Improvement of *Jatropha curcas* L. through Marker-Assisted Breeding for High Seed Yield and Oil Content

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INTRODUCTION

- Increasing the prices of world fuel at the end of 2005, has affected the production cost of national and home industrial sectors in Indonesia
- This also influenced the availability and distribution of national energy.

- Development of bio-fuel became an alternative that has to be considered which fit to Indonesian condition, since we have many bio-resources with biodiversity for plant producing oil.

- One of the potential plant is *Jatropha curcas*. The seed contains about 20-33% oil.

- The new green fuel extracted from the seeds of the *Jatropha* plant has an ideal characteristic as future alternative biodiesel and also environmentally friendly

- Developing biodiesel from *Jatropha curcas* is one alternative solution for the availability of energy, land conservation, as well as the opportunity for raising the farmer's income.

Jatropha has potential benefits:

- For conservation of critical and marginal land with low level of fertility.
- As bio-kerosene and biodiesel sources.
- By product: glycerin and soap.
- Sediment / fruit trunk contain high concentration of N, P and K, as an organic fertilizer or compost sources.
- Part of plant as bio-pesticide.

Problems Formulation

- The cultivation of *Jatropha curcas* in Indonesia is short of high quality clones.
- There is huge variation in seed yield capacity and oil concentration in the seeds among the seedling used for cultivation or plantation.

Problems Formulation

- The Agriculture Dept.: *Jatropha* tree with the fruits number of 10-15 in one brunch is considered as the above average quality mother plant (high productivity).

- However that kind of trees are rare.

Problems Formulation

- Fruits of *Jatropha* in one brunch ripe at different time. It causes difficulty during harvesting, that farmer has to select ripening fruits only. This makes harvesting time slow and high cost.
- Uniformity of maturity will reduce harvesting cost.

Problems Formulation

- Some diseases and insects affect the growth of *Jatropha*
- Disease and insect resistance clones is needed to maintain high productivity


Spots on leaf


Insect in *Jatropha*


Curly leaves

Problems Formulation

- **Crude *Jatropha* oil has moderate-high viscosity, reducing its viscosity may extend its utilization to all diesel engines.**

- Crude Jatropha Oil (CJO) was tested into car engine. Converter has to be added into machine to warm up and reduce CJO viscosity

Flight Testing of Jatropha
In cooperation with aircraft manufacturer Boeing, **Air New Zealand** has scheduled flight tests using a 747-400 equipped with Rolls Royce engines for later in 2008 pending regulatory approval. This will be the world's first flight test using jatropha fuel on large passenger aircraft.

Monte Hawkins prepares to remove the fuel line attached to a Continental Airlines jet for the first biofuel-powered demonstration flight of a U.S. commercial airliner Wednesday, Jan. 7, 2009, at Bush Intercontinental Airport in Houston.
2:45 p.m. ET, 1/7/09

David J. Phillip / AP

Problems Formulation

- **The oil contains toxic substances that limits its uses. Reducing such substances in the oil may broaden its application.**

Problem Solving

- Since some economical traits are quantitatively inherited, using appropriate methods such as quantitative trait loci analysis, combined with marker-assisted selection, however, would enhance the breeding methods for *Jatropha*.

- As a result, plant varieties may be produced with combination of traits previously too difficult or time-consuming to produce otherwise.

Research Objectives:

- Clones with superior Characters
- Vigor and high seed yield
- High seed oil content and seed composition
- Fruit uniformity of maturation
- Disease and insect resistance
- Environmental stress resistance
- Low viscosity of crude *Jatropha* oil
- Low toxicity substances

METHODOLOGY

- Mother plants collection with specific characters
- DNA finger printing method to characterize the mother plants
- Develop the F_2 or backcross population based on the selected mother plants
- Construct the linkage mapping using DNA markers

DNA MARKERS USED IN ANALYSIS

- RFLP (Restriction Fragment Length Polymorphisms)
- RAPD (Random Amplified Polymorphic DNA)
- SSR (Simple Sequence Repeats)
- AFLP (Amplified Fragment Length Polymorphisms)
- Caps (Cleaved Amplified Polymorphic Sequences)

- Identified the QTLs that are associated with seed yield, seed oil content, and seed composition
- Identified the putative clones that associated with specific or superior characteristics, such as high seed yield and oil content.

Mother plants collected in Biotech Center

- *Jatropha* from Gunung Kidul, Yogyakarta, grows well in dry stone-soil, leaf dark-green in color and resistance to salt.
- *Jatropha* from Bengkulu and Central Sulawesi, have high concentration of seed oil content (37-39%).
- *Jatropha* from Sukabumi, West Java, and Mataram, NTB have superior high seed yield (more than 20 fruit per brunch).
- *Jatropha* from Bogor, West Java, has characteristic in uniformity for fruits maturity.
- *Jatropha* from NTB with vigor, fast growing tree and wide leaf, however very low seed yield (2-3 fruit per brunch).

Development of Breeding Population

- DNA characterization is applied to all collected mother plant, as well as DNA finger printing analysis.
- Artificial hybridization is employed between selected mother plants
- F_2 and/or Backcross population

QTL Analysis

- The presence of QTLs and their effects are identified by three methods.
- These are:
- single-factor analysis of variance,
- interval mapping and
- composite interval mapping, as a combination of interval mapping and multiple regression

Research Design

No.	Year	Activities
1.	One	Mother plants identification and characterization Phenotypic and genotypic data collection
		DNA marker detection (AFLP, SSR, RAPD) DNA finger printing analysis Mother plant hybridization
2.	Two	F2 and / or Backcross population Phenotypic and genotypic data collection
3.	Three	*Jatropha* Linkage map and QTL analysis Clones identification on high seed yield and oil content

Preliminary Results

Mother plants identification and characterization

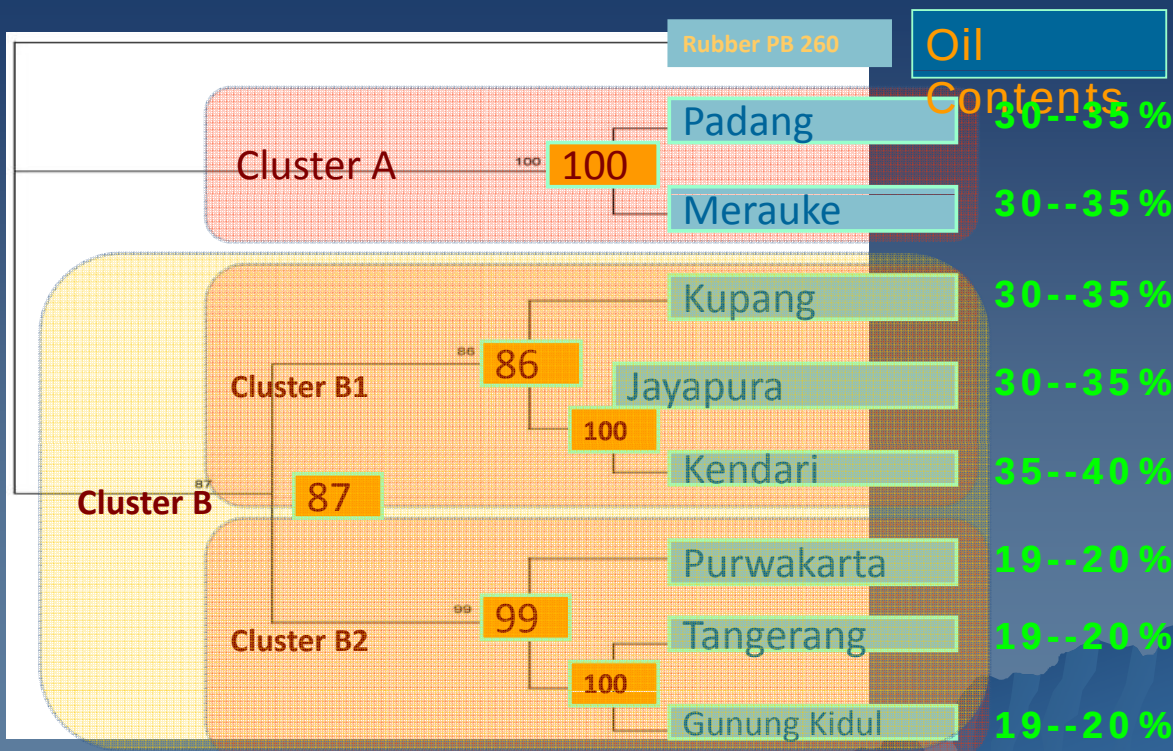
1 2 3 4 5 6 7 8 9 M1 M2

Polyacrylamide Gel 6%, 40 W, 3 hours 40 minute

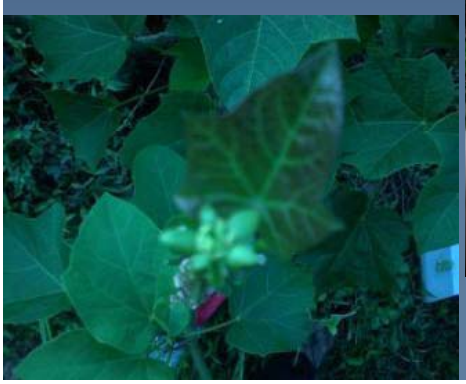
Note:

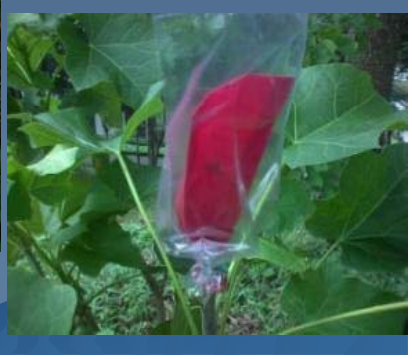
M1= 1 kb *ladder; M2= 50 bp ladder; 1= Padang;*
2= NTT; 3= Merauke;
4=Jayapura; 5= Kendari;
6= Teluk Naga; 7= Gunung Kidul; 8= Purwakarta;
9= Rubber Clone PB 260.
Red lines = specific bands

Phylogenetic study on mother plants using AFLP markers



Artificial crossing on selected mother plants






for your kind attention