Genetic diversity analysis of *Piper betle* from eight accessions of Indonesia based on SRAP markers

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Abstract. *Nabilla S, Safira UM, Puspita PJ, Subositi D, Maruzy A, Artika IM. 2021. Genetic diversity analysis of* Piper betle *from eight accessions of Indonesia based on SRAP markers. Biodiversitas 22: 3401-3408.* Leaves of betel vine (*Piper betle* Linn.) have been used as a traditional medicine in various regions in Indonesia. However, the genetic diversity of this plant has not been well recorded. Considering its diverse usage in traditional and folk medicine, it is essential to analyze and document the genetic diversity of *Piper betle* L. with the aim to collect the scientific data of betel vine genetics in Indonesia. This study aims at analyzing the population structure and genetic diversity of betel vine from the Singkil, Gayo Serbajadi, Baduy, Bandung, Hutan, Kalisusu, Kaledupa, and Mekongga accession groups of Indonesia using the sequence-related-amplified polymorphism (SRAP) marker technique. Genomic DNA was isolated from each accession and then used as a template for DNA amplification using PCR. As many as 16 SRAP primer combinations were screened for the genetic diversity analysis. Optimization of primer combinations resulted in 7 selected combinations based on their ability to generate clear amplification patterns and polymorphic bands. These were then employed for genetic diversity analysis. The genetic distance dendrogram showed the lowest similarity coefficient was 0.62 and that the betel vine grouping pattern was not based on genotype. The Singkil population had the highest genetic diversity and the Hutan population had the lowest. The mean value of Nei's genetic diversity index was 0.0985, while Shannon's information index was 0.01459 and the percentage of polymorphic loci was 25.81. This study concluded that the level of betel vine diversity is low.

Keywords: Betel vine, genetic diversity, *Piper betle*, SRAP

INTRODUCTION

Betel vine (*Piper betle* Linn.) is a perennial dioecious plant with leaves possessing numerous bioactive components (Patra and Pradhan 2018). It has been widely cultivated and used in traditional herbal medicines in many Asian countries, such as India, China, Taiwan, Thailand, Srilanka, Bangladesh, Burma, Nepal, Indonesia, etc. (Ali et al. 2010; Rai et al. 2011; Durani et al. 2017; Andrianto et al. 2020). Traditionally, betel vine leaves are used as a mouth freshener, an anthelmintic, an anti-allergic, an antiacne, in contraception and to treat eye infection and postpartum infection (Ghosh and Bhattacharya 2005; Andrianto et al. 2020). In addition, the leaves of *Piper betle* L. are also reported to have antioxidant, anti-inflammatory, anti-apoptotic, anti-cancer and anti-microbial activities (Das et al. 2016). Studies on profiling phenolic compounds of *Piper betle* L showed that the leaves of this plant contain twelve phenolic compounds, consisting of a phenylpropanoid, five cinnamoyl, and six flavonoids, with hydroxychavicol as the main compound (Ferreres et al. 2014).

Information on plant genetic diversity is very useful for the development of new or improved cultivars with more desirable characteristics (Govindaraj et al. 2015). In addition, genetic diversity analysis is important for our understanding of genetic traits, population genetics, molecular breeding and disease control programs (Li et al. 2014). Several studies have been conducted in order to analyze the molecular genetic diversity of *Piper betle* L. For example, Verma et al. (2004) employed the random amplified polymorphic DNA (RAPD) method to analyze the genetic diversity of several Indian betel vine varieties. Based on eleven RAPD primers, they found that the varieties belonging to the Kapoori group were genetically the most diverse (Verma et al. 2004). Data on genetic diversity of Indonesian *Piper betle* L. are limited. The present study was aimed at analyzing the genetic diversity of *Piper betle* L. amongst 8 betel vine accessions from Indonesia based on sequence-related-amplified-polymorphism (SRAP) markers. SRAP markers have been used to amplify coding regions of DNA with primers targeting open reading frames. These markers are found to be robust, highly variable, and user-friendly. SRAP markers find wide applications in agronomic and horticultural fields as well as in the analysis of genetic diversity of large germplasm collections (Li et al. 2014; Robarts and Wolfe 2014).

MATERIALS AND METHODS

Preparation of samples

As many as 20 samples from 8 accessions, from 4 different regions of Indonesia (Table 1) were employed in this study. Samples of 0.04 to 0.05 g dry leaf were placed in a labelled zip-lock plastic bag. The samples were stored at –20ºC overnight or for 1 hour in an ultralow temperature freezer. Each sample was then ground using a sterile mortar and pestle.

Isolation of DNA

DNA isolation was carried out using the Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit #K0791, following the manufacturer's instructions. As much as 0.04-0.05 g of sample was ground in the presence of liquid nitrogen. After washing with STE solution (0.25 M sucrose, 0.03 M Tris, 0.05 M EDTA) or HEPES buffer, the mixture was centrifuged twice at 2000 x g for 10 min followed by centrifugation at 3000 x g for 5 min. The pellet was transferred into a 1.5 mL microcentrifuge tube followed by the addition 350 μL lysis buffer A, 50 μL lysis buffer B and 20 μL RNase A. The mixture was vortexed for 1 min followed by incubation at 65ºC for 10 min with occasional vortexing. Then 130 μL of precipitating solution was added and the mixture was homogenized, incubated in ice for 5 min and then centrifuged at ≥20.000 x g for 5 min. A 500 μL of supernatant was transferred into a new microcentrifuge tube followed by the addition of 400 μL plant gDNA-binding-solution and 400 μL ethanol 96% v/v. As much as 600 μL of the mixture was transferred to the spin column and then centrifuged for 1 minute at 6000 x g. The flow-through solution was discarded and the remaining mixture was applied onto the same column and centrifuged again for 1 minute at a speed of 6000 x g and the flow-through was discarded. As much as 500 μL of wash buffer I was added to the column followed by centrifugation at 8000 x g for 1 min and the flow-through solution was discarded. A total of 500 μL of wash buffer II was added to the column followed by centrifugation. The flow-through solution was discarded and the purification column was placed back into the tube and re-spun for 3 minutes at a maximum of ≥20,000 x g and the flow-through solution was discarded. The column was inserted into a sterile 1.5 mL microcentrifuge tube. A total of 100 μL of elution buffer was added to the middle of the column followed by incubation for 5 minutes at room temperature. The column was centrifuged for 1 minute at a speed of 8000 x g. A second elution was performed in the same way. To determine purity, the absorbance of the isolated genomic DNA was measured at a wavelength of 260 nm and 280 nm ($\lambda 260 / 280$). The DNA was stored at -20°C.

Amplification of DNA

DNA amplification was carried out using the polymerase chain reaction (PCR) technique employing SRAP primers. The SRAP primers used consisted of 4 forward primers (Me1-Me4) and 4 reverse primers (Em1-Em4), as listed in Table 2. In this study, 16 primer combinations (Table 3) were generated and these were then screened for use in the analysis based on their ability to generate clear polymorphic bands from all of the betel vine accessions.

DNA amplification was conducted according to a modified method of Li and Quiros (2001). PCR was carried out in a 15 μl reaction volume containing 1 μl of DNA template DNA (20 ng/ μl), 0.7 μl forward primer (10-20 μM), 0.7 μl reverse primer (20 μM), 7.5 μl PCR mixture, and 5.1 μl nuclease-free water. Our PCR procedures included initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 1 min, phase I primer annealing at sequentially increasing temperature from 33.0°C, 33.7°C, 35.2°C, 37.7°C, 40.5°C, 42.4°C, 44.0°C, to 45.0°C for 1 min, and primer extension at 72°C for 1 min, which were conducted for 5 cycles. These were followed by the second PCR cycle with denaturation at 94°C for 1 min and phase II primer annealing at increasing temperature from 45°C, 45.9°C, 47.5°C, 50.0°C, 53.0°C, 55.3°C, 57.0°C, to 58°C for 1 min, primer extension at 72ºC for 1 min, which was conducted for 35 cycles. The final step was to hold the reaction at 4ºC for 15 min (modified from Li and Quiros 2001).

Electrophoresis of DNA

Separation of genomic DNA, or DNA fragments, was carried out essentially using a standard agarose-gel electrophoresis method (Montero-Campos et al. 2015; Lee et al. 2012). Genomic DNA was separated using 1% (w/v) agarose gel at 100 Volts for 30 min in 45 mM Tris-borate, 1 mM EDTA (TBE) running buffer. PCR products were separated using 2.5% (w/v) agarose gel at 50 Volts for 60 min in TBE running buffer. DNA molecules were stained using PeqDye and visualization was conducted using a UV transillumination or Gel Doc™ EZ System.

Table 1. Samples used

Sample code	Accession	Province
PB-AC-A1	Singkil	Aceh
PB-AC-E1	Singkil	Aceh
PB-AC-E2	Singkil	Aceh
PB-AC-E3	Singkil	Aceh
PB-AC-E4	Singkil	Aceh
PB-AC-F1	Gayo Serbajadi	Aceh
PB-AC-G1	Gayo Serbajadi	Aceh
PB-RI-B1	Hutan	Riau
PB-RI-B2	Hutan	Riau
PB-JB-A2	Baduy	West Java
PB-JB-A3	Baduy	West Java
PB-JB-A4	Baduy	West Java
PB-JB-B4	Bandung	West Java
PB-LG-A1	Kalisusu	Southeast Sulawesi
PB-LG-A4	Kalisusu	Southeast Sulawesi
PB-LG-B2	Kaledupa	Southeast Sulawesi
PB-LG-C1	Mekongga	Southeast Sulawesi
PB-LG-C2	Mekongga	Southeast Sulawesi
PB-LG-D1	Mekongga	Southeast Sulawesi
PB-LG-D2	Mekongga	Southeast Sulawesi

Table 2. SRAP primers used

Primer code	Type	Primer sequence (5'→3')
Me1	forward	TGA GTC CAA ACC CGA TA
Me2	forward	TGA GTC CAA ACC CGA GC
Me3	forward	TGA GTC CAA ACC CGA AT
Me4	forward	TGA GTC CAA ACC CGA CC
Em1	reverse	GAC TGC GTA CGA ATT AAT
Em2	reverse	GAC TGC GTA CGA ATT TGC
Em3	reverse	GAC TGC GTA CGA ATT GAC
Em4	reverse	GAC TGC GTA CGA ATT TG

Table 3. SRAP primer combinations generated from the SRAP primer sets

No.	Primer combination	No.	Primer combination
1	Me1-Em1	9	Me3-Em1
2	Me1-Em2	10	Me3-Em2
3	Me1-Em3	11	Me3-Em3
4	Me1-Em4	12	Me3-Em4
5	Me2-Em1	13	Me4-Em1
6	Me2-Em2	14	Me4-Em2
7	Me2-Em3	15	Me4-Em3
8	Me2-Em4	16	Me4-Em4

Analysis of genetic diversity

DNA fragments that were generated from each betel vine accession using each primer combination were measured and documented. The existence of bands from each betel vine accession was scored (1 for presence, 0 for absence). The similarity index was calculated using the Dice similarity index formula. Cluster analysis and dendrogram construction were carried out using the unweighted pair group method using the arithmetic mean (UPGMA). The coordinate principle of each individual was analyzed. Population genetic diversity was analyzed. Data obtained was analyzed using the NTSYS version 2.02 and PopGen 32 computer programs.

RESULTS AND DISCUSSION

Concentration and purity of genomic DNA

The concentration and level of purity of the isolated genomic DNA is shown in Table 4.

Selected SRAP primer combination

Sixteen SRAP primer combinations were screened based on their ability to generate a clear amplification pattern and show polymorphism in all betel vine tested using the gradient-PCR technique at various temperature combinations. Results showed that 7 primer combinations, i.e., combinations 2 (Me1-Em2), 3 (Me1-Em3), 5 (Me2-Em1), 7 (Me2-Em3), 13 (Me4-Em1), 14 (M24-Em2), 15 (Me4-Em3) have the ability to amplify betel vine DNA and generate clear bands at various annealing temperatures as shown in Figure 1. These primer combinations were selected for genetic diversity analysis. The other combinations did not produce sufficiently clear bands.

When primer combinations 5, 6, and 7 were further tested using sample PB-LG-C1, the results were consistent, in that, primer combinations 5 and 7 generated clear polymorphic bands while primer combination 6 did not. Primer combination 5 generated bands at annealing temperatures of 42.4ºC and 55.3ºC. Similarly, primer combination 7 generated bands at annealing temperatures of 45.0ºC and 58.0ºC (Figure 2).

Genetic diversity based on SRAP markers

The extent of genetic diversity of the betel vine was revealed by the 7 selected SRAP primer combinations. The number of bands generated by each primer combination ranged from 3 to 7. The size of the bands ranged from 75 to 700 bp. The total bands generated by the 7 primer combinations were 31 with average 3.43 bands produced by each primer combination. The total polymorphic bands produced were 24 with average 3.43 polymorphic bands generated by each primer combination. The polymorphic bands were identified based on their size. It was observed that the proportion of the polymorphic bands was consistently above 66.7%. Among the primer combinations employed, primer combination 7 generated the highest level of polymorphic bands, being 85.71%. Overall, the primer used generated an average of polymorphic bands of 74.86% as shown in Table 5.

The number of amplifiable amplicons ranging from 3-7, with a mean of 4.43 amplicons per primary combination (Table 5) was lower than the study conducted by Bhatt et al. (2017). These results indicated that there was a substantial amount of unamplified polymorphism at the SRAP locus studied.

Table 4. Concentration and purity of isolated genomic DNA from betel vine

Sample code	DNA Concentration (ng/µL)	Purity ($A_{260/280}$)
PB-AC-A1	7.3	2.39
PB-AC-E1	48.2	1.98
PB-AC-E2	23.6	1.95
PB-AC-E3	57.4	1.92
PB-AC-E4	72.1	1.87
PB-AC-F1	40.6	1.92
PB-AC-G1	58.6	1.75
PB-RI-B1	5.4	2.36
PB-RI-B2	13.3	1.77
PB-JB-A2	56.6	1.87
PB-JB-A3	42.8	1.94
PB-JB-A4	85.0	1.82
PB-JB-B4	83.0	1.80
PB-LG-A1	70.2	1.80
PB-LG-A4	48.4	1.89
PB-LG-B2	63.8	1.80
PB-LG-C1	123.4	1.77
PB-LG-C2	106.3	1.79
PB-LG-D1	91.9	1.78
PB-LG-D2	94.4	1.73
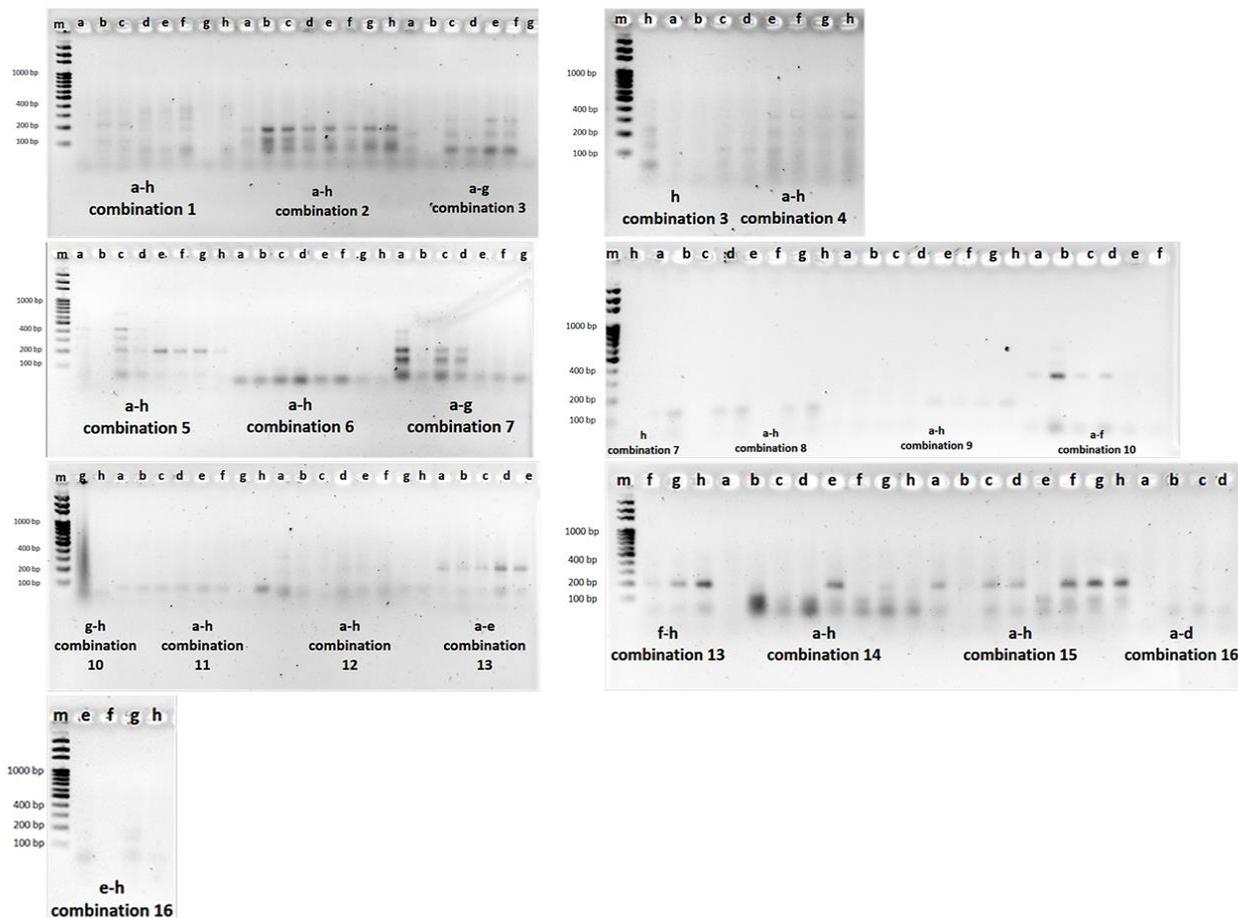
Figure 1. Electrophoregrams showing optimization of PCR conditions for 16 SRAP primer combinations. a: annealing temperature I 45.0˚C, annealing temperature II 58.0˚C; b: annealing temperature I 44.0˚C, annealing temperature II 57.0˚C; c: annealing temperature I 42.4˚C, annealing temperature II 55.3˚C; d: annealing temperature I 40.5˚C, annealing temperature II 53.0˚C; e: annealing temperature I 37.7˚C, annealing temperature II 50.0˚C; f: annealing temperature I 35.0˚C, annealing II 47.5˚C; g: annealing temperature I 33.7˚C, annealing temperature II 45.9˚C; h: annealing temperature I 33.0˚C, annealing temperature II 45.0˚C.

The number of DNA bands amplified from the PCR technique was 31 bands, with 24 polymorphic bands. Polymorphism results from the variations in the DNA sequences and it is positively correlated with genetic diversity (Abdelaziz et al. 2020). In addition, high polymorphism obtained indicates that the techniques used in evaluating the samples genetic diversity are efficient (Hamouda 2019). The most informative primary combination among the 7 primary combinations used was the combination of 7 and 2 with the resulting polymorphic band percentage of 85.71% and 83.33% respectively (Table 5). The primer that amplified the most was primer 7 (Figure 3). The electropherogram was then translated into binary form (Table 6). Code 1 designates the occurrence of DNA bands and code 0 the absence of bands.

Genetic diversity based on a selected SRAP primer combination

As primer combination 7 produced the most polymorphic bands, it was further tested using samples PB-JB-A3, PB-AC-E1 and PB-AC-E4. Results of PCR amplification are shown in Figure 3. The amplicon bands were then translated into binary codes, 1 for presence and 0 for absence of bands as shown in Table 6.

Genetic relationship of betel vine based on SRAP marker (cluster analysis)

Results for the betel vine accessions genetic relationship showed the genetic similarity coefficients ranged from 0.62 to 0.94. This indicated that the genetic diversity of the betel vine ranged from 6 to 38%. The genetic similarity of the betel vine formed two major groups, group I and II, with coefficients of 0.62 and 0.724 respectively. The two groups consisted of 3 distinct clusters, A, B, and C as shown in Figure 4. Group I consisted of two small clusters (A and B) and group II consisted of one cluster (C). Group A includes 10 populations from 5 regions, Aceh, Riau, West Java, and Sulawesi. Group B consisted of 4 populations from Sulawesi. Group C consisted of 6 populations from West Java and Aceh. Principal Component Analysis showed that the 20 betel vine populations formed 3 main clusters, A, B, and C, with cumulative diversity values of 84.20% (PC1 73.61% and PC2 10.59%). As shown in Figure 5.
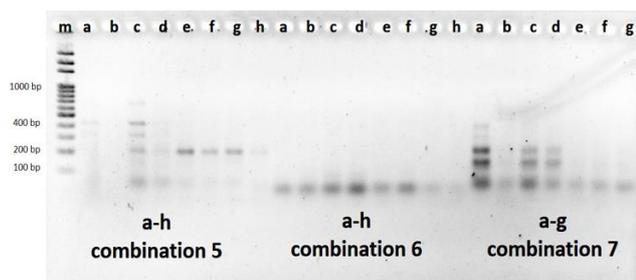
Figure 2. Electrophoregram of PCR products for temperature optimization of primer combinations 5, 6 and 7. Annealing temperature (a) (I:45.0˚C, II:58.0˚C); (b) (I:44.0˚C, II:57.0˚C); (c) (I:42.4˚C, II:55.3˚C); (d) (I:40.5˚C, II:53.0˚C); (e) (I:37.7˚C, II:50.0˚C); (f) (I:35.2˚C, II:47.5˚C); (g) (I:33.7˚C, II:45.9˚C); (h) (I:33.0˚C, II:45.0˚C).
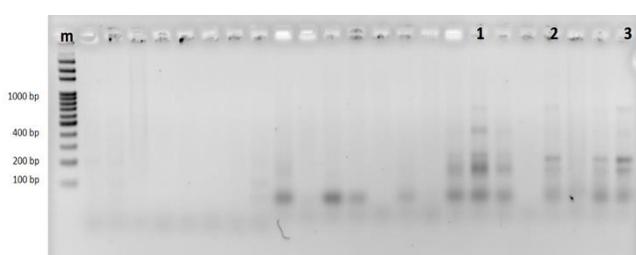
Figure 3. Electropherogram of PCR amplicons of samples PB-JB-A3 (1), PB-AC-E (2) and PB-AC-E4 (3) using primer combination 7. The genomic DNA of the samples PB-JB-A3, PB-AC-E1 and PB-AC-E4 were isolated using the Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit #K0791. Each of the isolated genomic DNA samples was used as a template for SRAP PCR amplification using primer combination 7.

Table 5. Profile of bands generated from PCR amplification of seven primer combinations using SRAP markers

Primer combination	Band size (bp)	No. of bands	No. of polymorphic bands	Percentage of polymorphic band (%)
2	75-260	6	5	83.33
3	75-140	3	2	66.67
5	75-600	5	4	80.00
7	75-700	7	6	85.71
13	75-200	3	2	66.67
14	75-200	3	2	66.67
15	75-200	4	3	75.00
Total		31	24	74.86
Average		4.43	3.43	

Clustering using principal component analysis (PCA) supported the results of analysis using statistical software NTSYSpc version 2.02 which grouped the samples into the 3 clusters A, B, and C. Cluster A featured the generation of

bands (75 bp) by all primer combinations. Cluster B produced bands by primer combinations 2 (80 and 150 bp), 13 (80 and 200 bp), and 15 (200 bp). Cluster C generated bands by primer combination 5 (200 bp), 7 (80, 150 and 210 bp), 13 (200 bp), 14 (100 and 200 bp) and 15 (200 bp). Both dendrogram and matrix plot formed 3 clusters regardless of the sample origin.

The data in Figure 4 shows the lowest morphological similarity coefficient (0.94) with the farthest genetic distance (0.62) resulting in a diversity percentage that was low (38%). Low diversity can be due to the similarity of genotype species and vegetative reproduction. The highest similarity coefficient (0.94) was in the two genotypes originating from the Hutan ethnic group and the other two genotypes originated from the Mekongga accession. A small genetic distance indicates that the individual is closely related. Another genetic distance (0.94) was also found in one genotype originating from the Gayo Serbajadi and Singkil ethnic groups. However, another sample that also comes from Singkil is in sub-group C and has a close relationship with Baduy.

The PCA results support the phylogenetic tree by forming 3 large groups (Figure 5). The fact that the grouping of the 3 major PCA groups is not based on the geographic origin of the genotype. This result is in line with a study by Xiang et al. (2020) who used PCA based on SRAP amplification results in analyzing genetic diversity of *Distylium chinense*. Principal Component Analysis supports the result of the UPGMA clustering above.

Genetic diversity within a plant population can occur even if plant material comes from the same accession. Location differences as to where it grows can affect the diversity of plant species genetically, morphologically, and the content of active compounds (Wu et al. 2013). Betel vine originating from Aceh, the Singkil ethnic group, is grouped with betel vine from West Java, the Baduy accession means that the two accessions are genetically the same but have different names. This might be caused by genetic drift, local adaptation, and founder effect processes (Kumar and Agrawal 2019).

Population genetic structure of betel vine of Indonesia

The variations observed between the populations could be influenced by the fluctuating macro and microclimatic habitat conditions (Agarwal et al. 2019). Parameters of the genetic structure analysis include the number of different alleles (Na), the number of effective alleles, Nei genetic diversity (h), the value of Shanon information index (I) and the percentage of polymorphic loci (PPL). The results of the population genetic structure as analyzed using PopGen 32 are shown in Table 7.

Table 6. Binary profile of PCR amplification products using SRAP primer combination 7

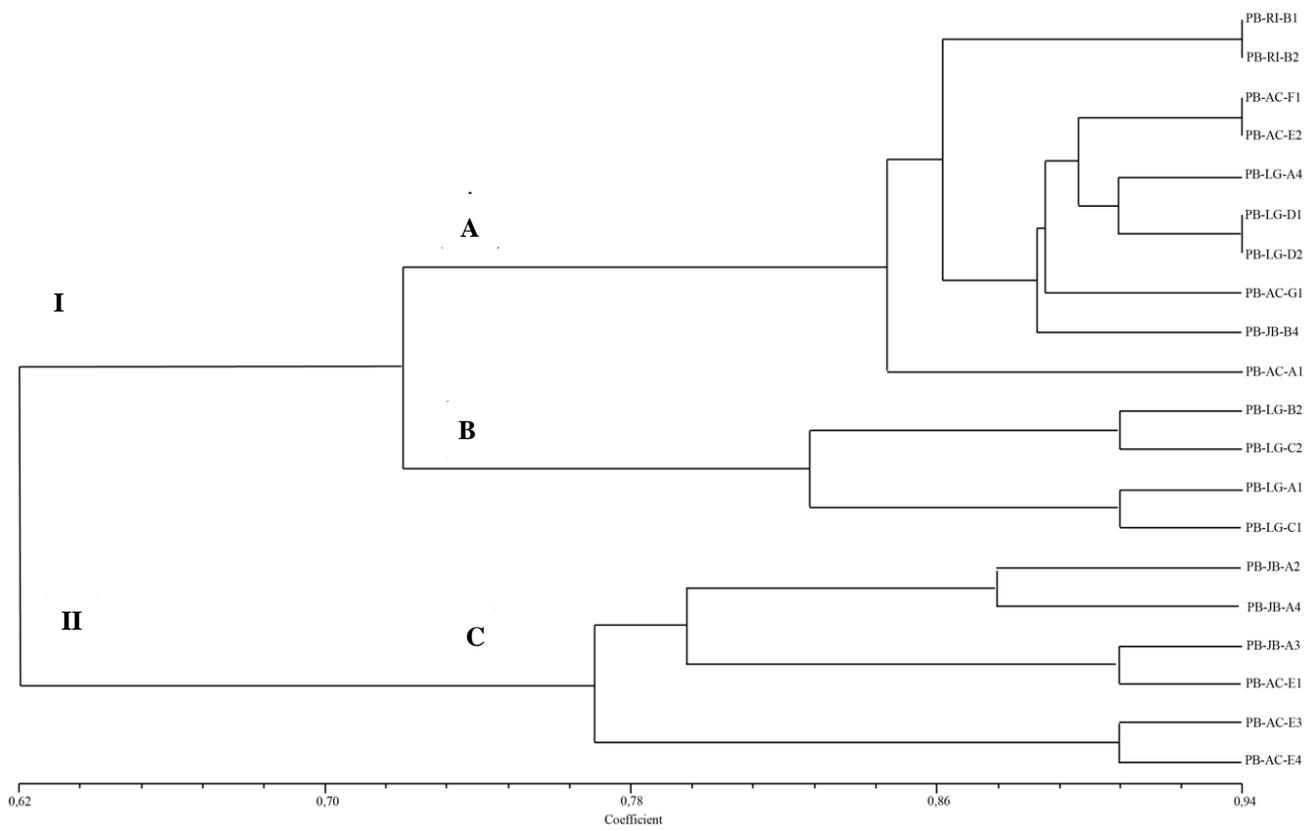| Sample	1 (700 bp)	2 (410 bp)	3 (210 bp)	4 (200 bp)	5 (150 bp)	6 (80 bp)	7 (75 bp)
PB-JB-A3	1	1	1	0	1	1	1
PB-AC-E1	1	1	1	0	1	1	1
PB-AC-E4	1	1	1	0	1	1	1

Figure 4. Phylogenetic tree for the twenty populations of *Piper betle* based on SRAP markers using the UPGMA method
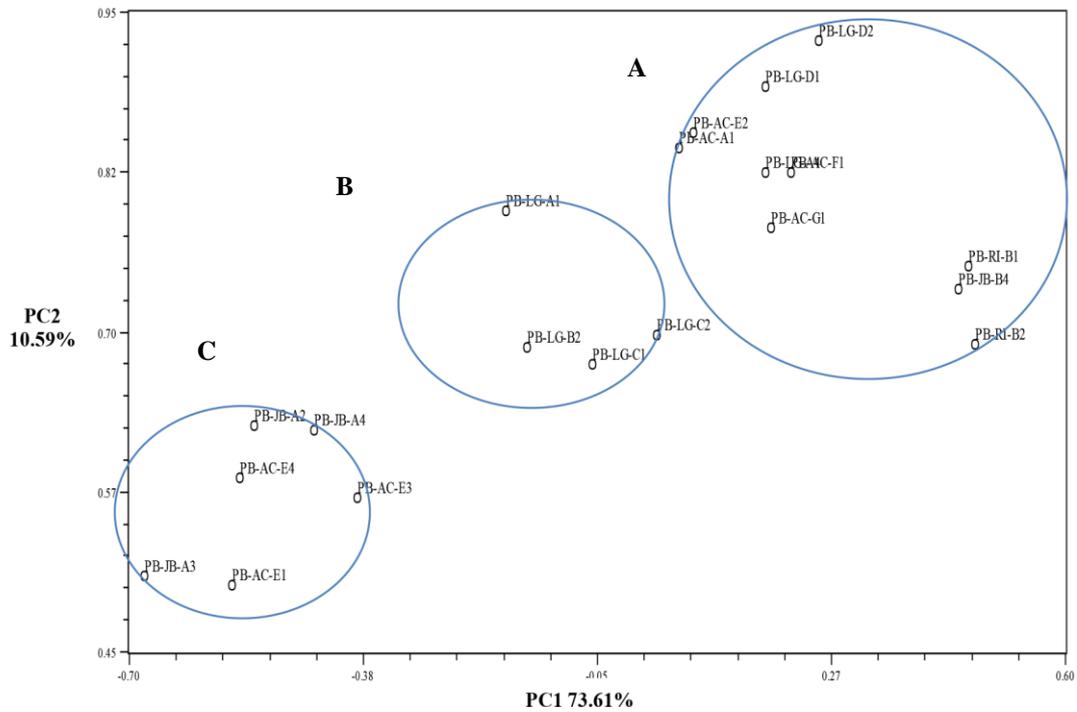

Figure 5. Matrix plot of principal component analysis of 20 betel vine populations using selected SRAP primer combinations

Table 7. Population genetic structure of six betel vine population of Indonesia

ID	Population	N	*Na*	*Ne*	*H*	*I*	*PPL* (%)
1	Hutan (Riau)	2	1.0642	1.0456	0.0267	0.0390	6.45
2	Singkil (Aceh)	5	1.5161	1.3466	0.2007	0.2957	51.61
3	Gayo Serbajadi (Aceh)	2	1.1290	1.0912	0.0534	0.0780	12.90
4	Baduy (West Java)	3	1.2258	1.1644	0.0920	0.1340	22.58
5	Kalisusu (Southeast Sulawesi)	2	1.2258	1.1597	0.0935	0.1366	22.58
6	Mekongga (Southeast Sulawesi)	4	1.3871	1.2012	0.1247	0.1922	38.71
	Mean	3	1.2580	1.1681	0.0985	0.1459	25.81

Note: N: number of individuals; *Na*: number alleles analyzed; *Ne*: number of effective alleles; *H*: Nei's genetic diversity; *I*: Shannon's information index, and *PPL*: percentage of polymorphic loci

Table 8. Value of Nei's genetic distance six betel vine population of Indonesia

ID Population	1	2	3	4	5	6
1	****					
2	0.2699	****				
3	0.1262	0.1958	****			
4	0.5921	0.1310	0.3688	****		
5	0.1590	0.1284	0.1219	0.3338	****	
6	0.1334	0.1321	0.1164	0.3124	0.0597	****

Note: Populations: 1. Hutan; 2. Singkil; 3. Gayo Serbajadi; 4. Baduy; 5. Kalisusu; 6. Mekongga
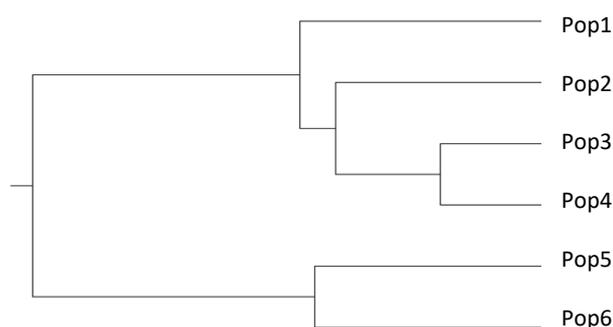
Figure 6. Dendrogram of genetic relationship between betel vine populations of Indonesia. Note: 1. Hutan; 2. Singkil; 3. Gayo Serbajadi; 4. Baduy; 5. Kalisusu; 6. Mekongga; pop: population.

Samples from Bandung and Kaledupa were not analyzed as each sample consists of only one individual so that did not meet our population criteria. The value of the observed number of alleles (*Na*) (mean 1.2580) was higher than the value of the number of effective alleles (*Ne*) (mean 1.1681). The Na value ranged from 1.0642 to 1.5161. The highest *Na* value, which indicates the highest number of different alleles, was shown in the Singkil population, while the lowest *Na* value was detected in the Hutan population. The *Ne* value ranged from 1.0456 to 1.3466. The highest value of Nei's gene diversity (*H*= 0.2007) was shown by the Singkil population, while the lowest value (*H*= 0.0267) was shown by the Hutan population. Similarly, the value of Shannon's information index was highest (*I*= 0.2957) in the Singkil population and lowest (*I*= 0.090) in the Hutan population. The percentage of polymorphic loci was also highest (*PPL*= 51.61%) in the Singkil population and lowest (*PPL*= 6.45%) in the Hutan population.

The value of the Nei's genetic distance indicates the relatedness between populations. Results showed that the Nei's genetic distance ranged from 0.0597 to 0.5921. Kalisusu and Mekongga populations showed lowest genetic distance (0.0597) while Hutan and Baduy populations showed highest genetic distance (0.5921) (Table 8). These values were supported by the dendrogram data which showed that Hutan and Baduy populations showed the most distant genes while Kalisusu and Mekongga populations showed the closest genetic relationship (Figure 6).

We report genetic diversity of betel vine plant leaves collected from 8 different accessions of Indonesia analyzed using DNA-based sequence-related-amplified-polymorphism (SRAP) molecular markers. Genomic DNA of each sample was successfully extracted using the GeneJET™ plant genomic DNA purification kit which was designed using a silica membrane for the isolation and purification of genomic DNA from a wide variety of plant species. This method has also been applied for isolation of genomic DNA from sorghum (*Sorghum bicolor* L. Moench) stem and root tissues (Maropola et al. 2015) as well as from pathogenic fungi of the family *Fusarium proliferatum* (Ferrara et al. 2019).

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