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Conservation and selection of plus trees of *Pongamia pinnata* in Bali, Indonesia

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Abstract. *Arpiwi NL, Wahyuni IGAS, Muksin IK, Sutomo. 2018. Conservation and selection of plus trees of* Pongamia pinnata *in Bali, Indonesia. Biodiversitas 19: 1607-1614. Pongamia pinnata* (L.) Pierre or commonly known as pongamia is a tropical legume tree produces oil seeds for biodiesel feedstock. The aims of present study were mapping growth sites of pongamia in Bali, counting the number of trees and selecting plus trees based on growth parameters such as total height, clear bole height, diameter at breast height, canopy width, and oil content. Method of plus tree selection was comparison tree where one candidate tree was compared with 5 nearby check trees from each village. A total of 126 pongamia trees were found in coastal beach of Bali. The majority of trees were in the north west of the island. Temperature ranges from 26-28°C, humidity ranges from 74-80% and altitude 5-50 meters above sea level. Eight pongamia plus trees were selected from 4 villages namely Kalibukbuk, Pengulon, Pemogan, and Sanur. In conclusion, pongamia was mostly distributed in the northern-west part of the Bali Island of Buleleng District. Lower number of trees was also found in the southern and western part of the island. Trees either scattered or grown in small groups. The number of pongamia tree in Bali is small and this needs further action for conserving the species.

Keywords: Comparison method, conservation, plus tree, *Pongamia pinnata*, selection

INTRODUCTION

Pongamia pinnata (L.) Pierre or commonly known as pongamia is a tropical legume with medium sized perennial tree producing seed oil. The height of the tree is 8-10 meters with trunk diameter about 50 cm and thick spreading branches (Sangwan et al. 2010). The flower is inflorescence long raceme, bisexual and zygomorphic with dark purple-brown calyx, light purple and white corolla (Raju and Rao 2006). The seed oil could be used as raw material for biodiesel (Arpiwi et al. 2013). Biodiesel is fatty acid methyl ester which is derived by reacting vegetable oils or animal fats with alcohol and catalyst known as transesterification reaction (Srivastava and Prasad 2000). The depletion of crude oil reserve due to vary rapid increases in oil consumption has led to the search for alternative renewable energy sources. Pongamia is one of the alternative energy sources that produce non-edible oil for biodiesel feedstock and can be grown on wasteland (Azam et al. 2005).

Pongamia has not been widely cultivated in Indonesia and its population in nature is declining (Jayusman 2017). Broadacre plantations of the species are needed to support biodiesel industry with supplies of feedstock (Scott et al. 2008). These also support the diversification program of renewable energy sources in Indonesia. Selection and improvement of the species is necessary to be studied before raising broad acre plantation with elite trees (Mukta and Sreevali 2010). It needs detailed information about the distribution of the species in nature as sources of breeding material. It is followed by selection of plus trees from natural population to obtain the best phenotype trees, which will be used as source of propagation (Arpiwi et al. 2017).

Data on distribution of pongamia in Indonesia especially in Bali is still very limited. Global Biodiversity Information Facility (GBIF) (https://www.gbif.org/species/2965911) does not have any records of pongamia row in Bali Island. In the red list of IUCN this species is included in the least concern category (Groom 2012). A research by Jayusman (2017) explores pongamia germplasm in Baluran Situbondo National Park of East Java, Alas Purwo Banyuwangi National Park of East Java and Ujung Kulon National Park, Banten Province. In the study, there is no explanation of the number of pongamia trees in each of the National Parks so that no clear picture of the existence of the species in their natural habitat is available. It is mentioned that In Baluran National Park the number of pongamia trees is very small, growing in coastal forest and brackish forest separated 0.5-1 km from each other.

Plus trees are trees that have superior morphological characteristics has advantages over similar tree-like trees, such as growth, height, stem diameter, yields, resistance to disease and oil content of the seeds. Oil content is one of the most important selection criteria because oil is used as raw material for biodiesel (Kesari et al. 2008). Selection of candidate plus tree is an initial and important step for tree breeding for improvement program (Zobel and Talbert 1984). The current research aims at (i) mapping growth sites of pongamia in Bali and; (ii) counting the number of

trees and selecting plus trees based on growth parameters and oil content. This research is also the first to attempt at mapping the distribution of *Pongamia pinnata* in Bali.

MATERIALS AND METHODS

Exploration and mapping of Pongamia

Exploration was carried out along the entire coast of Bali Island, Indonesia to find the growth location of pongamia. The number of trees in each location (village) was counted. Coordinates where each tree grows and the elevation were measured using GPS and weather data including temperature and humidity were recorded. Distribution of pongamia was mapped based on coordinate growth locations. Dried mature pods were taken (2 kg) from each tree for measurement of oil content. Each tree was measured for total height (TH), clear bole height (CBH), diameter at breast height (DBH), canopy width, oil content and observation of pests and diseases affecting the trees.

Oil extraction

Pod samples were dried under the sun for one week. Pods were extracted to obtain the seeds which were then oven dried at 65ºC for one week. Dried seeds were ground and then sieved to be homogeneous. Ten (10) grams of fine seed powder was wrapped in a filter paper then placed into a soxhlet for oil extraction. Hexane was used as a solvent and extraction was performed at 65ºC for one hour. After the extraction of hexane-mixed oil is separated by distillation. The oil was oven dried at 65ºC for one day to evaporate the remaining hexane. The oil yield was expressed as percent dry weight (%w/w).

Selection of plus trees

Selection of plus trees was conducted using comparison trees method. For each village with a minimum of 6 trees, one candidate tree and 5 check trees were selected. The number of pongamia trees in each village varies greatly, ranging from 1 to 30 trees, so that some villages are not represented in the selection of candidate trees because they have less than 4 trees. With respect to candidate and check trees, the following measurements are made: total height, clear bole height, diameter at breast height, canopy width and pest and diseases were measured. Data of check trees and candidate trees were then scored as in Table 1.

Growth parameters

Total height (TH) was measured from the base to the tip of the tree by using software ImajeJ. Clear bole height (CBH) was measured from the base to just under the first branch of a tree. Diameter at breast height (DBH) was measured at 130 cm from the base using a meter tape around the main stem. Canopy width (CW) was measured from the widest distance between two ends of canopy. Pests and diseases were observed on each tree.

The value of each growth parameter for candidate plus tree (CPT) was obtained by dividing the value of CPT by the mean value of check tree (CT) using the following formula:

$$\text{Total Height (TH)} = \frac{\text{THcpt}}{\text{THct}} \times 100$$

$$\text{Clear Bole Height (CBH)} = \frac{\text{CBHcpt}}{\text{CBHct}} \times 100$$

$$\text{Diameter on Breast Height (DBH)} = \frac{\text{DBHcpt}}{\text{DBH ct}} \times 100$$

$$\text{Crown Width (CW)} = \frac{\text{CWcpt}}{\text{CWct}} \times 100$$

$$\text{Oil Content (OC)} = \frac{\text{OCcpt}}{\text{OCct}} \times 100$$

Where
cpt: candidate plus trees
ct : check trees

Data analysis

Growth data and oil content were subjected to cluster analysis with alogarithm paired group and *similarity measure* Euclidean and presented as a dendrogram. Growth data including total height, clear bole height, diameter at breast height and canopy width were correlated with oil content using Statspearman. Weather data including temperature, humidity, and elevation were subjected to analysis of similarity (ANOSIM), non-metric multi-dimensional scaling (NMDS) and principal component analysis (PCA) using Primer 6 software.

Table 1. Scoring CPTs by comparison trees method (Modified from Soeparno 2013)

Characters	Value	Ranges	Score
Total Height (TH)	15	<100%	3
		100-115%	6
		116-131%	9
		132-147%	12
		>147%	15
Clear Bole Height (CBH)	20	<100%	4
		100-140%	8
		141-181%	12
		182-222%	16
		>222	20
Diameter at Breast Height (DBH)	15	<60%	3
		60-85%	6
		86-111%	9
		112-137%	12
		>137%	15
Canopy Width (CW)	15	<69%	3
		69-99%	6
		100-130%	9
		131-161%	12
		>161%	15
Oil Content (OC)	30	<103%	5
		103-105%	10
		106-108%	15
		109-111%	20
		112-114%	25
		>114%	30
Pests and diseases		Absent	5
		Present	0

RESULTS AND DISCUSSION

Distribution of *Pongamia pinnata* in Bali

Pongamia pinnata were found in coastal beach of Bali, Indonesia with a total number of 126 trees and mostly in the north west of the island. Most trees grow naturally. The number of trees in each village varies considerably from 1 to 30 trees. Temperature ranges from 26-28°C, humidity ranges from 74-80% and altitude 5-50 meters above sea level (m asl.). Growth location of *P. pinnata*, the number of trees in each location, temperature, humidity, and elevation were presented in Table 2.

Table 2. Location, weather data and number of *Pongamia pinnata* trees in Bali, Indonesia

Village	Sub-district	District	Number of tress	Temp. (°C)	Humidity (%)	Elevation (m asl)
Penarukan	Buleleng	Buleleng	6	27	75	9
Pemaron	Buleleng	Buleleng	11	28	74	17-20
Kalibukbuk	Buleleng	Buleleng	30	28	74	5-25
Temukus	Banjar	Buleleng	4	28	74	11-28
Dencarik	Banjar	Buleleng	3	28	74	11
Tangguwisia	Seririt	Buleleng	2	28	74	6
Uma Anyar	Seririt	Buleleng	6	27	74	12-14
Kalisada	Seririt	Buleleng	7	27	74	14-20
Celukan Bawang	Gerokgak	Buleleng	4	27	74	13-18
Pengulon	Gerokgak	Buleleng	9	27	74	40-50
Banyu Poh	Gerokgak	Buleleng	1	27	74	17
Sumber Klampok	Gerokgak	Buleleng	14	27	74	8-20
Serangan	Denpasar Selatan	Denpasar	5	28	69	16-23
Pemogan	Denpasar Selatan	Denpasar	11	28	74	5-17
Sanur	Denpasar Selatan	Denpasar	8	27	74	9-12
Kedonganan	Kuta	Badung	1	26	80	9
Tanjung Benoa	Kuta Selatan	Badung	4	28	74	13-18
Total			**126**			

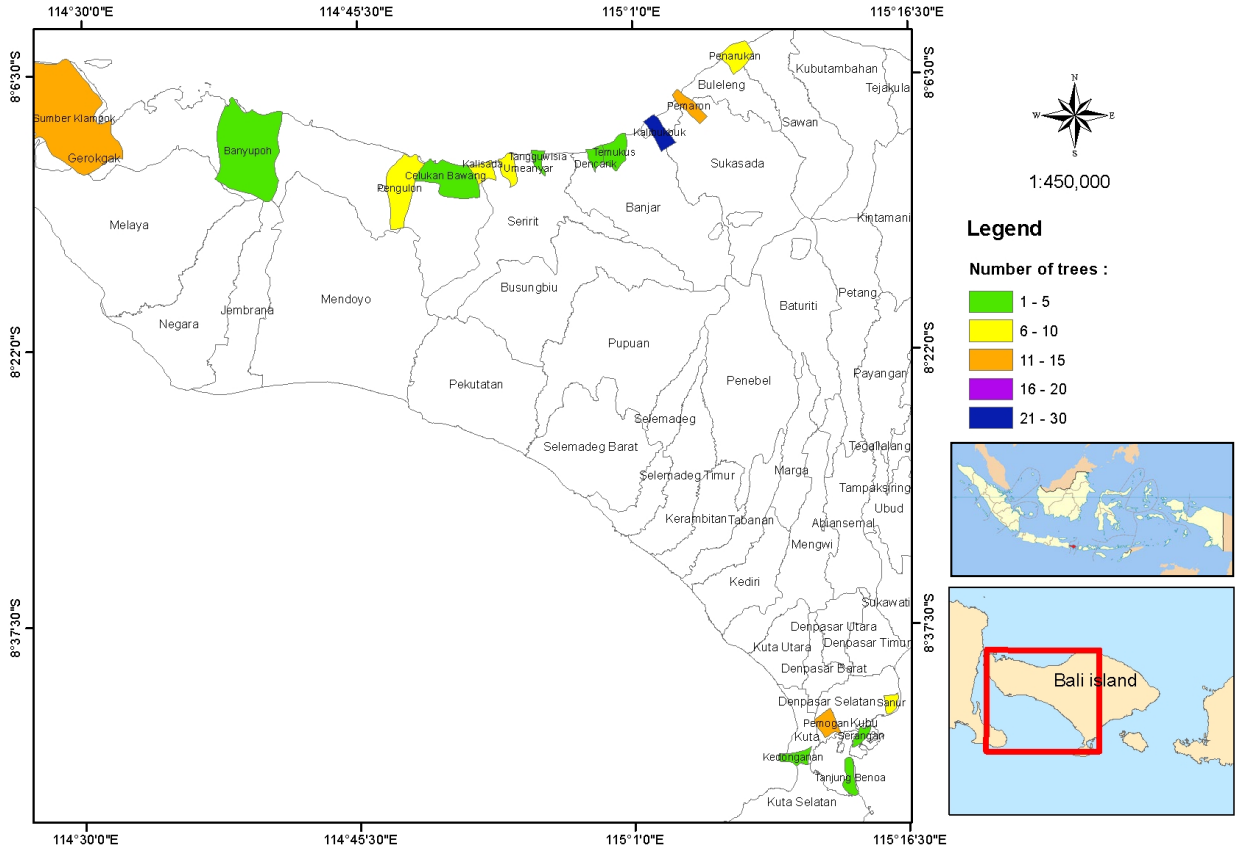

Figure 1. A map of distribution of *Pongamia pinnata* in Bali, Indonesia

Trees were found in 3 districts of Bali, namely Buleleng, Badung and Denpasar. The trees mostly found along the north coast of Buleleng District covering 11 villages, ranging from east to west, namely: Penarukan, Pemaron, Kalibukbuk, Temukus, Tangguwisia, Uma Anyar, Kalisada, Celukan Bawang, Pengulon, Banyupoh and Sumber Klampok Villages. In Badung District, pongamia was found in Kedonganan and Tanjung Benoa Villages. In Denpasar, pongamia was found in the Serangan, Pemogan and Sanur Villages. The map of distribution of pongamia in Bali is presented in Figure 1.

Selection of plus trees

Selection of candidate trees was conducted in villages with a minimum of 6 pongamia trees. Five trees were selected as check trees (CT) and 1-5 as candidate trees depending on the number of trees in each village. From 126 pongamia trees found in Bali, 17 trees were chosen as candidate trees from 9 villages, namely: Penarukan, Pemaron, Kalibukbuk, Uma Anyar, Kalisada, Pengulon, Sumber Klampok, Pemogan, and Sanur. Growth data and oil content of both candidate and check trees are presented in Table 3.

Growth parameters of the 17 candidate trees were rank from the lowest to the highest. Total height was 7.50-13.60 m, clear bole height was 1.40-6.13 m, diameter at breast height was 20.70-63.69 cm, crown width was 6.00-20.00 m, and oil content were 26.00-32.00%. Scores of candidate trees are presented in Table 4.

Tabel 4 showed scores for 17 candidate trees from 9 villages in Bali. Candidate trees with scores more than 60% were selected as plus trees. Eight pongamia plus trees were selected from 4 villages, namely CPT1, 2, 4 and 5 from Kalibukbuk Village, CPT2 from Pengulon Village, CPT1 and 2 from Pemogan Village, and one CPT from Sanur Village.

Cluster analysis

The 17 candidate trees were clustered based on growth parameters and oil content (Figure 2). As can be seen from Figure 2 that the candidate trees were grouped into 2 with a distance of 24. Group A consisted of most of candidate trees while group B consisted of trees from Buleleng District (Pemaron, Kalibukbuk, Kalisada Villages) and Denpasar (Sanur Village). At the distance of 16, group A was divided into 2 subgroups, namely A1 and A2. Group A1 consisted of trees from Buleleng District (Pemaron, Sumber Klampok Kalibukbuk and Kalisada Villages) and Denpasar (Pemogan Village). Group A2 consisted of trees from Buleleng District (Kalibukbuk, Uma Anyar, Pengulon, Penarukan and Sumber Klampok Villages) and Denpasar (Pemogan Village).

Table 3. Growth data and oil content of check (CT) and candidate plus (CPT) trees

Village	Tree code	Total height (m)	CBH (m)	DBH (cm)	Canopy width (m)	Oil content (%)	Pests and diseases
Penarukan	CT	10.14	2.59	55.41	13.20	27.80	A
	CPT	9.32	2.53	46.18	20.00	29.00	A
Pemaron	CT	10.33	3.70	55.10	15.00	28.00	A
	CPT	9.32	4.46	29.30	8.50	28.00	A
Kalibukbuk	CT	11.20	1.46	40.45	10.36	27.90	A
	CPT1	13.60	1.90	41.40	10.00	32.00	A
	CPT2	12.00	2.30	33.44	11.50	32.00	A
	CPT3	9.60	2.69	47.77	7.50	30.00	A
	CPT4	9.55	3.69	63.69	7.50	30.00	A
	CPT5	12.00	2.90	45.54	13.50	30.00	A
Uma Anyar	CT	9.92	2.36	35.22	9.40	30.40	A
	CPT	10.60	3.40	41.40	10.00	32.00	A
Kalisada	CT	12.66	3.23	55.61	14.30	30.20	A
	CPT1	10.70	6.13	61.15	11.00	31.00	A
	CPT2	9.53	2.96	20.70	10.60	32.00	A
Pengulon	CT	10.22	2.00	51.46	10.90	27.60	A
	CPT1	10.90	2.50	40.76	7.00	30.00	A
	CPT2	11.49	5.00	47.77	14.00	30.00	A
Sumber Klampok	CT	8.02	1.54	29.49	9.80	24.20	A
	CPT1	7.50	1.40	29.62	8.00	28.00	A
	CPT2	9.50	1.80	37.90	6.00	26.00	A
Pemogan	CT	7.46	1.68	26.82	7.80	24.00	A
	CPT1	9.30	2.10	28.66	8.00	28.00	A
	CPT2	11.80	1.70	42.99	14.00	27.00	A
Sanur	CT	11.10	2.06	45.86	11.00	26.20	A
	CPT	12.40	3.60	63.06	16.00	29.00	A

Note: A = Absent

Table 4. Sores of candidate trees

Village	CPTs	Total height	CBH	DBH	Canopy width	Oil content	Pests & diseases	Total score
Penarukan	CPT	3	4	6	12	10	5	40
Pemaron	CPT1	9	8	9	9	5	5	45
	CPT2	6	12	3	3	5	5	34
Kalibukbuk	**CPT1**	**9**	**8**	9	**6**	**25**	**5**	**62**
	CPT2	**6**	**12**	6	**9**	**25**	**5**	**63**
	CPT3	3	16	9	6	15	5	54
	CPT4	**3**	**20**	15	**6**	**15**	**5**	**64**
	CPT5	**6**	**16**	12	**9**	**15**	**5**	**63**
Uma Anyar	CPT	6	12	12	9	10	5	54
Kalisada	CPT1	3	16	9	6	5	5	44
	CPT2	3	4	3	6	10	5	31
Pengulon	CPT1	6	8	6	3	15	5	43
	CPT2	**6**	**20**	9	**9**	**15**	**5**	**64**
Sumber Klampok	CPT1	3	4	9	6	30	5	57
	CPT2	9	8	12	6	15	5	55
Pemogan	**CPT1**	**9**	**8**	9	**9**	**30**	**5**	**70**
	CPT2	**15**	**8**	15	**15**	**25**	**5**	**83**
Sanur	**CPT**	**6**	**12**	12	**12**	**20**	**5**	**67**

Note: Bold indicated candidate trees with total scores more than 60% regarded as plus trees.

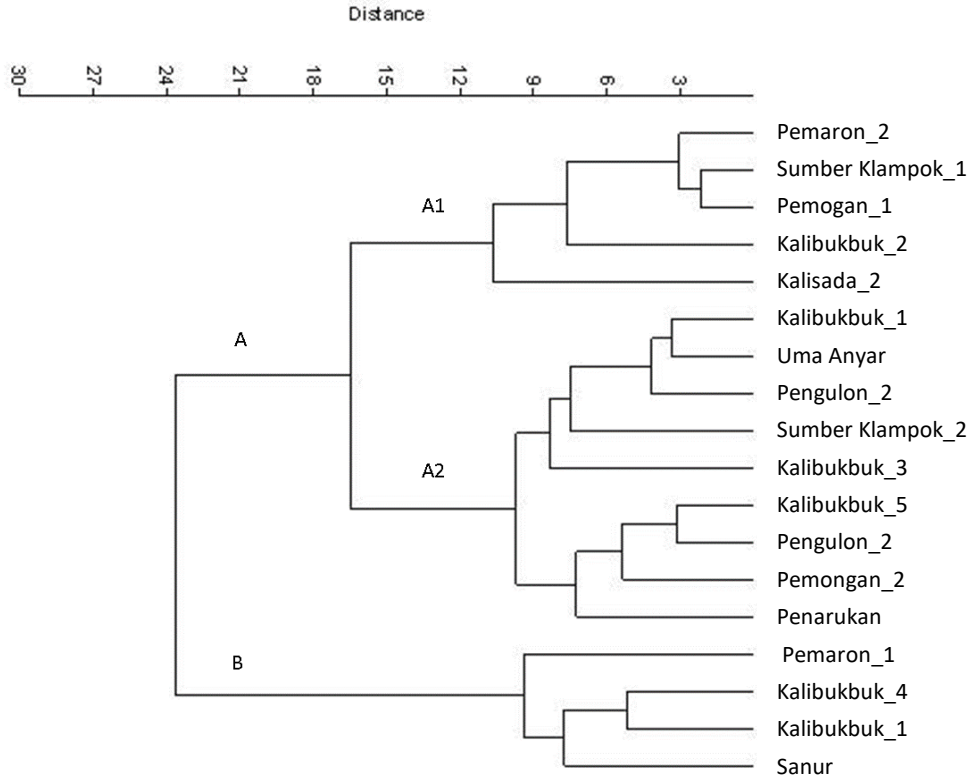

Figure 2. A dendrogram of cluster analysis of CPTs based on growth data and oil content

Table 5. Correlation between oil content and growth parameters

Growth parameters	Oil content		
	Spearman's Rho	df	P value
Canopy width (CW)	0.101	124	0.2594
Diameter at breast height (DBH)	0.204	124	0.022
Total height (TH)	0.267	124	0.00025
Clear bole height (CBH)	0.305	124	0.002516

Note: df = degree of freedom

Correlations

Correlation between oil content and growth parameters including total height, clear bole height, diameter at breast height, and canopy width is presented in Table 5. It is showed that oil is significantly correlated with total diameter at breast height (P = 0.022), total height height (P = 0.00025), clear bole height (P = 0.002516).

Weather data and elevation

Multivariate analysis of the environmental factors including temperature, relative humidity and elevation indicated that there were differences in those factors among sites. Analysis of similarity (Figure 3) showed global R ANOSIM of 0.6-0.7 indicating significant difference in environmental factors among sites (villages).

Sites were clustered based on the environmental factors using Nonmetric Multidimensional Scaling (NMDS) (Figure 4). There are 4 cluster sites, namely Pengulon, Serangan, Sumber Klampok and mixture of sites.

Principal component analysis (PCA) of the sites based on temperature, relative humidity and elevation (Figure 5) indicating most sites had similar temperature and relative humidity respectively. The difference among sites was due to elevation. The only one site had differences in both temperature and RH was Serangan Village.

Discussion

Pongamia were mostly distributed in the northern-west of Bali Island in Buleleng District (Figure 1). Trees mostly grow in small group of 2-14 trees and vary rarely in a big group. The only big group of 30 trees was found in Kalibukbuk Village which is a tourism area for Dolphin attraction. Pongamia in Kalibukbuk Village was grown in the parking area, between art shop, and along the beach for shading. Total of 126 pongamia trees was found in Bali and mostly distributed in the north-west of the Island. This is relatively small number of pongamia trees compared to population of the species in other islands. For example, population of pongamia in Kutai Kartanegara District of Kalimantan Island is about 121.000 trees with high density (Sidiyasa et al. 2012).

The existence of pongamia in Kutai Kartanegara District of Kalimantan Island is supported by biotic and abiotic factors. Pongamia grows well on sandy beaches either directly face the sea or bordered by muddy mangrove trees. Pongamia is mostly found in undisturbed habitats such as area with no abrasion and no human disturbance (Sandiyasa et al. 2012). In the case of the small number of pongamia in Bali probably due to human activities such as development of residential areas, development of tourism facilities, hotels, and restaurants in most beaches in Bali. These may have largely contributed to habitat loss of pongamia and threatening the population of the species. Jayusman (2017) stated that population of pongamia is relatively stable in conservation areas, such as Ujung Kulon, Baluran and Alas Purwo National Parks. While in the areas with bad sea abrasion such as in the southern beaches of West Java, population of pongamia is small. Many big pongamia trees have killed due to intrusion of sea water.

Bali Island which located on the west of Wallacea line has abundance species of flora as part of its biodiversity. At the moment, an array of threats are thought to threaten this diversity whether it is natural such as climate change, natural disasters or anthropogenic such as land conversion from forests to agricultural fields, settlements, roads, mining, etc. On the other hand, humans are relying on plants for timber, foods, medicines, etc. In order to sustain the availability, the practice of plant conservation efforts is needed. Plant conservation basically can be grouped as in-situ and ex-situ.

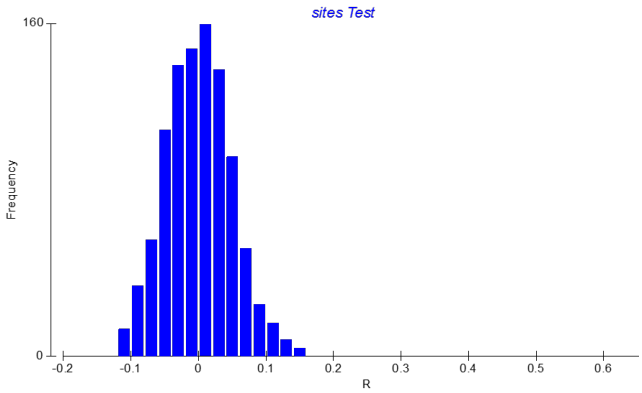

Figure 3. Analysis of similarity (ANOSIM) based on temperature, humidity, and elevation of site studied

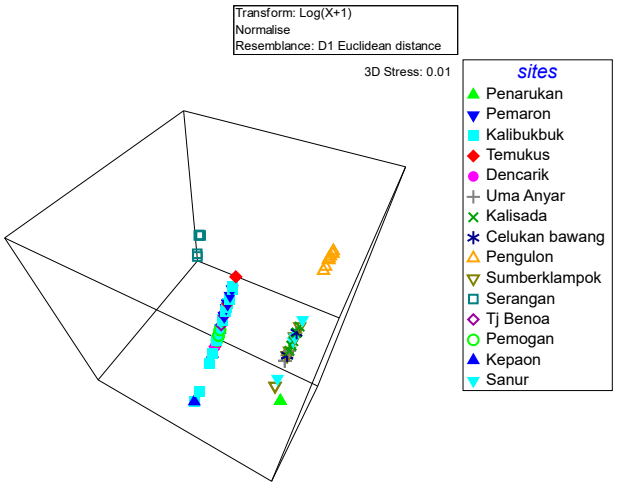

Figure 4. Nonmetric Multidimensional Scaling of sites based on temperature, relative humidity, and elevation

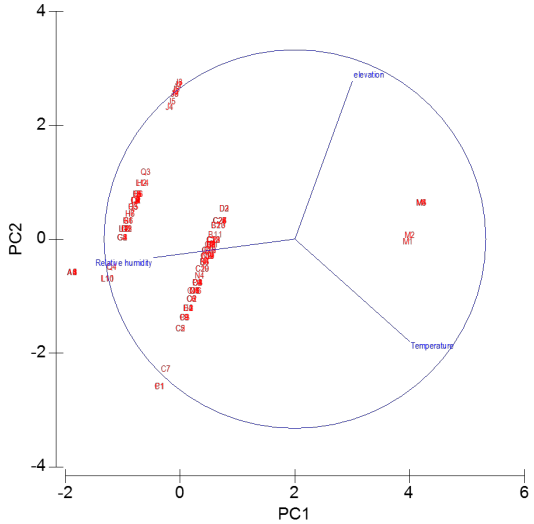

Figure 5. Principal component analysis (PCA) of the sites based on temperature, relative humidity, and elevation

Conservation of pongamia in Bali is urgently needed to conserve the threatened genetic material of the species. Both in-situ and ex-situ conservations should be done to rescue the existence pongamia trees. For the purpose of ex-situ conservation where plant species are research, propagate and planted inside a botanical garden, the research on its ecology (relationship with environmental factors in its native habitat) and also selection of plus tree species of pongamia is needed. Through this selection, its hoped that the acclimatization and propagation processes can be done effectively so that the final goal of ex-situ conservation (which is reintroduction or enrichment planting to its natural habitat) can be achieved.

From 126 pongamia trees counted and observed in Bali, 17 candidate trees were selected from 9 villages based on growth characters and oil content. Selection criteria of plus trees depend on the purpose of selection. Oil content is one of important criteria for selecting biodiesel crops (Kaushik et al. 2007; Kesari et al. 2008; Raut et al. 2010). In the present study range of oil content of candidate trees was 26-32%. The highest oil content of 32% was found on candidate trees from Kalibukbuk, Uma Anyar dan Kalisada. There were 8 pongamia plus trees selected out of 17 candidate trees from 4 villages in Bali, namely Kalibukbuk, Pengulon, Pemogan, and Sanur. Oil content of plus trees ranged from 27 to 32%. Oil content of the present study was lower than those CPTs selected from Haryana, India with 32-44% oil (Kaushik et al. 2007). Variability in oil content is observed among trees within the same location and between locations (Arpiwi et al. 2013). The existence of variability in character studied can be utilized for selection of elite trees especially CPTs with high oil content can be used for developing high oil-yielding line (Mukta et al. 2009).

Total height and diameter at breast height of the CPTs rank from 7.50-13.60 m and 20.70-63.69 cm respectively indicating differences in age of the trees (Simbolon et al. 2003). Selection of CPTs from natural pongamia populations in Bali is proven hard due to limited number of trees. The nature of scattering grow instead of colony grow was another limiting factor in choosing base population for selection of pongamia plus trees in Bali. Therefore, some villages with less than 6 pongamia trees were not included in selection of plus trees, for example, Temukus, Dencarik, Tangguwisia, Celukan Bawang, Banyu Poh, Tanjung Benoa and Kepaon (data not shown). □

The dendrogram of individual trees from different locations grouped them into 3 at the taxonomic distance of 12 based on growth parameters such as, total height; clear bole height, diameter on breast height, canopy width and oil content. Each group consisted of individuals from different locations in Bali indicating narrow morphological variations. Trees were very closely related or they might come from less and very closely related mother trees, however, this needs further study on molecular genetic diversity.

Correlation between growth parameters and oil content indicated that the most significant factor influencing oil content was clear bole height ($P = 0.002516$ and Spearman's Rho = 0.305) followed by total height ($P = 0.00025$ and Spearman's Rho = 0.267). In terms of pongamia, it is observed in our field sampling and laboratory analysis that the highest oil content was found in trees which are tall and have high clear bole height. Clear bole height is associated with total tree height. These two parameters (beside diameter) are indicator of the maturity of tree (Simon, 1996). Significant positive correlation between oil content and tree height are found in *Madhuca latifolia* Macb (Divakara 2014) and sunflower (Kaya et al. 2007). However, another study by Rao et al. (2011) do not find significant correlation between oil content and plant height in pongamia. The discrepancy between the present study and thus by Rao et.al. (2011) on the correlation between oil content and plant height probably due to the difference in the age of trees. Rao et al (2011) clearly restricts the age of trees in their study above 10 years old while in the present study the age of trees varied as indicated by variable tree height among samples. Local environmental parameters that we observed in our sites fall within the normal description as to where pongamia is usually found. *Pongamia pinnata* is found in coastal areas, often along beaches or rivers and in thickets close to sea level (Groom 2012). Orwa et al. (2009) wrote that pongamia has biophysical limit. It grows only at an altitude range of 0-1200 m asl with mean annual temperature of 1-16°C to 27-38°C and mean annual rainfall of 500-2,500 mm.

In conclusion, this research is the first to attempt at mapping the distribution of *Pongamia pinnata* in Bali. *Pongamia pinnata* was mostly grown in the north-west part of the Bali Island of Buleleng District. Lower number of trees was also found in the southern and western part of the island. Trees either scattered, grown in small groups and very rarely grown in big groups. The number of pongamia tree in Bali is small and this needs further action for conserving the species. Eight trees were selected as pongamia plus trees from 4 villages, namely Kalibukbuk and Pengulon (Buleleng District), Pemogan (Denpasar) and Sanur (Badung District).

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