In vitro regeneration of *Jatropha curcas* L, - a first step towards its genetic improvement.

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Some facts about Jatropha

- Perennial shrub of the family Euphorbiaceae
- Poisonous – branches, seeds, leaves cannot be eaten by humans or animals
- Native to Central America/Mexico
- Currently also found throughout the Tropics in Africa and Asia
- Produces a tap root plus lateral roots when propagated from seed
- Produces only lateral roots when propagated from cuttings
- Seed contain high-quality oil, convertible as biodiesel for use in standard diesel engines
- Seed loses viability in storage

Other Claims

- Tolerant to drought
- Has pesticidal and fungicidal properties
- Has medicinal properties
- Grows well under marginal conditions
- Seeds contain 27 – 40% oil
- Jatropha oil is environmentally superior to petroleum diesel

- "Biodiesel miracle tree" - holds the key towards solving future energy problems of developing countries

Fig. 1 Fruited plant of *Jatropha curcas L.*

Courtesy : FACT FOUNDATION (2007)

Issues with Jatropha as a biofuel

- Seed viability
- Productivity - Seed/Oil yield
- Land use
- Production cost and selling price
- **Others**

Table 1:Seed yield compared to other crops

Crop	Seed yield/ha (kg)
Jatropha	**2500 - 3000**
Coffee	800 - 3000
Cashew	800
Cocoa	300 – 800

Table 2: Oil yield compared to other crops

Crop	Oil yield/ha (kg)
Oil palm	5000
Coconut	2260
Jatropha	***1600***
Rapeseed	1000
Sunflower	800
Soybean	375

Applications of *in vitro* Laboratory at BNARI

- Routine micropropagation
- Disease elimination
- Germplasm storage
- Induction of somatic embryos

- **Potential for genetic transformation**

*** Since 2007, lab has been involved in some preliminary investigations on Jatropha**

Exp 1: Effect of stage of fruit maturity on *in vitro* viability of *Jatropha curcas* L.

- Seeds obtained from different stages of fruit maturity (green, yellow and black) were collected

- Sterilised with 0.1% mercuric chloride.

- Zygotic embryos were excised

- Cultured on MS medium supplemented with BAP (0-1 mg/l) or KIN (0-1 mg/l), or 2iP (0-1 mg/l) in test tubes.

- Incubated in the dark for three days.

- Transferred to growth room.

- Number of embryos that germinated was recorded.

Exp 2: Effect of BAP, Kinetin or 2iP on regeneration of plantlets from apical shoot tips and meristems

- **Juvenile shoots harvested from decapitated plants of *Jatropha curcas***
- **Immersed in 10% commercial bleach containing 5% sodium hydroxide for 10 minutes**
- **Washed in three changes of sterile distilled water**
- **Shoot tips again immersed in 70% ethanol**
- **Washed with three changes of sterilized distilled water**
- **Cultured on MS medium supplemented with 0-3mg/l BAP, kinetin or 2iP.**
- **Meristems were dissected under microscope and cultured**

The number of explants that developed shoots was recorded four weeks after culture.

Exp 3: Induction of somatic embryos from cotyledons and leaf lobe explants

- Fruits from green, yellow and black stage fruits were collected
- Similarly, young leaf lobes from juvenile shoots were also collected
- Explants sterilised with commercial bleach
- Cultured on MS basal salts supplemented with varying concentrations of 2,4-D and picloram
- Incubated in dark
- Transferred to MS medium supplemented with 1 mg/l BAP for embryo maturation

Table 3. Effect of BAP, Kinetin and 2iP on viability of seeds at green stage of maturity

Maturity stage	Growth regulator	Concentration	Germination (%)
Green		0.0	90.00 ± 3.16
	BAP	0.5	98.33 ± 1.70
		1.0	100.00 ± 0.00
		0.0	90.00 ± 3.16
	Kinetin	0.5	93.33 ± 2.76
		1.0	100.00 ± 0.00
		0.0	90.00 ± 3.16
	2iP	0.5	88.33 ± 3.22
		1.0	98.33 ± 1.70

Twenty seeds were cultured per treatment; 3 Reps per treatment

Table 4: Effect of BAP, Kinetin and 2iP on viability of seeds at yellow stage of maturity

Maturity stage	Growth regulator	Concentration	Germination (%)
Yellow		0.0	93.33 ± 2.76
	BAP	0.5	98.33 ± 1.70
		1.0	100.00 ± 0.00
		0.0	93.33 ± 2.76
	Kinetin	0.5	100.00 ± 0.00
		1.0	98.33 ± 1.76
		0.0	93.33 ± 2.76
	2iP	0.5	88.33 ± 3.22
		1.0	96.67 ± 2.40

Twenty seeds were cultured per treatment; 3 Reps/ Treatment

Table 5: Effect of BAP, Kinetin and 2iP on viability of seeds at black stage of maturity

Maturity stage	Growth regulator	Concentration	Germination (%)
Black		0.0	55.00 ± 3.63g
	BAP	0.5	78.33 ± 2.76gh
		1.0	88.33 ± 3.54h
		0.0	55.00 ± 3.63i
	Kinetin	0.5	96.67 ± 2.40j
		1.0	83.33 ± 3.54j
		0.0	55.00 ± 3.63k
	2iP	0.5	51.67 ± 3.39k
		1.0	61.67 ± 3.79k

Twenty seeds were cultured per treatment; 3 Reps/Treatment

Table 6: Effect of BAP on shoot regeneration from apical shoot tips and meristem explants

Concentration (mg/l)	Percentage shoot regeneration (%)	
	Shoot tip	Meristem
0.0	50.00 ± 0.00a	85.00 ± 1.16a
0.5	55.00 ± 0.67ab	55.00 ± 0.67a
1.0	80.00 ± 1.05b	80.00 ± 1.05a
1.5	65.00 ± 0.95ab	70.00 ± 0.67a
2.0	65.00 ± 0.95ab	65.00 ± 0.95a
2.5	50.00 ± 0.00a	65.00 ± 0.95a
3.0	50.00 ± 0.00a	65.00 ± 0.95a

Table 7. Effect of kinetin on shoot regeneration from shoot tip and meristem explants of *Jatropha*

Concentration (mg/l)	Percentage Shoot regeneration	
	Shoot tip	Meristem
0.0	50.00 ± 0.00b	85.00 ± 1.58b
0.5	75.00 ± 0.67bc	85.00 ± 1.58b
1.0	50.00 ± 1.05b	30.00 ± 0.91c
1.5	55.00 ± 0.95b	40.00 ± 0.74cd
2.0	70.00 ± 0.95bc	40.00 ± 0.74cd
2.5	60.00 ± 0.00b	70.00 ± 0.67bd
3.0	40.00 ± 0.00bd	40.00 ± 0.74c

Table 8. Effect of 2iP on shoot regeneration from apical shoot tips and meristem explants of *Jatropha*

Concentration (mg/l)	Percentage shoot regenerations(%)	
	Shoot tip	Meristem
0.0	50.00 ± 0.00e	85.00 ± 1.58e
0.5	75.00 ± 0.00ef	90.00 ± 1.58e
1.0	60.00 ± 1.29ef	80.00 ± 1.05e
1.5	85.00 ± 0.95f	90.00 ± 0.95e
2.0	85.00 ± 0.95f	80.00 ± 1.05e
2.5	50.00 ± 0.00e	95.00 ± 0.67e
3.0	40.00 ± 1.45e	80.00 ± 1.05e

Somatic embryo induction

Table 9. Effect of 2,4-D and picloram on callus development from cotyledon explants of *Jatropha curcas*

Fruit maturity stage	Growth regulator Conc'n (mg/l)	Percentage calli development 2,4-D	Picloram
Green	0	0.00 ± 0.00e	0.00 ± 0.00a
	4	90.0 ± 0.67f	75.00 ± 0.00b
	8	15.00 ± 0.95e	95.00 ± 0.67bc
	16	0.00 ± 0.00e	95.00 ± 0.67bc
	24	0.00 ± 0.00e	60.00 ±1.16bd
Yellow	0	0.00 ± 0.00j	0.00 ± 0.00g
	4	70.00 ± 0.67k	95.00 ± 0.67h
	8	0.00 ± 0.00m	75.00 ± 0.00i
	16	0.00 ± 0.00k	85.00 ± 0.74hi
	24	0.00 ± 0.00m	95.00 ± 0.67h

Table 8. Effect of 2,4-D and picloram on callus development from leaf lobe explants of *Jatropha curcas*

Growth regulator	Percentage calli development	
Conc'n (mg/l)	2,4-D	Picloram
0	0.00 ± 0.00d	0.00 ± 0.00 a
4	25.00 ± 0.67e	35.00 ± 0.95b
8	60.00 ± 0.95f	95.00 ± 0.67c
16	20.00 ± 0.00de	55.00 ± 0.67b
24	15.00 ± 0.00de	45.00 ±0.67b

Fig 2:Effect of (a) Kin, (b) BAP and (c) 2iP on germination of green stage zygotic embryos

Embryos were cultured on 1mg/l of Kin, BAP or 2iP.

Fig3: Plantlets regenerated from shoot tips

Plantlets regenerated from shoot tips cultured on MS medium supplemented with 2 mg/l 2iP

Fig 4: Effect of picloram on callus initiation (Step 1)

Callus formation on picloram

Fig 5: Effect of BAP on calli development (Step 2)

No somatic embryos were developed

Main findings

1.Percent germination was lowest in embryos obtained from seeds at black stage of maturity;

2. Addition of BAP or Kinetin significantly enhanced germination in embryos from seeds at black stage of maturity;

3. For embryos obtained from seeds at green or yellow stages of maturity, supplementary cytokinin in the growth medium was not needed;

3. None of BAP, Kinetin or 2iP required for shoot regeneration from meristems;

Main findings cont'd

4. However, when shoot tips are used the same cytokinins (0.5 – 1.0 mg/l) were required to enhance shoot regeneration

5. Both 2,4-D and picloram induced calli from cotyledon and leaf lobe explants;

6. Calli did not develop somatic embryos on transfer to maturation medium indicating that calli were not embryogenically competent;

Discussion

- Regenerated plants from both meristems and shoot tips;
- Basic MS medium without additional cytokinins supports in vitro germination of embryos at green and yellow stages of maturity;
- However, embryos at black stage of maturity require supplement of BAP or Kinetin to enhance germination;
- Successful callus initiation, but calli not embryogenically competent.

Future investigation

- Improve upon callus induction
- Intensify efforts at somatic embryo development

Prospects for genetic transformation

Way forward

- Areas of improvement desired
  - - High seed yield/ha
  - - High oil yield per tonne
- Ready to collaborate with other researchers/labs

Thank you