Genetic Transformation of *Simarouba glauca*

Rafiyat Adeyiga¹, Behnam Tabatabai², and Sairam Rudrabhatla²

¹Cheyney University of Pennsylvania, 1837 University Circle Cheyney, Pennsylvania 19319.
²School of Science, Engineering and Technology, Penn State Harrisburg, 777 W. Harrisburg Pike Middletown, Pennsylvania 17057.

**Abstract**

*Simarouba glauca* is a relatively fast growing multipurpose tree, with many medicinal purposes. *Simarouba* is a medium-sized tree that grows up to 20 meters high and is well suited for warm, humid, tropical regions. Its seeds contain about 60-70% oil that can be converted to biodiesel. *Simarouba glauca* explants were transformed with *Agrobacterium tumefaciens*, carrying beta-glucuronidase (GUS) or green fluorescent protein (GFP) genes. The explants were then cultured on co-cultivation medium (MS w/v+vitamins+2 mg/L Thidiazuron (TDZ) +2 mg/L IBA). Thidiazuron (TDZ) was used at 26 mg/L IBA+ 4% sucrose +0.8% Agar at pH 5.7) for three days at 26-28°C and on callus induction medium containing MS+ vitamins+2 mg/L Thidiazuron (TDZ) + 2 mg/L IBA. After 2 weeks, the rest of the explants were washed with 0.1%HgCl solution to remove the wax on leaves, and plated on callus induction media 1.

**Materials and Methods**

Surface Sterilization of *Simarouba glauca* leaf explants

Experiments were conducted on various explants of the leaf explants. When placed on callus induction medium for 20 days, the rest of the explants were washed with 0.1%HgCl solution and then placed on callus induction media 1. After 2 weeks, the rest of the explants were washed with 0.1%HgCl solution and then placed on callus induction media 1.

**Preliminary Results-3 weeks**

**Protocol 1**

Transformation of *Simarouba glauca*

The leaf explants were placed in a sterile flask and then washed with 0.1%HgCl for 3 hrs. Then, the explants were plated on callus induction media 1.

**Protocol 2**

Transformation of *Simarouba*-

Scraped explants were also used for the transformation experiments. Preliminary results indicated that *Agrobacterium*-mediated transformation was more efficient than particle bombardment experiments. High frequency of GUS/GFP expression was observed in the adaxial surface of the leaf explants.

**Protocol 3**

Transformation of *Simarouba*-

Plasmolysis

Everything was same as Protocol 1 except the leaf explants were plated in both abaxial and adaxial position on plasmolysis media for 4hrs before *Agrobacterium* transformation.

**Protocol 4**

Transformation of *Simarouba*-

Vacuum

Everything was same as Protocol 1 except it was placed in a vacuum for 20 minutes while submerge in Agrobacterium solution.

**Protocol 5**

Transformation of *Simarouba*-

Particle Bombardment

The gold particles were coated with plasmid DNA of interest, loaded onto the microcarriers. Using high amount of pressure, the gene gun is used to fire the gold particles coated with foreign gene of interest onto the leaf explants.

**Conclusions**

- *Agrobacterium*-mediated transformation is more efficient than particle bombardment.
- Scarping wax off leaves and plasmolysis lead to GFP expression.
- Best GFP expression in vacuum with scraped leaves.

**References**


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