

# Improvement of Biodiesel Quality of *Jatropha curcas* Through Genetic Engineering Plant

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## Background



- *Jatropha curcas*: small tree, originated from Central America
- Seed cake and oil: toxic, non-edible due to phorbol ester
- Seed Oil Content: 30 %, FA composition:
  - Palmitic acid 14 %
  - Stearic acid 6 %
  - Oleic acid 42 %
  - Linoleic acid 35 %
- Jatropha Biodiesel:- Cetane Number (CN): 46.3
  - Oxidation Stability (OS): 3 hour
- Specifications:
  - in Europe EN14214, CN: 51, OS: 6 h
  - in USA ASTM D6751, CN: 47, OS: 3 h
- How Genetic engineering to improve Jatropha Biodiesel Quality ?

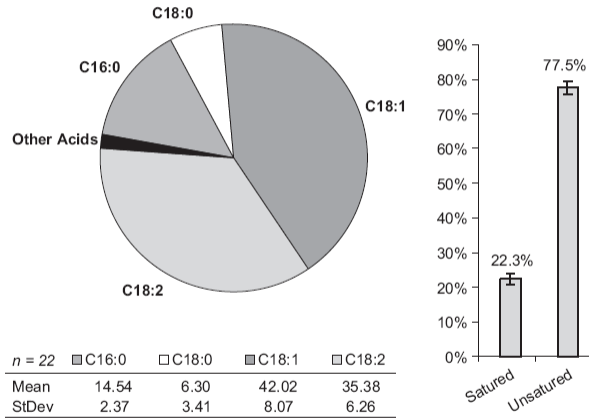


Fig. 6 - Fatty acid composition (%). C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 = oleic acid; C18:2 = linoleic acid. Other acids containing capric acid, myristic acid (C14:0), palmitoleic acid (C16:1), linolenic acid (C18:3), arachidic acid (C20:0), behenic acid (C22:0), cis-11-eicosenoic acid (C20:1) and cis-11,14-eicosadienoic acid (C20:2). n = number of observations used. Sources: [1,13,26,56,71,75,77-79,82].

Source: Achten *et al.*, 2008

## The Facts



Table 4. Cetane number (CN) of pure fatty acid methyl esters

CN	Lauric	Mystic	Palmitic	Stearic	Palmitoleic	Oleic	Linoleic	Linolenic
61.1	100	0	0	0	0	0	0	0
69.9	0	100	0	0	0	0	0	0
74.4	0	0	100	0	0	0	0	0
76.3	0	0	0	100	0	0	0	0
51.0	0	0	0	0	100	0	0	0
57.2	0	0	0	0	0	100	0	0
36.8	0	0	0	0	0	0	100	0
21.6	0	0	0	0	0	0	0	100

Source: Bamgboye and Hansen, 2008

Jatropha Biodiesel: - pure Methyl Oleate, CN : 57.2  
 - pure Methyl Linoleate, CN : 36.8

$$94:2 = 47$$

Methyl Linoleate ↘ CN Value

## The Facts

### Oxidative rate

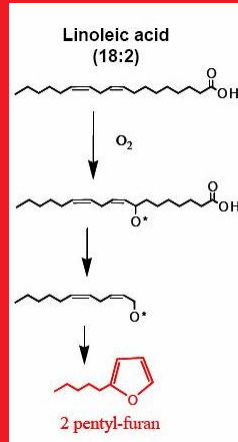
#### Fatty acid:

- Oleic acid 1 X
- Linoleic acid 10 X

#### Methyl ester:

- Methyl oleate 1 X
- Methyl linoleate 49 X

Source: Frankel, 2005



## The Facts

**Table 5. Melting Points, Kinematic Viscosity, and Oxidative Stability of Neat Fatty Esters**

ester	mp (°C) <sup>b</sup>	kinematic viscosity (mm <sup>2</sup> /s) <sup>c</sup>			oxidative stability
		40 °C	0 °C	-10 °C	
methyl octanoate	-37.3 (-40)	1.20	2.31	3.04	>24
ethyl octanoate	-44.5 (-43.1)	1.32	2.68	3.46	>24
methyl decanoate	-13.1 (-18)	1.71 <sup>e</sup>	4.04 <sup>e</sup>	4.04	>24
ethyl decanoate	-19.8 (-20)	1.87	4.28	4.28	>24
methyl laurate	4.6 (5.2)	2.43 <sup>e</sup>	solid	solid	>24
methyl myristoleate	-52.2	2.73 <sup>e</sup>	7.01 <sup>e</sup>	9.92	nd
ethyl myristoleate	-64.9	nd	nd	nd	nd
methyl palmitate	(30)	4.38 <sup>e</sup>	solid	solid	>24
methyl palmitoleate	-33.9	3.67	10.15	14.77	2.11 (0.11) <sup>e</sup>
ethyl palmitoleate	-36.6	nd	nd	nd	nd
methyl stearate	(39)	5.85	solid	solid	>24
methyl oleate	-19.5 (-19.9)	4.51	14.03	21.33	2.79 (0.21) <sup>e</sup>
ethyl oleate	-20.06	4.73	14.49	22.18	2.68 (0.18)
methyl ricinoleate <sup>d</sup>	-5.85	15.29 <sup>e</sup>	123.83 <sup>e</sup>	182.36	0.67 (0.02)
methyl linoleate	(-35)	3.65	9.84	14.10	0.94 (0.10)
methyl linolenate	(-52)	3.14	7.33	10.19	0.00 (0.00)

Source: Knothe, 2008

OS, Methyl oleate : 2.79 hour

OS, Methyl linoleate: 0.94 hour

Methyl linolenate ↓ OS value

## To increase oleic acid and to decrease linoleic acid for *Jatropha* 2 methods

### Hydrogenation



### Transgenic *Jatropha*



Soybean → Biodiesel → Partial Hydrogenation  
Additional cost 0,04 US \$/liter (Moser *et al.*, 2007) + investment cost

## Purpose of Research

- The main goal of this reserach proposal is to improve *Jatropha* biodiesel quality through genetic engineering.
- To modify fatty acid composition, especially to increase oleic acid and to decrease linoleic acid content of *Jatropha* oil, the strategy applied is to insert the silenced gene of  $\Delta 12$  fatty acid desaturase (FAD2) in antisense construction to *Jatropha* genome.



## Procedures



Primer design : To isolate complete gene FAD2 (DNA and mRNA)

Target Promoter : CaMV35S pada *pCambia1302*

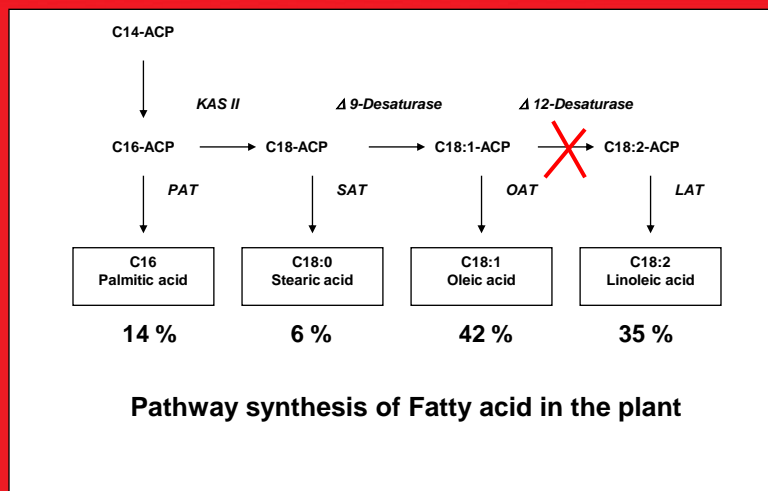
Gene target :  $\Delta$  12 fatty acid desaturase (FAD2) *J. curcas*

Construction : Antisense

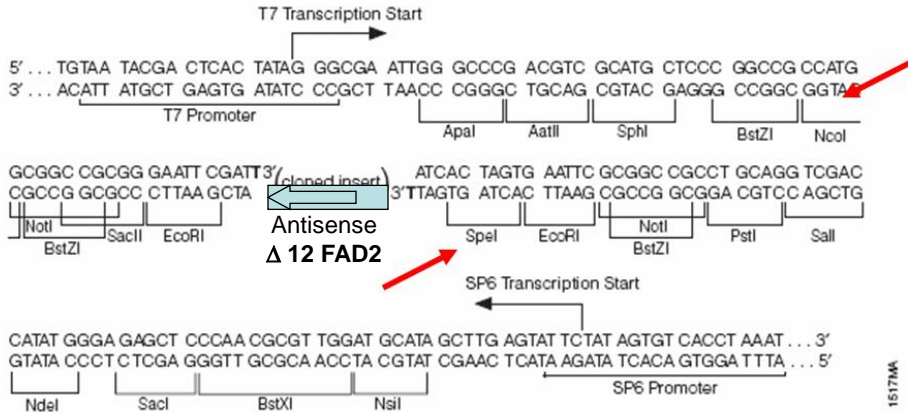
Gene Transformation: *Agrobacterium tumefaciens*

Oil Content Analysis : HPLC, GC

Propagation: Stem Cutting, Micro-Cutting, Tissue Culture

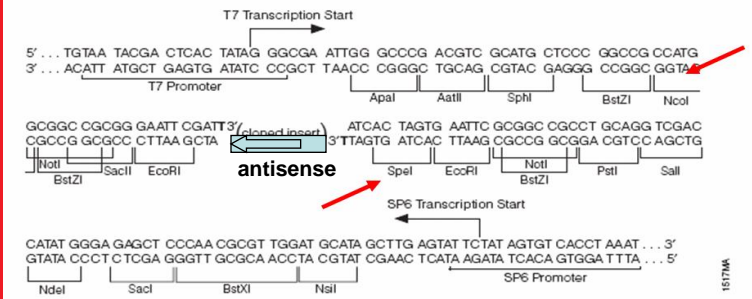


**pGEM®-T Easy Vector**

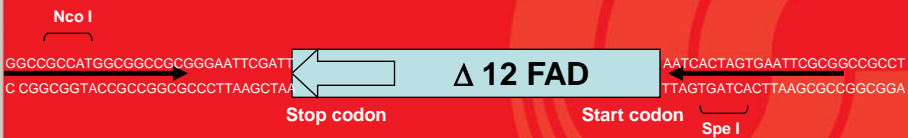


1517MA

**pGEM®-T Easy Vector**



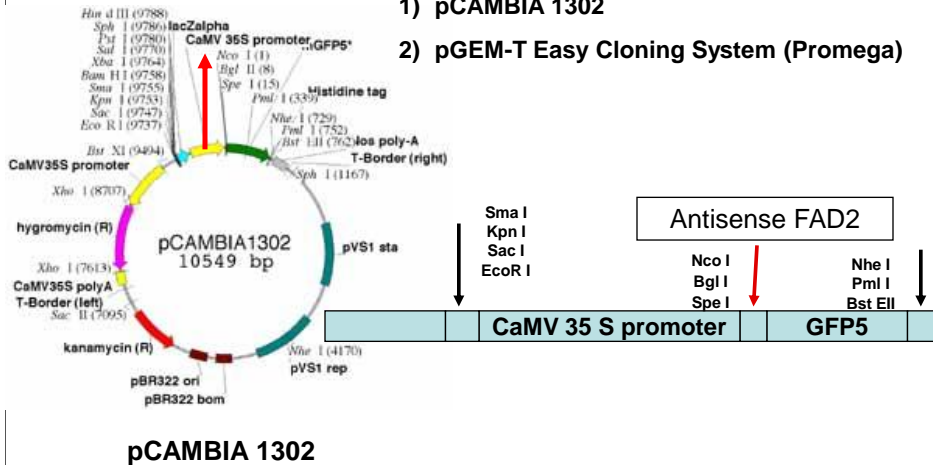
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**Antisense**

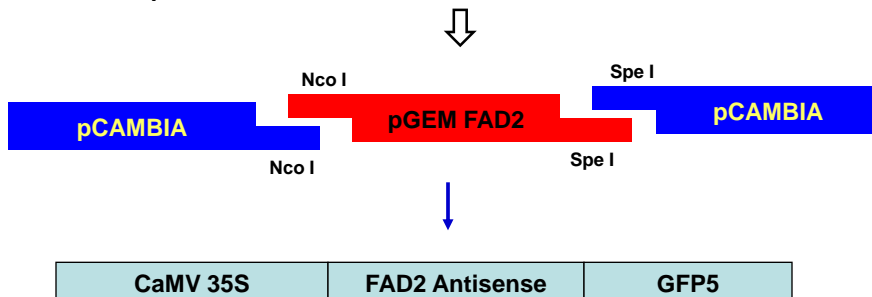
## Plasmids:

- 1) pCAMBIA 1302
- 2) pGEM-T Easy Cloning System (Promega)



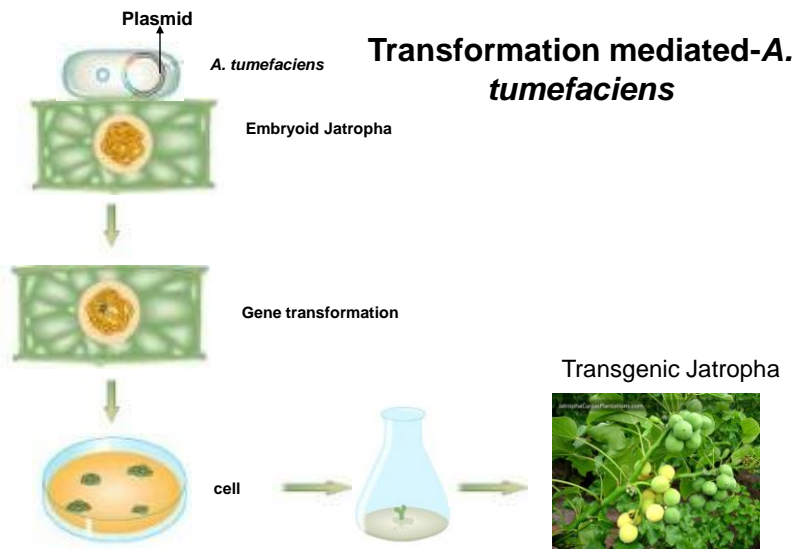
## Construction Gene with promoter CaMV 35S in plasmid pCambia 1302

- Amplify pGem by primer NcoI dan SpeI
- PCR product cut by using enzymes Nco I dan Spe I
- The fragment ligate in restriction sites of Nco I dan Spe I in pCambia 1302



Construct	DNA structure	PTGS
Cosuppression		10%
Antisense		15%
Inverted Repeat		69%
Intron-spliced Inverted Repeat		100%

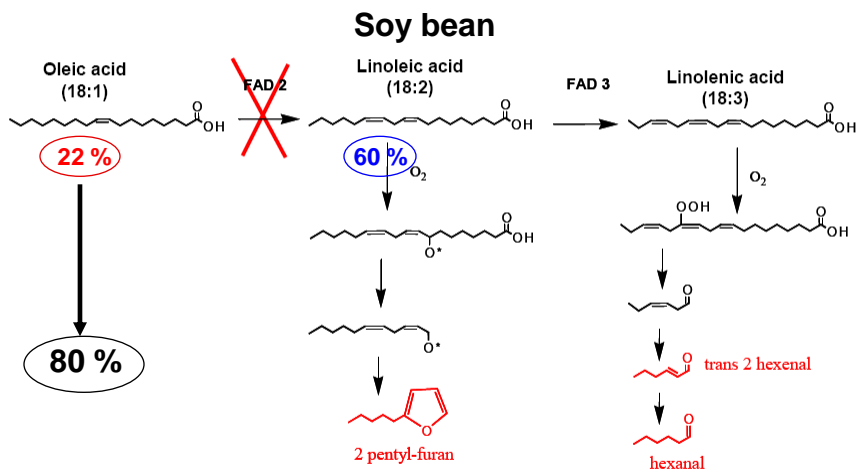
Various constructions of gene silencing



## Timelines

Activities	Year 1						Year 2					
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>
Isolation and confirmation gene	■	■										
Plasmid construction		■	■									
<i>Agrobacterium</i> transformation				■	■							
PCR transformant detection						■						
Plantlet growing							■	■	■			
Analysis of fatty acid content									■	■		
Propagation											■	■


## Improvement of oil quality for Soy bean




14 / 58 83% Find

## High Oleic Acid Trait is Environmentally Stable

Location	Avg. Oleic Acid Content		
	<i>Elite</i>	<i>G168-12</i>	<i>L2494*H07-9</i>
Ames, Iowa	20.3%	86.7%	56.0%
Cedar Rapids, Iowa	22.9%	85.2%	39.4%
Iowa City, Iowa	20.6%	85.7%	44.6%
Kalamazoo, Michigan	19.2%	83.9%	44.2%
Stine, Delaware	21.3%	85.0%	51.1%



### Estimated cost and yield



#### Resources for Biodiesel

Yield per hectare in liter\*

Oil palm	7,133
Coconut	3,223
Jatropha	2,268
Olive	1,450
Canola (Rape)	1,427
Sunflower	1,146
Soy bean	541
Corn (Malze)	200


\*Under optimal conditions Quelle: Phillips McDougall, January 2008 - Copyright © Bayer CropScience

#### Relative costs of biofuels from various feedstocks

Estimated cost per barrel of fuel

Cellulose	\$305
Wheat	\$125
Rapeseed	\$125
Soybean	\$122
Sugar beets	\$100
Corn	\$83
Sugar cane	\$45
Jatropha	\$43

Data source: Goldman Sachs via the Wall Street Journal (Aug 24, 2007) chart by mongabay.com



A 50:50 blend of the oily nut from the jatropa tree and Jet A1 fuel



December 30, 2008, 11:33 am  
**Air New Zealand Flies on Engine With  
Jatropha Biofuel Blend**  
By James Karner



Air New Zealand's Capt. David Morgan with vials of jatropa oil and Air New Zealand's biofuel blend.



# Thank You