

Selection of High Oil Yielding Trees of *Millettia pinnata* (L.) Panigrahi, Vegetative Propagation and Growth in the Field

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ABSTRACT

Millettia pinnata (L.) Panigrahi is a potential legume tree that produces seed oil for biodiesel feedstock. The initial step for raising a large-scale plantation of the species is selection of high oil yielding trees from the natural habitat. This is followed by vegetative propagation of the selected trees and then testing the growth of the clone in the field. The aim of the present study was to select high-oil yielding trees of *M. pinnata*, to propagate the selected trees by budding and to evaluate the survival and growth of budded plants in the field. Pods were collected from 30 trees in Lovina Beach, Buleleng Regency, Bali. Oil was extracted from seeds using soxhlet with hexane as a solvent. The high oil yielding trees were propagated by budding using root stocks grown from *M. pinnata* seeds. Scions were taken from young branches of selected trees. Incision was made on rootstock and the same size of cut was made on a scion containing a single bud. The scion was inserted to the incision of rootstock then closed tightly using plastic strips. The plastic was removed when the scion grew into a little green shoot. One month after plastic removal, the scion union grew into a single shoot and then the budded plants were removed to polybags. Budded plants were planted in the field of Bukit Jimbaran, Badung Regency, Bali with 4 × 4 spacing. Results showed all budded plants successfully grow new shoots. Two months after planting the survival of budded plants was 100%. Plant height increased by 22.13 cm, stem diameter increased by 2.43 mm and the number of compound leaf increased by 2.08. It can be concluded that four high oil yielding trees were selected from Lovina Beach and successfully propagated by budding. Survival of budded plants was 100% with vigorous growth.

Keywords: *Oil content, selected trees, scion, rootstock, survival, mother of tree*

INTRODUCTION

Fossil fuel is the main source of the world energy. However, it is not renewable and will be used up in coming decades if the trend of consumption continues to increase [6]. Rapid population growth has contributed to high energy demand and this needs to be anticipated to ensure the availability. The search for renewable, sustainable and environmentally friendly energy sources has begun worldwide. One of energy source falls in this category is biodiesel from seed-oil of non-food crops [2], such as *Millettia pinnata*. *M. pinnata* (L.) Panigrahi also known as *Pongamia pinnata* is a perennial leguminous tree capable of living on marginal land such as less fertile

land. This is due to the ability of the species to absorb nitrogen from the air through symbiosis with nitrogen-fixing bacteria called rhizobia [5]. The nitrogen fixation capable to reduce the need of nitrogen fertilizer for plant growth and this saves the production cost.

Ecologically, *M. pinnata* able to grow on most soil types include sandy soil, rocky soil, and clay making it easier for the critical land use. The plant can also grow in saline and waterlogged land [4] such as coastal areas, river banks, and estuaries. Distribution ranging from the beach to a height of 1,200 meters above sea level, annual rainfall of 500 – 2500 mm, a temperature above 0°C to 38°C [12]. The species able to grow on marginal land

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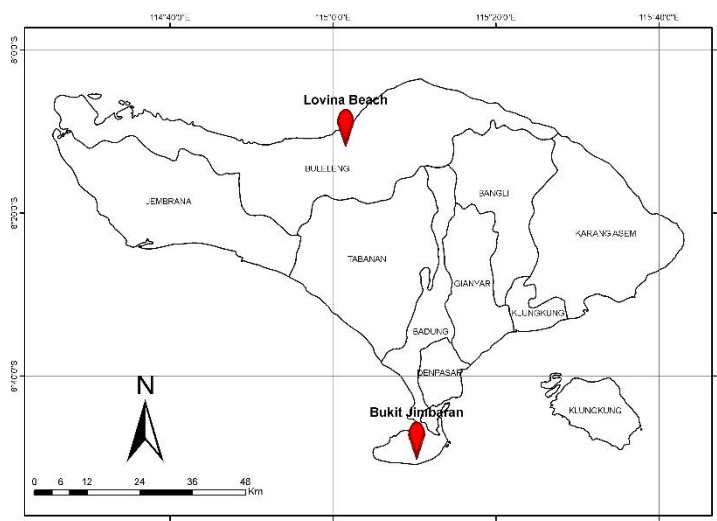


Figure 1. Location of the research in Lovina Beach, Buleleng Regency Bali where pods of *M. pinnata* were collected and Bukit Jimbaran, Badung Regency, Bali, Indonesia where the budded plants were grown

therefore the cultivation of the species would not compete with arable land so it will not disturb the productivity of agricultural crops.

M. pinnata is an oil-producing species suitable for biodiesel feedstock [4]. Oil content is the most important factor that needs to be considered when choosing tree species for biodiesel feedstock. Species with high seed-oil content is desirable because it will give profit on biodiesel production. Another important factor is seed yield [1]. Therefore, species with high oil content couple with high seed yield are most desirable for biodiesel feedstock [11].

There are so many plant species that have been used for biodiesel feedstock, such as soybean, rapeseed, palm oil, sunflower oil, and peanut oil [14]. However, these are edible and the utility of the plant for biodiesel feedstock will compete with human consumption and it disturb food security of a country. Non-food plant species have been used as biodiesel feedstocks, such as *Jatropha curcas*. The species has high oil yield [13] but low seed yield [7] and low productivity [16]. Another non-food plant species, *M. pinnata* also has potential for biodiesel feed stock [4].

Oil content of *M. pinnata* from natural habitat need to be measured to find high oil yielding trees for propagation materials. One of the populations of *M. pinnata* in Bali is in Lovina Beach, Buleleng Regency. The potency of this locally and naturally growing species need to be measured for the development and deployment of biodiesel industry in Indonesia.

The initial step to support the development of *M. pinnata* for biodiesel feedstock is to raise large scale plantation. This urgently needs seedlings that propaga-

ted from high-oil yielding trees by vegetative propagation. Vegetative propagation means that the progenies are the true copy of mother trees [10] and it is easy, cheap and replicable method [8]. *M. pinnata* can also be propagated via seeds as a mean of sexual reproduction, however it takes longer [3]. Research on production of seedlings from high-oil yielding trees is very urgently needed. Budding is vegetative propagation technique that unites a scion and the root system (rootstock) to form one unified plant. The scion usually contains a single bud. The plant that propagated by budding is called budded plant [10].

The aim of the present study was to select high-oil yielding trees of *M. pinnata*, to propagate the selected trees by budding and to evaluate the survival and growth of budded plants in the field.

MATERIALS AND METHODS

Location of Research

Mature pods were collected from 30 trees of *M. pinnata* grown in Lovina Beach (08°09'36.5" and 115°01'32.3"), Buleleng Regency, Bali, Indonesia (Figure 1).

One kilogram of pods was collected from each tree and placed in a plastic bag. All bags were labelled with sampling date and a code indicating which tree is the sample collected from. Seeds were manually separated from pods and dried in an oven at 60°C for one week.

Oil extraction

Seeds were ground using mortar and pestle to fine powder. One gram of the powder was packed in a thimble and placed in soxhlet extractor. Extraction was per-

formed at 60°C for 60 minutes using n-hexane as a solvent. Oil was separated from n-hexane by distillation. Oil content was expressed as percentage of dry weight (%w/w).

Budding

Rootstocks were grown in a field from *M. pinnata* seeds. Bud sticks were taken from selected trees in Lovina Beach, Buleleng Regency, Bali, Indonesia. The bark area of two months old rootstock was chosen for making incision at 15 cm high from the ground and the size was 1 × 1 cm. The top of root stocks was left intact. A scion bud was removed from a bud stick with the size slightly smaller than the incision on the rootstock. A single bud scion was inserted gently to the incision with proper contact between cambial layer of scion and stock. The bud union was enclosed tightly with plastic strip. This is to hold the bud union properly and to protect it from water. Three weeks after budding the plastics were removed. The green colour of the scion indicating survival. This was left for another month to grow the scions. The top of root stocks was removed when the scion bud has grown about 5 cm high, allowing the only scion bud grow as one composite plant.

Budded plants were transplanted to polybag using soil and compost with ratio 1 : 1 as media. A hundred seedlings were transplanted and were left under shading for three weeks to strengthen the new growing plant.

Planting budded plants in the field

A hundred budded plants were planted in the field of Bukit Jimbaran (08°47'56.8" and 115°10'15.2"), Badung Regency, Bali (Figure 1) with spacing 4 × 4 m. Compost (200 gram) was given to each plant followed by watering to the field capacity. Plants were irrigated twice a week and weeds were removed regularly. Fertilizer containing nitrogen (N), phosphorous (P) and Potassium (K) was applied one month after planting.

Data collection and analysis

Mean of oil content per tree was presented and standard deviation (SD) was calculated from 30 trees. Oil content were grouped into three: low ($< x - 1s$), medium ($x - 1s$ to $x + 1s$) and high ($> x + 1s$), where x is mean of oil content and s is standard deviation respectively [4]. Survival of scion unions to grow into composite plants was counted. Measurements in the field was done twice, namely at planting and two months after planting. Parameter measures included survival, plant height, plant diameter and the number of

compound leaf. Data was analysed using *Microsoft Excel*.

RESULTS AND DISCUSSION

Oil content

Oil content of each tree ranged from 28 to 31. The mean of oil content of all trees was 28.83 (± 1.44). Oil content was classified as low ($< 27.39\%$), medium (27.39 – 30.27%) and high ($> 30.27\%$). Among the 30 trees studied, there were 3 trees with low oil content, 23 trees with medium oil content and 4 trees with high oil content. In other studies oil content of *M. pinnata* varies greatly from one location to another and between trees within a location. For example, oil content of 30 *M. pinnata* trees in Kununurra, Western Australia are 31 – 45%. Among these, one tree has low oil, 24 trees have high oil and 5 trees have high oil content [4]. In the State of Haryana, India oil content are 32.57 – 44.07% [9], in Andhra Pradesh and Orissa, India oil content are 14.9 – 42.6% [15]. It is clear that environment plays important role on oil content.

Since the studied trees grow in the similar environment, variation in oil content among trees within location is probably influenced by genotypes according to [9] the influence of environment on oil content is very



Figure 2. Four selected trees of *M. pinnata* with high oil content for sources of vegetative propagation

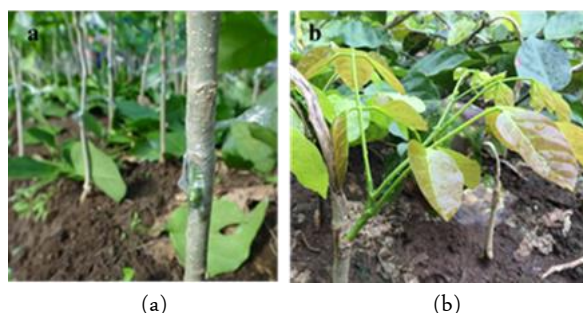


Figure 3. After 3 weeks, budded plants showed green scions (a) and it grew into a new shoot (b)



Figure 4. Two months after planting, *M. pinnata* plants grew well in the field of Bukit Jimbaran, Badung, Bali

Table 2. Survival and growth parameters of *M. pinnata* at the day of planting and two months after planting in the field. Values are means of 100 plants \pm standard deviation (SD)

Parameters	At the day of planting	Two months after planting
Survival	100	100
Plant height (cm)	16.2 (\pm 1.86)	38.33 (\pm 4.76)
Stem diameter (mm)	9.57 (\pm 1.74)	12.02 (\pm 2.01)
Number of compound leaves	7.06 (\pm 0.86)	9.14 (\pm 1.38)

low. Therefore selection of high oil yielding trees is useful for finding trees from natural habitat that will be used as sources for vegetative propagation. The four trees who have highest oil content were selected for vegetative propagation (Figure 2).

Budding

All budded plants had green scions after three weeks indicating 100% plants survival (Figure 3a). These grew into new shoots (Figure 3b) and resulted in 100 budded plants from four selected trees. All budded plants grew shoots meaning that the techniques used successfully propagate the selected trees with 100% survival.

Evaluation of growth of budded plants in the field indicated 100% survival two months after planting (Figure 4). The successful of budding is influenced by several factors, such as compatibility of scion and rootstock and scion orientation [10]. Both rootstock and scion are of the same species therefore they are compatible. Scion orientation was upward and this is the normal orientation.

Other growth parameters, such as plant height, stem diameter and the number of compound leaf increased (Table 2). Plant height increased from 16.2 (\pm 1.86) to 38.33 (\pm 4.76) cm, stem diameter increased from 9.57 (1.74) to 12.02 (\pm 2.01) mm and the number of compound leaf increased from 7.06 (\pm 0.86) to 9.14 (\pm 1.38). The vigorous growth of budded plants in the early growth stage due to the suitability of the environment, such as temperature, water availability and fertilizer.

CONCLUSION

There were four trees with high oil content ($>30.27\%$ w/w) of *M. pinnata* growing in Lovina Beach, Buleleng Regency, Bali, Indonesia. Those were selected for vegetative propagation by budding. The propagation technique was suitable for *M. pinnata* with 100% scion union to grow into composite plants. The survival of plants in the field was 100% with vigorous growth. All growth parameters measured increased, including plant height, stem diameter and the number of compound leaf.

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