Optimizing Tissue Culture Methods in Diverse Nightshade Species

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Introduction

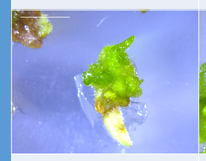
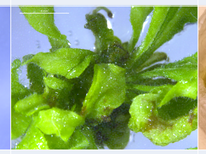
- *In vitro* propagation is essential for the generation of stable transgenic plant lines in *Solanaceae* and for propagation of sterile lines.
- We are interested studying fruit evolution by creating transgenic knockout lines in nightshade species with diverse fruit types.
- We have adapted a tomato (*Solanum lycopersicum*) *in vitro* tissue culture and transformation protocol from the Boyce Thompson institute for use in a diverse group of *Solanaceae* species with useful phylogenetic placement:
  - Fleshy Fruited Species:
    - Wild tomato (*Solanum pimpinellifolium*)
    - *Cestrum spp.* & *Brunfelsia*, fleshy-fruited species in the dry-fruited grade
  - Dry-fruited Species:
    - Synthetic tobacco (*N. tabacum*) hybrids
    - Desert tobacco (*Nicotiana obtusifolia*), a small diploid congener of *N. tabacum* more amenable to greenhouse growth conditions
    - Jimsonweed (*Datura stramonium*), a dry-fruited species in the fleshy *Solanoideae* clade
    - *Schizanthus grahamii*, a very early diverging species within *Solanaceae*

Methods

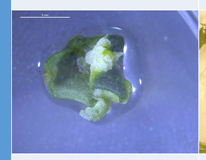
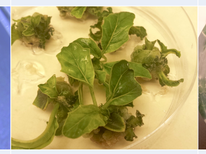
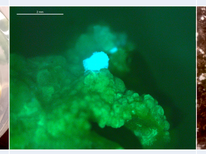
All explants were grown on MS media supplemented with Nitsch vitamins in 16h light at 22°C. Filter-sterilized plant growth regulators including zeatin and indole-3-acetic acid were added at the concentrations indicated after autoclaving. Initial explants were grown on 15mm-deep petri dishes; after the establishment of callus, they were transferred to 16oz plastic containers.

Results

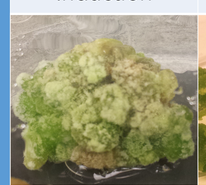
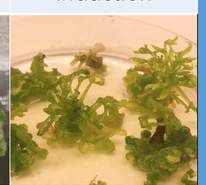
Desert Tobacco (*Nicotiana obtusifolia*)

Callus Induction	Shoot Induction	Root Induction	Agro Transformation	Transfer to Soil
				
Cotyledons 0.2$^{mg}/_{L}$ 2,4-D	2$^{mg}/_{L}$ zeatin then 1$^{mg}/_{L}$ zeatin	0.5-1.0$^{mg}/_{L}$ zeatin	Success with kan^{R}	Success

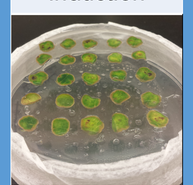
Jimsonweed (*D. stramonium*)

Callus Induction	Shoot Induction	Root Induction	Agro Transformation	Transfer to Soil
				
Cotyledons 0.2mg/L 2,4-D	2$^{mg}/_{L}$ zeatin then 1$^{mg}/_{L}$ zeatin	1$^{mg}/_{L}$ IAA	Success with erGFP	Success

Schizanthus

Callus Induction	Shoot Induction
	
Cotyledons 0.2mg/L 2,4-D	2mg/L zeatin then 0.5mg/L zeatin

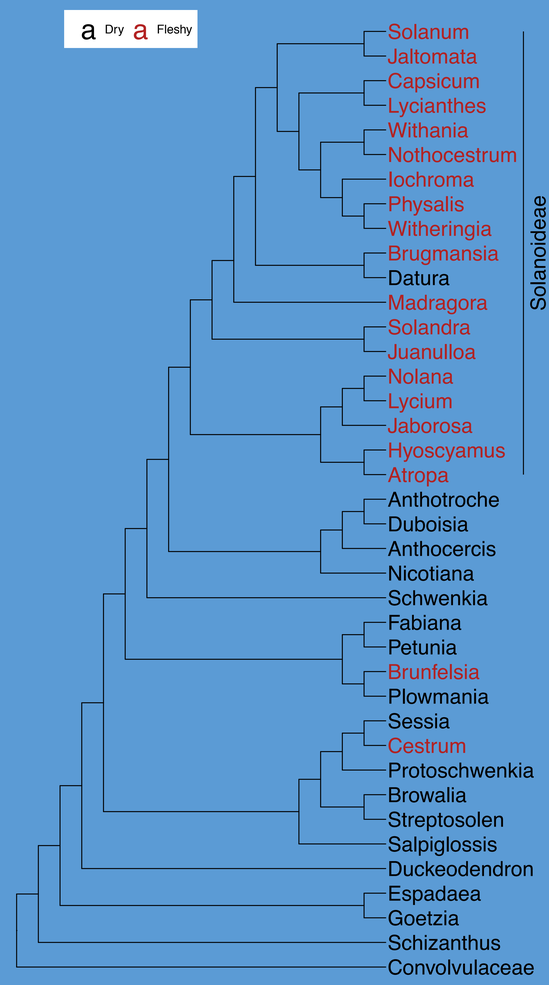
Brunfelsia

Callus Induction

Leaf Disc 0.2mg/L 2,4-D

Cestrum

Callus Induction

Leaf Disc 0.2mg/L 2,4-D



Conclusions and Future Work

- Viable *N. obtusifolia*, *Nicotiana* hybrids, and *D. stramonium* have been regenerated from callus.
- *Schizanthus*, *Cestrum*, and *Brunfelsia* are still in progress, but show promise.

Acknowledgements

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