



# MAINTENANCE OF GUAYULE IN TISSUE CULTURE



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## Objectives

- Maintain healthy plants in tissue culture including:
  1. Guayule
  2. Tobacco
  3. Russian Dandelion
- Prepare different kinds of media varying in concentrations and hormone levels.

## Background



Guayule has been known as a source of rubber since the pre-Columbian times. In the early 1900s, guayule was first considered as an alternative source of natural rubber in the United States because of the high price of *Hevea* rubber from the Amazon region, with efforts centered in Arizona and California. Guayule is a native desert plant that could be farmed as a domestic source of bio-energy as well as high-quality latex.

Guayule is adapted to hot desert environments, and sites with well-drained calcareous soils and relatively low concentrations of nutrients. Sandy-loam soil are most suitable since root diseases, which are exacerbated by standing water, are one of the few problems encountered in guayule cultivation. High temperature does not appear to affect growth, but temperatures below 4°C induce semi-dormancy and extended freezing temperatures can cause plant death.

The Crop Improvement & Utilization research unit (CIU) conducts research that integrates biochemical & molecular approaches with biotechnological strategies. Goals in rubber research are:

**Objective 1:** Develop germplasm that enables domestic commercial production of natural rubber by metabolic engineering and conventional breeding.

**Objective 2:** Develop technologies to enable the domestic production of natural rubber with properties as good as or better than existing sources for industrial applications.

## Method: Prepare Media

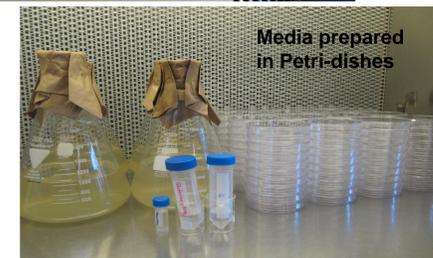
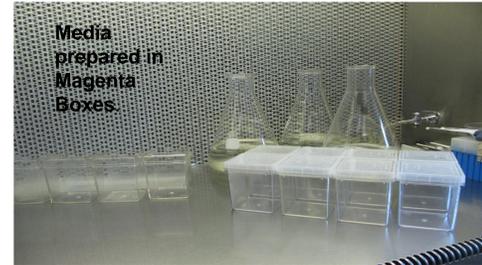
- Prepare 4 liters for each time autoclaving, autoclave at liquid 45 minute.
- For Magenta boxes, each liter pours into 12 boxes, and 4 liters pour into 48 boxes.
- For Petri Dishes, each liter pours into 40 plates, and 4 liters pour into 160 plates.

Different kinds of media:  
**Co-culture media:**  
 MSB2P0.1

**Recovery media**  
 NDB1T, DKWB1T

**Shoot media:**  
 - Shoot initiation media:  
 ½ MSB1TK30  
 - Shoot elongation media:  
 MSB1TS3, MSB1TS5, MSB1TS10  
 MSB1TK100, MSB1TK30, DKWB1TK30,  
 Kan selection MSB1TK30,  
 MSB1TG0, MSB1TG1, MSB1TG3

**Plant Rooting & maintenance media:**  
 S5, S10  
 K0, K10, K50  
 G0, G1, G2, G3



## Methods: Transform Plant & Schedule

### Petri dishes:

**Inoculation** Cut the leaves from the small plant in the Magenta box  
 Cut leaves into strips in *Agrobact.* + *Co-Culture Solution*

### Co-Cultivation

Blot and transfer leaves to empty petri-dish. Seal with Parafilm.  
 Keep in dark for 3 days

**Recovery** Keep in lab. for 5 days

**Shoot Initiation** Keep in low light for 2 weeks

**Shoot Elongation** Keep in bright light

Transfer the green tissues every 2 weeks (Totally 8-24 weeks)

**Rooting** Transfer the shoots 1 cm or longer (4 weeks)

**Maintenance** Transfer the shoot tips every 4 weeks



### Magenta Box:

In each bundle, We have different lines from line A to line Z = 26 lines  
 Each line contains 8 boxes \* 26 lines = 208 boxes  
 And at least one control line = 8 boxes

Total each bundle = 216 boxes

Each bundle needs 18 liters media to complete the transferring.

Each bundle also need to be transfer on the same day, in the same condition (lighting, temperature)

Maintenance: Transfer tips every 2 months



### Dry for analysis after transferred

Lyophilized 2011: ---X bundle samples

- Each sample harvests from 9 lines, including control line, each line collects from 8 boxes of plants

Lyophilized 2012: ...X bundle samples



## Materials

### Plants under care in culture

**Room 0109** ( see hand out list of plants)



#### \*Guayule

-Wild type:  
 -Genetic Modify:

#### \*Tobacco

-Wild type:  
 -Genetic Modify:

#### \*Russian Dandelion

-Wild type  
 -Genetic Modify

### Plants under care on second floor- Treatment Growth Chamber

➤ **Guayule (Genetic modify)**

I noticed that in this chamber, fungus are easy growing since there were more contaminated plants issue.



### Transgenic Plants Transferred and Maintenance in Green House

- Guayule Plants in Rooting Medium for 2 months.
- Transfers into pellets of soil w/ B5 and water in Magenta boxes for 2 months.
- Then transfer to pots in Green House.



## Results and Conclusion

1. We maintain healthy plants.
2. No major contamination.
3. The advances of maintenance of healthy plants and tissue culture are supporting scientists in researching and save their time in studying experiments.

Example: We have created guayule plants with G7-11C +PND34B show resistance to Sulfadiazine (at 0.5 mL/L or 1.0 mL/L).

However, G7-11C wild type can also be resistant in this media!  
 AZ2 does not show resistance to this media.

The next step will be GUS staining to confirm presence of the transgene. Picture b shows the genes expression with Gus staining in blue color of 22 transgenic plants which were grown in media contained Sulfadiazine. This means most of the transgenic plants (from A to V) transformed and resistant to Sulfadiazine (at 0.5 ml/L or 1.0 ml/L). However, there were 2 cells did not show blue color, nor resistance to Sulfadiazine. (Picture a). The 2 possibilities are:

- 1.They are transformed partially, or 2. they didn't transform.
4. Successful maintenance of in vitro plants allows:
  - Supply of material for genetic engineering studies.
  - Preservation of valuable genetic resources. (transformed plants).
  - Optimization of guayule transformation processes.

