Genetic Modify Guayule

than existing sources for industrial applications.

production of natural rubber with properties as good as or better

and conventional breeding.

biotechnological strategies. Goals in rubber research are:

research that integrates biochemical & molecular approached with

cause plant death.

induce semi

does not appear to affect growth, but temperatures below 4

nutrients. Sandy

Guayule is adapted to hot desert environments, and sites with well

reasons in Arizona and California. Guayule is a native

rubber from the Amazon region,

Wild Guayule

Hevea

as an alternative source of natural rubber in the United States

because of the high price of

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
do not affect to 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 approached 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 or better

than existing sources for industrial applications.

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

Genetic Modify:

Wild type:

*Guayule

*Russian Dandelion

*Tobacco

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

GUAYULE IN TISSUE CULTURE

Trinh Huynh, Niu Dong, Colleen McMahan

RUBBER LAB - AGRICULTURAL RESEARCH CENTER, WESTERN REGIONAL SERVICE, 800 BUCHANAN ST, ALBANY, CA 94710

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.

Method: Prepare Media

Methods: Transform Plant & Schedule

Results and Conclusion

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

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 +PN3D4B 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 this staining in blue color of 22 transgenic plants which were grown in media containing Sulfadiazine. This means most of the transgenic plants (from A to V) transformed and resistant to Sulfadiazin (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.

- Growth for gene expression studies.

- Production of elite lines from transgenic plants.

- Large scale production of material for different purposes.