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Variation in seed morpho-physiological and biochemical traits of Java olive populations originated from Java, Bali, Lombok, and Timor Islands, Indonesia

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Abstract. *Sudrajat DJ, Suwandhi I, Siregar IZ, Siregar UJ. 2018. Variation in seed morpho-physiological and biochemical traits of Java olive populations originated from Java, Bali, Lombok, and Timor Islands, Indonesia. Biodiversitas 19: 1004-1012.* Java olive (*Sterculia foetida* L.) is one of the promising non-edible feedstocks for biodiesel production. Utilization of this tree species for plantation is limited due to knowledge gaps in many fundamental aspects of seed properties. The aim of the study was to determine the variation that may exist among java olive seed collected from Bogor, Palimanan, Tuban, Gilimanuk, Pemenang and Naioni populations, and to analyze correlations between one or many seed morpho-physiological and biochemical traits. Analysis of variance showed significant differences of seed morphological and biochemical traits, except for the germination capacity. Seed morphological traits were not significantly correlated with agro-climate factors, while some of biochemical traits revealed significant correlation with some agro-climate factors. Genotypic variance and genotypic coefficient of variation were higher for fruit length, fruit width, carbohydrate, fat, protein and oil contents, indicating higher genetic contribution on the variance of these traits. High heritability values combined with high genetic gain was found for protein content, fruit width and oil content, that revealed the characters having highly genetic origin with a good number of heritable components. Population clustering exhibited that most of the geographically distant population are genetically related. Pemenang population was found highly associated with oil content, indicating that the population have high potency to develop as the feedstocks for for biodiesel production.

Keywords: *Sterculia foetida*, biodiesel, genotypic, heritabilitas, oil content, phenotypic

INTRODUCTION

The diminishing supply of fossil fuels reserves and the growing environmental concerns have made renewable energy an attractive alternative source for the future (Demirbas 2009; Mahanta and Shrivastava 2011; Atabani et al. 2013). Biodiesel is a promising alternative fuel and can make a substantial contribution to the future energy demands of the domestic and commercial economies (Silitonga et al. 2013). Some forest tree species have been found to be suitable species for biodiesel and one of the most prospective is java olive (*Sterculia foetida* L.) that produced about 200-350 kg of seed per plant annually (Munarso 2010) with a total oil content of about 34% (Kirtikar and Basu 1999). Moreover, the properties of the produced java olive oil methyl esters and all the measured properties are within the limits of ASTM D 6751 and EN 14214 specifications (Silitonga et al. 2013), and from emission and combustion characteristics, java olive methyl ester oil closely followed the standard of diesel oil (Devan and Mahalakshmi 2009). Engine running with biodiesel from java olive seed as fuel is 14.2% higher efficient than diesel fuel due to low frictional losses (Rao et al. 2015). Therefore, java olive oil is one of the promising non-edible feedstocks for biodiesel production.

Java olive is a large, straight, deciduous tree which can grow up to 40 m in height and 3 m in girth (diameter of trees is around 100-120 cm) (Vipunngeun and Palanuvej 2009). It is a wild plant and well adapted to tropical and subtropical area (30° North Latitude to 35° South Latitude). In Indonesia, the species is distributed on almost all islands, and it is often found in primer and secondary forest, rocky and sandy areas, and coastal areas (Lemmens et al. 1995). Java olive has good adaptability to variety of soil, but probably needs soils with sufficient moisture for optimum development (Orwa et al. 2009). The plant has an average life span of more than 100 years (Munarso 2010). The species produces timber for light constructions and various parts have bioactive compounds for medicine (Orwa et al. 2009) and insecticide properties (Orwa et al. 2009; Rani and Rajasekharreddy 2009).

Cultivation of java olive in Indonesia is still limited and only planted as garden filler plants and hedges (Lemmens et al. 1995). However, to increase the plantation area and biodiesel production, it is important to domesticate the species initiated by identifying suitable genotypes or populations of java olive bearing high oil-yielding seeds, including information of morphological, physiological, and biochemical characteristics of java olive seeds across wide range of populations. Population variation with respect to seed morphophysiological and biochemical traits has been studied in many species and locations, such as *Garcinia kola* in Cameroon (Kanmegne et al. 2010), *Pinus wallichiana* in India (Rawat and Bakshi 2011), *Jatropa curcas* in West Nusa Tenggara (Santoso 2011), *Cedrus deodara* in Jammu and Kashmir (Mughal and Thapliyal 2012), *Pongamia pinnata* in India (Kumari et al. 2013), and *Anthocephalus cadamba* in Indonesia (Sudrajat, 2016). In several tree species, significant correlations have been shown to exist between specific seed attributes and specific environmental and geo-climate factors (Ghildiyal et al. 2009; Tauchen et al. 2011). Such correlations help to identify the environmental factors influencing plant physiology and morphology.

Morphological traits are useful for preliminary evaluation because they facilitate fast and simple evaluation and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions (Čolić et al. 2012). Morphological traits combined with physiological and biochemical traits were commonly applied for characterization accessions or populations (Kumari et al. 2013). With respect to java olive, there are still lack of baseline information on many important traits such particularly as morpho-physiological and biochemical traits. The objective of this study was to determine the variability of morpho-physiological and biochemical traits of java olive seeds from 6 populations, to analyze the correlation among traits, and to identify the most useful variables for discrimination among populations, and finally to detect relationships among populations. The study will help selection criteria for characters of interest within population traits as they are necessary for further genetic improvement program.

MATERIALS AND METHODS

Materials

Seeds originating from six populations were pre-identified covering distribution areas of java olive in Bogor, Palimanan (West Java), Tuban (East Java), Gilimanuk (Bali), Pemenang (West Nusa Tenggara) and Naioni (East Nusa Tenggara). The information of geographic origin of seed collection locations were shown in Figure 1 and Table 1.

Table 1. Geo-climate origin of the studied populations

Geo-climate characteristics	Population					
	Bogor, West Java	Palimanan, West Java	Tuban, East Java	Gilimanuk, Bali	Pemenang, Lombok	Naioni, Timor
Latitude (S)	6°35’43”	6°41’50”	6°55’04”	8°11’16”	8°24’09”	10°15’19”
Longitude (E)	106°48’09”	108°24’18”	112°02’50”	114°26’29”	116°06’22”	123°36’05”
Altitude (m asl)	260	20	42	10	17	230
Precipitation (mm/year)	3500	2300	2600	1559	1722	1290
Temperature (°C)	26	26.7	27.1	27.2	27.2	27.4
Relative humidity (%)	83	77	74	73.9	74	73

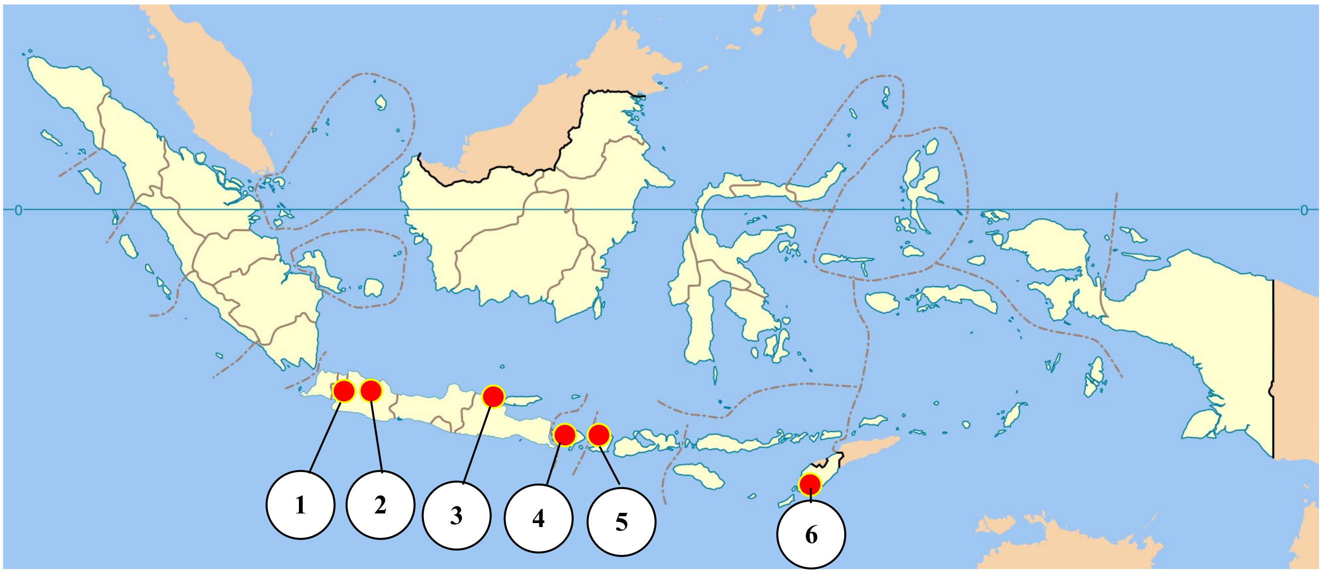

Figure 1. Samples distribution of Java olive in southern Indonesia. 1. Bogor (West Java), 2. Palimanan (West Java), 3. Tuban (East Java), 4. Gilimanuk (Bali), 5. Pemenang (West Nusa Tenggara), 6. Naioni (East Nusa Tenggara)

Seeds at each of the location were collected from 10 average trees found about 50 m apart from each other in order to avoid family structure due to relatedness or inbreeding events. Seed processing was carried out in laboratory of seed technology, Forest Tree Seed Technology Research and Development Center, Bogor. Soil samples were taken from 20 cm depth from each site. Soil pH, soil texture, soil nutrients, base saturation, and cation exchange capacity were analyzed at Soil and Plant Nutrition Laboratory, SEAMEO BIOTROP, Bogor.

Morpho-physiological data

The sampling method and design were the same for each population site. From each population, 200 undamaged fruits were randomly collected and measured for length and diameter of fruits (Rawat and Bakshi 2011). The seeds were extracted manually by cutting in half of fruits. The number of seed per fruit was counted. Equal weights of seeds composed the samples from individual trees, and seeds were bulked by population for the experiments. The seeds were dried in room condition (temperature 25-30º C, relative humidity 70-80%) for 4-5 days before measurement (Sudrajat et al. 2011). One hundred and fifty (150) seeds from each of populations were measured for their length and width using a digital caliper. The same sample was also used to determine seed weight using weight digital scale (AR3130, Ohaus). Seed moisture content was determined by the constant-temperature oven method at 103±2° C for 17 hours (ISTA 2012).

Seed germination studies were carried out in greenhouse conditions (temperature 29-34° C, relative humidity 60-75%) using sand media with four replications of 100 seeds from each population. Seeds were treated by soaking in water for 24 hours before sowing, and then seeds were buried in the sand media in about 2/3 part of the seed. A seed was considered to be germinated when a pair of leaves developed. Germination was assessed every 24 hours for 21 days, and data were recorded for germination capacity.

Biochemical data

The total crude oil content was determined using manual hot press hydraulic (Sudradjat et al. 2010; Handayani et al. 2015) at temperature 70°C for 1 hour. Pressing process was conducted two time to produce maximum yield. The oil produced by the process was weighed to measurement of oil content. For biochemical traits, two grams (2.0 g) of the seed sample were weighed and prepared for crude fat and protein analysis. Crude fat was determined based on the Soxhlet extraction method of AOAC (1990). Crude protein content was calculated using the Kjeldahl method. Total percentage carbohydrate was determined by the different method as reported by Onyeike et al. (1995).

Stastistical analysis

Seed morpho-physiological and biochemical traits were analyzed by using SPSS (version 21) for analysis of variance and Duncan multiple range test to determine the differences between populations. Linear correlation coefficients (Pearson) at 5% level of significant were calculated among the studied characters and agro-climate factors (latitude, longitude, altitude, precipitation, temperature and relative humidity). Genotypic (GV), phenotypic (PV) and environmental variances (EV) were calculated using the following equations according to Burton (1952).

$$GV = (Mt-Me)/r;\ PV = GV + Me;\ EV = Me$$

Where: Mt = mean square for treatments, Me = mean error variance, and r = number of replicates.

Phenotypic (PVC), genotypic (GVC), and environmental coefficients of variation (ECV) were calculated by using the formula as suggested by Burton (1952):

$$PCV = \sqrt{PV}/x;\ GCV = \sqrt{GV}/x;\ ECV = \sqrt{EV}/x$$

Where: x = population mean for each trait

Heritability in broad sense (H^2B), the ratio of genetic variance to the total phenotypic variance, was estimated as suggested by Allard (1999). Genetic advance (GA) is the expected increase in the magnitude of a particular character when a selection pressure of chosen intensity (i) is applied.

$$GA = GV/\ PV \times i \times \sqrt{PV}$$

Where: i = selection intensity (2.06)

Genetic gain (GG) expressed in percentage of mean was computed by using the formula given by Johnson et al. (1955). Hierarchical cluster analysis was used to explain the pattern of variation among the populations based on morpho-physiological and biochemical traits (Tomsone et al. 2012). Bi-plot analysis (Mustafa et al. 2015) was used to identify the performance and to cluster the java olive populations based on oil content and some key of seed morphological traits and geo-climate factors using SPSS (versi 22) for categorical principle component analysis.

RESULTS AND DISCUSSION

Variation of morphophysiological and biochemical traits

There were significant (P<0.05) differences in all of the morphological traits of fruit and seed of the populations. Bogor population showed higher values in all observed traits of fruit and seed morphological fruits (fruit length, fruit width, number of seed per fruit, seed length, seed width, and seed weight) (Table 2). Larger seed possibly due to a longer growing season (Totland and Birks 1996) and higher soil nutrition in Bogor population habitat (Table 3). Some studies reported a tendency for larger seed in drier habitats (Sorenson and Miles 1978; Stromberg and Patten 1990). The variation in fruit and seed traits among populations is probably due to influence of different intensities of natural constraints and also genetic factors.

Significant variations in fruit and seed morphological traits were also reported in other studies, viz. *Pinus wallichiana* (Rawat and Bakshi 2011), *Cedrus deodara* (Mughal and Thapliyal 2012), *Pongamia pinnata* (Kumari et al. 2013), *Jatropha curcas* (Khadidiatou et al. 2013) and *Anthocephalus cadamba* (Sudrajat 2016).

Physiological seed trait (germination capacity) was not significantly different among populations indicating all populations had relative similar physiological trait with germination capacity ranged between 76.3-88%. On the other side, all biochemical seed traits differed significantly (P<0.05) among populations. Bogor population provided the highest moisture content (29.5%) and carbohydrate (14.90%), while for fat content, Naioni population resulted the maximum value (34.52%) followed by Pemenang population (33.87%). The protein content was recorded maximum in Naioni population (15.20%) followed by Gilimanuk population (14.40%). The mean oil content varied from 17.54% (Bogor population) to 27.53% (Pemenang population) among the populations (Table 2). According to Sudradjat et al. (2010), oil content of java olive seed is about 25.5%, lower than the seed oil content from Pemenang and Naioni populations in this study. Kale et al. (2011) reported the higher total oil of java olive seed that is about 32.4% on seed moisture content 9.2% and even Kirtikar and Basu (1999) stated that the oil content could reach 34%. The lower oil content in this study could be caused by the high seed moisture content (17.1-29.5%) and less effective of press manual hydraulic device to extract oil from the seeds. Sudradjat et al. (2010) recommended the extruder press mechanisms device to extract maximally seed oil content.

Fat was the highest biochemical composition (30.32%) followed by oil (23.04%), carbohydrate (12.84%), and protein (12.84%). Kavitha et al. (2015) reported the similar result that java olive seeds were rich in fat (30-36%) and protein 11.4%. Bureau of Plant Industry (BPI, 2010) also reported that the main compositions of java olive seeds are fats and protein which are 51.78% and 21.61% respectively. Variation in seed biochemical traits (carbohydrate, protein, fat) were observed in *Pongamia pinnata* (Jiang et al. 2013; Kumari et al. 2013) and *Jatropha curcas* ((Kumar and Singh 2014). The seed biochemical contents in the selected genotypes also indicate towards some promising candidates for development and utilization in large scale plantation drives.

Table 2. Morphological traits of java olive fruit and seed from 6 populations

Populations	Fruit length (cm)	Fruit width (cm)	Number of seed per fruit	Seed length (mm)	Seed width (mm)	Seed weight (g)
Bogor	14.76±0.85 a	13.47±0.73 a	15±2.33 a	28.3±2.3 a	16.4±3.6 a	4.05±0.52 a
Palimanan	9.92±0.69 e	8.00±0.64 e	14±3.45 ab	25.7±0.7 bc	14.7±0.5 c	3.22±0.51 b
Tuban	12.13±0.55 c	11.45±0.56 c	15±1.69 a	25.4±1.1 c	13.7±0.8 e	2.89±0.64 c
Gili Manuk	13.15±1.79 b	12.25±1.20 b	10±4.05 c	25.8±1.4 bc	14.7±0.9 c	3.31±0.36 b
Pemenang	10.33±1.18 e	9.47±0.89 d	11±2.51 c	25.1±1.3 d	14.2±0.6 d	3.42±0.54 b
Naioni	11.03±0.85 d	9.67±0.80 d	13±1.91 b	25.9±1.2 b	15.3±1.1 b	3.33±0.39 b
F-test	88.785**	177.360**	21.192**	95.297**	49.267**	28.658**
Range	8.00-17.00	6.50-15.00	1-19	18.14-36.95	11.04-16.59	2.01-4.87
Mean	11.89	10.71	13.43	26.04	14.84	3.37
SD	1.98	2.02	3.48	1.76	1.82	0.61
CV (%)	16.65	18.86	25.91	6.75	12.26	19.76

Note: the data shown are mean ± standard error of six replicates; Different letters a, b, c, d and e denote significant difference ($p \leq 0.05$) between different treatments; **: Significant at $p < 0.01$

Table 3. Soil fertility condition of 6 java olive population in this study

Populations	Soil texture (SL-MU-TT-10, Hydrometer)			pH H_2O (SNI 03-6787-2002)	Organic matter		C/N	P (P2O5) (SL-MU-TT-05; Bray I/II)	K (SL-MU-TT-07C: Extraction of buffer NH_4OAc 1,0 N pH 7,0)	KTK (SL-MU-TT-07C: Extraction of buffer NH_4OAc 1,0 N pH 7,0)
	Sand (%)	Dust (%)	Clay (%)		C (%) (SNI 13-4720-1998: Walkey and Black)	N (%) (SNI 13-4721-1998: Kjeldah)				
Bogor	26.20	21.60	52.20	5.12	2.25	0.35	7.30	24.50	98.00	19.16
Palimanan	41.33	30.67	28.00	6.70	3.99	0.28	14.25	13.33	137.33	33.11
Tuban	30.67	41.33	28.00	6.96	1.64	0.15	10.46	102.67	602.00	29.00
Gili Manuk	54.67	28.33	17.00	7.83	2.02	0.16	12.41	13.00	694.33	23.17
Pemenang	59.67	28.67	11.67	6.83	1.20	0.09	12.44	68.33	412.33	16.42
Naioni	17.00	42.67	40.33	6.60	1.79	0.14	12.51	90.67	421.33	44.68

Note: SNI: Indonesian national standard

The highest coefficient of variation was recorded for number of seed per fruit (25.91%), moisture content (22.41%), seed weight (19.76%) followed by protein (19.09%) (Table 2 and Table 4). All of morpho-physiological and biochemical traits of java olive seeds showed significant differences among populations, confirming the hypothesis that java olive possesses strong differentiation between its populations, and indicating the existence of genetic variability. The variation of seed morpho-physiological and biochemical traits may due to both internal (maternal, hereditary) and external (environmental) conditions operating at the time of seed development (Harper et al. 1970; Kaushik et al. 2007). Changes of environment during evolution the species, habitat fragmentation and excessive exploitation may lead to a low gene flow, which in turn resulted in the differences in seed morpho-physiological and biochemical traits among populations.

Correlation among seed morpho-chemical traits and geo-climate factors

Fruit length exhibited positive significant correlation with fruit width (0.977). Significant correlations were found between seed length and seed width (0.905). Seed weight also had significant correlarion with seed length (0.856) and seed width (0.890). The correlation has been exposed previously by Bagchi and Dobriyal (1990) on *Acacia nilotica*, Rawat and Bakshi (2011) on *Pinus wallichiana,* and Sudrajat (2016) on *Anthocephalus cadamba*, indicating that the traits are interdependent on each other. Significant correlation among traits are main interest in the improvement activities, because the improvement of one trait my cause simultaneous effects in the other trait (Divakara et al. 2010). In biochemical seed traits, a strong negative relationship of fat content with moisture content (-0.920) and carbohydrate (-0.815) was revealed. Fat content of java olive seeds showed positive correlation with oil content (0.841). Oil content also revealed a negative significant correlation with moisture content (-0.919) (Table 5).

Table 4. Physiological and biochemical traits of java olive seed from 6 populations

Populations	Germination capacity (%)	Moisture content (%)	Carbohydrate (%)	Fat (%)	Protein (%)	Oil content (%)
Bogor	76.3± 5.7	29.5±3.7 a	14.90±0.81 a	27.20±1.63 b	11.50±0.89 b	17.54±1.89 d
Palimanan	88.0± 5.6	25.3±2.6 b	14.72±0.78 a	27.97±2.08 b	9.17±0.36 d	19.17±1.26 d
Tuban	85.0±11.5	24.3±3.1 c	13.60±0.82 b	28.67±2.40 b	14.40±0.97 a	24.76±2.29 bc
Gili Manuk	81.0±14.1	23.3±6.7 d	13.77±0.23 b	29.67±1.75 b	9.87±0.28 cd	23.30±0.81 c
Pemenang	80.0±11.7	17.1±3.3 f	13.37±0.85 c	33.87±2.40 a	11.82±0.29 bc	27.53±0.96 a
Naioni	79.5±10.2	19.9±1.4 e	9.73±0.53 d	34.52±1.59 a	15.20±0.24 a	25.97±1.84 a
F-test	0.665ns	4.968*	23.880**	26.901**	9.636**	64.925**
Range	61.0-93.0	13.2-34.2	9.10-15.90	25.20-36.60	8.70-15.60	15.20-28.62
Mean	81.62	23.2	12.84	30.32	11.99	23.04
SD	9.93	5.2	1.82	3.40	2.29	3.92
CV (%)	12.17	22.41	14.17	11.21	19.09	17.01

Note: the data shown are mean±standard error of six replicates, different letters a, b, c, d and e denote significant difference ($P \leq 0.05$) between different treatments, **: significant at $p<0.01$, *: significant at $p<0.05$, ns: not significant.

Table 5. Simple correlation (Pearson) between fruit and seed traits of java olive

Parameters	FL	FW	NS	SL	SW	SWE	GC	MC	CH	FT	PT
FW	0.977**										
NS	0.186	0.101									
SL	0.784	0.656	0.457								
SW	0.569	0.415	0.244	0.905*							
SWE	0.575	0.472	0.083	0.856*	0.890*						
GC	-0.608	-0.617	0.173	0.173	0.597	0.397					
MC	0.671	0.541	0.635	0.635	0.765	-0.772	0.005				
CH	0.314	0.270	0.186	0.186	0.303	0.238	0.222	0.611			
FT	-0.512	-0.419	-0.521	-0.521	-0.501	-0.157	-0.279	-0.920**	-0.815*		
PT	-0.020	0.069	0.307	0.307	-0.107	-0.236	-0.247	-0.311	-0.734	0.492	
OL	-0.469	-0.293	-0.515	-0.515	-0.750	-0.522	-0.040	-0.919**	-0.646	0.841*	0.549

Note: FL: fruit length, FW: Fruit width, NS: number of seed per fruit, SL: seed length, SW: seed width, SWE: seed weight, MC: moisture content, GC: germination capacity, CH: carbohydrate content, F: fat content, PT: protein, OL: oil content, **: significant at $p < 0.01$, *: significant at $p < 0.05$.

Table 6. Simple correlation (Pearson) between fruit and seed traits studied and geo-climate factors

Parameters	Latitude (S)	Longitude (E)	Altitude (m asl)	Precipitation (mm/year)	Temperature (° C)	Relative humidity (%)
Fruit length	-0.293	-0.389	0.320	0.551	-0.564	0.562
Fruit width	-0.242	-0.301	0.235	0.417	-0.429	0.434
Number of seed per fruit	-0.547	-0.467	0.373	0.478	-0.577	0.497
Seed length	-0.328	-0.492	0.602	0.871*	-0.855*	0.877*
Seed width	0.016	-0.202	0.745	0.731	-0.669	0.728
Seed weight	-0.093	-0.312	0.518	0.816*	-0.719	0.820*
Seed moisture content	-0.734	-0.802	0.166	0.759	-0.842*	0.748
Germination capacity	-0.358	-0.210	-0.619	-0.340	0.228	-0.365
Carbohydrate	-0.912*	-0.946**	-0.557	0.641	-0.685	0.624
Fat	0.901*	0.918**	0.231	-0.636	0.740	-0.618
Protein	0.468	0.609	0.554	-0.408	0.391	-0.362
Oil content	0.684	0.816*	-0.140	-0.845*	0.891*	-0.822*

Note: **: significant at $p < 0.01$, *: significant at $p < 0.05$

Table 6 showed that all of seed morpho-physiological traits, moisture content and proteint had not significant correlation with latitude, longitude and altitude. Some significant correlations were shown between precipitation and seed length (r = 0.871) and seed weight (r = 0.816). Similar with precipitation, relative humidity revealed significant positive corelation with seed length (r = 0.877) and seed weight (r = 0.820). The lower precipitation intensity may reduce seed mass as a result acceleration in seed growth rate (dry matter accumulation) and reduction in the duration of seed filling (Weigand and Cueller 1981; Spears et al. 1997). In this study, temperature of maternal habitat also significantly correlated with seed length (r = -0.855) (Table 6). According to Li et al. (2015), seed size and weight variation may be the result of temperature variation during seed development.

Some agro-climate factors also exhibited the significant correlation with biochemical traits of java oil seeds. Latitute and longitude were significantly correlated with carbohydrate and fat content. Carbohydrate and fat tend to increase on the southern and eastern java olive populations. Longitude also had negative significant correlation with oil content. The effect of geographic position on seed biochemical traits is accumulation impact of geoclimate factors incorrelation with seed adaptation and development. Temperature exhibited the negative significant correlation with seed moiture content. Temperature, relative humidity and precipitation also showed the significant correlation with seed oil content (Table 6). Population had higher precipitation produced seed with relative higher moisture content and lower oil content. Studies of oil content variation also were reported by some researchers such as in *Jatropha curcas*. Ginwal et al. (2004) reported highest oil content (58.12%) in Chhindwara accession of *J. curcas* with less rainfall and moderately high temperature. In contrary, Khadidiatou et al. (2013) reported that the seeds from wet region (Bignona) in Senegal recorded the highest oil content. Other study reported by Santoso (2011) using the narrow habitat of *J. curcas* in West Nusa Tenggara, there was not significant different oil content among populations. In this study, the seed samples were collected from relative large distribution from West Java to East Nusa Tenggara with the result that the seed variation including oil content was significant.

Coefficient of variation and heritability

The genotypic variance and genotypic coefficient of variation were higher for several traits, such as fruit length, fruit width, carbohydrate, fat, protein and oil contents, compared with environment variance and environment coefficient of variance. The results indicated that the genotypic component provided the major contributions to the total variance of fruit and seed biochemical traits. The variability among genotypes is generally used to estimate the total genetic variation to determine the degree of genetic control for a particular character (Giwali et al. 2015). Genetic control on the biochemical traits (carbohydrate, protein, oil content) also has been observed for several provenances of *Pinus wallichiana* in India (Rawat and Bakshi 2011) and several candidates plus tree of *J. curcas* in Eastern India (Kumar and Singh 2014). On the other hand, variation of seed morpho-physiological traits (number of seed per fruit, seed length, seed width, seed weight, germination capacity, moisture content) was more controlled by environmental factors.

In tree improvement and selection of suitable tree species, heritability of a certain desirable characters is important consideration. Broad sense heritability is defined as the ratio of all of the genetic variation to the phenotypic variation (Zobel and Talbert 1984) and it is important in predicting the amount of genetic gain. The high heritability value reveals that the offspring has a greater chance of resulting an expression of the trait that is similar to the trait of the parents; contrarily, a low heritability indicates a lower level of similarity (Kumari et al. 2013). High predictions of heritability value suggested that these traits may serve as important genetic markers in estimation the selection of best individuals or provenances. The highest heritability was revealed by protein (0.94), carbohydrate (0.87), oil content (0.85 and fruit width (0.85). High heritability values combined with high genetic gain was found for protein content, fruit width and oil content, revealed the characters have highly genetic origin with a good amount of additive genetic effect (heritable components). Rawat and Bakshi (2011) explained that high heritability value coupled with high genetic gain on some characters is important information for selecting the best population. Conversely, some characters such as seed length, seed width and germination capacity had lower genotypic coefficient of variance and genetic gain indicating that the contribution of non-additive genetic effects were higher than additive genetic effect.

Population clustering

Morphological characterization combined with multivariate statistical methods, such as cluster analysis, are useful tools for screening accessions (Akintobi et al. 2002; Golparvar 2013; Adebisi et al. 2013). Hierarchical cluster analysis separated the java olive populations based on the fruit, seed morphological and biochemical characteristics into three groups, i.e. Group 1: Pemenang and Naioni, Group 2: Tuban, Gilimanuk and Paliman, and Group 3: Bogor. The geographically distance among populations affected to the similarity of morphological and biochemical traits of java olive and belonging in the same cluster (Figure 2). Pamenang and Naioni populations lied in same cluster as representation of eastern populations of Indonesian java olive with higher seed oil content and lower seed moisture content. Some populations might be formed a mixture population such as Palimanan, Tuban, and Gilimanuk populations might be the mixture populations as a result of seed or genetic materials transfer among populations, whereas the Bogor population was separated from other populations representing the true population from relative wet site in western Java island.

Table 7. Mean coefficient of variability and genetic estimates of seed traits of java olive

Parameters	GV	EV	PV	PCV	ECV	GCV	H^2B	GA	GG%
Fruit length	3.36	1.15	4.50	2.12	1.07	1.83	0.75	3.26	27.40
Fruit width	4.08	0.69	4.77	2.18	0.83	2.02	0.85	3.84	35.90
Number of seed per fruit	5.24	7.78	13.02	3.61	2.79	2.29	0.40	2.99	22.26
Seed length	1.29	2.05	3.34	1.83	1.43	1.13	0.39	1.45	5.58
Seed width	0.86	2.69	3.55	1.88	1.64	0.93	0.24	0.95	6.37
Seed weight	0.13	0.15	0.28	0.53	0.38	0.36	0.46	0.50	14.54
Germination capacity	8.91	106.43	115.34	10.74	10.32	2.99	0.08	1.71	2.09
Seed moisture content	14.83	14.95	29.79	5.46	3.87	3.85	0.50	5.60	24.13
Carbohydrate	3.26	0.50	3.77	1.94	0.71	1.81	0.87	3.46	26.97
Fat	8.72	4.04	12.77	3.57	2.01	2.95	0.68	5.03	16.59
Protein	5.67	0.35	6.02	2.45	0.60	2.38	0.94	4.76	39.68
Oil content	14.76	2.58	17.34	4.16	1.61	3.84	0.85	7.30	31.69

Note: GV: Genotypic variance, EV: environmental variance, PV: phenotypic variance, PCV: Phenotypic coefficient of variation, ECV: environmental coefficient of variation, GCV genotypic coefficient of variance, H2B: board sense heritability, GA: genetic advance, GG: genetic gain

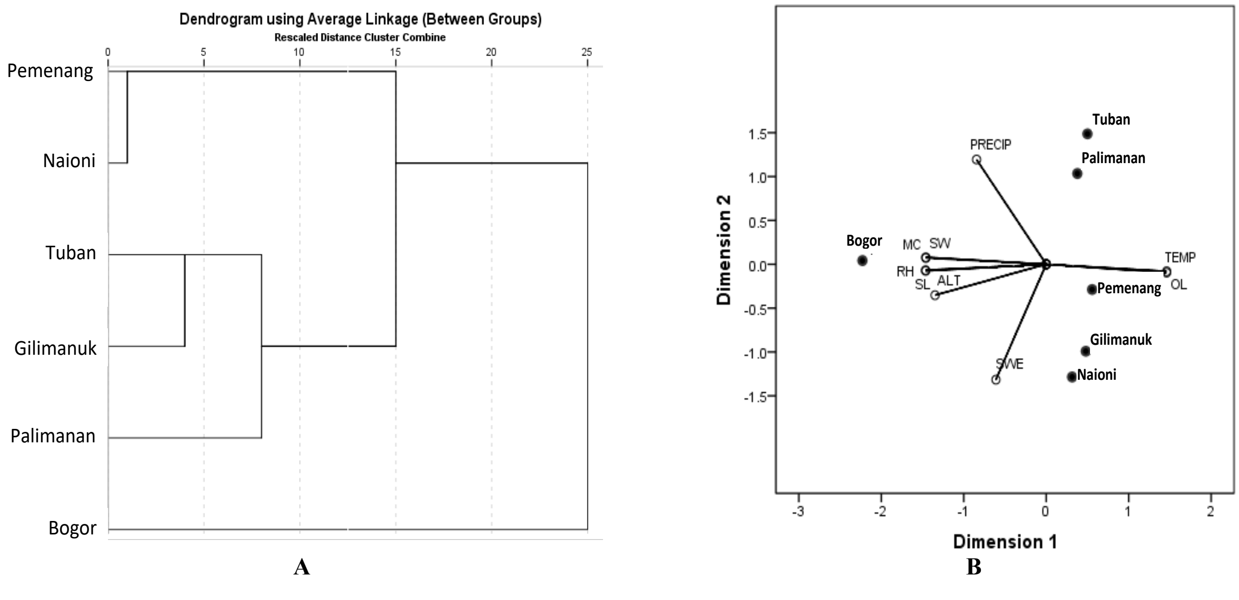

Figure 2. A. Cluster analysis graphical of 6 java olive populations based on fruit and seed morphophysiological and biochemical traits; B. Biplot of 6 java olive populations of seed width (SW), seed weight (SWE), seed length (SL), seed moisture content (MC), oil content (OL), altitude (ALT), precipitation (PRECIP), temperature (TEMP), and relative humidity (RH)

The oil content, seed production and seed characters (e.g. seed size, width and moisture content) are the important parameters in the plant improvement program especially in the correlation with biodiesel production. In this study, the seed production was not analyzed because the population samples are the nature populations which has different age and stand density. Biplot analysis including some agro-climate factors (altitude, precipitation, temperature and relative humidity) combined by seed traits revealed that oil content formed obtuse angle with seed length, seed width, seed weight, and seed moisture content. The oil content also formed the obtuse angle with some agro-climate factors, such as altitude, relative humidity and precipitation, while as the sharp angle was formed by oil content and temperature. The obtuse angle revealed a negative correlation between characters, and conversely the sharp angle showed a positive correlation (Nurhasybi et al. 2009; Rohandi et al. 2017). Pemenang population associated with oil content, indicating the population have high trait of oil content, and opposite each other with Bogor population that have high seed length, seed width and moisture content. The characteristic of oil content is opposite with the seed size and weight and the information could be a consideration in selection of java olive in correlation with the oil content as material for biodiesel production.

This study had some weaknesses, such as limited number of populations, no metabolomic approaches using LCMS-GCMS etc. Nevertheless, for preliminary evaluation, the study could recommend the java olive potential population for biodiesel feedstock. Some populations such as Pemenang and Naioni had higher oil content that can become option populations for cultivation. Moreover, location of cultivation should be a consideration in relation with cost of production and appropriate environment for biodiesel production. According BPPESDM (2018), based on economic feasibility, development of renewable energy is more focused in outside of Java island because the local production cost of fossil oil in outside of Java island is higher than the national production cost. This study recommended that the java olive cultivation is carried out in Nusa Tenggara islands, such as Lombok and Timor islands.

Conclusion

Java olive from 6 populations in Java, Bali, Lombok and Timor islands exposed the diversity in seed morphological and biochemical traits. Seed morphological and biochemical traits revealed significant differences, except for the germination capacity. Most of seed morphological traits were not significantly correlated with agro-climate factors (except for seed lenght and seed weight), while some of biochemical traits (seed moiture content, carbohydrate, fat and oil content) revealed significant correlation with some agro-climate factors. Genotypic variance and genotypic coefficient of variation were higher for several traits, such as fruit length, fruit width, carbohydrate, fat, protein and oil contents, compared with environment variance and environment coefficient of variance, indicating that the genetic has the higher contribution on the variance of these traits. High heritability values combined with high genetic gain was found for protein content, fruit width and oil content, showed the characters have highly genetic origin with a good amount of additive genetic effect (heritable components). Hierarchical cluster analysis separated the java olive populations based on the fruit, seed morphological and biochemical characteristics into three groups, i.e. Group 1: Pemenang and Naioni, Group 2: Tuban, Gilimanuk and Paliman, and Group 3: Bogor. Pemenang population associated with oil content, indicating the population have high trait of oil content, and opposite each other with Bogor population that have high seed length, seed width and moisture content. It exhibited that the existence of substantial variation can be utilized for genetic resource conservation and further genetic tree improvement programs of the species.

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