Jatropha curcas In Vitro Propagation

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MILLS

Jatropha curcas

- *Euphorbiaceae* (5m tall)
- Native to tropical areas
- Oil yielding seeds
- 50 year life-span

Benefits

- Drought Tolerant
- Can grow in poor soil conditions
- Non-food crop
- High oil yield
- Varied Uses

Challenges

- Cold Sensitive

Objectives

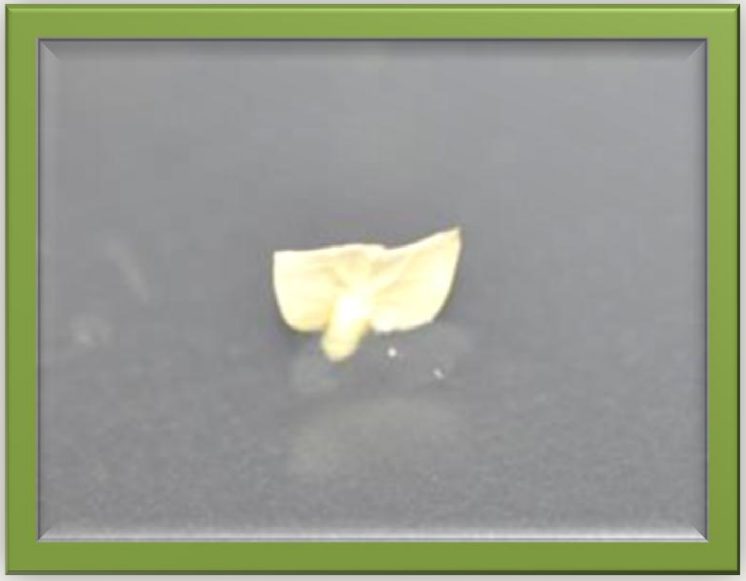
- Short Term:
  - Develop an efficient tissue culture protocol
- Long Term:
  - To develop an efficient transformation protocol
  - Activation of cold tolerance genes through genetic engineering
  - Wide-scale cultivation on temperate land

Materials and Methods

Jatropha Embryo Germination

✓ *Jatropha* NBM and MC seeds

- Seeds were sterilized (rinsing with sterile water between steps) utilizing:
  - Tween-20 and 10% Bleach
  - 0.1% Mercury Chloride



- Seeds were plated:
  - On JEG5 media
  - 10 embryos per plate
  - Incubated in light

Materials and Methods

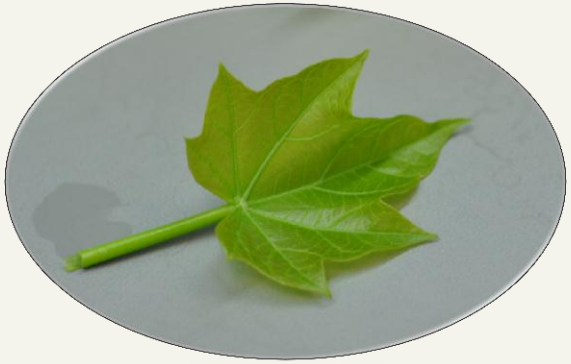
✓ **JCI: Hypocotyls & Cotyledons**

- Hypocotyls and cotyledons were excised from 2 week old seedling
- These were plated:
  - JCI #1 Sucrose Media
  - From 5 cotyledons or 10 hypocotyls explants per plate
  - Incubated in dark

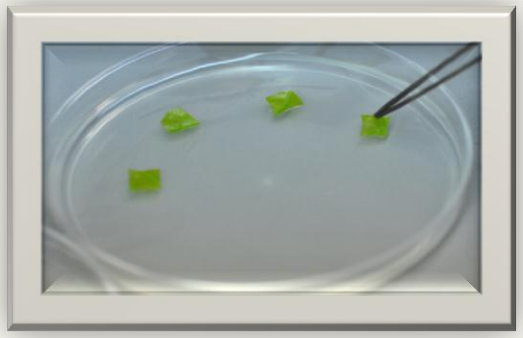
Materials and Methods

Jatropha Callus Induction

✓ **JCI: New Growth Leaves**



- Surface sterilized with:
  - Tween-20 and tap water
  - 70% Ethanol
  - 0.1% Mercury Chloride

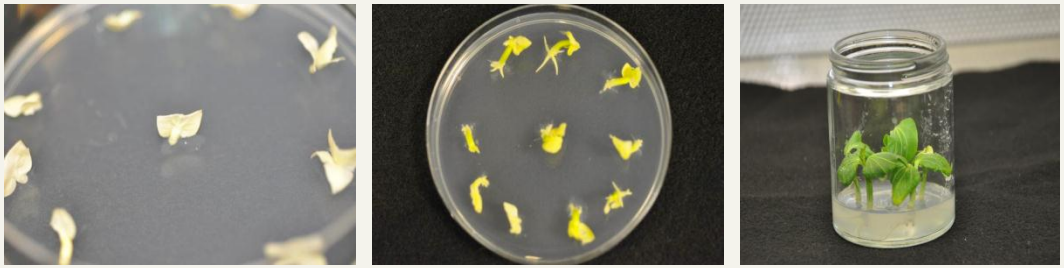


- Leaves were plated:
  - On JCI media
  - From 5 to 10 leaf explants/plate
  - Incubated in dark

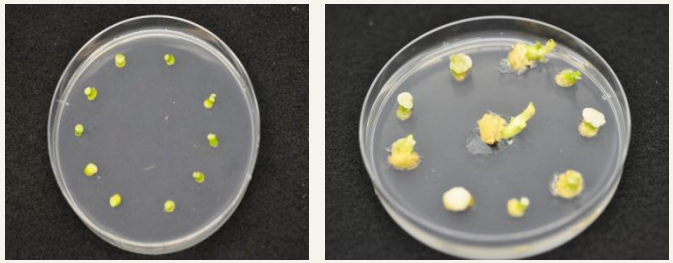
JCI Media

- JCI #1
  - ✓ (MS Medium; 1.5mg/L BAP; 0.05mg/L IBA; 30g/L; sucrose/maltose; 7g/L agar)
- JCI #2
  - ✓ (MS Medium; 1.0mg/L BAP; 0.05mg/L IBA; 8mg/L CuSO4; 100mg/L Casein; 200mg/L L-glutamine; 30g/L sucrose/maltose; 7g/L agar)
- JCI #3
  - ✓ (MS Medium; 3mg/L BAP; 0.01mg/L IBA; 30g/L sucrose/maltose; 7g/L agar)
- JCI #5
  - ✓ (MS Medium; 5mg/L BAP; 1mg/L; 30g/L sucrose/maltose; 7g/L agar)

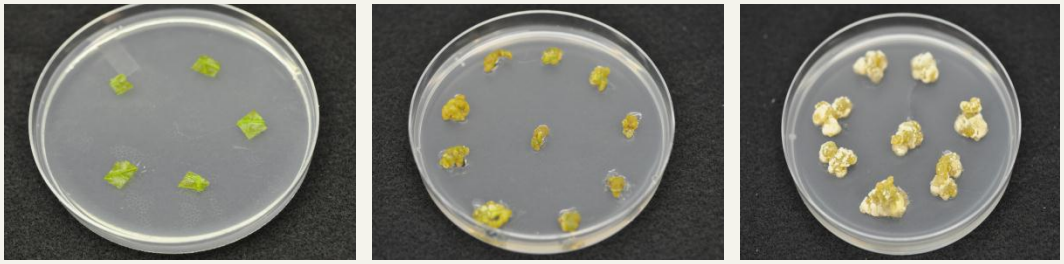
Results



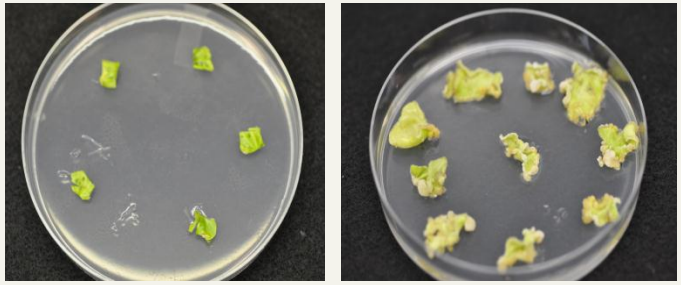
Embryo Germination (2 weeks)



Callus induction form hypocotyl explant

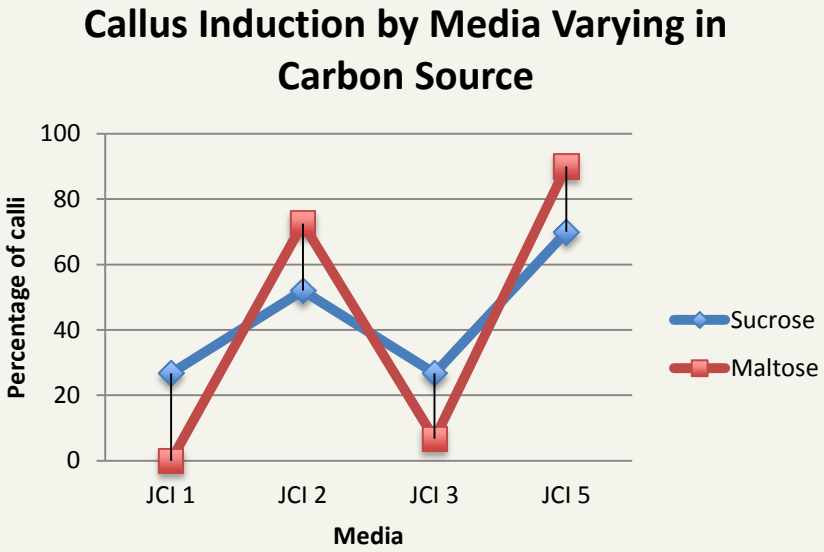


Callus induction from leaf explant (8 weeks)

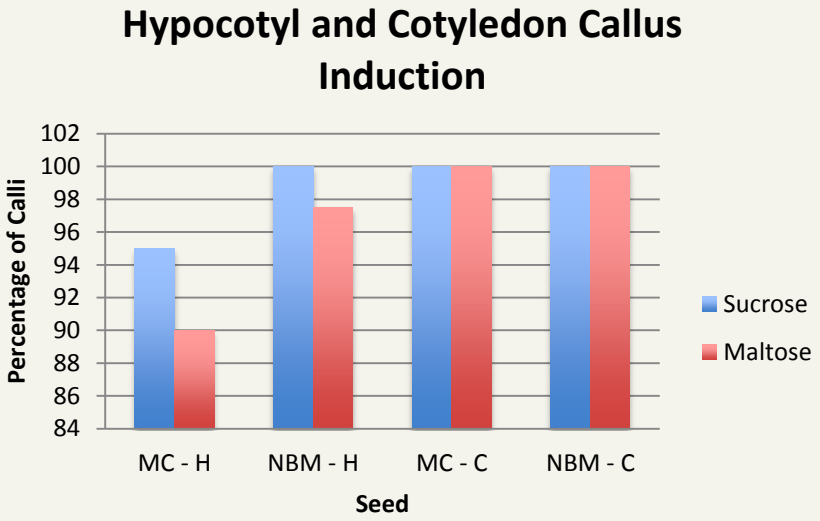


Callus induction form cotyledon explant

Results



A) Comparison between JCI media varying in carbon source for new growth leaves



B) Comparison between hypocotyls and cotyledons with varying carbon and seed source

Summary

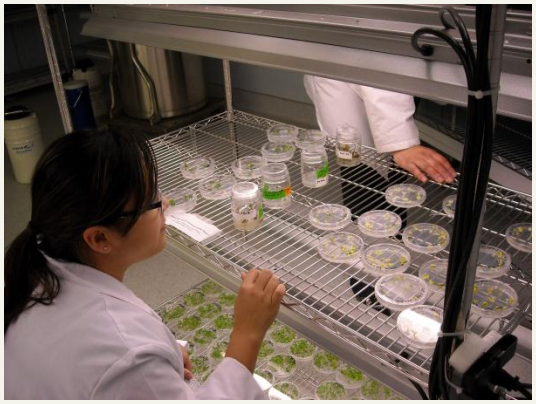
- Callus Induction:
  - 98% cotyledon and hypocotyl explants
  - Hypocotyl explants responded slightly better in sucrose medium
- For leaf explants callus formation:
  - JCI 5M 90% ★
  - JCI 5S 70%
  - JCI 2M 72.5% ★
  - JCI 2S 52%

Future Works

- Regeneration of *Jatropha curcas* from callus
- Optimization of transformation protocol to introduce cold-tolerance gene (*CBF3*)
- Regeneration of transgenic plants

Techniques Learned

- Media Making
- Sterile Technique
- Tissue Culture
  - Embryo Germination
  - Callus Induction
- *Agrobacterium* Transformation
- Particle Bombardment



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References

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