RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
51 (4) 390-404, 2019

GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSES IN *BACCAUREA ANGULATA* ACCESSIONS USING ISSR PRIMERS

GUNAWAN[1,2], T. CHIKMAWATI[1*], SOBIR[3] and SULISTIJORINI[1]

[1] Department of Biology, Bogor Agricultural University, Bogor, Indonesia
[2] Department of Biology, Lambung Mangkurat University, Banjarbaru, Indonesia
[3] Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
*Corresponding author's email: tchikmawati@yahoo.com
Email addresses of coauthors: gunawan@ulm.ac.id, sobir@ipb.ac.id, sulistijorini@gmail.com

SUMMARY

Genetic diversity and population structure analyses are important tools which can be used towards improving the plant species. *Baccaurea angulata* commonly known as red star fruit, belongs to the family Phyllantacheae, and used as an underutilized species and source for food and medicine. However, the genetic information about the said species are lacking because no such detailed studies have been carried out before. Present study aims to determine the genetic diversity in different accession of *B. angulata* which can provide important about the base line for further improvement and also to conserve for future. Twenty-five inter-simple sequence repeat (ISSR) primers were used to investigate the genetic diversity in 75 accessions of *B. angulata* collected from different locations in the Kalimantan and Natuna Islands, Indonesia. Analysis of 75 accessions of *B. angulata* through 25 ISSR primers produced 234 loci with 230 polymorphic loci (98.28%). The markers were highly informative with average polymorphic information content (PIC) of 0.90. The genetic diversity of *B. angulata* was categorized as high [Nei's genetic diversity (Na) = 1.20 – 1.70, effective number of alleles (Ne) = 1.12–1.46, Shannon's information index (I) = 0.07 – 0.26, gene diversity (H) = 0.11–0.38]. The genetic diversity within the population was higher (60%) than that among populations of different areas (40%). Cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) method which divided the *B. angulata* accessions into four different groups at a similarity coefficient index of 0.67, and to cluster the accessions according to their geographic distribution. Similarly, population genetic structure analysis using the Bayesian method grouped the 75 accessions of *B. angulata* into four gene pools. This study emphasizes that there is need for more attention to study such minor and underutilized species for utilization and future conservation.

Key words: *Baccaurea angulata*, genetic diversity, ISSR primers, population structure Kalimantan and Natuna Island, Indonesia

Key findings: Genetic diversity was studied in different accessions of *B. angulata* procured from Kalimantan and Natuna Islands, Indonesia. Based on analysis through inter-simple sequence repeat (ISSR) primers useful information and future strategies were developed for further improvement in *B. angulata* through breeding and conservation.

Manuscript received: May 9, 2019; Decision on manuscript: August 30, 2019; Accepted: September 4, 2019.

Communicating Editor: Dr. Himmah Rustiami

INTRODUCTION

Baccaurea is genus of flowering plant belonging to the family Phyllantacheae, and the said genus comprises over 100 species, distributed from Indomalesia to the West Pacific. *Baccaurea angulata,* locally known as *'belimbing dayak'* or *'belimbing hutan'* is an underutilized fruit, belongs to the family and is widely distributed in Borneo and Natuna Islands, Indonesia (Haegens, 2000; Gunawan *et al.,* 2018a). The said fruit in fresh form is usually locally used as a food and as traditional medicine in West Kalimantan, Indonesia. The phytochemical analysis of *B. angulata* fruit peelsshowed that it contains protein, carbohydrates, fiber, minerals, vitamin A, C, E, and anthocyanin compounds (Norazlanshah *et al*., 2015). Past studies reported that *B. angulata* juice can be used to prevent atherosclerosis, inhibit lipid peroxidase activity, and induce antioxidant enzyme activity (Mikail *et al*. 2014; 2016). It also has a potential to be used a source of natural ingredients for cancer treatment (Adam and Rasad, 2015) and has additionally shown antimicrobial activity (Momand *et al*., 2014).

The *B. angulata* naturally grows in the primary and secondary forests at 40 to 800 masl (Haegens, 2000). Importantly, more detailed information about this species is needed for its further development and conservation for future. Information about its distribution (Haegens, 2000; Gunawan *et al*., 2018a) and ecology (Gunawan *et al*., 2018b) revealed that the data pertaining to its genetic diversity and population structure have not yet been conducted and reported. Knowledge about the genetic diversity of a plant species is very important and exclusively needed for its genetic improvement, the development of agricultural and forest products and conservation strategies for future (Monfared *et al*., 2018).

Plant genetic diversity had been investigated using several markers including morphological, cytological, molecular, and biochemical (Mondini *et al*., 2009; Pu *et al*., 2016) ones. Molecular marking is an effective technique for analyzing genetic diversity. Certain molecular markers are often used for studying the plant genetic diversity, such as random amplified polymorphic (RAP) DNA, amplification fragment length polymorphism (AFLP), simple sequence repeat (SSR), next

generation sequencing, and inter simple sequence repeat (ISSR) (Mondini *et al*., 2009; Moulin *et al*., 2012). However, ISSR is a dominant marker and offers many advantages such as being highly reproducible, requiring a small amount of DNA, producing high genetic variability and costs low (Moulin *et al*., 2012).

Several studies have shown that the ISSR marker is an effective tool and has a significant role in

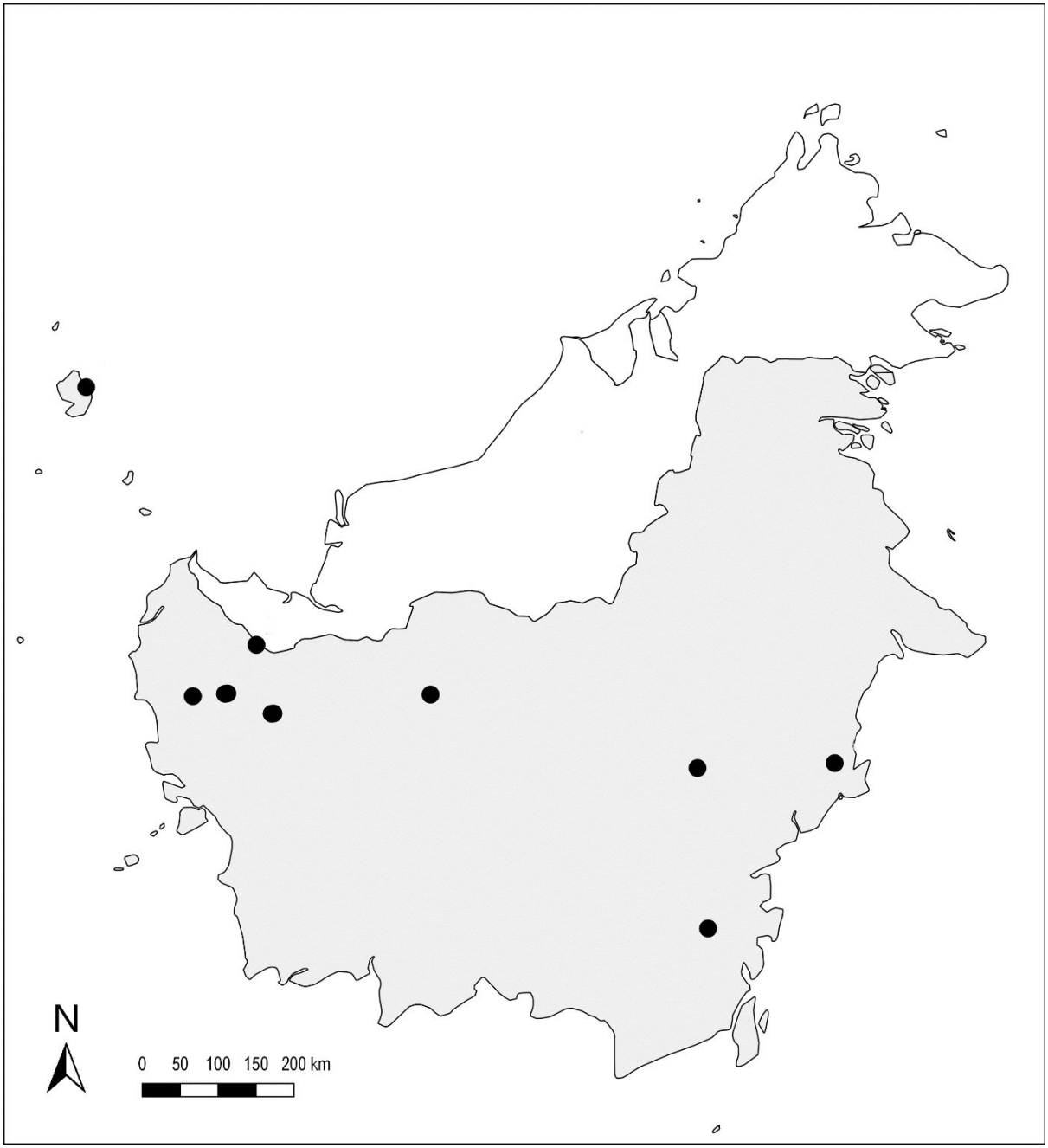

Figure 1. Map of origin and locations for the accessions of *B. angulata* collected from Kalimantan and Natuna Islands, Indonesia.

revealing the genetic diversity and population structure of *Salvadora persica* L., and *Capparis spinosa* L. (Monfared *et al*., 2018; Ahmadi and Saeidi, 2018). Therefore, the present study was planned with objective to study genetic diversity and population structure analyses in different accessions of *B. angulata* using ISSR markers collected from the Kalimantan and Natuna Islands, Indonesia. The said investigation is might be the first study report on the genetic diversity and population structure analysis in *B. angulata.*

MATERIALS AND METHODS

Plant material and DNA isolation

Seventy-five accessions of *B. angulata* (locally known as *'belimbing dayak'* or *'belimbing hutan'*) comprising 64 genotypes of *B. angulata* var. *angulata* and 11 accessions of *B. angulata* var. *globulus* were examined to study the genetic diversity and population structure analyses based on ISSR primers. Fresh leaf samples of all the accessions belongs to *B. angulata* var. *angulata* were collected from 10 localities of different regions of the Kalimantan and Natuna Islands while for genotypes of *B. angulata* var. *globulus*, the samples were obtained from Kalimantan Island, Indonesia especifically (Figure 1). All the accessions of *B. angulata* representing six different populations namely, Natuna (NTA), Entikong (ENT), Ngabang (NGA), Sintang (SIN), Samarinda (SMR), and Marajai (MRJ). Details about locality, sample size, latitude, and longitude are provided in Table 1. Each population was composed of plants found in the same district. The leaf samples of all the accession were stored dry at room temperature over silica gel at the time of collection. Genomic DNA was isolated from the silica dried plant tissue following the CTAB protocol (Doyle and Doyle, 1990). The quality and concentration of the extracted DNA were estimated at 1.5% agarose gel, stained in PeqGreen with a lambda DNA as a standard.

Polymerase chain reaction (PCR) amplification

A total of 25 ISSR primers were used for PCR amplification of the DNA templates (Table 2). The PCR mixtures were composed of a 25-µL final volume consisting of 2.5 µL of DNA, 1 µL of ISSR primer, 12.5 µL of DreamTaq Green PCR Master (0.4 mM of dATP, 0.4 mM of dGTP, 0.4 mM of dCTP, 0.4 mM of dTTP, and 4 mM if $MgCl_2$), and 9 µL of free nuclease water (Promega, Madison, WI, USA). PCR amplification was performed using the Swift™ Maxi model SWT-MY-BLC-7 (ESCO Corp., Portland, OR, USA). The PCR cycles consisted of 94°C for three minutes; followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C to 51.9°C (depending on the Tm of the primers) for 45 seconds, and extension at 72°C for 50 seconds; and then maintenance at 72°C for five minutes followed by cooling at 15°C for four minutes with little modification (Biyosi *et al*., 2010; Pardhe *et al*., 2011; Khajudparn *et al*., 2012; Hariri *et al*., 2017). Amplicons were separated by electrophoresis on 1.5% agarose gels submerged in 1 × TAE buffer and then stained with peqGreen (Tabin *et al.*, 2016). The DNAs were visualized and documented using UV transilluminator (WiseDoc, Germany).

Table 1. Origin and locations for accessions of *B. angulata* and their geographical coordinates.

Populations name	Locality	Sample size	Latitude	Longitude
NTA	Limau Manis	16	03° 59'59. 003"	108° 20'25. 006"
ENT	Entikong	8	00° 56'49. 793"	110° 21'31. 193"
	Sanggau	1	00° 07'54. 290"	110° 32'24. 425"
NGA	Ngabang	26	00° 22'21. 385"	110° 00'51. 802"
	Sompak	1	00° 20'21. 370"	109° 36'16. 542"
	Serumbi	1	00° 22'04. 207"	109° 59'11. 960"
SIN	Sintang	6	00° 21'31. 549"	112° 25'26. 518"
	Nangapinoh	1	00° 08'03. 721"	110° 33'40. 665"
SMR	Krus	3	00° 27'11. 228"	117° 13'04. 228"
MRJ	Marajai	11	02 ° 24'00. 043"	115° 42'57. 003"
	Barabai	1	00° 30'41.410"	115° 35'23. 896"

NTA: Natuna, ENT: Entikong , NGA: Ngabang, SIN: Sintang, SMR: Samarinda, MRJ: Marajai

Table 2. Inter-simple sequence repeat (ISSR) primers used for profiling the loci in accessions of *B. angulata*.

Primers	Sequences	Total number of bands	Number of polymorphic bands	Annealing temperature (°C)
ISSR 1	$(GT)_7ACAG$	9	9	49.9
ISSR 2	$(GA)_8A$	7	7	45.6
ISSR 3	$(CT)_7C$	7	7	45.6
ISSR 4	(TG)7CAC	9	8	45.7
ISSR 5	(GA)7CTC	8	7	45.6
ISSR 6	(AG)8YT	11	11	51.9
ISSR 7	$(GA)_9T$	11	11	48.6
ISSR 8	$(CT)_8T$	11	11	49.9
ISSR 9	$(GT)_8T$	6	6	50.9
ISSR 10	$(AG)_8T$	11	11	51.7
ISSR 11	$(AG)_8C$	11	10	49.7
ISSR 12	$(AC)_8T$	8	8	51.9
ISSR 13	$(AG)_8TC$	9	9	51.7
ISSR 14	$(AG)_8CTA$	7	7	48.4
ISSR 15	$(GA)_8CTC$	8	8	48.4
ISSR 16	$(CA)_8AGC$	10	10	45.7
ISSR 17	$(AC)_8CTA$	9	9	49.9
ISSR 18	$(AC)_8CTG$	12	12	49.7
ISSR 19	$(TG)_8AGT$	12	12	50.9
ISSR 20	$(AGG)_5$	11	11	46.4
ISSR 21	$(GAG)_5AC$	9	9	48.1
PKBT 2	$(AC)_8TT$	10	10	51.7
PKBT 3	$(GA)_9$	12	12	49.9
PKBT 7	$(AG)_8TT$	9	9	48.1
PKBT 12	$(GT)_9T$	7	7	53.9

Table 3. Details of ISSR primers used for diversity study in accessions of *B. angulata*.

Primers	Heterozygosity	Number of effective alleles	Shannon's information index	PIC
ISSR 1	0.28	1.44	0.41	0.86
ISSR 2	0.33	1.40	0.46	0.91
ISSR 3	0.25	1.37	0.38	0.92
ISSR 4	0.29	1.40	0.45	0.90
ISSR 5	0.27	1.42	0.36	0.87
ISSR 6	0.30	1.40	0.42	0.91
ISSR 7	0.27	1.35	0.38	0.91
ISSR 8	0.22	1.32	0.35	0.93
ISSR 9	0.29	1.46	0.42	0.86
ISSR 10	0.27	1.38	0.35	0.92
ISSR 11	0.29	1.46	0.42	0.86
ISSR 12	0.31	1.48	0.44	0.87
ISSR 13	0.31	1.45	0.42	0.88
ISSR 14	0.26	1.40	0.38	0.90
ISSR 15	0.28	1.48	0.42	0.85
ISSR 16	0.24	1.32	0.36	0.94
ISSR 17	0.21	1.37	0.35	0.88
ISSR 18	0.24	1.34	0.37	0.92
ISSR 19	0.16	1.23	0.26	0.94
ISSR 20	0.25	1.33	0.31	0.91
ISSR 21	0.25	1.39	0.38	0.90
PKBT 2	0.19	1.27	0.31	0.94
PKBT 3	0.21	1.32	0.34	0.92
PKBT 7	0.37	1.49	0.48	0.89
PKBT 12	0.25	1.44	0.39	0.86
Means	0.26	1.38	0.38	0.90

Data analysis

All ISSR fragments were scored manually and converted into binary data, based on the presence (1) or absence (0) of bands for each primer (Tabin *et al*., 2016). The data then were used to calculate the genetic similarity matrix by employing the simple matching coefficient using NTSys-PC version 2.1.1a (Rohlf, 1998). The average number of different alleles (Na), average number of effective alleles (Ne), genetic diversity, the Shannon information index (I), the number of polymorphic loci, and the percentage of polymorphic loci were analyzed using POPGENE version 1.32 (Yeh *et al*., 2000). Analysis of molecular variance (AMOVA) and genetic distance were performed using GenAlex 6.5 (Peakall and Smouse, 2012). The polymorphic information content (PIC) was calculated following the protocol of Botstein *et al*. (1980). The population structure was analyzed using STRUCTURE 2.3.4 with a burning period of 30,000 and 100,000 Markov Chain Monte-Carlo replications (Pritchard *et al*., 2000). The K value was set at 1 to 6 and each with 10 iterations. The best value of K (genetic groups) was determined using Evanno method (Evanno *et al.,* 2005) using the Harvester Structure Online Program V6.0.94 (Earl and Von-Holdt, 2012).

Table 4. Nei's genetic distance between pair-wise populations of *B. angulata*.

Populations	NTA	ENT	NGA	SIN	SMR	MRJ
NTA	0.000					
ENT	0.222	0.000				
NGA	0.276	0.218	0.000			
SIN	0.255	0.216	0.026	0.000		
SMR	0.243	0.237	0.138	0.123	0.000	
MRJ	0.193	0.233	0.227	0.218	0.178	0.000

NTA: Natuna, ENT: Entikong , NGA: Ngabang, SIN: Sintang, SMR: Samarinda, MRJ: Marajai

Table 5. Summary of population genetic diversity revealed through ISSR markers among six populations of *B. angulata.*

Populations	Na	Ne	I	H	PL (%)
NTA	1.57	1.39	0.21	0.31	57.26
ENT	1.54	1.34	0.19	0.29	54.70
NGA	1.68	1.12	0.09	0.17	68.80
SIN	1.54	1.19	0.13	0.21	54.27
SMR	1.20	1.12	0.07	0.11	20.94
MRJ	1.70	1.46	0.26	0.38	70.94
Means	1.53	1.30	0.15	0.24	54.48

NTA: Natuna, ENT: Entikong , NGA: Ngabang, SIN: Sintang, SMR: Samarinda, MRJ: Marajai
Na: Number of observed alleles, Ne: effective number of alleles, I: Shannon's information index, H: Nei's gene diversity, PL: Percentage of polymorphic loci

RESULTS

ISSR polymorphism and genetic diversity

Twenty-five ISSR primers produced a total of 234 bands, with 230 polymorphic bands (98.28%), and the average band for each primer was 9.4 bands (Table 2). The higher polymorphic band was produced when DNA was amplified using ISSR primers 18, 19, and primer PKBT-3, while the low polymorphic band was produced by ISSR primer 9. The polymorphic information of 25 ISSR primers is shown in Table 3. The mean of heterozygosity and the number of effective alleles were 0.26 and 1.38, respectively. Polymorphic information content (PIC) was used to determine the level of polymorphism of a molecular marker (Botstein *et al*., 1980). A polymorphism level is considered high if the PIC value is ≥ 0.50, medium if the PIC value is between 0.25 and 0.50, and low (PIC = ≤ 0.25) (Botstein *et al*., 1980). The PIC values of the observed markers ranged from 0.85 to 0.95, while the primer PKBT-2 produced the highest PIC value. The mean effective number of alleles was 1.4, with the highest number of effective alleles being produced by primer PKBT-7 (Ne = 1.5), while the lowest number of an effective allele being produced by the primer PKBT-2 (Ne = 1.27). The primer PKBT-7 additionally had the highest values of heterozygosity level and Shannon information index.

The parameters for genetic variation of *B. angulata* varied among six populations (Table 5). The number

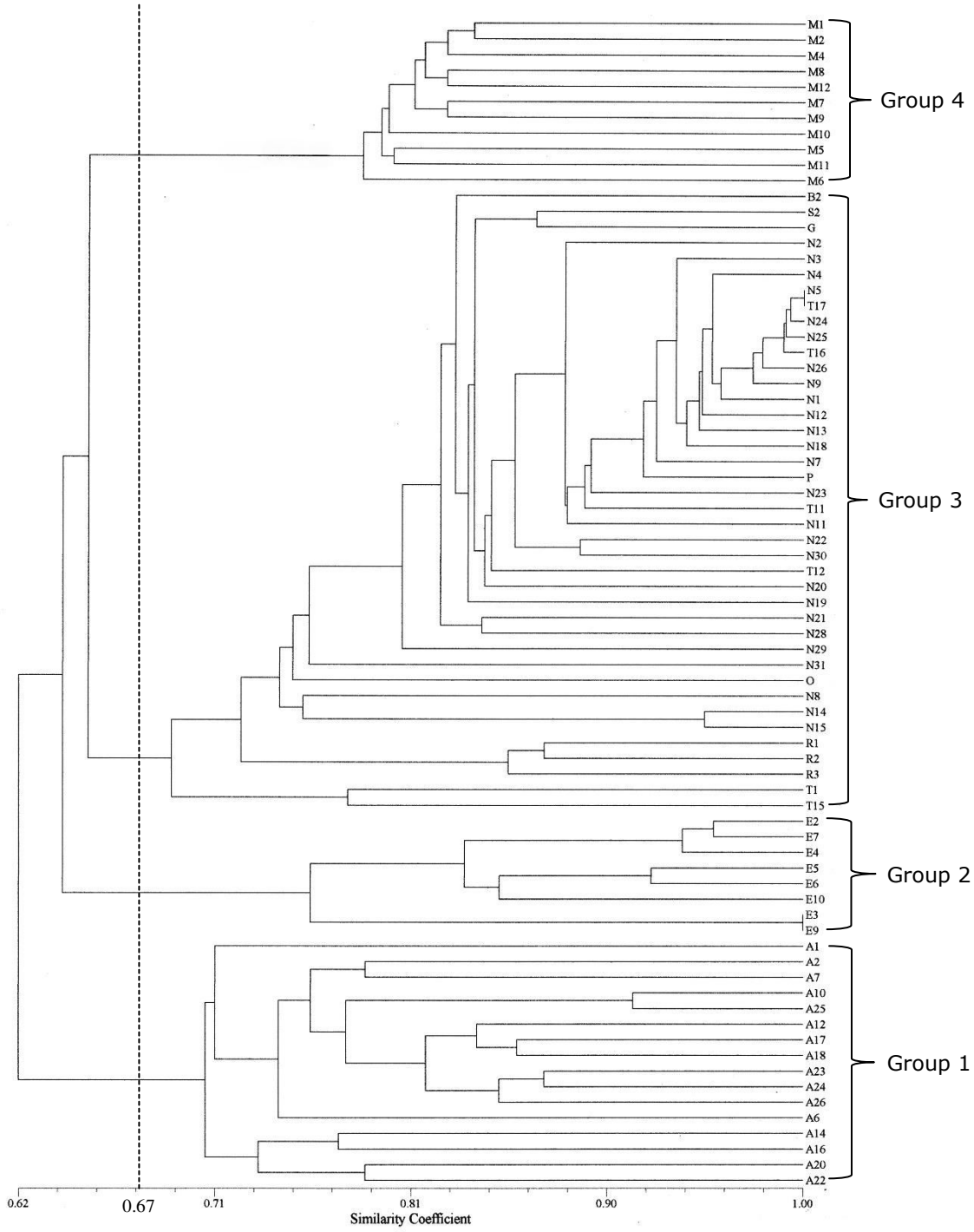

Figure 2. Dendrogram for 75 accessions of *B. angulata* based on ISSR data using simple matchingcoefficient and UPGMA method; group 1, 2, and 3: *B. angulata* var. *angulata* accession; group 4: *B. angulata* var. *globulus*; A. Limau Manis; B. Barabai; E. Entikong; G. Sanggau; M. Marajai; N. Ngabang; O. Nangapinoh; P. Sompak; R. Krus; S. Serumbi and T. Sintang.

of different alleles (Na) observed ranged from 1.20 to 1.70 with an average of 1.53. Additionally, the number of effective alleles (Ne) varied from 1.12 to 1.46 with an average of 1.30, Shannon Information index (I) ranged from 0.07 to 0.26 with an average of 0.15, and Nei's genetic diversity (H) observed ranged from 0.11 to 0.38 with an average of 0.24. Also, the percentage of polymorphic loci (PL%) varied from 20.94 to 70.94% with an average of 54.48%. Population Marajai (MRJ) showed the highest level of genetic diversity (I = 0.26, H = 0.38), while population Samarinda (SMR) exhibited the lowest (I = 0.11, H = 0.07).

Genetic distance can be used to analyze the genetic similarities between different populations. The values for highest and lowest genetic distance were owned by the populations i.e., NTA – NGA (0.27) and NGA – SIN (0.02), respectively (Table 4). AMOVA further showed that the genetic variation within a population was higher (60%) than among the populations (40%).

Cluster analysis

Cluster analysis using the UPGMA method based on the simple matching similarity coefficient grouped all the 75 accessions of *B. angulata* into four groups at the coefficient similarity of 0.6 to 1.0, according to sampling locations (Figure 2). Group I included 11 accessions of *B. angulata* var. *angulata* collected from Natuna Island with a similarity coefficient index of 0.71; group II consisted of 8 accessions of *B. angulata* var. *angulata* procured from Entikong with a similarity coefficient index of 0.75; and group III contained 40 accessions of *B. angulata* var. *angulata* gathered from Ngabang, Sompak, Nangapinoh, Sanggau, Barabai, Sarumbi, Sintang, and Krus, Indonesia with a similarity coefficient index of 0.69. The last group (group IV) consisted of 11 accessions of *B. angulata* var. *globulus* from Marajai, Indonesia with a similarity coefficient index of 0.78. Accessions of *B. angulata* which were originated from different locations were grouped into one group, and were fit in Nei's genetic distance.

Population structure analysis using the STRUCTURE program produced the best ΔK value = 4, indicating that 75 accessions of *B. angulata* were also divided into four genetic groups. The STRUCTURE program produced the same group as the UPGMA method. Most of the accessions were grouped according to their origin and geographic location and the accessions collected from same locality were grouped also in same genetic group. Group 1, denoted in blue color, consisted of the accessions collected from Natuna Island, Indonesia; group 2, denoted with yellow color included all the accessions from Entikong; group 3 symbolized with green color consisted of all the accessions originated from Ngabang, Sompak, Nangapinoh, Sanggau, Barabai, Serimbu, Sintang, and Krus, Indonesia; and the last group (group 4) shown with red color having the accessions from Marajai (Figure 3).

DISCUSSION

Genetic diversity in a species an important role and impact on the plant development programs and conservation efforts in the future, since the intensity of genetic diversity depends on the environmental

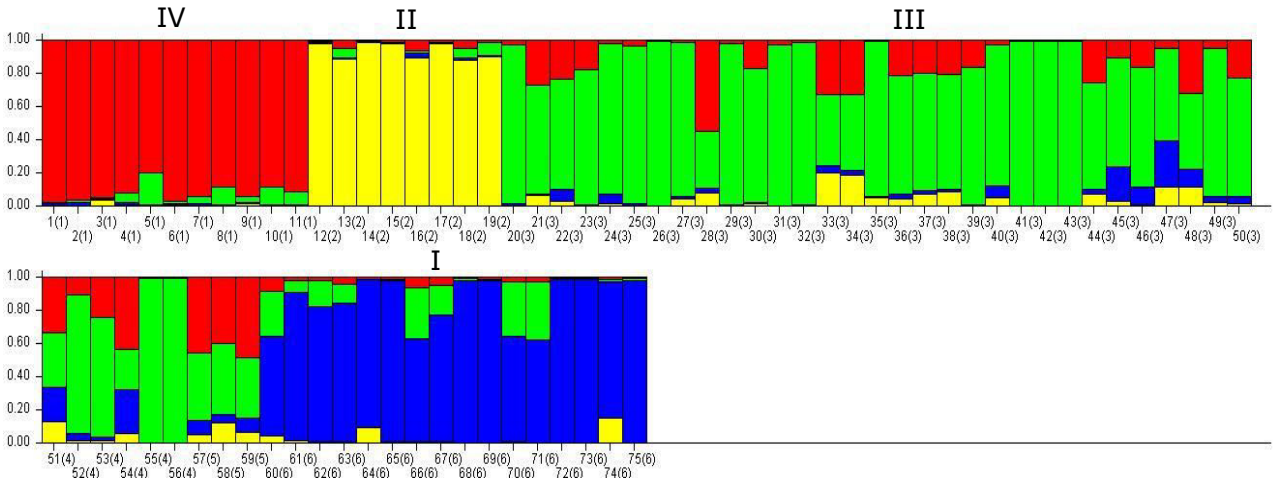

Figure 3. STRUCTURE plot for 75 accessions of *B. angulata* into K = 4. The different color bars referred to four different genetic groups.

conditions, human activities, breeding systems, evolutionary steps, and plant distribution (Rao and Hodgkin, 2002). The genetic diversity was evaluated in different accessions of *B. angulata* belongs to Kalimantan and Natuna Islands, Indonesia. Knowledge of the genetic diversity of a species can provide an idea of how the species responds to environmental and climatic changes in its habitat.

Molecular markers are an effective option for studying genetic diversity because of having no influence from epistatic effects and environmental factors (Ni *et al*., 2018). Molecular markers has a wide potential and application in varietal and parentage identification through genetic mapping (Chaudhary *et al*., 2018). In this study the ISSR markers were used for evaluating the diverse genetic and population structure of the accession of *B. angulata* collected in the Kalimantan and Natuna Islands, Indonesia. However, ISSR primers are of low cost, its ability to produce a high polymorphic level, and its ease of being used. The said markers have also been successfully employed to identify the genetic variation in other members of Phyllantacheae family i.e., *Phyllanthus* (Senapati *et al*., 2011).

The PIC values showed the level polymorphism or informativeness of the molecular marker. The values of PIC depend on the number of detectable alleles, and the distribution of their frequency is equivalent to gene diversity (Ghehsareh *et al*., 2015). The PIC value of a marker is categorized as informative when the PIC value is ≥ 0.5 and highly informative when the PIC value is > 0.7 (Pourkhaloee *et al*., 2018). The mean PIC value of this study was 0.90. These results indicated that the ISSR markers are capable of producing high polymorphic alleles and is highly informative for analyzing the genetic diversity of *B. angulata*.

Information on genetic diversity and population structure are necessary for selecting plant materials for cultivation and to use as stock for further development in the species

(Kuwi *et al*., 2018). Moreover, this information can also be used for conservation of the genotypes for future generations. Present study revealed that average value of Shannon's information index was 0.3, Nei's genetic diversity (H) was 0.19, and the polymorphic loci was 58.62% (Table 5). Some populations have different values of Nei's genetic diversity (H) and Shannon's information index (I). The information of 'H' and 'I' indices for each population is very crucial for managing the future breeding strategies (Kumar and Agrawal, 2017). The H and I index were two important indicators for revealing genetic diversity in *Marphysa sanguinea* (Zhao, 2015). The value of Shannon information index ranges from 0 to 1, with a value far from 0 indicating high genetic diversity (Silva *et al*., 2015). Thus, the genetic diversity in the present accessions of *B. angulata* is categorized as high. The value of genetic diversity in natural populations may be caused by ype of pollination, dispersal propagation material (seeds and other vegetative propagules) (Tabin *et al*., 2016), and habitat diversity (Nam *et al*., 2016).

Heterozygosity can be used as a parameter for determination of the genetic diversity within a population (Nei, 1978). The average value of heterozygosity in the populations of *B. angulata* was 0.26 which suggested that the genetic variation within the accessions of *B. angulata* is considered high (Table 3). Past finding revealed that higher heterozygosity values suggested that the high genetic diversity (Vilas *et al*., 2015). These results are in accordance with the finding of AMOVA analysis that revealed genetic variation within the population of *B. angulata* was higher than among the populations. This species is a dioecious plant, which makes it easy to have cross-pollination. Results further authenticated that allele exchanges within the population were more prevalent than among populations. The level of genetic variation within a population is also affected by genetic drift, mutation, and environmental conditions (Young *et al*., 2000). Variations within and between the population are very important for planning the strategies for conserving the plant genetic resources for future use (Tiwari *et al*., 2016). Based on the values of the AMOVA the *in-situ* conservation strategy is proposed is to avoid the losses to *B. angulata* germplasm. In addition, the information on the genetic variation within a population can be used in preparing the future breeding strategies for *B. angulata.*

Genetic distance intensities showed the size of the substituted alleles in each locus during the evolutionary process between two populations or species (Woldesenbet *et al*., 2015). The Nei's genetic distance between pairwise populations of *B. angulata* has the higher value for NTA and NGA populations, and the lower for NGA and SIN populations. Thus the high Nei's genetic distance indicated low genetic similarity between both populations (Kuwi *et al*., 2018). Past findings are in agreement with present results that on dendrogram the *B. angulata* showed that NTA population was far from NGA population. The close genetic distance between two populations can be caused by interspecific hybridization during the evolutionary process which favors alleles sharing (Cidade *et al*., 2013).

Cluster analysis using a simple matching coefficient and the UPGMA method revealed that 75 accessions of *B. angulata* were divided into four groups at a similarity coefficient of 0.69. Grouping using the STRUCTURE analysis also resulted in a similar pattern, which grouped all the *B. angulata* accessions into four genetic gene pools. The accession in groups 1, 2, and 4 had a correlation with the origin location. Result further indicated that each location of accession has specific ecological conditions. Group three contained some accessions from different locations (i.e., Ngabang, Sompak, Nangapinoh, Sanggau, Barabai, Serimbu, Sintang, and Krus). However, the accessions from Barabai, Nangapinoh, Sanggau, and Serumbi were colledted from the yard and roadside. Bayesian model was used to classify accessions into different genetic groups based on the genetic variation and diversity (Pourkhaloee *et al.*, 2018).

Presence of some accessions in the locations was probably due to human intervention in the seed dispersal and domestication, and human activities also contributed to exploration of plant genetic diversity. A previous study on *Capparis spinosa* L. reported that human activities through seed dispersal caused genetical admixes within population (Ahmadi and Saeidi, 2018). Grouping some accessions from different locations may also indicate that the accessions share the same allelic pool (Souza *et al*., 2010). Similarly, Kuwi *et al*. (2018) reported that the same accessions of *Urochloa* grass from different geographical origins in Tanzania could be grouped together. In addition, similar microclimate conditions (e.g., humidity, temperature, and light intensity) in the origin growing location of the accessions in group three also have an influence on genetic diversity. Thus, the differentiation of genetic diversity is the result of adaptation to a certain environmental condition as a response to changes in the environment and the surrounding climate. Previous studies revealed that *Boehmeria nivea* plants will transmute the alleles as an adaptation effort to environmental changes (Ni *et al*., 2018). Data on the genetic diversity and grouping correlations with environmental differences have also been reported in the previous studies (Ahmadi and Saeidi, 2018).

CONCLUSION

The ISSR markers used in the present study were found very effective in assessing the genetic diversity in different accessions of *B. angulata*. Cluster analysis using the UPGMA method and population structure, divided all the accessions of *B. angulata* into four groups. Cluster three was the largest group consisted of some accessions of *B. angulata* from different geographical origins, which revealed that the genetic admixture was also the highest among all the groups. Genetic diversity and population structure analyses could play an important role in the management of conservation and bio-prospecting of *B. angulata* accessions in the future.

ACKNOWLEDGEMENT

The authors would like to thank to Ministry of Research, Technology, and Higher Education for providing BPPDN scholarship. Present research is supported by *Penelitian Desertasi*

Doktor (PDD) Grant 2018. I also pay thanks to all parties involved with me in present study.

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