The Genetic Transformation of *Jatropha curcas*

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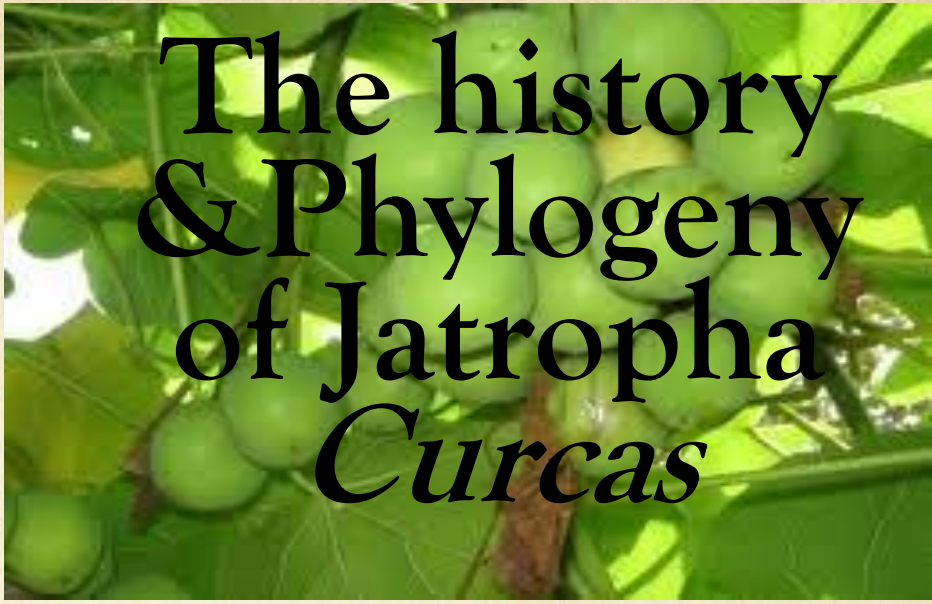
The history & Phylogeny of Jatropha *Curcas*



KINGDOM Plantae	ORDER Malpighiales
FAMILY Euphorbiaceae	GENUS *Jatropha*
SPECIES *J. curcas*	

- Tropical & subtropical regions
- High degree of aridity
- 30-40 % oil content
- Grows on marginal land
- **USED AS BIODIESEL**

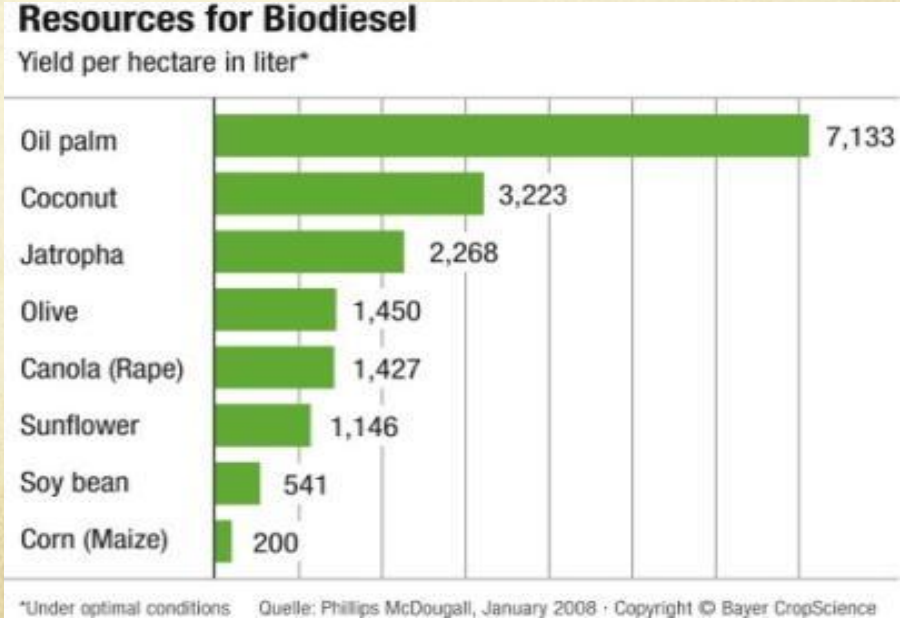
What is transformation and why is it important and helpful?

Biotechnology

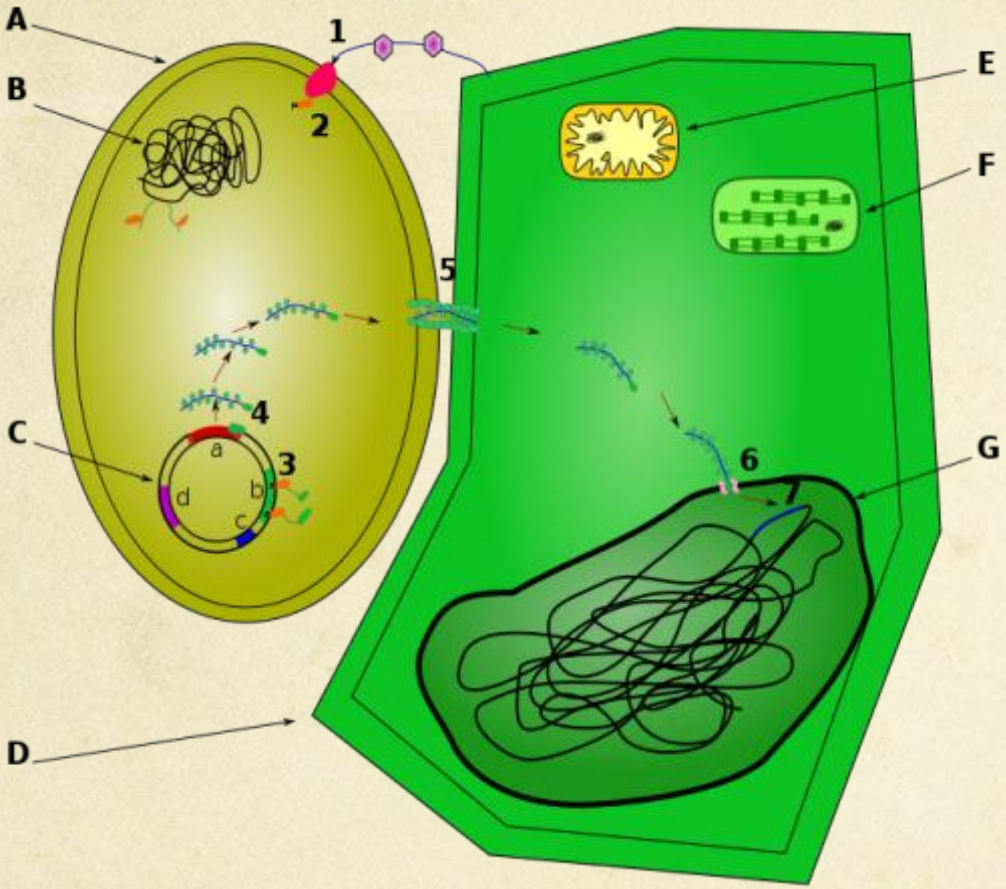
- A desired(single) gene of interest
- Introduce foreign genes
- Generate plants with useful phenotypes
- It allows the commercial value of improved plant lines to be captured by those investing in the research. (**ME AND YOU**)

Traditional Breeding

- Combines multiple genes at once
- Mendelian Genetics (same species)

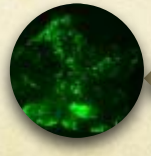


What is GUS gene & CBF_3?



A. Agrobacterium Cell
B. Agrobacterium DNA
C. Ti Plasmid
D. Plant Cell
E. Plant Mitochondria
F. Plant Chloroplast
G. Plant Nucleus
a. T-DNA
b. Vir genes
c. Replication origin

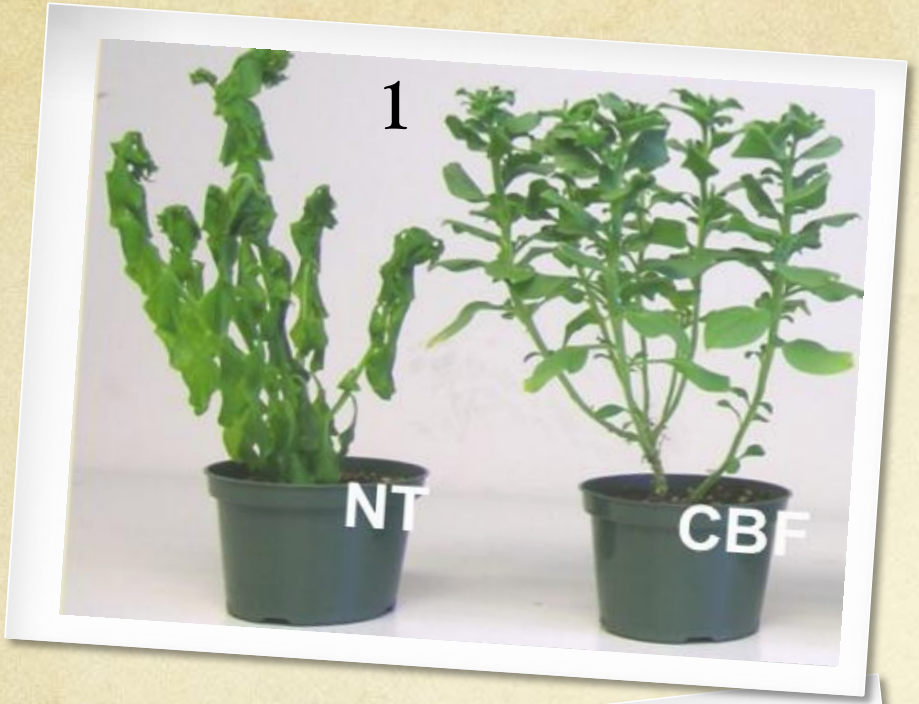
GUS GENE (beta-glucuronidase)



GFP (green fluorescent protein)

CBF3 (cold binding factor)

http://en.wikipedia.org/wiki/File:Transfection_by_Agrobacterium.svg



Proof of concept for cold and drought tolerance demonstrated in *Petunia* and corn (Sairam et al, 2008)

Plant 1:

- After exposure to -6 C (22 F)

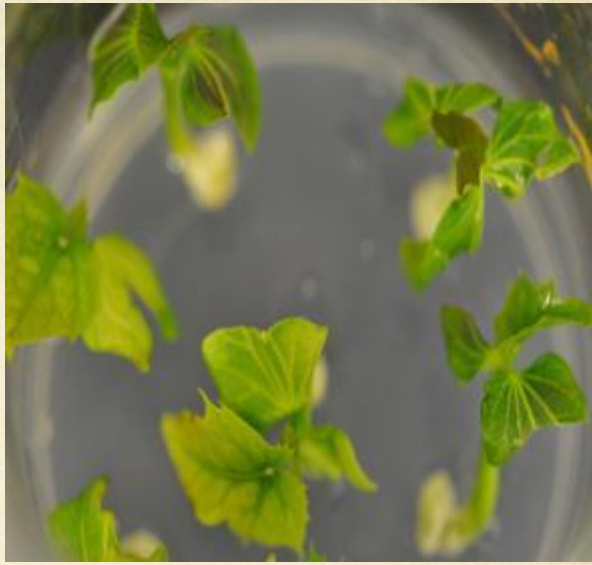
Plant 2:

- Watered to saturation
- No watering for 15 days
- 24 hours after watering

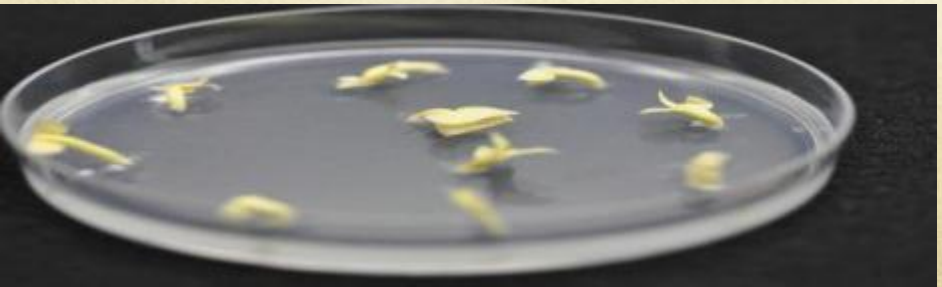
Sairam R.V., Al-Abed, D., Johnson, J., Muzynski, M., Raab, M., Reddy, T.V. and Goldman, S.L. Maize. (2008) In: Kole, C., Hall, T.C. (eds) A Compendium of Transgenic Crop Plants 49-82.

Procedure for *agrobacterium* transformation process

- Pour cultures containing GUS and CBF_3 into 2 separate/ sterile petri dishes

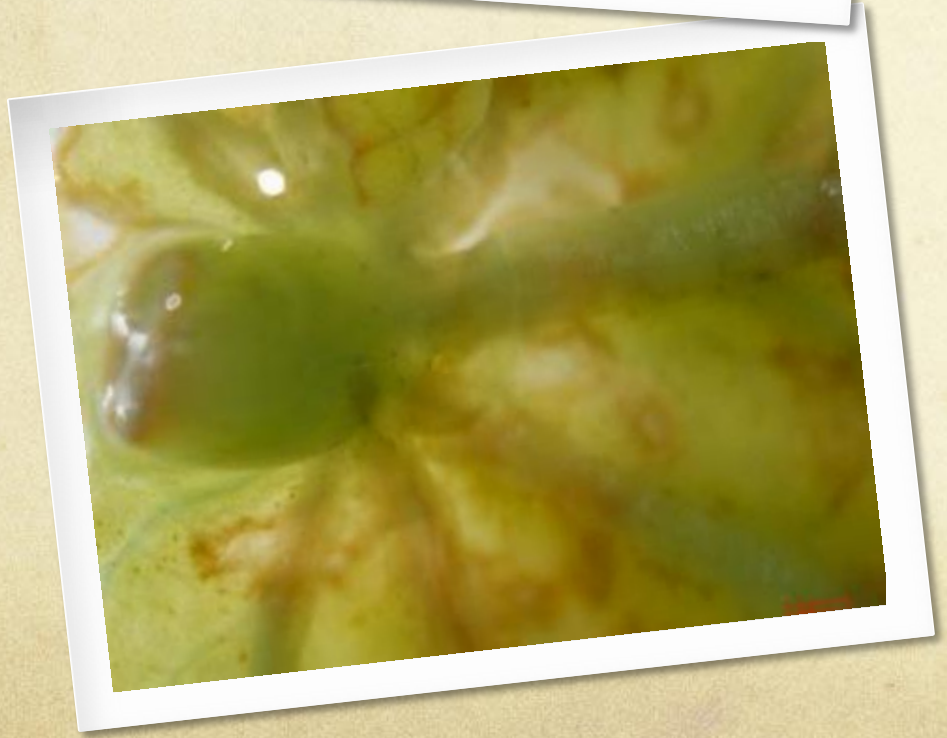


- Different explants used
  - Jat embryos- directly from seed- **sterilized**
    - 2-3 drops of Tween 20 (10 mins)
    - 5 Min 10% bleach
    - 5 min 0.1 % $HgCl_2$
    - Cut seeds and remove embryo
  - Jat Leaves- invitro(from shoots in JSM2 media)
    - Lightly poke
- Immerse explants in bacterial solutions 20 mins
- Plate leaves to co-cultivation
- Store in dark for 72 hours in growth incubator (25 C)



Procedure for GUS staining solution

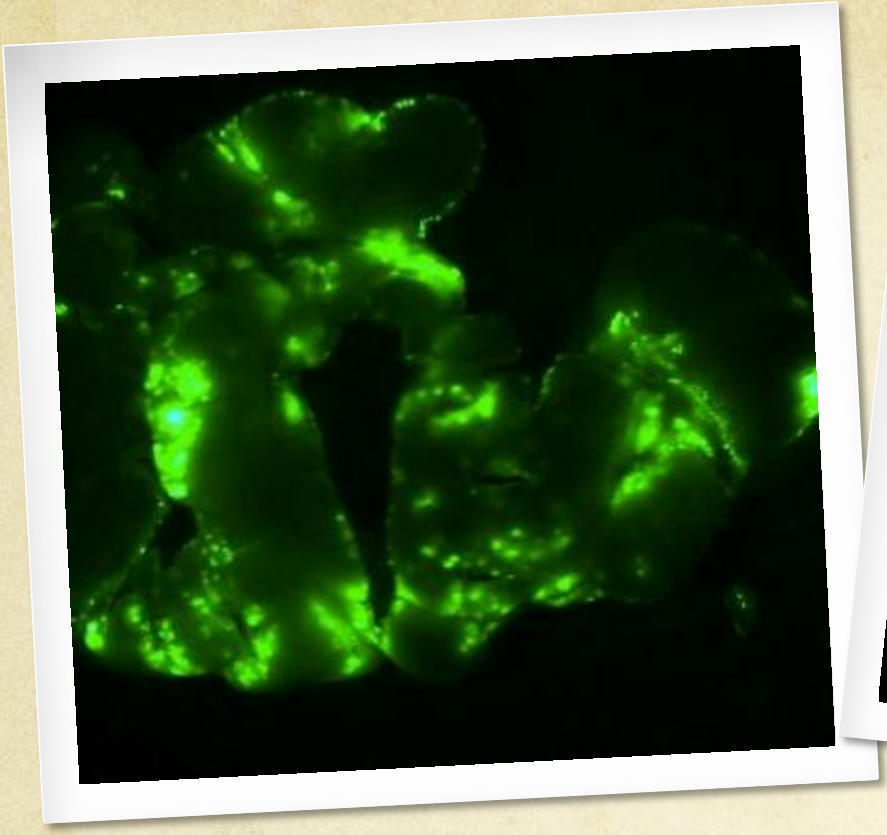
- Wash explants-MS w/ Vit + 500 **µ**L of Cefotaxime in sterile petri dish.
- Thaw staining solution on ice
- Place explants in 1.5 mL tubes
- Pour GUS staining solution enough to cover explants
- Cover with foil and leave in dark at 37 C for 24 hours
- Bleach with 70 % ethanol 1-2 hours
- Blot and put on petri dish with DH_2O and take PICTURES!

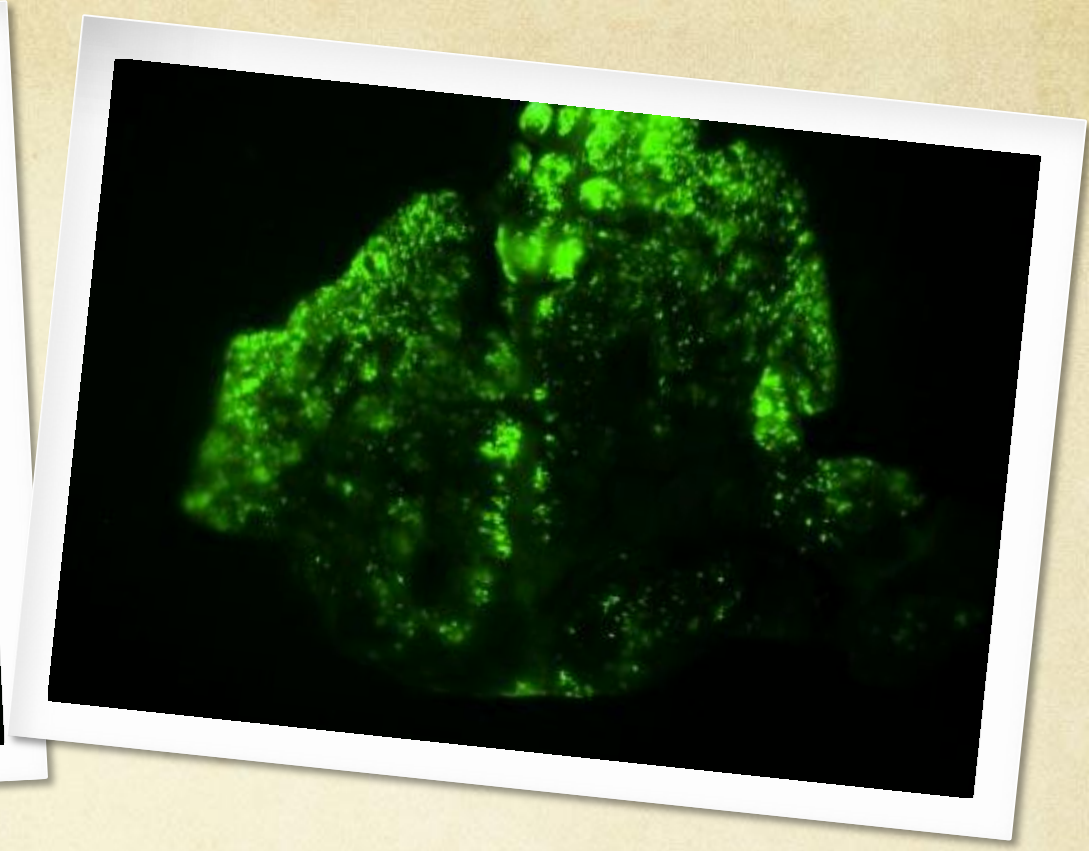


Transformation with GUS gene

- 6/17 explant shows more GUS
- 7/15 explant shows less GUS
- BOTH explants express the gene

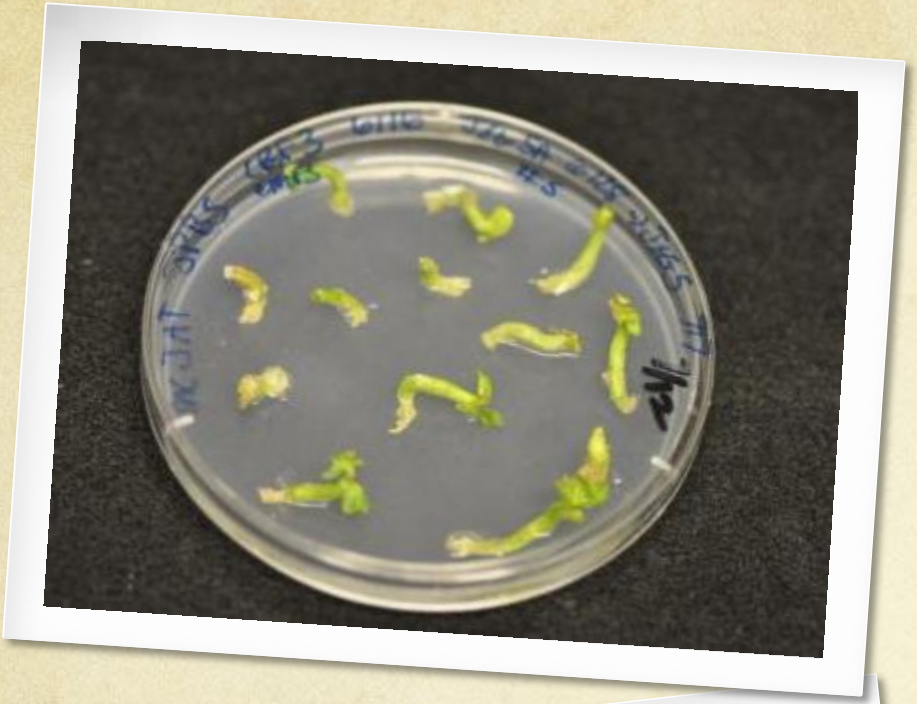
Explants from JAT: 1st picture- 6/17 Jat Embryo. 2nd picture- 7/15 Jat leaf invitro.





Transformation with GFP

EXPLANT: Leaf

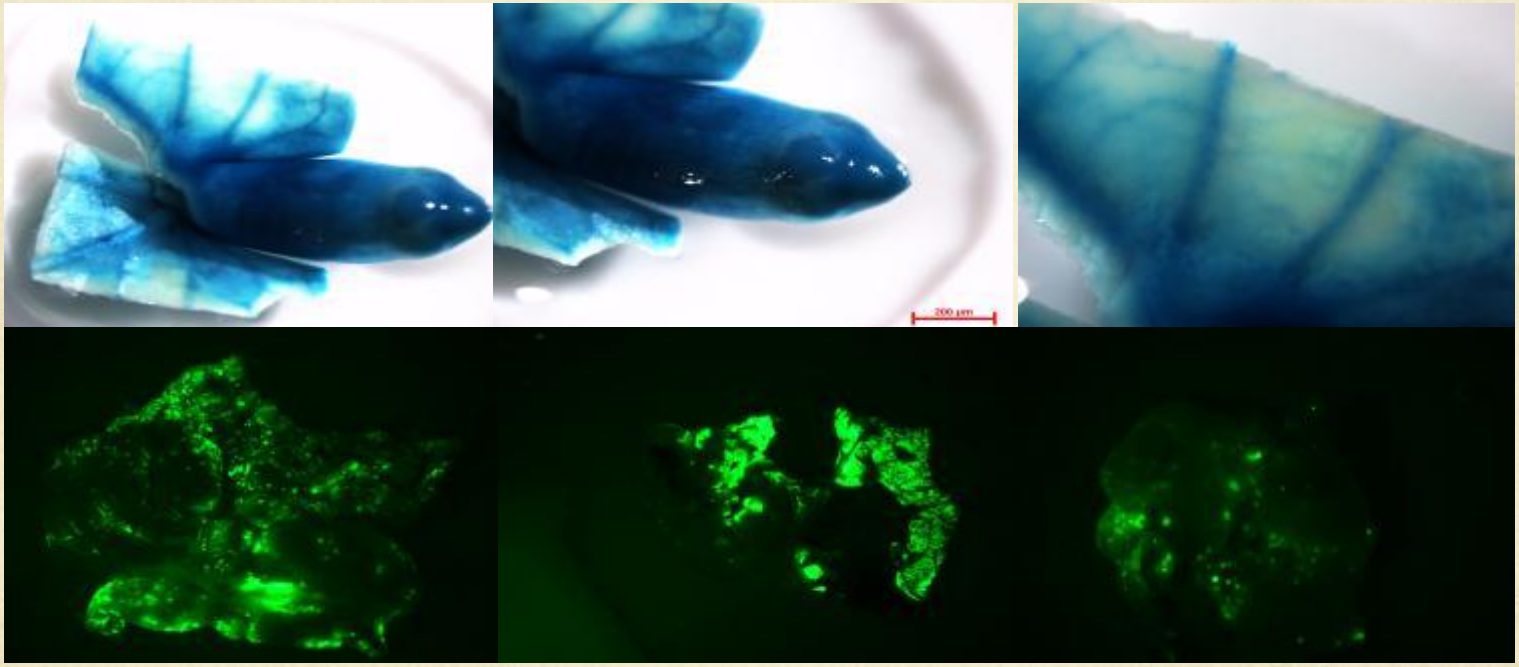


Transformation with CBF_3

Explants from 6/17/11 JPB exp.

Conclusion & Future Research

- Jatropha embryos were successfully transferred with GUS gene



- Molecular analyses (PCR, Southern blot) have to be done to confirm if transformation with CBF3 has been successful.

Industrial use of Jatropha Oil

On January 7, 2009 Continental Airlines successfully completed a test flight from Houston, Texas using a 50/50 mixture of algae/Jatropha oil-derived biofuel & traditional Jet fuel in one of the 2 engines.

http://phx.corporate-ir.net/phoenix.zhtml?c=85779&p=irol-newsArticle&ID=1241576

Work Cited

http://phx.corporate-ir.net/phoenix.zhtml?c=85779&p=irol-newsArticle&ID=1241576

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Sairam R.V., Al-Abed, D., Johnson, J., Muzynski, M., Raab, M., Reddy, T.V. and Goldman, S.L. Maize. (2008) In: Kole, C., Hall, T.C. (eds) A Compendium of Transgenic Crop Plants 49-82.

http://en.wikipedia.org/wiki/File:Transfection_by_Agrobacterium.svg(Image)

http://gas2.org/2008/10/02/jatropha-from-haitian-voodoo-to-biodiesel-holy-grail/ (Images)

http://www.biodiesel.org/pdf_files/fuelfactsheets/emissions.pdf