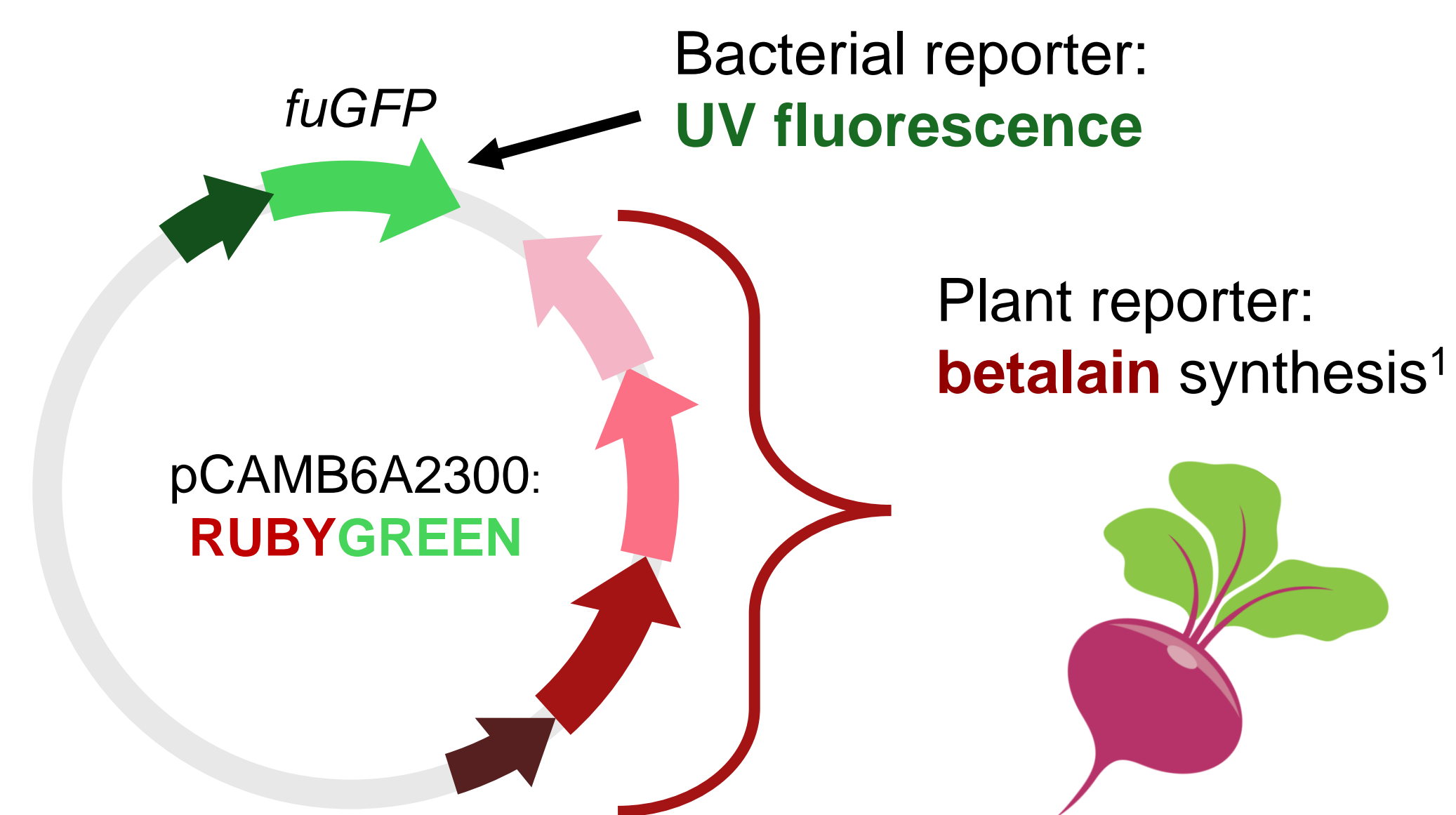
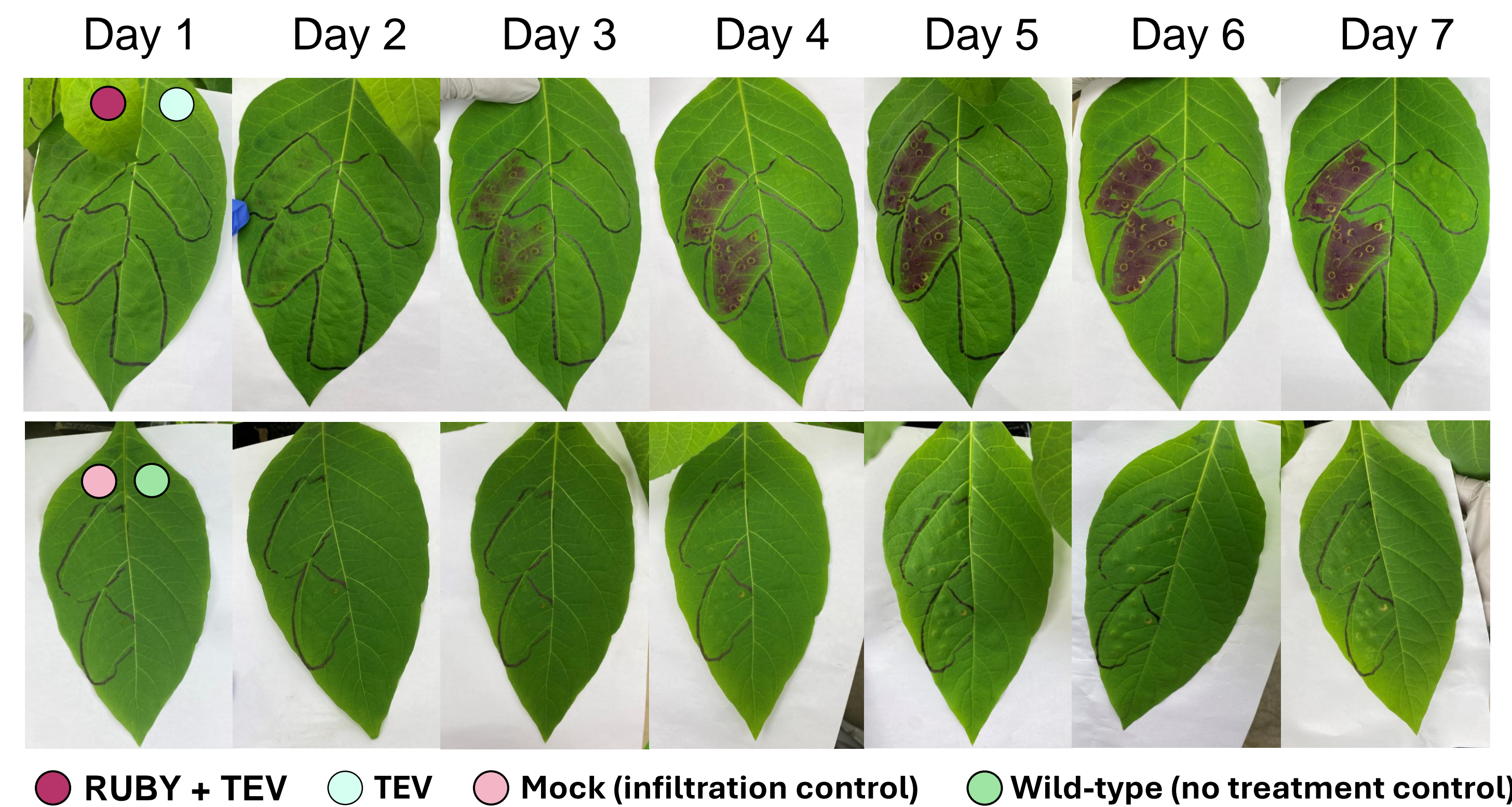


Introduction: RUBY encodes enzymes to synthesize betalain

- Atropa belladonna* (Deadly Nightshade) produces important medicinal compounds, such as tropane alkaloids (atropine and scopolamine)
- Transgenic methods are commonly used to increase the production of medicinal compounds; however, first a transformation protocol must be established
- To create protocols for these transformations, the visual reporter **RUBY** was introduced into *A. belladonna* using *Agrobacterium tumefaciens*



Transient RUBY expression accumulates betalain in 5 days



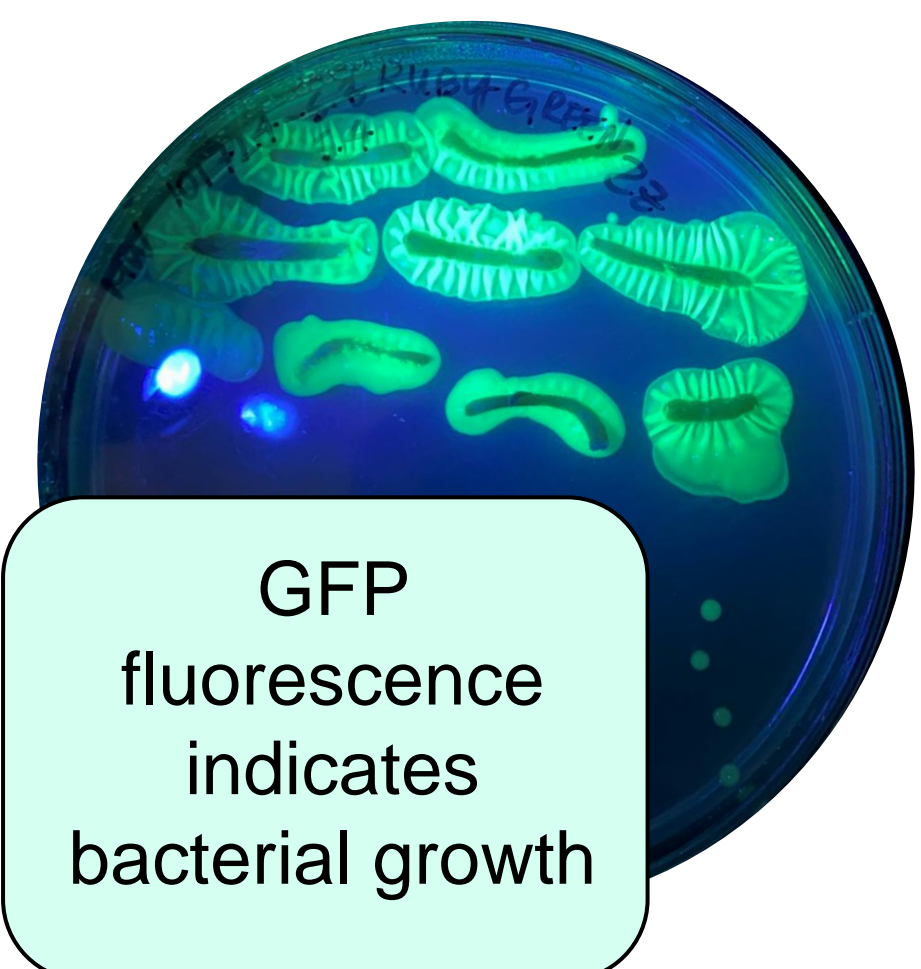
- Transient transformation with RUBY was successful
- RUBY was co-expressed with TEV (RNA silencing suppressor) to enhance expression
- Betalain** starts accumulating by day 2, then peaks at day 5 post-infiltration

Conclusions

- The visual reporter RUBY was helpful to develop new protocols for transient and stable transformation of *A. belladonna*
- Tissue for metabolite extractions can be harvested at day 5 in future studies with medicinal components of *A. belladonna*
- RUBY can identify positive transformants at the **callus**, **shoot** and **root** induction, and **mature** plant developmental stages
- Decreased **betalain** accumulation in newer leaves of transgenic plants suggests that there is **reduced** expression of the **betalain** biosynthetic enzymes over time

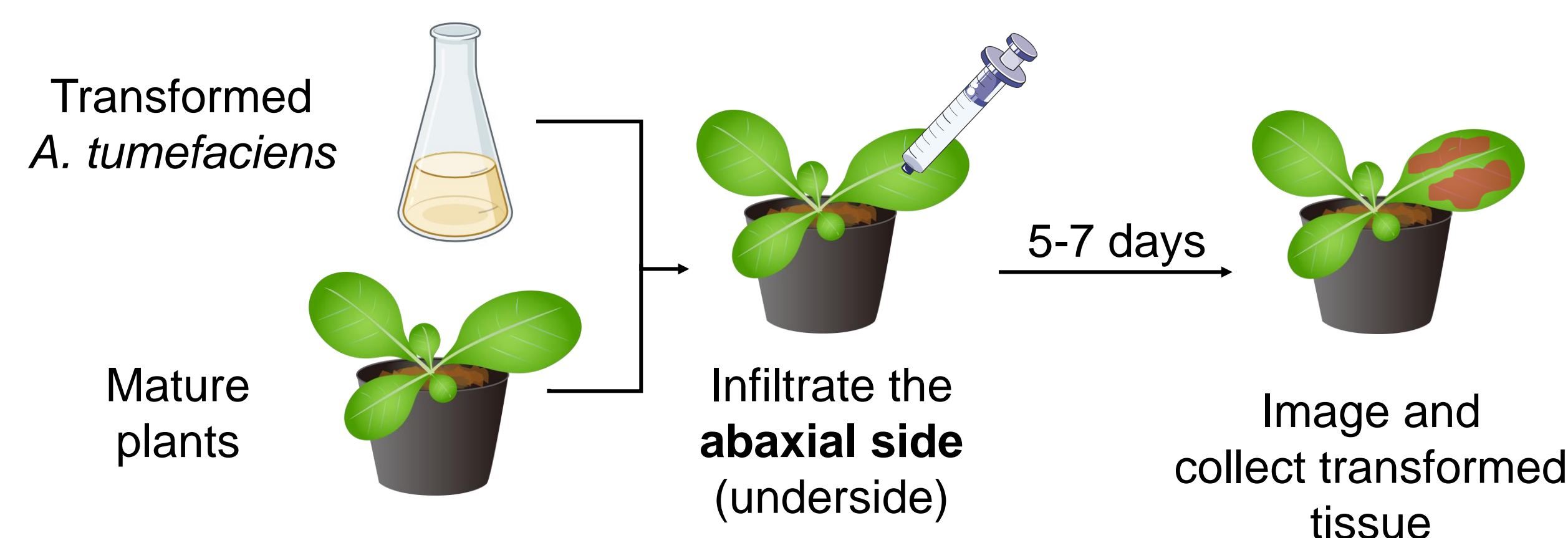
RUBY as a teaching tool in undergraduate laboratories

- These experiments were developed to be incorporated into a fourth-year undergraduate course, and can be adapted to weekly lab schedule
- UV fluorescence** visualizes RUBY-transformed *A. tumefaciens*, important for identifying contamination or transformation
- Visualization of metabolite accumulation promotes better understanding of plant transformations and applications for medicinal plants

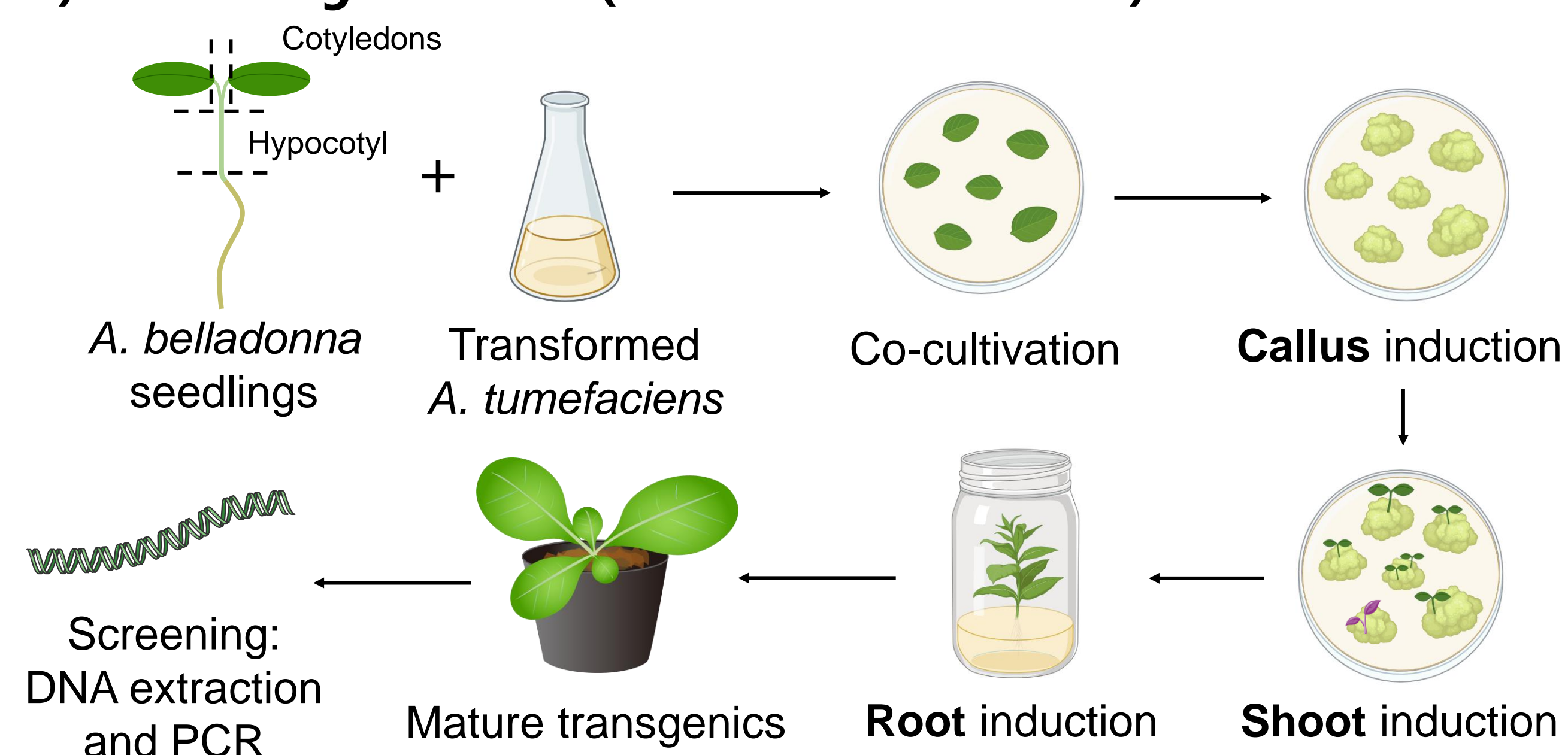


Approach: transient and stable transformations

1) Transient transformation via infiltration

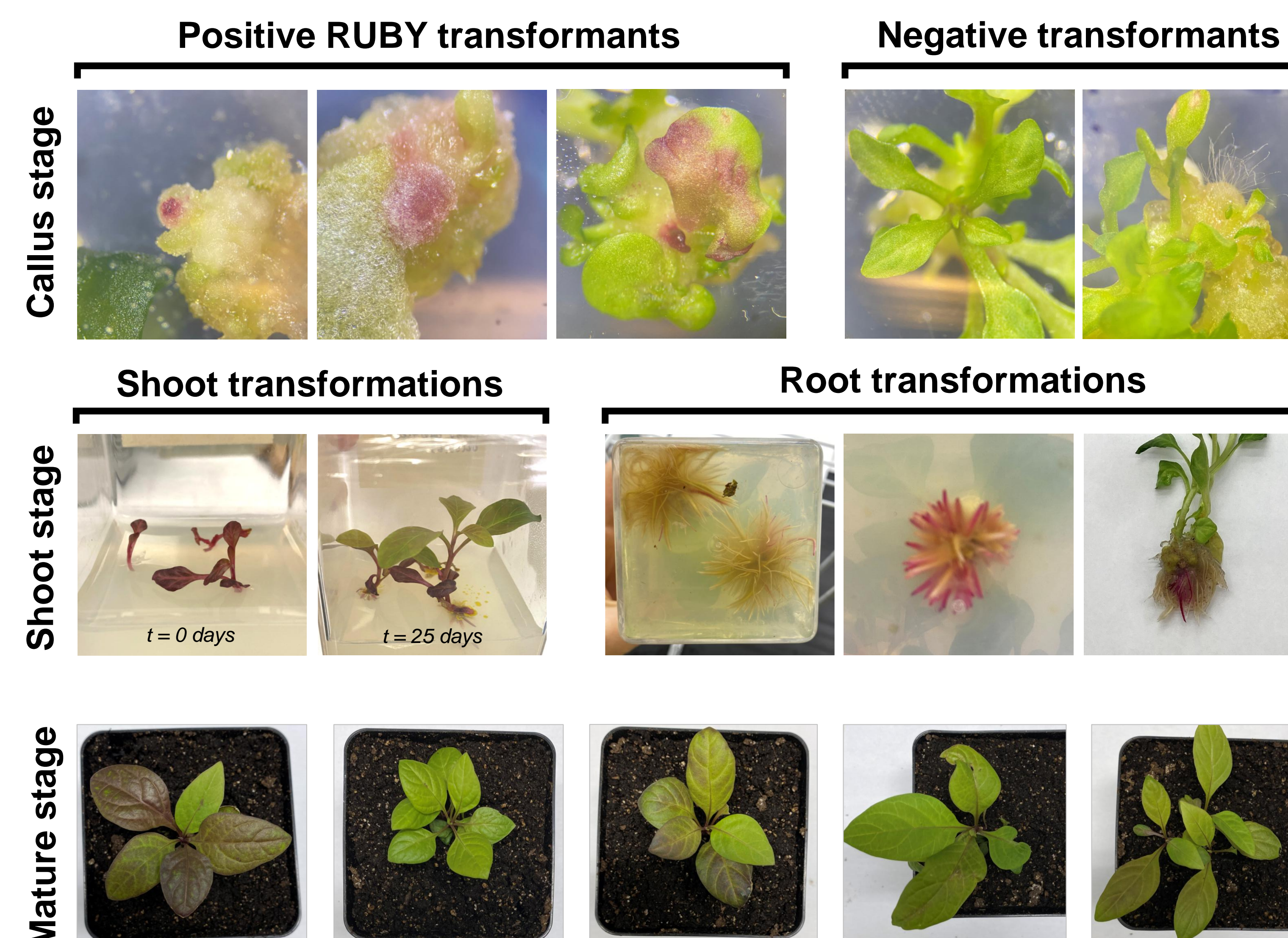


2) In-vivo regeneration (callus transformation)



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Transgenic *A. belladonna* have varied RUBY phenotypes



- A. belladonna* was successfully transformed using RUBY, confirmed by PCR
- Accumulation of **betalain** was patchy and expression **decreased** over time

Future directions

- Study the varied RUBY phenotypes by characterizing the **expression pattern** of the genes encoding the **betalain** biosynthetic enzymes
- Used protocols developed using RUBY for transient and stable transformations related to increasing **tropane alkaloid** accumulation in *A. belladonna*

References and acknowledgements

We would like to thank the teaching labs from the Biological Sciences department for the use of their dissecting microscope.

- He Y, Zhang T, Sun H, Zhan H, Zhao Y. 2020. A reporter for noninvasively monitoring gene expression and plant transformation. Horticulture Research. 7:152. doi:[10.1038/s41438-020-00390-1](https://doi.org/10.1038/s41438-020-00390-1).