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Seeds and Seedlings Production of Bioenergy Tree Species Malapari (*Pongamia pinnata* (L.)Pierre)

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Abstract

The study was aimed to determine the potency of seeds and seedlings production of malapari in relation to the procurement of planting stocks for plantation programmes. The methods were observing of flowering and fruiting development, measuring of seeds production and examining of seedlings quality and quantity. It was concluded that a proper fruit collecting time can be determined from the period of flowering and fruiting development and reproductive potency can be utilized for estimating the potency of seeds production. Stem diameter of the tree can be used to predict the produced seeds and seedlings. Seedlings quality can be improved by giving a treatment of 2.5 g mycorrhiza + 1.0 g NPK fertilizer.

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Keywords: bioenergy tree species; flowering; fruiting*;* Fungi Mycorrhiza Arbusculla *; Pongamia pinnata;* seed production

Nomenclature

a.s.l = above sea level	m = meter
yr = year	
d = days	
mo = month	
cm = centimeter	

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1. Introduction

National's policy on the continuation of energy is based on three core dimensions: energy security, social equity and environmental impact mitigation. These three goals require firm action to turn the national energy system onto sustainable and environmentally sensitive energy system [1]. Hence, to face the challenge on the demand of energy in the future, there is a need to develop bio-energy instead of fossil energy. It is predicted that Indonesia will run out of fossil energy sources in the near future due to the limited supply of natural gas, the increasing of oil price, inefficient use of energy [2]. Therefore, the sustainable energy sources should be concerned and one of the strategy to meet increasing demand is to accelerate the development of clean energy such as renewable and alternative energy[1]. Indonesia has been targeting a new and renewable share of 23 % in the national energy mix up in year 2025 [3]. In supporting the demand of new and renewable energy, the use of biomass-based biofuels are needed and several forest tree species can be utilized as a source of raw materials.

Malapari (*Pongamia pinnata* (L.)Pierre) is a bio-energy tree legume with the potential for high oil seed production and the added benefit of the ability to grow on marginal land [4]. Currently, the existing stands of malapari in Indonesia are naturaly grown. One of constraints that limit its large-scale production and availability to meet the need of bio-energy production, is lack of knowledge on its cultivation. The successful of plantation program is strongly dependent on the existence of seeds and seedlings procurement. The procurement of seeds and seedlings continuously need basic information on the period of flowering and fruiting, the potency of produced seeds and seedlings, as well as seedling techniques of the species.

The nature phenomenon occurs on a plant life cycle such as leave flushing, flowering and fruiting vary according to environmental condition, especially when the species are enable to live in a wide range of edaphic and climate types [5,6] Any species of trees possess different characteristics of flowering and fruiting pattern, but mostly begins with the appearance of flower buds and ends with fruit ripening [7]. Observation on a floral phenology of *Swietenia macrophylla* topped with the evaluation of fruit ripening level [8].

Seed production of a stand or a tree in each periods of fruiting will fluctuate that causing miss estimation of the produced seeds. This is due to inappropriate of seed collecting time and inefficient of the implementation. Therefore, in measuring seed production it should be correlated with geographical conditions, growth performances and tree ages [9,10]. The size of tree and form of the canopy are factors that influence the frequency, intensity and periods of fruiting, as well as the factors of ages, healthy, light and climates [11,12]. In relation to achieve a high quality and quantity of seedling production, it is necessary to pay attention to the seedling growth in a nursery which are concerning on growing media. This review assesses and integrates the floral phenology, seed production and seedling handling of malapari (*Pongamia pinnata*) in relation to determine their potency for plantation program.

2. Material and method

2.1. Study site

The study was carried out at a research plot in Batukaras, District of Ciamis, West Java Province with the altitude of (0 to 1) m a.s.l, latitude of 108°30'00"E and 07°43'55"S, rainfall of (1 200 to1 600) mm $\cdot$ yr^{-1} temperature of 30 °C to 33 °C, RH of 50 % to 86 % and soil type of sandy loam.

2.2. Reproductive development and fruit set

The inflorescences were observed individually for flowering and fruiting development including flower buds, developing flower and fruit development (from fruit initiation until maturation). Five branches that bearing inflorescences at the west and east of the tree canopy were randomly tagged with colored ribbon and every developed inflorescence was labeled. Any changes of flowering to fruiting structures were recorded by taking notes of time (date and period), shape and color [13]. The number of flowers and fruits were calculated for measuring the fruit set and evaluating its potency. Data were analyzed by using paired t-test. Comparative test between two-paired values of western and eastern of inflorescence-bearing branches was employed for each sample tree [14].

2.3. Seed and seedling production

Sample trees of 25 were selected to assess seed production of malapari. Tree dimension of total height, stem diameter and canopy width were measured. Data were analyzed by using correlation test to evaluate the relationship between tree dimensions and the number of produced seeds. Seedling production was measured by calculating the numbers of seeds per tree multiply by the number of normally germinated seeds and survived seedlings.

2.4. Seedling techniques

There are two treatments applied onto the one month old seedlings of malapari, i.e. :

- anorganic fertilizers of NPK 15 : 15 : 15 (0, 0.5 and 1.0) g for each seedling
- innoculation of Fungi Miccorhyzza Arbusculla (FMA) (mixture of *Glomus* + *Aculospora* + *Gigaspora* 1 : 1 : 1) of (0, 2.5 and 5.0) g for each seedling.

The experiment was arranged in a factorial randomized-completed design with 9 replications. Five months after treatments, growth were assessed by measuring seedling growth, inoculants effectiveness and infectivity and P-uptake. Root colonization was measured to find out their infectivity in the root [15] and formulated as follows [16] :

$$\text{Infected root} = \frac{\sum \text{infected root sample}}{\sum \text{observed root sample}} \times 100\ \% \qquad (1)$$

Samples were wet oxidized with HNO_3 and $HCLO_4$. The extract was used to analyse P element which was using spectrophotometry method [17] carried out at SEAMEO-BIOTROP service laboratory.

3. Results and discussion

3.1. Fruit/flower ratio

The number of flowers and fruits in the west are less than those in the east (Table 1). Randomly sampled, the ratio of fruits/flowers (fruit set) of malapari in the west is around 38 % to 88 % and in the east is 43 % to 89 %. Thus, the potency of flowers to fruits in average at the west and east are 63 % and 66 %, respectively. These values are much higher than that of another biofuel species such as nyamplung (*Callophylum inophylum*) at Batukaras i.e 29 % in the west and 37 % in the east. Each species will genetically produce different values of fruit set that refer to the characteristics of flowering and fruiting it self. Plants with more abundance flowers may have less pollen limitation [18]. A greater variance of fruit set over years may occur when pollination was carried out by the majority of floral visitors [19]. In general, fruit set is influenced by many factors of environments, breeding system evolution, pollination system and biogeography [19,20].

Table 1. Number of flowers and fruits per inflorescence of malapari at Batukaras research plot

No	West			East		
	(yield ± se) flowers · inflorescence $^{-1}$	(yield ± se) fruit · inflorescence $^{-1}$	(Fruit set yield ± se)	(yield ± se) flowers · inflorescence $^{-1}$	(yield ± se) fruit · inflorescence $^{-1}$	(Fruit set yield ± se)
1	(52.2 ± 7.3)	(24.6 ± 8.2)	(0.48 ± 0.1)	(56.8 ± 9.6)	(45.2 ± 7.3)	(0.79 ± 0.0)
2	(53.8 ± 13.8)	(40.4 ± 6.8)	(0.77 ± 0.1)	(57.2 ± 7.5)	(37.0 ± 8.2)	(0.65 ± 0.1)
3	(55.8 ± 8.4)	(24.5 ± 8.8)	(0.47 ± 0.2)	(53.0 ± 7.1)	(36.0 ± 1.4)	(0.68 ± 0.1)
4	(56.6 ± 7.5)	(39.8 ± 6.6)	(0.71 ± 0.1)	(53.4 ± 11.1)	(31.2 ± 6.7)	(0.58 ± 0.1)
5	(54.6 ± 13.8)	(36.2 ± 4.2)	(0.70 ± 0.2)	(51.7 ± 4.0)	(30.7 ± 5.5)	(0.59 ± 0.1)
Mean	(54.6 ± 1.7)	(33.1 ± 7.9)	(0.63 ± 0.2)	(54.4 ± 2.4)	(36.01 ± 5.8)	(0.66 ± 0.2)

- se = standart error

Statistics analysis showed that there is no significantly different between flowering in the West and East in relation to the reproductive variables such as number of flower, number of fruit and fruit set (Table 2).

Table 2. Paired t-test of the influence of two-sun directions on reproductive variables of malapari

Variables	West	East
number of flowers	54.6 a	54.4 a
number of fruits	33.1 a	36.0 a
Fruit set	0.63 a	0.66 a

Notes: Mean values followed by the same alphabet are not significantly different at α : 0.05 according to Duncan's

3.2. Flowering and fruiting development

Floral development from generative buds to the appearance of flower buds that grew at the West and East of the branches took about 19 d to 23 d and 18 d to 23 d, respectively. The changes from flower buds to the developed ovaries and small young fruits in the west and east were approximately of 44 d to 66 d and 35 d to 73 d (Table 3).

Table 3 .The periods of flowering-fruiting development (days) of malapari at the West and East of branches

Development stages	No of trees				
	1	2	3	4	5
West					
(Flower buds periods ± se)	(20.0 ± 1.5)	(22.0 ± 1.52)	(22.0 ± 6.3)	(23.0 ± 5.9)	(19.0 ± 6.2)
(Developed ovary periods ± se)	(6.6 ± 1.1)	(7.6 ± 2.07)	(7.2 ± 2.4)	(10.0 ± 3.1)	(7.0 ± 2.16)
(Small young fruit periods ± se)	(36.6 ± 10.7)	(56.2 ± 14.4)	(42.2 ± 17.4)	(41.4 ± 9.5)	(39.2 ± 9.8)
(Total days ± se)	(63.2 ± 14.4)	(85.8 ± 20.6)	(71.4 ± 15.2)	(74.4 ± 14.9)	(65.2 ± 13.7)
East					
(Flower buds periods ± se)	(21.6 ± 2.7)	(19.2 ± 2.8)	(20 ± 5.4)	(22.6 ± 5.8)	(17.6 ± 5.3)
(Developed ovary periods ± se)	(5.0 ± 1.2)	(6.8 ± 0.8)	(6.6 ± 2.1)	(6.4 ± 2.0)	(6.33 ± 1.7)
(Small young fruit periods ± se)	(32.8 ± 2.1)	(44.0 ± 1.4)	(50.1 ± 6.7)	(35.4 ± 10.7)	(17.1 ± 1.2)
(Total days ± se)	(59.4 ± 11.8)	(70.0 ± 15.7)	(76.6 ± 18.5)	(64.4 ± 12.8)	(65.6 ± 15.2)

- Se = standart error

The periods of each stage of flowering and fruiting development located in the west and east of the branches did not show a significant difference following paired t-test (P > 0.05) (Table 4).

Table 4. Paired t-test of the influence of two-sun direction on the flowering and fruiting periods of malapari

Development stages	West	East
Flower buds (d)	21.20 a	20.20 a
Developed ovary (d)	7.68 a	6.23 a
Small young fruit (d)	43.13 a	40.78 a

Note: Mean values followed by the same alphabet are not significantly different at α : 0.05

The ratio of flowers to fruits of malapari and the periods of flowering and fruiting development stages do not distinguish the behaviour of flowering and fruiting in two different sun directions of the West and East. The sun's rays are emitted in all directions and only a small percentage received by the earth. Geographically, the west and east receive relatively the same light intensity. The differences occur when the cloud or rain impedes solar radiaton [21]. In addition, planting distances of malapari in Batukaras are relatively closed each other (2.5 m × 1.0 m) and canopy closure are dense, thus the incoming light is more or less the same. Therefore, the growth and development of floral and fruit of malapari in the West and East in the same tree receive a similar intensity of solar radiation.

Regardless the sun direction of the West and East, mostly generative buds of malapari occur in March-April and developed to be flower bloom in May that proceeds for about 5 d to 7 d. When, the flowers pollinated, the ovaries become bigger and developed to be young fruits. This phase was seen in June-July and become mature fruits in October-November. Overall, the cycle of flowering and fruiting of malapari lasts for 7 mo to 8 mo.

3.3. Seed and seedling production

The age of malapari natural stands in Batukaras is approximately more than 20 yr old, with the stem diameter and height total of 21.53 cm ± 6.7 cm and 6.36 m ± 1.4 m in average, respectively. The number of seed production collected in 2012 were 1.41 kg tree^{-1} ± 1,56 kg tree^{-1} or around 2.0 kg tree^{-1} to 3.3 kg kg tree^{-1}. Seed production was negatively correlated to the stem diameter ($P < 0.05$). Thus, the increase of stem diameter would be followed by the reduction of the produced seeds (Figure 1). This might be due to the relatively closed of planting distance among the trees, i.e. 2.49 m ± 0.80 m [22].

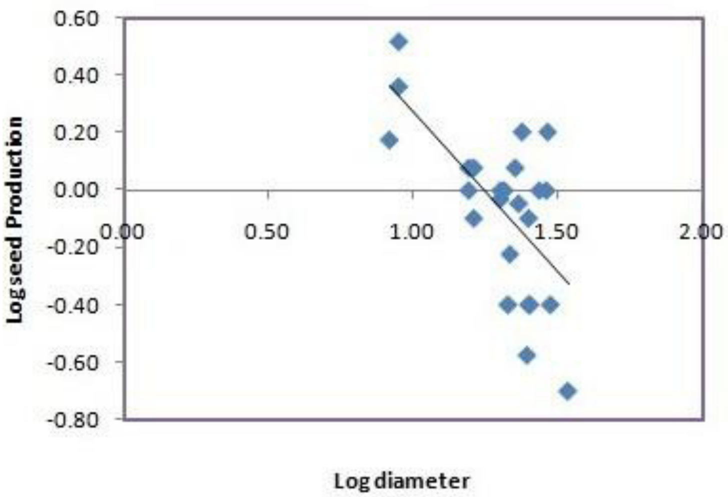

Fig.1. Seed production versus diameter of Malapari at Batukaras research plot

The dense of planting distance causes the tree crowns do not develop well and make a limitation to the fruit bearing branches, eventually. The supply of energy to set up fruit production is depend on the green leaf surface area. Indirectly stem diameter affect to the seed production, but tree crown does directly [23]. Therefore, the approachment of silvicultural techniques application for malapari should pay attention to the most influence factors on tree productivity, among others are planting distance and fertilization arrangement. It was suggested that planting distance of 8 m was enable to produce seeds of malapari of around 10 kg tree^{-1} to 250 kg tree^{-1} [24]. Thus, it is possible to increase the production of malapari seeds in Batukaras by thinning, so that the distance among trees would be wider.

The allometric equation for the estimation of malapari seed production in Batukaras is log Y = 1.334 – 1.06 log X_1, where Y is seed production (kg $tree^{-1}$) and X_1 is stem diameter (cm). Based on determination coefficient (R^2adj), it was found that 31.12 % of seed production variation was influenced by stem diameter. In this case, there were others independent variables affected that cannot be explained by such regression equation. The factors are including soil and climate conditions.

Seedling production of malapari was measured based on the estimation of seed production. It was found out that seed production of malapari per tree in Batukaras was 0.2 kg to 3.3 kg or equal to 155.8 seeds to 2570.7 seeds (the number of seed per kg is 799 seeds). Germination testing of the seeds resulted a germination capacity of 60 % [25] and survived seedlings of 95 % [26]. Considering of these values, it was counted that the number of seedlings produced from one tree was around 89 seedlings to 1 465 seedlings. Thus, to fulfill the demand of malapari seedlings for plantation of e.g. one hectare with a planting distance of 3 m × 3 m, the number of seeds needed were about 2.5 kg, which come from the calculation of:

$$\text{Number of seeds} = \frac{\text{NS's}}{\%\,\text{GC x}\,\%\,\text{SS x}\sum \text{seeds.kg}^{-1}} \qquad (2)$$

where:
NS's : Number of seedlings
GC : Germination capacity
SS : Survived seedlings

Two and half kg of seeds can be gathered from only (1 to 2) trees. However, concerning of a wide genetic variation of the seeds that should be used for gaining a better quality stand, a bulk of seeds is preferably harvested from a lot of improved mother trees [27].

3.4. Seedling quality

3.4.1. Seedling growth

Five months after treatments, the survived seedlings of malapari were high and gave a significant difference on the growth of the seedlings height and diameter. The interaction between treatment of 2.5 g mycorrhiza and fertilizer of 1.0 g NPK gave diameter value of 10.47 mm or around 152.3 % increasing compared to the value of non treatment. The combination of 5 g mycorrhiza and 0.5 g NPK fertilizer gave a high value of root colonization (84.96 %) but showed no difference with other treatments (Table 5).

Table 5**.** Mean values of the effect of myccorhiza and fertilizer on the seedlings growth of five months old malapari

Treatments	Seedling height (cm)	Seedling diameter (cm)	Survival percentage (%)	Dry organic matters (g)	Root colonization (%)
Untreated seedlings	13.9	4.15 b	90.64	3.18	15.70
0 g mycorrhiza + 0.5 g fertilizer	17.4	4.77 b	94.64	3.12	78.83
0 g mycorrhiza +1.0 g fertilizer	16.7	4.34 b	97.32	5.00	67.00
0 g fertilizer + 2.5 g mycorrhiza	11.1	3.23 b	79.96	3.65	54.43
0 g fertilizer + 5.0 g mycorrhiza	12.0	3.78 b	85.30	2.77	34.96
2.5 g mycorrhiza + 0.5 g fertilizer	16.8	4.06 b	89.32	3.29	52.20
2.5 g mycorrhiza +1.0 g fertilizer	11.8	10.47 a	87.96	3.48	70.53
5.0 g mycorrhiza +.0.5 g fertilizer	17.0	3.30 b	77.28	2.70	84.96
5.0 g mycorrhiza +1.0 g fertilizer	15.7	4.00 b	86.64	3.62	75.50

Notes: Mean values followed by the same alphabet are not significantly different at α : 0.05 according to Duncan's

3.4.2. Inoculants infectivity and effectiveness to the seedling growth

The obtanined values of inoculant infectivity and effectiveness of the treatments were compared with the values of seedlings growth without treatment (control). Seedling height, diameter and dry organic matters of the untreated seedlings were 4.00 cm, 86.64 mm and 3.62 %, respectively. Some of the treatments were unable to increase the seedlings growth of malapari determined from the negative values (Table 6).

Table 6. The infectivity and effectiveness of inoculants on the increment growth of malapari seedlings

Treatments	Height increment (%)	Diameter increment (%)	Dry organic matters increment (%)
0 g mycorrhiza + 0.5 g fertilizer	25.17	14.93	-1.88
0 g mycorrhiza +1.0 g fertilizer	20.14	4.57	57.23
0 g fertilizer + 2.5 g mycorrhiza	-20.14	-22.16	14.77
0 g fertilizer + 5.0 g mycorrhiza	20.86	-2.16	3.45
2.5 g mycorrhiza + 0.5 g fertilizer	-15.10	152.28	9.43
2.5 g mycorrhiza +1.0 g fertilizer	-13.66	-8.91	-12.89
5.0 g mycorrhiza +.0.5 g fertilizer	22.30	-20.48	-15.09
5.0 g mycorrhiza +1.0 g fertilizer	12.94	-3.61	35.79

Dry organic matters of the seedlings is an indicator of the success of seedling growth. The mass of dry matters imply the occurrence of net photosynthesis that can be deposited after drying of moisture content [28]. The increase of biomass shows the productivity of rapid cell tissues establishment [29].

The effectiveness of mycorrhizza utilization on the root colonization and P-uptake were formulated as follows [30]:

$$\frac{(\text{treated} - \text{untreated})}{\text{untreated}} \times 100\% \tag{3}$$

and measured by calculating their relative increment (Table 7).

Table 7. Innoculant effectiveness of the increment of P-uptake

Treatments	Root colonization increment (%)	P-uptake increment (%)
Untreated	57.86	0.28
Fertilizer 0.5g	37.3	30.5
Fertilizer 1.0 g	15.69	-94.91
Mycorrhiza 2.5 g	-5.98	118.64
Mycorrhiza 2.5 g +Fertilizer 0.5 g	-37.57	66.1
Mycorrhiza 2.5 g +Fertilizer 1.0 g	-10.29	220.33
Mycorrhiza 5.0 g	25.05	-30.5
Mycorrhiza 5.0 g +Fertilizer 0.5 g	46.45	13.55
Mycorrhiza 5.0 g +Fertilizer 1.0 g	33.41	-1 642.37

FMA (Fungi Mycorrhiza Arbusculla) colonization will increase the uptake of phospate due to the availability of phospatase enzyme roled in mineralization of organic phosphate [28]. In this study the improvement of relative phosphate uptake by the seedlings treated with 2.5 g mycorrhiza at concentration of 1.0 g fertilizer shows that the mycorrhiza could create FMA colonization. Similarly, the increase of phosphate element uptake in *Vitex cofassus* seedlings was due to the presence of FMA [28]. Phosphate element accelerates cell division especially on the development of plant meristem tissues which is important in the growth of seedlings.

4. Conclusion

There were no different characteristics of flowering and fruiting of malapari regarding to the sun direction of the west and east located in Batukaras-Ciamis. The potency of flowers to fruits in average in the west and east were 63 % and 66 %, respectively. In general, the period of flowering and fruiting development took around 7 mo to 8 mo that began in March-April and lasted as matured fruits in October-November. There was negative correlation between seed production and stem diameter. The allometric equation for the estimation of malapari seed production in Batukaras was log Y = 1.334 – 1.06 log X. Seedling production of malapari can be calculated based on the estimation of seed production. The number of seedlings per tree was around (89 to 1 465) seedlings. Combination of 2.5 g myccorhiza and 1.0 g NPK fertilizer treatments given to the seedlings of malapari would increase phosphate uptake of 220.33 % compared to the untreated seedlings.

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