BIODIVERSITAS
Volume 21, Number 7, July 2020
Pages: 2923-2934

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210708

Phenotypic diversity and plasticity index of *Eurycoma apiculata* populations in Eastern Sumatra, Indonesia based on leaves morphology

ZULFAHMI[1,4,♥], EDI PURWANTO[2], PARJANTO[2], AHMAD YUNUS[2,3]

[1]Doctoral Program of Agriculture Science, Graduate School, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta, 57126, Central Java, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta, 57126, Central Java, Indonesia
[3]Research Center for Biotechnology and Biodiversity, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta, 57126, Central Java, Indonesia
[4]Department of Agrotechnology, Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau. Jl. H.R. Subrantas Km 15 No. 155, Pekanbaru 28293, Riau, Indonesia. Tel.: +62-761-562051; Fax.: +62-761-562051, ♥email: zulfahmi@uin-suska.ac.id

Manuscript received: 18 May 2020. Revision accepted: 7 June 2020.

Abstract. *Zulfahmi, Purwanto E, Parjanto, Yunus A. 2020. Phenotypic diversity and plasticity index of* Eurycoma apiculata *populations in Eastern Sumatra, Indonesia based on leaves morphology. Biodiversitas 21: 2923-2934. Eurycoma apiculata* A.W. Benn. is a protected species in Indonesia, but diversity information of this species is limited. The objective of this study was to assess the phenotypic diversity, phenotypic plasticity index, and phenotypic differentiation among populations of *E. apiculata* in Eastern Sumatra, Indonesia based on leaves morphology. A total of 45 traits were measured on leaves from six populations studied. The result of this found that the phenotypic variation coefficient (CV) of the characters was ranged from 7.41% to 36.97%, revealed the abundant phenotypic variation in the species. The phenotypic CV values of the population varied from 13.95% to 24.10%. The CV values of all populations from the mainland Sumatra (17.75%) were lower than that from the Riau archipelago (23.61%), which revealed that phenotypic traits in mainland Sumatra were more stable compared to populations in the Riau archipelago. The population phenotypic plasticity index value of populations ranged from 0.41 to 0.51, and it was classified as a moderate level. The phenotypic differentiation coefficient among populations in this study was relatively low (V_{ST} = 21.06%), indicating a lower phenotypic variation among populations than within populations. The scatter plot of principal component analysis and UPGMA dendrogram divided the six populations studies into two groups. The findings of this study recommend that the *in-situ* conservation method is an effective protection strategy for *E. apiculata* while *ex-situ* conservation method can be implemented as a supplementary method.

Keywords: Differentiation, *Eurycoma apiculata*, phenotypic, variation

INTRODUCTION

Eurycoma apiculata A.W. Benn. is a member genus *Eurycoma* in the Simaroubaceae family. This species is identified as Pasak bumi daun runcing in Indonesia. *E. apiculata* is distributed in Sumatra and Malaysian Peninsular (Nooteboom 1962; Padua et al. 1999; Nordin 2014; Lee et al. 2015). Recently *E. apiculata* has found in the Lingga island, Riau Archipelago Province during the field exploration in 2019. *E. apiculata* is small tree or shrub that grows reaching 5 m in height. *E. apiculata* is well grown on acid and sandy soil in primary and secondary of tropical forest (Nooteboom 1962; Padua et al. 1999; Zulfahmi et al. 2019). *E. apiculata* is economically an important species as a source of herbal medicine. Traditionally, the extract of the root of this species is used to as drink to febrifuge, diarrhea, tonic, and to reduce the boneaches. The bark is externally used to recover wounds and ulcers, and to reduce pain head, meanwhile, a decoction of the leaves is used to reduce the itchiness of the skin (Nooteboom 1962; Padua et al. 1999; Zulfahmi et al. 2019).

The land and forest degradation in Indonesia has been continued every year due to forest fire, and conversion to other uses for housing, agricultural development, and mining, which in recent years has totaled about 439.439 ha/year (Ministry of Environment and Forestry 2019). These activities greatly contributed to species rarity in the wild population, a decline of population size, habitat fragmentation, lack of natural regeneration and reduction of genetic diversity of the species (Naito et al. 2005; Naito et al. 2008; Leonardi et al. 2012; Matesanz et al. 2017; Semizer-Cuming et al. 2017). *E. apiculata* is a rare species and difficult to find in natural habitat so that the Ministry of Environmental and Forestry has established *E. apiculata* as a protected species in Indonesia based on regulation No: P.20/MENLHK/SETJEN/KUM.1/6/2018. Therefore, both in-situ and ex-situ conservations of this species were urgent to be implemented, and one of the critical issues for the conservation of genetic resources is to obtain a better knowledge of its genetic variation. Eastern Sumatra region is one of the *E. apiculata* distribution areas of reported in Sumatra (Zulfahmi et al. 2018, 2019) so that this region may be a consideration to be a target for the conservation area of this species in future.

The diversity of *E. apiculata* in Indonesia and Malaysia, either using morphological, biochemical, and genetic markers have not been reported. As a preliminary study, we will assess the phenotypic diversity of *E. apiculata* in a larger geographical scale based on the leaflet morphometric analysis. However, the main disadvantage in using solely a morphometric approach is the difficulty to distinguish observed variation in natural populations

whether originate from genetic diversity or phenotypic plasticity, but this method is still frequently used researchers to estimate the morphological variability of plant species (Kajba et al. 2015; Poljak et al. 2015; Yang et al. 2015; Stojnic et al. 2016; Romeo et al. 2016; Yang et al. 2016; Han et al. 2017; Sheng et al. 2017; Bijarpasi et al. 2019). The morphological traits of leaves in tree species can provide rich information about the evolution of genetics and phenotypic diversity. Phenotypic diversity in plants is the basic feature of life system, it is needed by the population to evolve in response to environmental changes, and its maintenance is very important for long time species survival (Han et al. 2017).

Plants can not move to other places when exposed to the heterogeneity environmental conditions including climate change and land-use change. The ability of the plant to adapt to environmental changes depends on the population genetic characteristics and phenotypic plasticity. Phenotypic plasticity is the capacity of certain genotypes to express different phenotypes in response to different environmental conditions (Alfaro et al. 2014). However, knowledge of phenotypic plasticity of a species or population is often overlooked. Therefore, a deep understanding of the phenotypic plastic response of a species or population is needed to estimate its full potential to adapt and/or evolve to changing environmental conditions. The plastic phenotypes possible to preserve the adaptive potential that might be important for survival in heterogeneous environments. Phenotypic plasticity of *E. apiculata* is important to know because the distribution of *E. apiculata* has rapidly changed due to degradation and fragmentation habitat by human activities so that it can help us to estimate the potential adaptation of this species to rapid environmental changes. Some researchers have been used morphological traits to measure of the plasticity of species, for example, *Solidago canadensis* L. (Du et al. 2017), *Stipa grandis* P.A. Smirn. (Gao et al. 2018), and *Physospermum cornubiense* (L.) DC. (Gentili et al. 2018). The objective of this study was to assess the phenotypic diversity, phenotypic plasticity index, and phenotypic differentiation among populations of *E. apiculata* in Eastern Sumatra, Indonesia based on leaves morphology. The results of this are expected to be useful as a scientific basis for formulating protection and conservation strategies of *E. apiculata* in their natural habitat.

MATERIALS AND METHODS

Samples collection

Six natural populations of *E. apiculata* were sampled in Eastern Sumatra, four populations in Riau Province, and two populations in Riau Archipelago Province (Figure 1). The geographic position and climatic data each population was exhibited in Table 1. For climatic data used secondary data. Each population was represented by 15 plants, the sampled individuals in each population were randomly chosen adult trees in which distance among trees was separated at least 20 m from each other to prevent sampling adult trees from the same parent. From each tree is chosen three fully developed leaves, healthy and undamaged leaves. After sampling, the leaves sampled were herbarised and sent to the laboratory. From each sample of the compound leaf of *E. apiculata*, three leaflets (terminal, lateral and basal leaflets) were taken to be measured, as shown in Figure 2.

Measurement of morphological traits

A total of 45 traits were measured on the leaves as shown in Figure 2 and Table 2 following Gonzalez-Rodrigues and Oyama (2005), Brus et al (2011), Jarni et al (2011), and Poljak et al (2015) methods. Leaflet measurements were conducted using a digital caliper with 0.01 mm accuracy of measurements.

Data analysis

The dispersion degree of phenotypic traits was obtained based on mean coefficient of variation (CV) analysis using the formula Yang et al. (2015):

$$CV = \frac{SD}{X} \times 100$$

Where; SD is standard deviation of character and X is the average value of character.

Table 1. Research sites characteristic of *Eurycoma apiculata* populations in Eastern Sumatra, Indonesia

Population	Population abbreviation	Status of research sites	Longitude	Latitude	Altitude (m asl.)	Temp. mean annually (ºC)	Precipitation mean annually (mm/year)
Pokomo-Kampar District, Riau Province	AP-PK	Protected forest	100°57'9" E	0°15'7" N	155	27.50[a]	225.17[a]
TAHURA, Siak District, Riau Province	AP-TH	Forest Park	101°25'46" E	0°40'21" N	59	27.50 [a]	208.60[a]
Rumbio, Kampar District, Riau Province	AP-RB	Protected forest	101°8'20" E	0°19'40" N	66	27.50[a]	225.17[a]
Lingga-1, Lingga District Riau Archipelago Province	AP-LG1	Natural forest	104°40'26" E	0°10'41"S	47	27.20[b]	236.50[b]
Lingga-2, Lingga District Riau Archipelago Province	AP-LG2	Protected forest	104°34'52" E	0°12'42"S	87	27.20[b]	236.50[b]
Sentajo, Kuantan Singingi District, Riau Province	AP-ST	Protected forest	101°34'2" E	0°31'37"S	106	27.50[a]	279.66[a]

Sources: [a]BPS (2019a); [b]BPS (2019b).

Figure 1. Location of *Eurycoma apiculata* sampling in Eastern Sumatra, Indonesia

Phenotypic Plasticity index (PPI) within population was accounted by population for each variable using followed formula Bruschi et al. (2003):

$$PPI = 1 - \left(\frac{x}{X}\right)$$

Where; x is the smallest mean value and X is the greatest mean value for any given leaflet character.

Partitioning of the total variance at among populations and within-population was determined with a nested analysis of variance using the following model:

$$Y_{ijk} = \mu + P_i + T(P)_{ij} + \varepsilon_{ijk}$$

Where; Y_{ijk} is the *k* replication observed in the *j* single plant of the *i* population, μ is the total mean value of samples, P_i is the population effect, $T(P)_{ij}$ is the nested effects of the single plant within population, and ε_{ijk} is the error (Yang et al. 2015; Bijarpasi et al. 2019). The populations were set as fixed factors, while a genotype was considered as random factor (Batos et al. 2010). The phenotypic differentiation coefficients (V_{ST}) among population were calculated using the following formula Zhang et al. (2015):

$$V_{ST} = \frac{\sigma^2_{t/s}}{\sigma^2_{t/s} + \sigma^2_s}$$

Where; $\sigma^2_{t/s}$ is the variance component value among populations and σ^2_s is the variance component value within population. Principle component analysis (PCA) was used to identify the major sources variation to the separation of the population. A principal component (PC) that is considered was eigenvalues greater than one. These analyses were performed using the SAS software (SAS Institute 2020). The morphological similarity coefficients according to Elucidean methods were calculated using the SIMQUAL program of numerical taxonomy multivariate analysis system (NTSYS) ver.2.01 (Rohlf 1998) and then dendrogram was constructed with a sequential agglomerative hierarchical nesting (SHAN) clustering program using unweighted pair group method with arithmetic mean (UPGMA).

Table 2. List of leaflet morphological traits examined

Traits (unit)	Descriptions
LLB (mm)	Basal leaflet length
LLL (mm)	Lateral leaflet length
LLT (mm)	Terminal leaflet length
LW1B (mm)	Lamina width from middle vein to the right edge, on 10% of the leaflet length from base to up of the basal leaflet.
LW1L (mm)	Lamina width from middle vein to the right edge, on 10% of the leaflet length from base to up of the lateral leaflet
LW1T (mm)	Lamina width from middle vein to the right edge, on 10% of the leaflet length from base to up of the terminal leaflet
LW2B (mm)	Lamina width from middle vein to the left edge, on 10% of the leaflet length from base to up of the basal leaflet
LW2L (mm)	Lamina width from middle vein to the left edge, on 10% of the leaflet length from base to up of the lateral leaflet
LW2T (mm)	Lamina width from middle vein to the left edge, on 10% of the leaflet length from base to up of the terminal leaflet
LW3B (mm)	Lamina width from middle vein to the right edge, on 50% of the leaflet length from base to up of the basal leaflet
LW3L (mm)	Lamina width from middle vein to the right edge, on 50% of the leaflet length from base to up of the lateral leaflet
LW3T (mm)	Lamina width from middle vein to the right edge, on 50% of the leaflet length from base to up of the terminal leaflet
LW4B (mm)	Lamina width from middle vein to the left edge, on 50% of the leaflet length from base to up of the basal leaflet
LW4L (mm)	Lamina width from middle vein to the left edge, on 50% of the leaflet length from base to up of the lateral leaflet
LW4T (mm)	Lamina width from middle vein to the left edge, on 50% of the leaflet length from base to up of the terminal leaflet
LW5B (mm)	Lamina width from middle vein to the right edge, on 90% of the leaflet length from base to up of the basal leaflet.
LW5L (mm)	Lamina width from middle vein to the right edge, on 90% of the leaflet length from base to up of the lateral leaflet
LW5T (mm)	Lamina width from middle vein to the right edge, on 90% of the leaflet length from base to up of the terminal leaflet
LW6B (mm)	Lamina width from middle vein to the left edge, on 90% of the leaflet length from base to up of the basal leaflet
LW6L (mm)	Lamina width from middle vein to the left edge, on 90% of the leaflet length from base to up of the lateral leaflet
LW6T (mm)	Lamina width from middle vein to the left edge, on 90% of the leaflet length from base to up of the terminal leaflet
LW10B (mm)	Lamina width at 10% of the leaflet length from base to up of the basal leaflet (LW10= LW1B+LW2B)
LW10L (mm)	Lamina width at 10% of leaflet length from the base to up of the lateral leaflet (LW10= LW1B+LW2B)
LW10T (mm)	Lamina width at 10% of leaflet length from the base to up of the terminal leaflet (LW10= LW1B+LW2B)
LW50B (mm)	Lamina width at 50% of leaflet length from the base to up of the basal leaflet (LW50B= LW3B+LW4B)
LW50L (mm)	Lamina width at 50% of leaflet length from the base to up of the lateral leaflet (LW50B= LW3B+LW4B)
LW50T (mm)	Lamina width at 50% of leaflet length from the base to up of the terminal leaflet (LW50B= LW3B+LW4B)
LW90B (mm)	Lamina width at 90% of leaflet length from the base to up of the basal leaflet (LW50B= LW5B+LW6B)
LW90L (mm)	Lamina width at 90% of leaflet length from the base to up of the lateral leaflet (LW50B= LW5B+LW6B)
LW90T (mm)	Lamina width at 90% of leaflet length from the base to up of the terminal leaflet (LW50B= LW5B+LW6B)
LMWB (mm)	Lamina maximum width of the basal leaflet
LMWL (mm)	Lamina maximum width of the lateral leaflet
LMWT (mm)	Lamina maximum width of the terminal leaflet
DMWB (mm)	Distance from the base to the point where LMWB was measured
DMWL (mm)	Distance from the base to the point where LMWL was measured
DMWT (mm)	Distance from the base to the point where LMWT was measured
LWMB/LLB	Ratio of lamina maximum width of the basal leaflet/basal length leaflet
LWML/LLL	Ratio of lamina maximum width of the lateral leaflet/lateral length leaflet
LWMT/LLT	Ratio of lamina maximum width of the terminal leaflet/terminal length leaflet
DMWB/LLB	Ratio of distance from the base to the point maximum width of the basal leaflet/basal length leaflet
DMWL/LLL	Ratio of distance from the base to the point maximum width of the lateral leaflet/lateral length leaflet
DMWT/LLT	Ratio of distance from lamina base to the point maximum width of the terminal leaflet/terminal length leaflet
LLB/LW50B	Ratio of leaflet length to lamina width at 50% of the basal leaflet
LLL/LW50L	Ratio of leaflet length to lamina width at 50% of the lateral leaflet
LLT/LW50T	Ratio of leaflet length to lamina width at 50% of the terminal leaflet

RESULTS AND DISCUSSION

Phenotypic diversity among traits and populations

The coefficient of variation (CV) of the phenotypic characters delineates the dispersion level of characters. The lower CV, the smaller the dispersion level of the characters and vise versa. The CV value is often considered as the main indicator of variability (Zhang et al. 2015; Stojnic et al. 2016). The CV values of all traits and whole populations were exhibited in Table 3. The CV mean value of all traits measured in *E. apiculata* was ranged from 7.41% to 36.91%, and the mean value was 19.71%. The lowest CV value was obtained in the DMWL/LLL trait and the highest CV value was observed in the LW6T trait, showing that the stability of DMWL/LLL trait was the highest while the LW6T was the lowest. Most of the characters measured in this study showed CV values <20%, indicating that these characters are less diverse than others due to a low environmental effect. This is in line with those reported by Liggarreto et al. (2011) in *Vaccinium meridionale* Swartz and Yang et al. (2015) in *Magnolia sprengeri* Pamp. Hounkpevi et al. (2016) also found most of the CV values of leaf morphology traits of the *Vitex doniana* Sw. species below 15%.

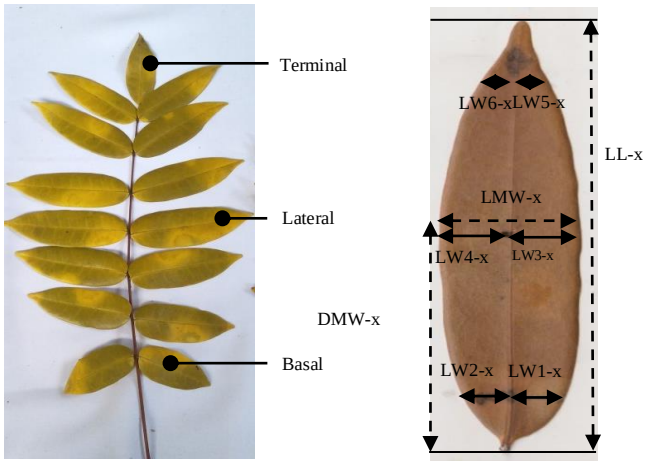

Figure 2. Illustration of the measured leaflet characters. Sign x replace the leaflet position (basal, lateral and terminal)

The CV mean of all traits in the lateral leaflet was 17.79% and that was lower than the CV mean of all traits in the basal (18.82%) and terminal leaflets (21.45%). This revealed that all traits studied in the lateral leaflet were less diverse (stable) compared to basal and terminal leaflets. Our results are similar to those reported by Brus et al. (2011) and Poljak et al. (2015) in *Sorbus domestica* L. For the efficiency of leaflet morphological measurements on compound leaves, we recommend using only lateral leaflet samples.

The CV values of the six populations studied varied from 13.95% to 24.10% and the mean value of all populations was 19.71%. The highest CV value was observed in the Lingga-2 population, followed by the Lingga-1 population, whereas the lowest CV value was observed in the Sentajo population. Sheng et al. (2017) explained that the CV value can indirectly delineate the richness of populations phenotypic diversity, in which a large CV indicated that the population trait variation range is high and the phenotypic diversity is rich, conversely, if the variation range is small then the phenotypic diversity is poor. Therefore, two populations (Lingga-2 and Lingga-1) have a relatively abundant diversity of phenotypes compared to the others. The geographical position of the two populations may be the reason, where both populations located in the middle of the sea, far from the mainland Sumatra with different environmental conditions and then provide superior requirements for higher phenotypic diversity. The same result was reported by Stenøien et al (2014) in *Sphagnum palustre* L. in which the diversity of phenotypes in the island population is higher compared to the mainland population due to higher environmental variability in the island than the mainland populations as well as genetic differences between both two group populations. The CV values of all populations from the mainland Sumatra were lower than that from the Riau archipelago, indicating that phenotypic traits in mainland Sumatra were more stable compared to populations in the Riau archipelago. The phenotypic diversity of *E. apiculata* in this study (19.71%) was higher than those reported by Yang et al. (2015) in the *Magnolia sprengeri* Pamp (15.55%), Yang et al. (2016) in *Rosa platyacantha* Schrenk (16.51%), Sheng et al. (2017) in *Crataegus songorica* K. Koch. (15.89%), and Goba et al. (2019) in the *Pterocarpus erinaceus* Poir (15.52%), but it was lower than the study reported by Kajba et al. (2015) in the *Populus nigra* L. (20.08%) and Poljak et al. (2015) in the *Sorbus domestica* L. (22.76%). These result differences are estimated due to variation in ecological and geographical conditions in the collecting sites of species as well as the difference in the genetic structure of the population each species.

Phenotypic plasticity index

Phenotypic plasticity is defined as the ability of the particular genotypes to express different phenotypic under different environmental conditions (Alfaro et al. 2014) and is considered as a major mechanism for plants to adapt to new environments (Pichancourt and van Klinken 2012). The phenotypic plasticity index (PPI) values for each character and each population are shown in Table 4. According to Valladares et al. (2006), the phenotypic plasticity index value ranged from 0 to 1, where 0 value indicates non-plastic and 1 value indicates very plastic. By using the heritability value classification approach according to Singh (2001), in this study, we classified the phenotypic index into four classes, i) PPI value above 0.80 is considered as very high plastic, PPI value between 0.60 to 0.79 is high plastic, PPI value between 0.40 to 0.59 is moderate plastic, and PPI value less than 0.40 is low plastic. The PPI average value of all characters was 0.47 and ranged from 0.23 to 0.69. The PPI average value of all characters in the lateral leaflet was (0.45) lower than the basal leaflets (0.47) and terminal leaflets (0.50), this confirms the results of CV value, and evident that lateral leaflet is more stable than both basal and terminal leaflets. In general, we see that the low value of the CV character, the character plasticity index value tends to also low and vice versa.

The average value of leaflet shape plasticity index (LWMB/LLB, DMWB/LLB, LLB/LW50B, LWML/LLL, DMWL/LLL, LLL/LW50L, LWMT/LLT, DMWT/LLT, LLT/LW50T) was at low plastic level (PPI < 0.40) and lower than other characters (Table 4), indicating that leaflet shape is less plastic to environmental factors. Out of the characters of leaflet shape, the lowest PPI value was observed in DMWL/LLL trait (0.23). Some researchers reported that in many cases leaf shape does not segregate as a single Mendelian trait, with F1 hybrid having a shape intermediate between two parents (Talukdar and Talukdar 2003; Atanasova and Mihov 2006; Toker et al. 2012; Nwofia and Emeka 2014). Only a few loci control variation of leaf shape between species or population. Some of the genes that control leaf shape determination are *PIN1*, *KNOX1*, *HD-ZIPIII*, *KANADI*, *YABBY*, *AGUSTIFOLIA3*, *WOX*, *TCP*, *LEAFY*, *MIR164A*, *CUC*, *DPA4*, and *APUM23* (Dhkar and Pareek 2014). Furthermore, Dhkar and Pareek (2014) affirmed that besides genetic control, environmental factors (mainly temperature and light) have significant contributions in the final adjustment of the shape of the leaf. Martinez et al. (2016) reported that plastic properties of leaf shape are caused by variation in lamina growth

along the axis of the leaf. Development of lamina linked to the distribution of plant growth regulator to initiating leaf primordia.

At the population level, PPI value ranged from 0.41 to 0.51, and the mean value was 0.47. The lowest PPI value is observed in the Rumbio population (0.41), followed by the Sentajo population (0.43), while the highest PPI value is observed in the Lingga-1 population (0.51). The level of plasticity of all populations is commonly classified as moderate plastic. The high PPI value in the Lingga-1 population may be caused by more variable environmental conditions compared to other populations. According to Kreyling et al. (2019), phenotypic plasticity is not related to neutral genetic diversity, but it is closely related to environmental conditions, mainly the climate of the population origin. The population that has a low PPI value tends to be phenotypically uniform if their genetic material is exposed to fluctuating environmental conditions. The selection of population and genotypes that demonstrate a good level of phenotypic plasticity can be appropriate management of action against climate change anticipation. The PPI value of *E. apiculata* in this study is higher than the results of the study of Vieira et al. (2014) in *Allophylus dullis* (St.-Hil.) Radlk, *Casearia sylvestris* Sw., *Cupania vernalis* Cambess, and *Luehea divaricata* Mart.

Table 3. The coefficient of variation (CV) of phenotypic traits of *Eurycoma apiculata*

Traits	Populations						Mean
	AP-PK	AP-LG1	AP-LG2	AP-RB	AP-TH	AP-ST	
LLB	12.60	19.32	20.70	12.72	12.87	25.58	17.30
LW10B	24.19	22.94	30.51	15.03	19.09	22.86	22.44
LW1B	22.26	22.47	35.15	17.00	19.32	21.13	22.89
LW2B	28.37	24.47	27.15	14.37	19.89	25.14	23.23
LW50B	16.00	20.21	20.21	11.26	12.40	22.23	17.05
LW3B	13.99	24.82	20.39	10.16	16.46	22.75	18.10
LW4B	18.59	18.17	21.22	13.46	11.29	22.75	17.59
LW90B	23.59	34.33	18.78	22.41	29.82	28.44	26.23
LW5B	23.42	33.23	22.11	19.72	30.45	26.72	25.94
LW6B	25.42	36.28	17.46	26.65	29.70	30.94	27.74
MWLB	12.58	20.21	22.84	11.04	13.19	22.12	17.00
DMWB	10.00	18.10	19.96	12.72	14.22	26.7	16.95
LWMB/LLB	14.84	7.71	11.83	13.03	11.65	13.23	12.05
DMWB/LLB	8.89	16.23	5.68	2.72	5.98	7.58	7.85
LLB/LW50B	13.26	8.41	9.55	12.02	12.73	12.33	11.38
LLL	14.12	18.69	18.58	11.22	13.07	13.44	14.85
LW10L	26.63	25.4	23.29	20.63	22.37	14.33	22.11
LW1L	25.64	29.13	27.05	16.37	22.23	14.28	22.45
LW2L	29.18	23.42	24.83	25.28	25.18	17.24	24.19
LW50L	22.38	21.64	16.50	10.27	11.39	13.19	15.90
LW3L	20.08	25.11	16.71	9.80	13.75	14.22	16.61
LW4L	25.08	19.63	18.34	11.26	11.39	14.22	16.65
LW90L	28.56	27.93	18.84	27.44	33.99	13.22	25.00
LW5L	26.17	25.67	21.60	25.20	35.32	13.98	24.66
LW6L	32.39	30.71	18.16	31.15	34.26	14.49	26.86
MWLL	13.37	21.64	18.81	10.22	10.78	12.95	14.63
DMWL	9.00	24.15	19.14	12.22	13.05	16.33	15.65
LWML/LLL	8.46	9.09	7.88	8.96	11.64	9.57	9.27
DMWL/LLL	9.66	12.05	7.01	3.78	6.51	5.42	7.41
LLL/LW50L	13.96	9.70	9.73	9.27	11.35	9.89	10.65
LLT	13.04	19.57	19.52	15.02	10.46	12.55	15.03
LW10T	32.92	27.93	33.56	28.08	20.45	13.32	26.04
LW1T	32.08	26.83	33.97	26.65	21.47	14.86	25.98
LW2T	35.49	30.95	35.11	30.80	21.77	16.52	28.44
LW50T	20.20	22.04	23.21	14.49	16.87	13.92	18.46
LW3T	19.10	27.60	23.80	15.98	18.36	13.67	19.75
LW4T	21.56	18.46	23.60	13.67	16.49	18.18	18.66
LW90T	28.89	38.25	61.20	19.20	38.03	20.63	34.37
LW5T	28.30	38.14	60.93	17.69	35.61	19.12	33.30
LW6T	31.11	39.16	62.05	21.98	42.24	24.92	36.91
MWLT	14.86	22.04	22.87	14.31	17.29	13.67	17.51
DMWT	13.77	21.03	16.54	14.52	11.12	14.77	15.29
LWMT/LLT	8.67	11.27	11.78	9.81	17.57	9.83	11.49
DMWT/LLT	10.26	11.94	9.81	5.71	7.10	4.88	8.28
LLT/LW50T	12.10	12.35	12.14	9.93	16.61	10.64	12.30
Population mean	20.94	23.12	24.10	16.40	19.72	13.95	19.71

Phenotypic differentiation among populations

The phenotypic differentiation coefficients (V_{ST}) among population in *E. apiculata* was shown in Table 4. The mean value of phenotypic differentiation coefficient among populations in the lateral leaflet was 33.83%, higher than that of the basal leaflets (14.23) and terminal leaflets (15.13%), showed that differentiation level of the lateral leaflet traits was higher than differentiation level of the terminal and basal leaflets. The mean value of phenotypic differentiation coefficient (V_{ST}) among populations for 45 traits of *E. apiculata* was 21.06%, indicating that mean of phenotypic variation among populations was lower than variation within population (78.94%).

Table 4. Mean values of phenotypic plasticity index, percentage of variance component and phenotypic differentiation among population of *Eurycoma apiculata*

Traits	Populations						PPI mean	Percentage variance component			V_{ST} (%)
	AP-PK	AP-LG1	AP-LG2	AP-RB	AP-TH	AP-ST		Among population	Within Population	Error	
LLB	0.37	0.45	0.46	0.33	0.37	0.58	0.43	11.83	33.47	54.70	26.124
LW10B	0.63	0.51	0.62	0.42	0.47	0.56	0.53	4.44	41.74	53.82	0.011
LW1B	0.62	0.51	0.68	0.41	0.48	0.58	0.55	5.68	39.28	55.04	0.014
LW2B	0.64	0.53	0.60	0.42	0.54	0.56	0.55	3.32	35.87	60.81	0.009
LW50B	0.38	0.43	0.46	0.32	0.36	0.56	0.42	0.00	44.06	55.94	-0.005
LW3B	0.37	0.59	0.53	0.26	0.46	0.57	0.46	0.19	38.11	61.70	0.000
LW4B	0.43	0.41	0.49	0.40	0.37	0.54	0.44	0.00	44.24	55.76	-0.006
LW90B	0.53	0.67	0.40	0.56	0.66	0.64	0.58	6.33	37.24	56.43	0.017
LW5B	0.53	0.66	0.45	0.47	0.66	0.65	0.57	2.65	34.96	62.39	7.093
LW6B	0.55	0.69	0.47	0.64	0.68	0.70	0.62	9.86	33.67	56.47	22.654
MWLB	0.37	0.43	0.53	0.32	0.37	0.55	0.43	0.00	47.32	52.68	-0.002
DMWB	0.34	0.44	0.47	0.34	0.39	0.64	0.44	18.86	30.23	50.91	38.422
LWMB/LLB	0.42	0.30	0.39	0.41	0.36	0.37	0.38	23.69	23.98	52.33	49.696
DMWB/LLB	0.25	0.44	0.20	0.10	0.20	0.25	0.24	8.14	19.70	72.16	29.227
LLB/LW50B	0.38	0.30	0.33	0.41	0.37	0.38	0.36	25.53	21.95	52.52	53.659
LLL	0.47	0.48	0.43	0.34	0.30	0.39	0.40	9.38	57.90	32.73	13.936
LW10L	0.66	0.60	0.53	0.57	0.56	0.39	0.55	94.58	2.38	3.03	97.541
LW1L	0.64	0.70	0.52	0.47	0.58	0.38	0.55	2.08	49.70	48.22	0.004
LW2L	0.68	0.59	0.59	0.65	0.61	0.49	0.60	7.34	56.65	36.01	0.013
LW50L	0.54	0.44	0.42	0.30	0.33	0.37	0.40	47.16	31.18	21.67	60.198
LW3L	0.52	0.49	0.39	0.29	0.41	0.40	0.42	2.28	36.74	60.98	0.006
LW4L	0.56	0.45	0.50	0.35	0.33	0.34	0.42	0.00	69.14	30.86	-0.005
LW90L	0.63	0.62	0.45	0.62	0.70	0.45	0.58	80.60	10.49	8.90	88.483
LW5L	0.61	0.57	0.49	0.59	0.71	0.43	0.57	12.24	33.48	54.28	26.788
LW6L	0.65	0.68	0.42	0.65	0.77	0.48	0.61	16.41	35.04	48.55	31.871
MWLL	0.40	0.44	0.47	0.30	0.32	0.36	0.38	0.00	61.97	38.03	0.000
DMWL	0.29	0.58	0.45	0.34	0.34	0.44	0.41	14.27	53.24	32.49	21.136
LWML/LLL	0.24	0.26	0.24	0.29	0.34	0.27	0.28	38.73	28.12	33.15	57.940
DMWL/LLL	0.30	0.40	0.20	0.13	0.19	0.17	0.23	13.16	38.35	48.49	25.546
LLL/LW50L	0.36	0.26	0.30	0.29	0.34	0.27	0.30	72.58	12.97	14.45	84.000
PDT	0.38	0.49	0.44	0.40	0.30	0.30	0.39	12.68	39.00	48.32	24.532
LW10T	0.73	0.58	0.74	0.64	0.52	0.42	0.61	7.48	36.50	56.02	0.020
LW1T	0.69	0.60	0.71	0.60	0.57	0.41	0.60	6.52	35.33	58.15	0.018
LW2T	0.79	0.65	0.77	0.69	0.51	0.47	0.64	8.24	23.39	68.37	26.039
LW50T	0.46	0.49	0.54	0.38	0.48	0.34	0.45	0.00	56.85	43.15	0.000
LW3T	0.44	0.58	0.54	0.44	0.52	0.34	0.48	1.69	46.33	51.98	0.004
LW4T	0.50	0.46	0.55	0.39	0.48	0.41	0.47	1.77	49.70	48.54	0.004
LW90T	0.67	0.73	0.76	0.50	0.72	0.53	0.65	2.61	27.37	70.02	0.010
LW5T	0.63	0.75	0.75	0.49	0.77	0.53	0.65	1.10	29.09	69.81	3.596
LW6T	0.71	0.77	0.78	0.54	0.71	0.62	0.69	3.84	22.87	73.29	14.394
MWLT	0.46	0.49	0.55	0.37	0.49	0.34	0.45	1.02	53.81	45.18	0.002
DMWT	0.43	0.56	0.38	0.34	0.33	0.38	0.40	16.78	40.01	43.21	29.549
LWMT/LLT	0.24	0.37	0.33	0.34	0.50	0.28	0.34	21.89	28.50	49.61	43.433
DMWT/LLT	0.32	0.35	0.30	0.19	0.21	0.14	0.25	15.49	33.13	51.38	31.860
LLT/LW50T	0.31	0.37	0.33	0.34	0.49	0.30	0.36	20.37	29.27	50.36	40.000
Average	0.49	0.51	0.49	0.41	0.47	0.43	0.47	14.51	36.76	48.73	21.063

These results revealed that source of phenotypic variation was originated from variation within population. The high variation within populations might be in part the expression of phenotypic plasticity and/or instability development due to micro-environmental conditions experienced by each tree, but it might be also the result of genotypes differences among individuals. In the studies of leaf morphometric analysis of widely distributed species, the greater variability is common existed within populations (Brus et al. 2011; Jarni et al. 2011; Poljak et al. 2015). High variability within population of *E. apiculata* might be caused by high gene flow between individuals. The mating system *E. apiculata* is outcrossing (Zulfahmi et al. 2019). The mating system that outcrossing among trees would increase variation among trees due to happened genetic recombination among them when miosis events, and that would be expressed in high variation phenotypically. In addition, the fact that these species have never been improved or selected intensively. High variation within population in *Eurycoma longifolia* species is also reported by Rosmaina and Zulfahmi (2013) and Rosmaina et al. (2015) used random amplified polymorphic DNA (RAPD) marker.

The phenotypic differentiation coefficient (V_{ST}) among populations of *E. apiculata* was relatively low, because of limited gene flow (pollen and seed) among populations due to geographically long distance among populations. Based on field observation, cross-pollination of this species assisted by thrips spp. and honeybees. Seed dispersal mechanism of *E. apiculata* is unknown. Many seedlings observed close to their maternal plant. Thus implies that most seeds are dispersed not far from the maternal plant of *E.apiculata*. Limitation gene flow during a sufficiently long period can result in geographical isolation (Reeves and Richards 2014; De-Vriendt et al. 2017). On the other hand, ecologically environments differentiation among populations also contributed to phenotypic plasticity of this species and would increase the value of variation among populations (Viscosi and Cardini 2011). The V_{ST} value among populations was lower than that in *Rosa praelucens* Byhouwer (V_{ST} = 69.56%) (Li et al. 2013), *Ulmus lamellosa* C. Wang & S. L. Chang (V_{ST} = 28.10%) (Zheng et al. 2013), *Rosa beggeriana* Schrenk ex Fisch & Mey. (V_{ST} = 35.76%) (Li et al. 2014), *Rosa platyacantha* Schrenk (V_{ST} = 27.50%) (Yang et al. 2016), *Paphiopedilum armeniacum* S.C. Chen & F.Y. Liu (V_{ST} = 35.76%) (Zhou et al. 2016), *Tetracentron sinense* Oliver (V_{ST} = 61.84%) (Han et al. 2017), *Paeonia rockii* (S.G. Haw & Lauener) T. Hong & J. J. Liu (V_{ST} = 71.07%) (Zhang et al. 2017), but it is higher than that in *Magnolia sprengeri* Pamp (V_{ST} = 12.50%) (Yang et al. (2015) and *Crataegus songorica* K. Koch (V_{ST} = 13.85%) (Sheng et al. 2017). At least, there are two factors that caused the difference result of this study with others. Firstly, breeding systems of species, in which selfing plants with relatively more homozygous individuals and reduced population size usually exhibited higher differentiation among populations than crossing plants. This is in line with those reported by Spielman et al. (2004). Secondly, geographical isolation in the population studied. It will reduce gene flow, resulting in loss of genetic variation and high differentiation among populations, especially when coupled with small population size. It is also in line with those reported by Han et al. (2017).

The results of the nested variance analysis of all traits of *E. apiculata* confirmed that higher variation within population (36.76%) was higher than among populations (14.51%) (Table 4). The mean value of percentage of variance component within-population was 35.05% for the basal leaflet, 38.49% for the lateral leaflet, and 36.74% for the terminal leaflet. All characters measured contributes significantly to variation within the population. The average values of the percentage of variance component among populations explained 8.03% of the total variation in the basal leaflet, 27.39% in the lateral leaflet, and 8.10% in the terminal leaflet. The characters that most strongly differentiated among populations were LLB/LW50B, LW10L, LW90L, LW50L, LWML/LLL, and LLL/LW50L. These characters showed less variation within population and vice versa. The percentage of variance component among populations of *E. apiculata* in this study was lower than those reported by Bruschi et al. (2003) in *Quercus petraea* (Matt.) Liebl. (19.50%), Brus et al. (2011) in *Sorbus domestica* L. (28.52%), and Jarni et al. (2011) in *Fraxinus angustifolia* Vahl (22%), but it is higher than those reported by Gonzalez-Rodriguez and Oyama (2005) in *Quercus affinis* Scheidweiler and *Quarces laurina* Humboldt (11%), and Yang et al. (2015) in *Magnolia sprengeri* Pamp (2.86%). The difference in this result is caused by the limited gene flow of each species in the population studied.

Principal component analysis and dendrogram

To determine the contribution of each variable to the separation of the population, only original variable that is used to principal component analysis (PCA) (Brus et al. 2011; Poljak et al. 2015) whereas derived variables were not used since they are highly correlated with original variables (data not shown). The five principal components (PC1-PC5) explained 100% of the total variance among populations, in which the first second main components accounted for 89.85% of the variation (Table 5). PC1 explained 74.19% of the total variation which highest contribution was DMWT, DMWL, DMWB, LLL, LLB, and LLT characters. PC2 explained 15.66% of the total variance which variable responsible for the differentiation along the PC2 was LLT, DMWT, LLB, and DMWB characters. The third PC accounted for 7.36% of the total variance. Characters that contributed to differentiation along the PC3 were LLB and DMWL. The fourth PC accounted for 1.77 % of the total variance. Characters that contribute to differentiation along PC4 are DMWB and MWLL. The fifth PC accounts for 1.03 % of the total variance. Characters that contribute to differentiation along PC5 are MWLT and MWLL. Among the leaflet morphological characters, we found character of leaflet length (LL) and distance to maximum width from the base (DMW) in the basal, lateral, and terminal leaflets that can discriminate among populations.

Table 5. Eigenvalues, percentage of the total variation and correlation between the investigated traits and the first five principal components (PCs) of *Eurycoma apiculata*

Traits	PC1	PC2	PC3	PC4	PC5
LLB	0.497	-0.360	0.483	-0.085	-0.060
LW1B	-0.016	-0.011	0.198	0.057	-0.138
LW2B	-0.019	-0.037	0.152	0.215	0.039
LW3B	-0.037	-0.037	0.168	-0.033	0.067
LW4B	0.013	-0.055	0.046	0.043	0.121
LW5B	-0.002	-0.011	0.053	0.128	0.195
LW6B	-0.002	-0.006	0.056	0.195	0.188
MWLB	-0.020	-0.084	0.279	-0.181	0.219
DMWB	0.303	-0.227	0.037	0.483	-0.212
LLL	0.554	-0.191	-0.116	-0.082	0.175
LW1L	0.002	0.022	0.146	0.062	-0.209
LW2L	-0.045	0.031	0.016	0.425	0.144
LW3L	-0.053	-0.018	0.146	-0.086	0.126
LW4L	0.004	-0.048	0.023	-0.029	0.181
LW5L	-0.002	-0.011	0.053	0.128	0.195
LW6L	-0.002	-0.006	0.057	0.194	0.188
MWLL	-0.051	-0.063	0.175	-0.197	0.367
DMWL	0.295	-0.128	-0.554	-0.172	0.323
LLT	0.423	0.780	0.245	-0.162	-0.012
LW1T	-0.002	0.080	0.092	0.216	-0.046
LW2T	-0.035	0.081	0.050	0.261	0.197
LW3T	-0.048	0.079	0.107	0.030	0.048
LW4T	0.009	0.108	-0.061	0.109	0.248
LW5T	-0.002	-0.011	0.053	0.128	0.195
LW6T	-0.002	-0.006	0.056	0.195	0.188
MWLT	-0.048	0.191	0.087	0.051	0.377
DMWT	0.269	0.263	-0.297	0.293	-0.110
Eigenvalue	98.957	20.886	9.813	2.360	1.369
% variation	74.19	15.66	7.36	1.77	1.03
Cumulative %	74.19	89.85	97.20	98.97	100.00

The scatter plot of the first two components from principal component analysis can separate the *E. apiculata* populations based on leaf characteristics (Figure 3). The first principal component (PC1) was grouped Lingga-2, Lingga-1, and pokomo populations into the first cluster, meanwhile, Rumbio, Sentajo and Tahura populations are grouped into second clusters (axis x). The second principal component (PC2) separated the Lingga-2 and Pokomo populations with Lingga-1 population, as well as Rumbio and Sentajo populations are separated with Tahura population (axis y). The scatter plot in Figure 2 exhibits the geometrical distances among populations in the plot that reflects a morphological similarity in terms of the studied leaf morphometric traits. Because PC1 and PC2 elucidated the majority of total variation (90%) among populations, the approximation of the real multivariate diversity of populations on both PC axis is considered to be acceptable for the most important discriminating traits. A similar result was reported in *Ziziphus jujuba* Mill. (total variation of PCI and PC2= 84%) (Li et al. 2015) and *Fagus sylvatica* L. (total variation of PCI and PC2= 81%) (Stojnic 2016).

The UPGMA dendrogram of *E. apiculata* based on the morphological similarity coefficient between populations is shown in Figure 4. The UPGMA dendrogram of *E. apiculata* congruent to the PCA scatter plot projection. The UPGMA dendrogram of *E. apiculata* also divided populations studied into two groups at morphological similarity coefficient between populations of 0.014, the first group consists of the Lingga-1, Lingga-2, and Pokomo populations, while the second groups consist of the Tahura, Sentajo, and Rumbio populations. When morphological similarity coefficient between populations was 0.022, the first group is divided into two subclusters, the first subcluster was Lingga-2 and Pokomo populations and the second subcluster was Lingga-1 population, meanwhile, the second groups also are divided into two subclusters, namely Rumbio and Sentajo populations as the first subcluster and Tahura population as the second subcluster.

Geographic clustering of *E. apiculata* is not obviously separated among islands. In this study, *E. apiculata* from the Pokomo population from the Sumatra mainland is joined with the Lingga-2 and Lingga-1 populations from the Riau archipelago region into one group, which reflect the migration between two regions that must have happened in the past (last glacial time) when both regions were connected in Sundaland (Bird et al. 2005; Slik et al. 2011). To prove that, molecular DNA analysis is required in further study.

Implications for conservation

The phenotypic variation of *E. apiculata* within the population was higher than the variation among populations. According to Rao and Hodgkin (2002), information on diversity within and among populations is a pivotal factor to be considered in deciding germplasm conservation methods. Our results emphasize that *in-situ* conservation is the most effective method for protecting *E. apiculata* plants in which the whole gene pool can be protected in the natural habitat. Wu et al (2015) and Guo et al. (2019) stated that *in-situ* conservation is considered as the most effective method to conserve endangered species. From all the study populations, only the Lingga-1 population is an unprotected area while other populations are protected areas (see Table 1). As a majority of the *E. apiculata* population is protected, these populations should be maintained. According to Xu et al. (2017), populations with high diversity should be chosen first for in situ conservation because they can maintain the greatest degree of diversity. Considering the result phenotypic diversity, the Lingga-1 population should be chosen for *in-situ* conservation after Lingga-2 population, but the status of this population is unprotected so that it may difficult to maintain in the long term. Therefore, seed collecting from the Lingga-1 population should be done to prevent the loss of unique alleles that exist in the population, and seedlings can be used as planting material for *ex-situ* conservation. The efforts of protection such as improvement regeneration, strictly prohibiting the harvesting of wild plants of *E. apiculata* and protecting natural habitat from illegal human activities, may be enough to maintain the population size and prevent the genetic homogeneity on *in-situ* population.

To establish *ex-situ* conservation of *E. apiculata*, germplasm garden, and plant propagation via tissue culture techniques can be alternative. Therefore, the collection of planting material is needed. Related to the higher variation found in the population in this study, seeds collection should be performed in each population to capture maximum alleles variation, but minimum collection of plant material can be implemented at two group populations as shown in the UPGMA dendrogram (Figure 3). The first location of collection represents the population of Lingga-1, Lingga-2, and Pokomo, and the second location represents the population of Tahura, Sentajo, and Rumbio. After ex-situ cultivation, seedlings produced should be introduced into originate population.

In conclusion, population phenotypic diversity mean value of *E. apiculata* was 19.71%, the population phenotypic plasticity index mean value of *E. apiculata* was 0.47, and the phenotypic differentiation coefficient among populations of *E. apiculata* was relatively low (V_{ST} = 21.06%). Finally, This study suggests using molecular DNA markers to completing genetic data of *E. apiculata* species so that protection and conservation strategies can be formulated comprehensively.

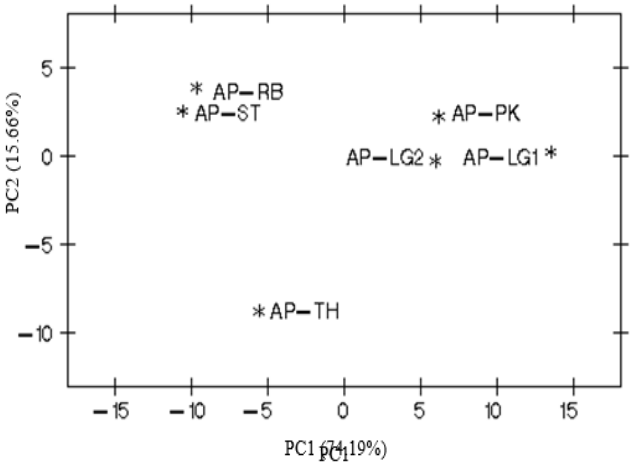

Figure 3. PCA Scatter plot of *E. apiculata* populations: Pokomo population [AP-PK], Lingga-1 population [AP-LG1], Lingga-2 population [AP-LG], Rumbio population [AP-RB], Tahura population [AP-TH], and Sentajo population [AP-ST].

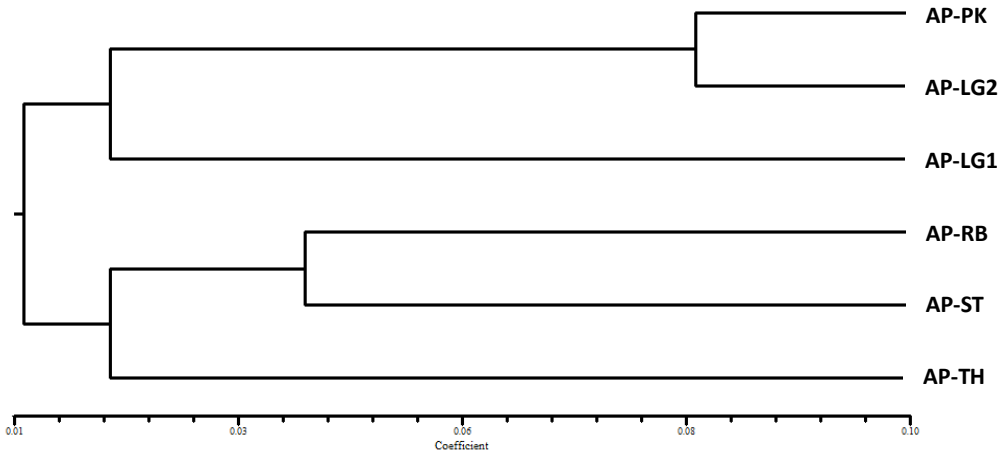

Figure 4. Dendrogram UPGMA based on the morphological similarity coefficient of *E. apiculata* populations: Pokomo population [AP-PK], Lingga-1 population [AP-LG1], Lingga-2 population [AP-LG], Rumbio population [AP-RB], Tahura population [AP-TH], and Sentajo population [AP-ST]

ACKNOWLEDGEMENTS

The authors would like to thank Educational Fund Management Board (LPDP), Ministry of Finance, Republic of Indonesia, for funding this research with contract number of PRJ-3/LPDP.4/2020. The authors thank Riau Province Forestry Services, Forest Park of Sultan Syarif Hashim Minas, Lingga District Agriculture and Plantation Services, Head of Sentajo Village, Kuantan Singingi District, Head of Rumbio Village and Head of Rimba Melintang Village, Kampar District for permission of collecting material in the field.

REFERENCES

Alfaro RI, Fady B, Vendramin GG, Dawson IK, Fleming RA, Sáenz-Romero C, Lindig-Cisneros RA, Murdock T, Vinceti B, Navarro CM, Skrøppa T, Baldinelli G, El-Kassaby YA, Loo J. 2014. The role of forest genetic resources in responding to biotic and abiotic factors in the context of anthropogenic climate change. For Ecol Manag 333: 76-87.

Atanasova D, Mihov M. 2006. Inheritance of flower color and leaf shape of chickpea (*Cicer arietinum* L.). Bulg J Agric Sci 12: 521-524

Batos B, Vilotić D, Orlović S, Miljković D. 2010. Inter and intra-population variation of leaf stomatal traits of *Quercus robur* L. in Northern Serbia. Arch Biol Sci 62: 1125-1136.

Bijarpasi MM, Shahraji TR, Lahiji HS. 2019. Genetic variability and heritability of some morphological and physiological traits in *Fagus orientalis* Lipsky along an elevation gradient in Hyrcanian forest. Folia Oecologia 46: 45-53.

Bird NI, Taylor D, Hunt C. 2005. Palaeoenvironments of insular Southeast Asia during the last glacial period: a savanna corridor in Sundaland? Quat Sci Rev 24: 2228-2242.

BPS. 2019a. Riau Province in Figure. BPS-Statistic of Riau Province, Pekanbaru [Indonesian]

BPS. 2019b. Lingga Regency in Figure. BPS-Statictic of Lingga Regency, Lingga. [Indonesian]

Bruschi P, Grossoni P, Bussotti F. 2003. Within-and among tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. Natural populations. Trees 17: 164-172.

Brus R, Ballian D, Bogunić F, Bobinac M, IdžOjtić M. 2011. Leaflet morphometric variation of service tree (*Sorbus domestica* L.) in the Balkan Peninsula. Plant Biosystems 145: 278-285.

De-Vriendt L, Lemay MA, Jean M, Renaut S, Pellerin S, Joly S, Belzile F, Poulin M. 2017. Population isolation shapes plant genetics, phenotype, and germination in naturally patchy ecosystems. J Plant Ecol 10: 649-659.

Dkhar J, Pareek A. 2014. What determines a leaf's shape? EvoDevo 5. DOI: 10.1186/2041-9139-5-47

Du L, Liu H, Yan M, Li J, Li J. 2017. Individual plasticity of the shade response of the invasive *Solidago canadensis* in China. PLoS ONE 12: e0170049. DOI: 10.1371/journal.pone.0170049

Gao SB, Mo LD, Zhang LH, Zhang JL, Wu JB, Wang JL, Zhao NX, Gao YB. 2018. Phenotypic plasticity vs local adaptation in quantitative traits differences of *Stipa grandis* in semiarid steppe, China. Scientific Report 8: 3148. DOI: 10.1038/s41598-018-21557-w

Gentili R, Solari A, Diekmann A, Duprè C, Monti GS, Armiraglio S, Assini S, Citterio S. 2018. Genetic differentiation, local adaptation, and phenotypic plasticity in fragmented populations of a rare forest herb. PeerJ 6: e4929. DOI: 10.7717/peerj.4929

Goba KAE, Kouonon LC, Koffi KG, Bony BS, Diomande I, Sie RS. 2019. Morphological diversity within *Pterocarpus erinaceus* Poir. (Fabaceae), an overexploited species in the savannahs of Cote d'Ivoire. Am J Plant Sci 10: 1675-1688.

Gonzalez-Rodriguez A, Oyama K. 2005. Leaf morphometric variation in *Quercus affinis* and *Quercus laurina* (Fagaceae), two hybridizing Mexican red oaks. Bot J Linn Soc 147: 427-435.

Guo JL, Cao WJ, Li ZM, Zhang YH, Volis S. 2019.Conservation implications of population genetic structure in a threatened orchid *Cypripedium tibeticum*. Plant Divers 41: 13-18.

Han H, Li S, Gan X, Zhang X. 2017. Phenotypic diversity in natural population of an endangered plant *Tetracentron sinense*. Bot Sci 95: 283-294.

Hounkpevi A, Azihou AF, Kouassi EK, Porembski S, Kakai RG. 2016. Climate-induced morphological variation of black plum (*Vitex doniana* Sw.) in Benin, West Africa. Genet Resour Crop Ev 63: 1073-1084.

Jarni K, Westergren M, Kraigher H, Brus R. 2011. Morphological variability of *Fraxinus angustifolia* Vahl in the north-western Balkans. Acta Soc Bot Pol 80: 245-252.

Kajba D, Ballian D, Idzojtic M, Poljak I. 2015. Leaf morphology variation of *Populus nigra* L. in natural populations along the rivers in Croatia and Bosnia and Herzegovina. South-East Eur For 6: 39-51.

Kreyling J, Puechmaille SJ, Malyshev AV, Valladeres F. 2019. Phenotypic plasticity closely linked to climate at origin and resulting in increased mortality under warming and frost stress in common grass. Ecol Evol 9: 1344-1352.

Lee TA, Kamal NM, Poay TH, Roslan I. 2015. Notes on morphological characteristics of *Eurycoma* Spp. and its status in Peninsular Malaysia. Reinwardtia 14: 259-263.

Leonardi S, Piovani P, Scalfi M, Piotti A, Giannini R, Menozzi P. 2012. Effect of habitat fragmentation on the genetic diversity and structure of peripheral populations of beech in Central Italy. J Hered 103: 408-417

Li S, Li C, Jian H, Li S, Xiong J, Li J, Tang K. 2013. Studies on phenotypic diversity of vulnerable *Rosa praelucens* endemic to Shangrila, Yunan. Acta Hort Sin 40: 924-932.

Li S, Yang S, Liu H, Guo N, Fu L, Ge H. 2014. Phenotypic diversity of *Rosa beggeriana* population in Tianshan Mountain of Xianjing. Acta Hort Sin 14: 1723-1730.

Li X, Li Y, Zhang Z, Li X. 2015. Influences of environmental factors on leaf morphology of Chinese Jujubes. PLoS ONE 10: e0127825. DOI: 10.1371/journal.pone.0127825

Ligarreto GA, del-Pilar PM, Magnitskiy SV. 2011. Phenotypic plasticity of *Vaccinium meridionale* (Ericaceae) in wild populations of mountain forests in Colombia. Rev Biol Trop 59: 569-583.

Martinez CC, Chitwood DH, Smith RS, Sinha NR. 2016. Left-right asymmetry in decussate and distichous phyllotactic systems. Phil Trans R Soc B 371: 20150412. DOI: 10.1098/rstb.2015.0412

Matesanz S, Rubio Teso ML, García-Fernández A, Escudero A. 2017. Habitat fragmentation differentially affects genetic variation, phenotypic plasticity and survival in populations of a Gypsum endemic. Front Plant Sci 8: 843 DOI:10.3389/fpls.2017.00843

Ministry of Environment and Forestry. 2019. Statistic of environmental and forestry 2018. Ministry of Environment and Forestry, Jakarta.

Naito Y, Konuma A, Iwata H, Suyama Y, Seiwa K, Okuda T, Lee SL, Muhammad N, Tsumura Y. 2005. Selfing and inbreeding depression in seeds and seedlings of *Neobalanocarpus heimii* (Dipterocarpaceae). J Plant Res 118: 423-430.

Naito Y, Kanzaki M, Iwata H, Obayashi K, Lee SL, Muhammad N, Okuda T, Tsumura Y. 2008. Density-dependent selfing and its effects on seed performance in a tropical canopy tree species, *Shorea acuminata* (Dipterocarpaceae). For Ecol Manag 256: 375-383.

Nooteboom HP. 1962. Simaroubaceae. In: van Steenis CGGJ (eds) Flora Malaysiana. Wolter Noordhoff, Groningen-Netherlands.

Nordin MS. 2014. Distribution of the population of tongkat ali (*Eurycoma* spp.) in Malaysia based on data tahen from herbarium records. Med Aromat Plants 3: 155. DOI: 10.4172/2167-0412.1000155

Nwofia, Emeka G. 2014. Inheritance of Leaf Shape, Pod Shape, Pod Colour and Seed Coat Colour in Cowpea (*Vigna unguiculata* L. Walp). World J Agric Sci 10: 178-184.

Padua LS, Bunyapraphatsara N, Lemmens RHMJ. 1999. Plant Resources of South-East Asia No.12(1): Medicinal and Poisonous Plants I. Backhuys Publisher, Leiden-Netherlands.

Pichancourt JB, van Klinken RD. 2012. Phenotypic Plasticity Influences the size, shape and dynamics of the geographic distribution of an invasive plant. PLoS One 7: e32323. DOI: 10.1371/journal.pone.0032323

Poljak I, Kajba D, Ljubic I, Idzojtic M. 2015. Morphological variability of leaves of *Sorbus domestica* L in Croatia. Acta Soc Bot Pol 84: 249-259

Rao VR, Hodgkin T. 2002. Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell Tiss Org 68: 1-19

Reeves PA, Richards CM. 2014. Effect of a geographic barrier on adaptation in the dwarf sunflower (*Helianthus pumilus* Nutt.). Intl J Plant Sci 175: 688-701.

Rohlf F. 1998. NTSYSpc: Numerical Taxonomy. Stony Brook: Department of Ecology and Evolution, State University of New York, New York.

Romeo DP, Piera DM, Medagli P, Giuseppe M, Giuseppe SN, Robert WP, Paola F. 2016. Evidence from multivariate morphometric study of the *Quercus pubescens* Complex in Southeast Italy. Bot Serb 40: 83-100.

Rosmaina R, Zulfahmi Z. 2013. Genetic diversity of *Eurycoma longifolia* Jack based on random amplified polymorphic DNA Marker. J Manaj Hut Trop 19: 138-144.

Rosmaina R, Azhari R, Zulfahmi Z. 2015. Genetic diversity of *Eurycoma longifolia* Jack using random amplified polymorphic DNA (RAPD) marker in forest reserve of Kenegerian Rumbio, Indonesia. Malays Appl Biol 44: 73-80.

SAS Institute. 2002. SAS/STAT User's Guide, Version 9.00. SAS Institute Inc, Cary, NC, USA.

Semizer-Cuming D, Kjær ED, Finkeldey R. 2017. Gene flow of common ash (*Fraxinus excelsior* L.) in a fragmented landscape. PLoS One 12: e0186757. DOI: 10.1371/journal.pone.0186757

Sheng F, Chen SY, Tian J, Li P, Qin X, Wang L, Luo SP, Li J. 2017. Morphological and ISSR molecular markers reveal genetic diversity of wild hawthorns (*Crataegus songorica* K. Koch.) in Xinjiang. J Integr Agric 16: 60345-7

Singh B. 2001. Plant Breeding: Principles and Methods. Kalyani Publishers. New Delhi, India.

Slik JWF, Aiba SI, Bastian M, Brearley FQ, Cannon CH, Eichhorn KAO, Fredriksson G, Kartawinata K, Laumonier Y, Mansor A, Marjokorpi A, Meijaard E, Morley RJ, Nagamasu H, Nilus R, Nurtjahya E, Payne J, Permana A, Poulsen AD, Raes N, Riswan S, Schaik CPV, Sheil D, Sidiyasa K, Suzuki E, Valkenburg JLCHV, Webb CO, Wich S, Yineda T, Zakaria R, Zweifel N. 2011. Soils on exposed Sunda Shelf shaped biogeographic patterns in the equatorial forests of Southeast Asia. Proc Natl Acad Sci USA 108: 12343-12347.

Spielman D, Brook BW, Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. Proc Natl Acad Sci USA 101: 15261-15264.

Stenøien HK, Hassel K, Segreto R, Gabriel R, Karlin EF, Shaw AJ, Flatberg KI. 2014. High morphological diversity in remote island populations of the peat moss Sphagnum palustre: glacial refugium, adaptive radiation or just plasticity? The Bryologist 117: 95-109.

Stojnic S, Orlovic S, Miljkovic D, von Wuehlisch G. 2016. Intra and inter provenance variations in leaf morphometric traits in European beech (*Fagus sylvatica* L.). Arch Biol Sci 68: 781-788.

Talukdar TL, Talukdar D. 2003. Inheritance of growth habit and leaf-shape in mungbean [*Vigna radiata* (L.) Wilczek.]. Indian J Genet 63: 165-166.

Toker C, Ceylan FO, Inci NE, Yildirim T, Cagirgan MI. 2012. Inheritance of leaf shape in the cultivated chickpea (*Cicer arietinum* L.). Turk J Field Crops 17: 16-18.

Valladares F, Sanchez-Gomez D, Zavala MA. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol 94: 1103-1116.

Vieira WL, Boeger MRT, Cosmo NL, Coan AI. 2014. Leaf morphological plasticity of tree species from two developmental stages in *Araucaria* forest. Braz Arch Biol Technol 57: 476-485.

Viscosi V, Cardini A. 2011. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. PLoS One 6: e25630. DOI: 10.1371/journal.pone.0025630.

Wu FQ, Shen SK, Zhang XJ, Wang YH, Sun WB. 2015. Genetic diversity and population structure of an extremely endangered species: the world's largest Rhododendron. AoB Plants 7: plu082. DOI: 10.1093/aobpla/plu082

Xu JJ, Zhang LY, Zhao B, Shen HF. 2017. Assessment of genetic diversity among six populations of *Rhododendron triflorum* in Tibet using ISSR and AFLP markers. S Afr J Bot 108: 175-183

Yang M, Shi SG, Liu W, Zhang M, Gou L, Kang YX, Liu JJ. 2015. Phenotypic variation and diversity of *Magnolia sprengeri* Pamp in native habitat. Genet Mol Res 14: 6495-6508.

Yang S, Guo N, Ge H. 2016. Morphological and AFLP based genetic diversity in *Rosa platyacantha* population in population in Eastern Tianshan Mountain of Northwestern China. Hortic Plant Sci 2: 55-60

Zhang QD, Jia RZ, Meng C, Ti CW, Wang YL. 2015. Diversity and population structure of a dominant deciduous tree based on morphological and genetic data. AoB Plants 7: plv103. DOI: 10.1093/aobpla/plv103.

Zhang X, Song C, Zhang Y, Niu L, Zhang Q, Xie L. 2017. Phenotypic Diversity of *Paeonia rockii* population in Qinling and Ziwuling Mountains areas. Acta Hort Sin 44: 139-150.

Zheng X, Meng C, Ji Z, Wang Y. 2013. Phenotypic Diversity of Leaves Morphologic Characteristics of *Ulmus lamellosa* natural populations in Shanxi. Acta Hort Sin 40: 1951-1960.

Zhou Y, Jia R, Yang S, Zhao X, Ge H, Luo W, Zhao X, Sang Y. 2016. Phenotypic diversity of *Paphiopedilum armeniacum* populations. Acta Hort Sin 43: 1337-1347.

Zulfahmi, Rahmawati A, Irfan M, Rosmaina, Nazir M. 2018. Chromosome numbers and karyotypes of *Eurycoma longifolia* Jack and *Eurycoma apiculata* A.W.Benn (Simaroubaceae). Pak J Biotechnol 15: 969-973.

Zulfahmi, Aryanti E, Rosmaina. 2019. New Record of *Eurycoma apiculata*, A.W. Benn (Simaroubaceae) from forest reserve of Kenegerian Rumbio, Riau, Indonesia. Berita Biologi 18: 365-371. [Indonesian]