



Genetic Transformation of *Simarouba glauca*

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Major: Chemistry

Explant Collection



NSF-REU Experience

- * Chemistry lab
 - * Synthesis of styrene oxide
 - * Chiral heteroscorpionate
 - * DPMPZM
- * Biofuels
 - * Techniques learnt during Summer 2011
 - a.* *Agrobacterium*-mediated transformation
 - b.* Particle bombardment (biolistic transformation)
 - c.* Stereomicroscopy
 - d.* Tissue culture /Sterile technique

General features of *Simarouba glauca*

- * *Simarouba glauca* is commonly known as paradise tree which belongs to a family Simaroubaceae. Its native to Central America and Florida.
- * This evergreen tree grows to a height of 12-15m
- * Flowers are bisexual inconspicuous
- * Pre-bearing period is 6-8years.
- * The ripe fruits are eaten by birds and play an important role in seed dispersal.
- * Indigenous tribes in Central America use the bark for fevers, malaria, dysentery and as a haemostatic agent to stop bleeding and as a tonic.
- * *Simarouba glauca* contains glaucarubin having antiamoebic property.

Background

- * *Simarouba glauca* has a long history in herbal medicine in many countries.
- * *Simarouba glauca* is also known for its pharmacology properties such as haemostatic, antihelmenthic, antiparasitic, antidysentric and anticancerous.
- * *Simarouba glauca* seed contains 60-70% oil.
- * *Simarouba glauca* is indigenous to southern Florida, the West Indies and Brazil.
- * Each well-grown tree can yield 15 to 30 Kg nutlets.
- * *Simarouba* can yield 1000-2000 Kg oil/hectare/year.
- * Can yield 1000-2000 Kg oilcake that can be used as a bio-fertilizer, and feedstock.

Objective

- * Optimization of genetic transformation in *Simarouba* via biolistic and *Agrobacterium*-mediated transformation.
- * The transformation could lead to transgenic *Simarouba* with enhanced abiotic stress tolerance, especially cold tolerance.

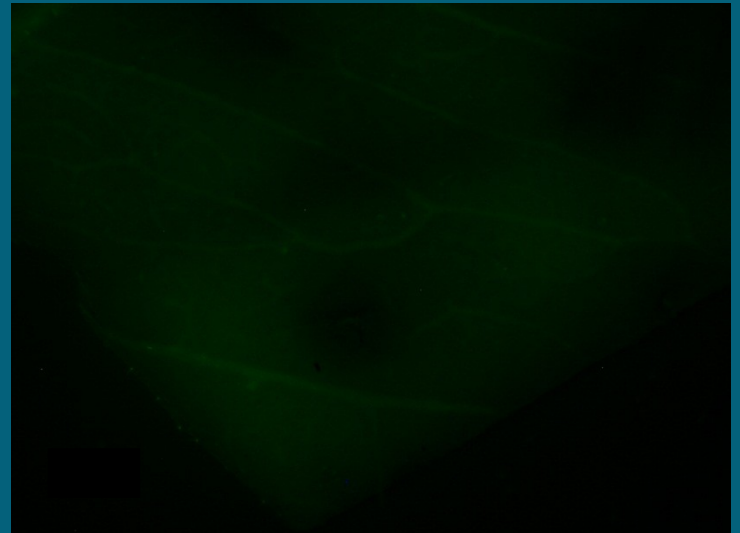
Transformation Protocol

- * Protocol 1:
 - * Sterilize explants with 70% EtOH and 0.1% HgCl₂,
 - * Submerge explants in *Agrobacterium* for 20 minutes, then blot dry and place in co-cultivation medium in dark at 25°C for 3 days.
 - * Place 2-3 *GUS*-putative transformed explants in *GUS* solution for 24 hours
 - * Wash rest of explants with liquid MS w/ vitamins + 500 mg/L Cefotaxime and place in callus induction medium 1
- * Protocol 2: Transformation of *Simarouba*: Scraped
 - * Everything as protocol 1 except the leaf explants were scraped to remove waxy cuticle.
- * Protocol 3: Transformation with Plasmolysis Treatment
 - * Everything as Protocol 1 except the leaf explants were plated in abaxial and adaxial position on plasmolysis medium for 4hrs
- * Protocol 4: Transformation of *Simarouba*: Vacuum Treatment
 - * Everything as Protocol 1 except it was placed in desiccator for 20min while keeping the explants in *Agrobacterium* solution.
- * Protocol 5: Particle Bombardment of *Simarouba*

Preliminary Results-3weeks



Transformation of *Simarouba* leaves
with no changes to the protocol.

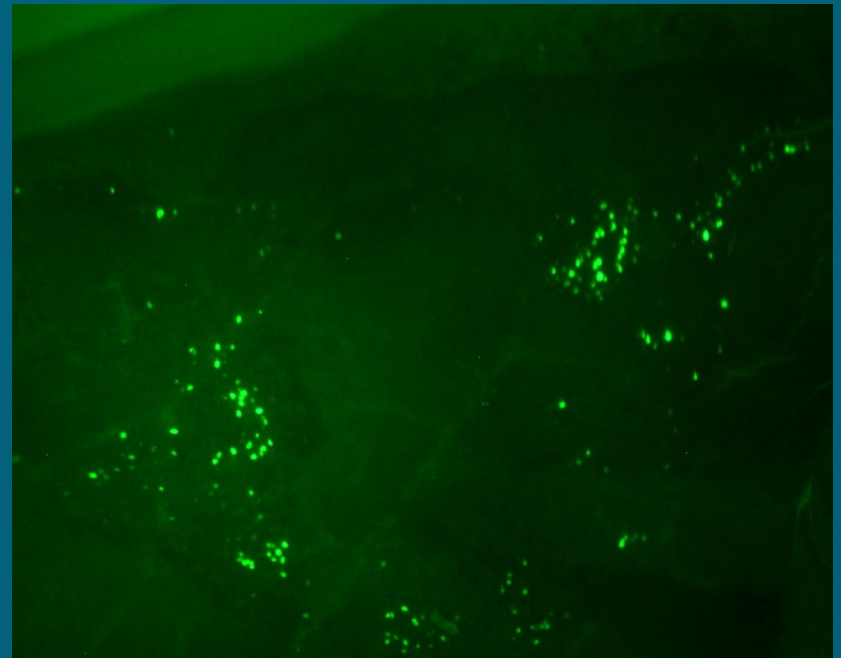


Conclusion: no GFP expression

Protocol 2

Scraped wax off leaf surface

Positive *GFP* expression in
leaf explants



Biolistic Transformation



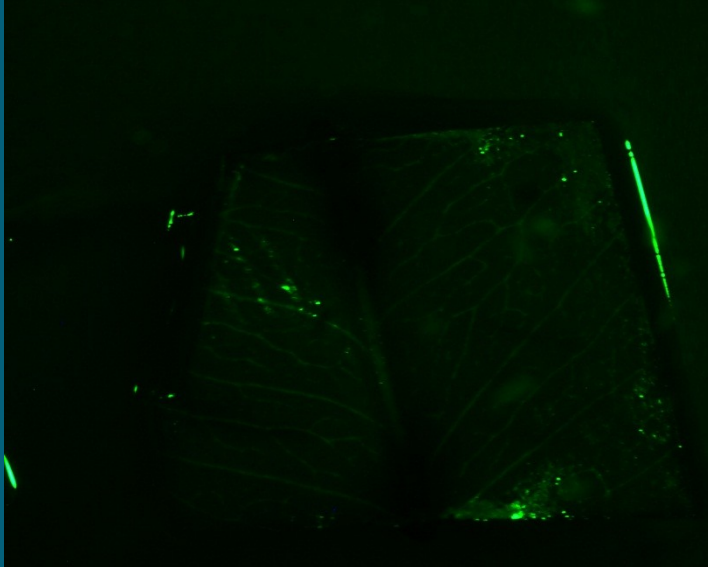
Particle Bombardment of *Simarouba* using gene gun



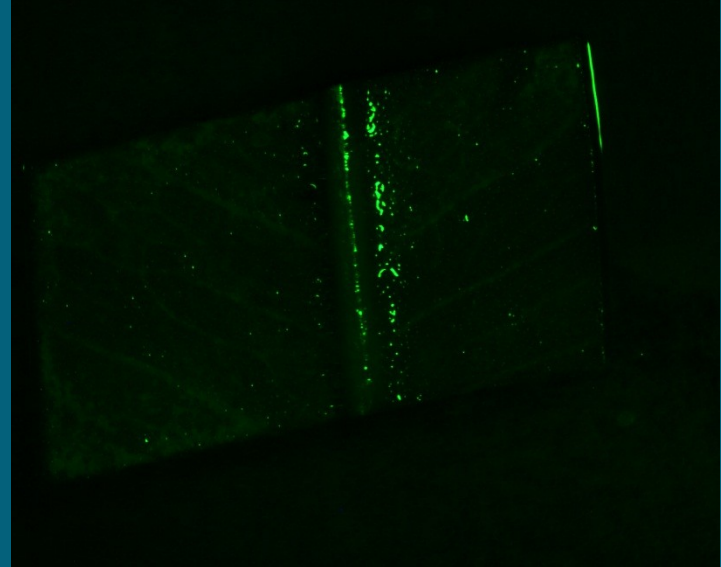
The transformation was negative and no GUS expression was observed

Plasmolysis(High sucrose and Maltose)

Scraped



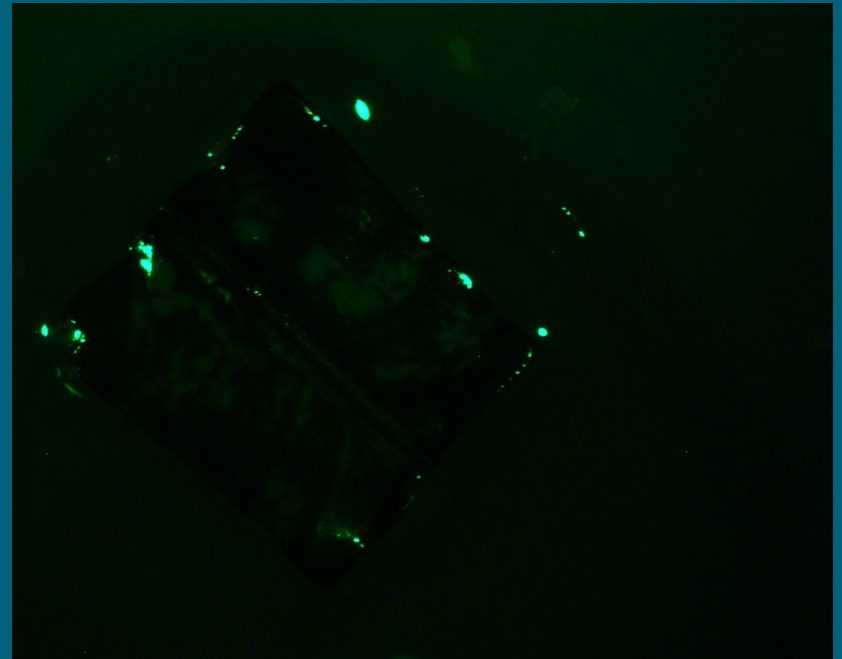
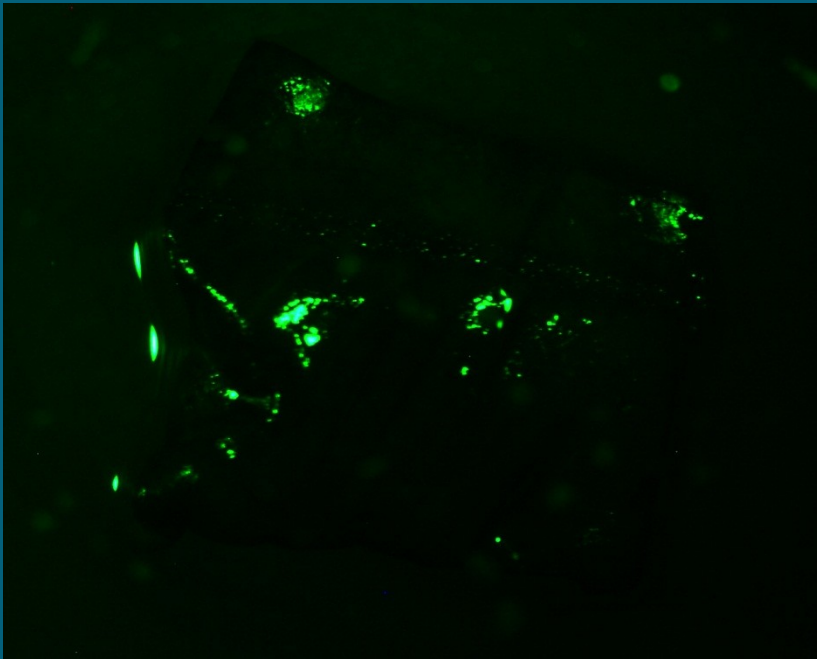
Not scraped



Vacuum

Scraped

Not Scraped



Summary

- * Preliminary results
 - * *Agrobacterium*-mediated more efficient than particle bombardment
 - * Scraping wax off leaves and plasmolysis lead to *GFP* expression
 - * Best *GFP* expression in vacuum with scraped leaves

Future work

- * Efficient regeneration protocol being optimized
- * Generate transgenic *Simarouba* resistant to abiotic stress (cold, drought, salinity, etc.) using the optimized transformation protocol.
 - * Potential gene of interest: *CBF3*

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Reference

Manasi, P. S., & Gaikwad, D. K. (2011, April 1). A Critical Review on Medicinally Important Oil Yielding Plant Laxmitaru (Simarouba Glauca DC.). *Pharmaceutical Sciences and Research*, 3(4), 1195-1213.

Questions

