Morphological characterization of *Calophyllum Inophyllum* as a biodiesel

E Yuniastuti\(^*\), I Anjani\(^2\), Nandariyah\(^3\) and M N I Delfianti\(^4\)

1 Department of Agrotechnology, Sebelas Maret University, Jl. Ir. Sutami 36 A Keningan, Surakarta, 57126, Indonesia  
2 Undergraduate Program in Agrotechnology, Graduate School, Sebelas Maret University, Jl. Ir. Sutami 36 A, Keningan, Surakarta, 57126, Indonesia  
3 Magister Program in Agronomy, Graduate School, Sebelas Maret University, Jl. Ir. Sutami 36 A, Keningan, Surakarta, 57126, Indonesia  
4 Corresponding author: yuniastutisibuea@staff.uns.ac.id

**Abstract.** Nyamplung (*Calophyllum inophyllum* Linn.) is one of the most potential forest plant species to be flourished. *C. inophyllum* seed can be deformed into oil as biofuel. Biofuel is very important to mitigate climate change impact as it reduces greenhouse gas emission from transportation sector. Mostly *C. inophyllum* grows along the coastline, especially in Ngombol and Grabag, Purworejo. There are a lot of *C. inophyllum* plant populations at these sites, but its varieties are not studied yet, therefore it is necessary to study the characterization of its morphology. This study aimed to obtain morphological character data and analyze *C. inophyllum* plants' diversity in Purworejo. Samples were selected intentionally (purposive sampling). Thus morphological data elaborated descriptively and the cluster was analyzed by NTSYS (Numerical Taxonomy and Multivariate Analysis System) program. The study showed the diversity and also the similarity of morphological characters. *C. inophyllum* fruit and seed's morphological character had its fruit length 2.1 – 3.1 cm, fruit diameter 1.93 – 1.95 cm, fruit weight 3.04 – 12.82 g, fruit shape is spherical/round, and fruit hardness 92.5 – 99.57 N. Moreover, it had seed length 1.16 – 2.22 cm, seed width 1.16 – 1.79 cm, seed weight 0.74 – 3.06 g, seed shape were spheroid, ellipsoid, reniform and the color was yellow. The morphological characters' variance was 26%, which was categorized as low and Grabag district had more potential for growing and production than Ngombol regency.

1. **Introduction**

Petroleum fuel is always needed, but these needs are very limited, so it is vital to have alternative energy as a fuel source such as biofuels. *C. inophyllum* is one of the plants that can be used as environmentally friendly biofuels. Biofuel is very important in mitigating climate change as it reduces greenhouse gas emissions from the transportation sector. *C. inophyllum* is a forest plant that grows along the coast throughout Indonesia. *C. inophyllum* can grow in coastal areas and the lowland forests of medium height. *C. inophyllum* is very potential to be developed. One of the benefits of being gained is *C. inophyllum* seed oil that can be used as a biofuel source with a higher economic value than kerosene and castor seed oil [1]. *C. inophyllum* seed is still underutilized and only wasted, whereas the oil content in the seed *C. inophyllum* between 40% - 73% [2].

Purworejo is one area that has a population of *C. inophyllum* of 5,000 hectares total area [3]. The research was conducted in Ngentak Village, Wero Village, and Pagak Village in Ngombol Sub-regency and Munggangsari Village and Kertojayan Village in Grabag Sub-regency, Purworejo Regency.
Selection of areas based on the region as the center of excellence *C. inophyllum* growth in Purworejo, located in coastal areas of the South Coast and in the region that was used as *C. inophyllum* fruit production centers for the biodiesel industry. *C. inophyllum* preservation has not been matched on the diversity and development of *C. inophyllum* cultivation optimally, especially in Purworejo. Purworejo is one area that has a large population of *C. inophyllum* so that it can be carried out an assessment of morphological characters and the diversity *C. inophyllum* for further development in the cultivation and utilization *C. inophyllum* as biofuel.

2. Methods
The study was conducted at Ngentak Village, Wero village, Pagak village in the Ngombol Sub-regency and in Munggangsari village and Kertojayan village in Grabag Sub-regency. Purworejo Regency. In this study, materials used were 20 *C. inophyllum* plants spread out in the Ngombol (NN) and Grabag (NG), Purworejo Regency.

The sample selection was intentional (purposive sampling). Sampling started from determining the location and amount of plant selected was 3-4 trees per location. The method used in this research was exploratory and descriptive methods. Data collection were in the form of field observations, interviews of respondents, documentation and literature study. Survey data observations rate were scored based on IBPGR (International Board for Plant Genetic Resources) modified descriptors’ guidelines correspond to *C. inophyllum* plant morphological characters.

Morphology data of each plant of *C. inophyllum* were rocessed using a similarity matrix method of Similarity for Qualitative Data (SimQual) with a similarity coefficient of SM (Simple matching). The further method used were Sequential and Nested Hierarchical Clustering (SHAN) with clustering analysis of plant samples using the Unweighted Pair Group Method Arithmetic Average (UPGMA). The program used was Numerical Taxonomy and Multivariate Analysis System (NTSYS) 2.02i version.

3. Result and discussion
3.1. General conditions of research
Geographically, Ngentak village, Wero village, Pagak village, in Ngombol Sub-regency, Purworejo located at coordinates of 7°51’67.6" S - 109°57’74.9" E at an altitude of 24 meters above sea level (masl) and within ± 1 km from the coast and Munggangsari and Kertojayan Village, Grabag Sub-regency, Purworejo is 7°49’84.2" S - 109°50’91.4" E at an altitude of 40 masl and within ± 500 m from the beach. These locations have a daily temperature of 28.9°C - 39.8°C and 56% - 74% of air humidity. Based on climate classification of Schmidt-Ferguson in Ngombol and Grabag Sub-district is currently on the climate type D which are located in areas of moderate or monsoon forest and soil types in these two areas, which Inceptisols. Indonesian Forestry Agency planted *C. inophyllum* tree in 1950 with the main objective is to put a halt to the wind speed and sea storms (windbreaker) that hit the shores. Population of *C. inophyllum* along the south coast regency of Purworejo area first recorded is 4.2 ha [4].

3.2. Tree morphology
*C. inophyllum* tree that was used as the sample aged 40-60 years. *C. inophyllum* tree ranged from 10.5 m – 16.34 m. More spacing is used, the higher and faster plants grow because each tries to find more sunlight [5]. The tree trunk's circumference was 65-182 cm. *C. inophyllum* trunk surface was varied between a bit rough, coarse and very coarse. *C. inophyllum* tree bark have gray and white exterior, shallow grooved and large-thin peeling [6]. Canopy diameter ranged from 2.72 to 11.5 m. Plants with a narrower canopy diameter has a higher plant height and vice versa. Canopy diameter is often greater than the tree height when the tree is in an open site to minimize the competition of sunlight absorbance [7]. *C. inophyllum* has pyramidal canopy shape, oblong shape, elliptical and spherical shape (Figure 1). Oblong and elliptical shape can produce higher fruit because can absorb higher light due to wide
area covering bottom to top, the wider of the canopy, the ability of plants capture sunlight getting high. Branching patterns are categorized as erect and semi-erect (angle less than 90°). The branching pattern is influenced by canopy shape, canopy diameter and plant height [8]. Fruit per tree in Ngombol regency was high but in Grabag regency was bear fruit frequently. Grabag regency closer to the beach what their content of salt (saline) was higher. Saline conditions can have positive effects for plants and this shows that despite having high salinity (sodium/Na element) but has a lot of cation content. Na element can replace K elements required by the plant. K is an element that can improve plant resistance to disease and drought [9].

3.3. Leaves morphology
C. inophyllum leaf length ranged from 7.96 to 12.27 cm. Leaf width ranged from 4.08 – 6.21 cm. The more large leaf, photosynthesis would be more optimal [10]. C. inophyllum leaves relatively thick, from 0.21 to 0.25 mm. The thick leaves can inhibit evaporation that the epidermis and cuticle also thicken. The highest leaves petiole length was 1.25–1.78 cm. Petiole width varied from 0.23 to 0.4 cm. C. inophyllum young leaf color is generally pale green, but there is a greenish-yellow and green. The whole old leaves of C. inophyllum is old dark green. Leaf age and physiological stages of a plant are factors that determine the chlorophyll content [11]. C. inophyllum leaf is oblong-shaped and the base leaf shape is acute and partly has rounded shape. The overall shape of the tip-shaped leaves is obtuse. The leaf edge is flat and the upper and lower leaf surface is glossy. The surface gloss of the petals or leaves is determined by the epidermis outermost layer (wax cuticle) and surface structure [12].

Figure 1. (a) Pyramidal canopy shape (b) Spherical canopy shape (c) Oblong canopy shape (d) Elliptical canopy shape
3.4. Flowers morphology

*C. inophyllum* plants in Purworejo can flower bloom during July - August and October - November. *C. inophyllum* flower is unlimited compound structure (inflorescemia racemosa) which blooms from the bottom to the top or from the edge to the center (centripetal) that shaped bunches with flower stalks grow from the stem tip [13]. *C. inophyllum* flower has a crown and petals each numbered four and both were white. *C. inophyllum* is a hermaphrodite flower, has numerous and yellow stamens and one, white pistils, bend-shaped and has shield-shaped anthers (Figure 3). The flower occurrence commonly derived from the end of the armpit, not the stalks and fragrant aroma. *C. inophyllum* flower has a high chance of self-pollination when the flower is enclosed and the distance between the pistils with stamens is close, but cross-pollination can also occur due to the wind and insects.

![Figure 3. Calophyllum inophyllum flower](image)

The cluster length of *C. inophyllum* was ranged (9.15 cm). The width of the flower cluster was also ranged from 2.95 to 7.5 cm. The width of flower cluster was determined by branches number that grew and the number of blooming flowers. The longer branches grown, wide flower cluster will be even greater. *C. inophyllum* plant has 5-15 flowers per cluster and (maximum) can reach 30 flowers per cluster [14]. The flower stalk's length ranged from 1.63 to 3.1 cm. The diameter of the highest flowers was 3.1 cm and the lowest flower diameter was 0.93 cm. Flowers can bloom optimally from young buds for 3 months and the flowers can last up to 2 weeks, but flowers do not bloom simultaneously.

3.5. Fruit morphology

*C. inophyllum* plants bear fruit twice a year around May and November and produced 100 kg of dried fruit/tree/year. These plants begin to bear fruit at the age of seven years and can last up to the age of 70 years [1]. Fruit can be harvested 3-4 months after the flowers bloom. Fruit length ranged from 2.5-3.5 cm [15]. Fruit diameter is 1.93 cm – 2.95 cm. Enlargement of fruit diameter from week 1 to week 8 was very high. Entering the 9th week, the increasing rate of fruit volume tends to be stable. This is
because the fruit undergoes cell division and cell enlargement during that period, then the growth rate decreased [16].

*C. inophyllum* fruit weight ranged from 3.04 to 12.82 g. Whole fruit shape is spherical/round (Figure 4). *C. inophyllum* fruit appearance was categorized as very poor, poor, intermediates and good. *C. inophyllum* old fruit had mature and large seeds with a very ugly appearance (very poor). Ripeness color varies dark green, light brown, dark brown and dark brown. Average fruit hardness ranged from 98.17 to 99.57 N. The drupe consists of a fleshy or thin exacerbated (such as skin) and a hard endocarp that wraps the seeds [17]. The fruit stalk length was 1.87–8.33 cm. The width of the stalk ranged from 0.1 to 0.23 cm. The color of the fruit stalk was yellow, green, brown, and black.

![Figure 4. Spherical/round shape of Calophyllum inophyllum fruit](image)

3.6. Seeds morphology

*C. inophyllum* seeds contain oil that is processed and used as biofuels (biodiesel). According to Dweck and Meadows [18] *C. inophyllum* seeds have a high oil content of 55% in the fresh seeds and 70.5% on dry beans. *C. inophyllum* seed productivity (20 ton/ha) is higher than jatropha (5 ton/ha), palm (6 tons/ha) and other vegetable crops [19].

The length of *C. inophyllum* seeds is 1.16 to 2.22 cm. *C. inophyllum* seed width is 1.16 to 1.79 cm. *C. inophyllum* seed can be obtained by cleaning the skin and husk of the seeds. In each 1 kg of seeds, there are about 100-200 seeds [20]. The highest seed weight was 3.06 g and lowest seed weight was 0.74 g. The weight and size of *C. inophyllum* fruit did not show a significant correlation with the seed size and weight. This is possible because the structure of *C. inophyllum* fruit consists of flesh, shell and seeds so that the variation among populations of *C. inophyllum* of these 3 sections would affect the weight and size of the fruit and seed *C. inophyllum*. The seed's size does not influence the rendement content of *C. inophyllum* because the sap's content in the seeds varies between the trees [6]. *C. inophyllum* seed forms diverse shapes such as a spheroid, ellipsoid and reniform (Figure 5) and the overall *C. inophyllum* seed color is yellow.

![Figure 5. (a) spheroid seed form (b) Ellipsoid seed form and (c) Reniform seed form](image)
3.7. Morphology characters

Morphological diversity does not necessarily reflect genetic diversity. The diversity of morphology occurs due to an interaction between genetic and environmental factors [21]. Environmental factors are believed to influence plants' morphological changes due to climate, temperature, soil type, soil conditions, altitude and humidity. If the environmental factors are stronger than genetic factors, then the plant in different places with different environmental conditions will have a varied morphology. However, when environmental factors are weaker than genetic factors, then there will be no variation in the morphology, although planted in different places [22].

![Figure 6. Dendrogram grouping of 20 plants based on morphological characters overall Calophyllum inophyllum with coefficient of SM](image)

Based on figure 6, the result of the dendrogram grouping of 20 C. inophyllum plants based morphological characters showed similarity which the highest morphological characters coefficient was 0.84 or 84% (degree of dissimilarity coefficient 16%). In contrast, the lowest similarity coefficient value was 0.74 or 74% (dissimilarity coefficient level 26%). According Cahyarini et al [23] and Damayati [24] 75% similarity distance is the distance of close resemblance and similarity distance. It is great if it is less than 60%. At the level of 0.76 or 76% similarity (with a 24% degree of dissimilarity), they grouped into 5 groups of A, B, C, D and E.

4. Conclusion

Diversity of plant morphology C. inophyllum is as low as 74%. It means that C. inophyllum potential economic to developed as biofuels. So, C. inophyllum is very important to mitigate the climate change impact as it reduces greenhouse gas emission from the transportation sector.

Acknowledgment

Authors are grateful to Ministry of Research and Technology for financial support.

References

[1] Yuniarti R 2014 Pengaruh minyak biji nyamplung pada bio-fux oil sebagai modifier asbuton butiran terhadap kinerja asbuton campuran panas J Teoretis dan Terapan Bidang Rekayasa Sipil 21 251-58.
[2] Elbert L, Little J R and Roger G 2003 Nyamplung (Calophyllum inophyllum). Agriculture Handbook, Tropical Agriculture and Human Resources, University of Hawaii, Manoa.
[3] Department of Forestry (MoF) 2008 Plant nyamplung as biofuel energy source. http://www. Indonesia.go.id.
[4] Mardiastuti A and Tonny S 2015 Development of forestry in Java, Madura, Bali, Nusa
Zea mays thiyah S, Desrial on and -

Calopyllum inophyllum

Tenggara: notes and learning 2007-2013 (Bogor (ID): Foundations Nata Samastha).

[5] Bilman W S 2001 Analysis of the growth of plants sweet corn (Zea mays), a shift in the composition of the weed in some plant spacing A Indonesian Agricultural Sciences 3 25-3

[6] Leksono B and Putri K P 2013 Variations in the size of the fruit-seed and physico-chemical properties of oil nyamplung (Calopyllum inophyllum ) From the six populations in Java.Proceedings of the National Seminar on Role of Research & Development of NTFP in Supporting Development of Forestry FORDA. Jakarta. 36: 321-334.

[7] Prabakaran K, and Britto S J 2012 Biology, agroforestry and medicinal value of Calophyllum inophyllum L. (Clusiaceae): a review International Journal of Natural Products Research 1 24-33.

[8] Maiti R, Humberto G, Rodriguez et al 2015 Branching pattern and leaf crown architecture of some tree and shrubs in northeast mexico. International Journal of Bio-resource and Stress Management 6 41-50.

[9] Hani A 2011 Influence of seawater against seedling watering nyamplung (Calophyllum inophyllum) J. Plant Forest Tekno 4 2. 79-84.

[10] Pertamawati 2010 The influence of photosynthesis on the growth of potato (Solanum tuberosum L.) in vitro photoautotrophs environment J. Science and Teknol Indo 12 1 31-37

[11] Biber PD 2007 Evaluating a chlorophyll content meter on three coastal wetland plant species J Agri Food and Environ Sci 1 1-11.

[12] Whitney H M, Sean A R, Nick J E and Allan G E 2012 A technique for measuring petal gloss with examples from the namaqualand flora. URL: http://dx.doi.org/10.1371/journal.pone.0029476.

[13] Estate Crops Research and Development Center (Puslitbangbun0 2009 Research and development of industrial plants controversy over the use oilcake distance (Ricinus communis) at the base of the stem rot disease and jaundice pepper News Industrial Crops Research and Development 15 1-32.

[14] Orwa C, Mutua A, Kindt R, Jamnadass R and Anthony S 2009 Agroforestrree database: a tree reference and selection guide version 4.0. Kenya (KE): World Agroforestry Centre.

[15] Putra F S K, Findra A F and Setiyo G 2012 Characterization and potential nyamplung oil (Calophyllum inophyllum L.) as a raw material for making biodiesel J Mechanical Pomits 1 1-5.

[16] Romadlon Z 2010 Studies flowering, fruit development and seed germination nyamplung (Calophyllum inophyllum L.), a biodiesel plants. Essay. Bogor (ID): Department of Biology, IPB.

[17] Hasnah TM 2014 Effect of seed scarification to germination and seedling growth of Nyamplung (Calophyllum inophyllum L.) Wana Benih 15 10-20.

[18] Dweck A C and Meadows T 2002 Tamanu (Calophyllum inophyllum) the African, Asian, Polynesian and Pacific Panaceae J Int. of Cosmetic Sci 24 1-8.

[19] Kartika I A, Fathiyah S, Desrial and Purwanto Y E S 2010 Nyamplung oil purification and application as a biofuel J Industries Agriculture 20 122-129.

[20] Friday J B, Okano and Dana 2006 Calophyllum inophyllum (Nyamplung). College of Tropical Agriculture and Human Resources.University of Hawai’i. Hawai’i. URL http://www.traditionaltree. org.

[21] Aharizad S, Rahimi M H and Moghadam M 2012 Study of genetic diversity in lemon balm (Melissa officinalis L.) Populations based on morphological traits and essential oils content Annals of Biological Research 3 5748-53.

[22] Suranto 2001 Isozyme studies on the morphological variation of Ranunculus nanus Populations Agrivita 23 139-46.

Subekti K and Firda A 2011 External genetic characteristics of chicken in the districtriver ceiling solok southern districts Scientific Journal of Animal Husbandry Sciences 14 2 74-87.

[23] Cahyarini R D, Yunus A and Purwanto E 2004 Identification of the genetic diversity of some
local soybean varieties in Java by isozyme analysis *Agrpsains* 6 79-83.

[24] Damayanti A D 2015 Studies of shallow ground water salinity in coastal areas north of the city of Makassar *Thesis* Universitas Hasanuddin (ID): Makassar.