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Genetic Diversity and Population Structure of IRRDB 1981 and Wickham Rubber Germplasm Based on EST-SSR

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ABSTRACT

The accession of the IRDB 1981 (PN'81) population is a newly introduced and an important rubber tree germplasm while the Wickham clone is a commercial variety one. The objectives of this study were to assess the genetic diversity and the population structure of PN'81 populations and the Wickham clones using 15 EST-SSR loci. Results of the analysis showed that the evaluated SSR primers yielded polymorphic markers. The gSSR 268 primer pairs yielded the most informative markers while HBE 280 primers generated the lowest ones. Results of the genetic diversity analysis supported that the PN'81 population belonged to a single large natural population of rubber trees while the Wickham clones belonged to a different group than that of PN'81. The population structure analysis of the rubber accessions was also in agreement with the results of the genetic diversity analysis. The experiment also indicated that PN'81 populations would be useful for future rubber breeding in Indonesia, especially as the sources of parent clones for rubber tree hybridization programs and rubber tree genetic resource conservation.

Keywords: Amazon germplasm; *Hevea brasiliensis*; IRRDB 1981; rubber breeding; Wickham population

INTRODUCTION

Rubber tree (*Hevea brasiliensis* Muell.Arg), a perennial plant of the *Euphorbiaceae* family, is the main commercial source of natural rubber production worldwide. Although it originated from Amazone basin, South America, rubber tree is extensively cultivated in Southern Asia and contributes more than 90 % of the world natural rubber production (Priyadarshan & de Souza Goncalves, 2003).

The rubber tree clones existed in Asia, including Indonesia, originated from 22 seeds collected by Henry Wickam from Brazil in 1876. They were the progenitor of the commercial rubbers known as Wickham clones. Wickham collected the stocks from one Brazilian site (Boim area), in the Western banks of the Tapajos River. Previous studies reported that those commercial rubber clones exhibited a very narrow genetic base (Besse et al., 1994; Lekawipat et al., 2003; Oktavia & Kuswanhadi, 2011) making current improvement of rubber clones difficult. Moreover, because of assortative mating, low fruit-set limitation, selection for the high latex yield character, and extensive use of particular clones as parents in rubber breeding programs, further reduce the genetic diversity of the commercial rubber germplasm (Priyadarshan, 2016). Researchers have reported the occurrences of inbreeding depression because the superior genotypes are repeatedly used in rubber tree breeding programs, resulting in low latex yield, high-risk exposures to disease and insects attack (Lopes & Marques, 2015), and low adaptability to environmental changes (Ellstrand & Elam, 1993). Therefore, broadening the genetic basis through the introduction of new germplasm from the natural population is necessary for the breeding program.

Attempt to introduce new genetic materials has been done in 1981 under the coordination of International Rubber Research Development Board (IRRDB). Scientists have done an expedition to collect new rubber genetic materials to the Acre, Matto Grosso and Rondonia district, the primary center of origin of rubber germplasm in the Amazone Basin. The collected rubber germplasm is known as IRRDB 1981 (PN'81) or the Amazonian rubber germplasm. For evaluation purposes, Indonesia received 7,700 accessions of the PN'81 population.

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After field evaluations, all accessions yielded lower latex than that of the Wickam clones (Huat, Othman, & Benong, 1995; Aidi,-D, 2009). However, some accessions have useful characters, such as robust growth and well-formed leaf canopy (Huat, Othman, & Benong, 1995; Aidi,-D, 2009). The chance to directly identify high yielding and superior clones from PN'81 is probably low (Huat, Othman, & Benong, 1995; Aidi,-D, 2009). However, they may be used as a donor for disease resistance and abiotic stress tolerance characters (Mercy, 2001; Le Guen, Garcia, Mattos, & Clément-Demange, 2002; Le Guen, Doaré, Weber, & Seguin, 2009; Mydin, Reju, Narayanan, & Abraham, 2012; Reghu, Mercy, & Lakshmanan, 2012). Exploitation of diverse sources of variation for the genetic enhancement of the current rubber clones were needed.

The genetic diversity plays a crucial role in supporting many plant breeding program, including the rubber trees. Such genetic diversity may be estimated either at phenotype or molecular levels. Unlike those phenotype-based characterizations, molecular marker-based evaluations of genetic diversity are more accurate since the molecular markers are not affected by environmental factors. Simple sequence repeats (SSR) or microsatellites is one of the markers widely distributed throughout the nuclear genome of eukaryotes (Bhargava & Fuentes, 2010). SSR marker is highly polymorphic and often use as genetic markers for population genetic analysis (Guichoux et al., 2011). Several genetic analysis has been done using SSR markers, such as in coconut (Larekeng, Maskromo, Purwito, Matjik, & Sudarsono, 2015; Maskromo et al., 2015) and oil palm (Tinche, Asmono, Dinarti, & Sudarsono, 2014). Scientists have also done the evaluation of rubber tree genetic diversity analysis using RAPD or SSR markers (Besse et al., 1994; Saha, Roy, & Nazeer, 2005; Lam, Thanh, Chi, & Tuy, 2009; Gouvêa, Rubiano, Chioratto, Zucchi, & de Souza Gonçalves, 2010). Diversity analysis using SSR markers is more beneficial than using other dominant markers since SSR markers can differentiate the homozygous and the heterozygous individuals and exhibit high polymorphism. Moreover, SSR markers are highly reproducible and transferable among related species, and they can differentiate closely related accessions (Mantello, Suzuki, Souza, Gonçalves, & Souza, 2012).

The EST-SSR (expressed sequence tag-SSR) is an SSR marker developed using sequences of the expressed genes; therefore, it can be utilized as functional markers (Varshney, Graner, & Sorrells, 2005). Although the EST-SSR markers tend to be less polymorphic than the genomic SSR, EST-SSR is better in their ability to differentiate accessions belonging to closely related species (Feng, Li, Huang, Wang, & Wu, 2009). Many research groups have developed the EST-SSR for rubber trees (Ko, Chow, & Han, 2003; Chow et al., 2007; An, Zhao, Cheng, Li, & Huang, 2009; Triwitayakorn et al., 2011; Xia et al., 2011; Li, Deng, Qin, Liu, & Men, 2012; Mantello, Suzuki, Souza, Gonçalves, & Souza, 2012; An et al., 2013; Cubry et al., 2014; Li, Deng, Guo, Xia, 2014; Mantello et al., 2014; Silva et al., 2014). We can readily evaluate the SSR marker informativeness for different rubber tree population. The objectives of this study were to assess genetic diversity and population structure of PN'81 rubber tree population and Wickham accessions using 15 EST-SSR loci. The generated data may subsequently be used to support rubber tree breeding program in Indonesia.

MATERIALS AND METHODS

Planting Materials

The genetic evaluation of the rubber trees was done in the Plant Molecular Biology (PMB) Lab., Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Bogor, Indonesia during January - September 2015. The researchers evaluated a total of 56 rubber tree accessions (Table 1) consisted of six Wickam clones (three from Indonesia, two from Malaysia, and one from Sri Lanka) and 50 PN'81 accessions (33 from Rondonia, 15 from Mato Grosso and two from Acre). One can trace back all of these rubber tree accessions to their original location at the Amazone Basin (Fig. 1). Six clones of the Wickham population are the commercial clones grown in Indonesia, Malaysia, and Sri Lanka while 50 accessions of the PN'81 are the newly introduced rubber accessions from International Rubber Research and Development Board (IRRDB) in 1981. Currently, all planting materials exist in the Indonesian Rubber Research Institute germplasm collection.

Table 1. List of plant materials, the populations and their origins in the Amazone Basin – used in this rubber tree genetic diversity and population structure analysis

Group	Acces-sions	Population/ Origin	Group	Acces-sions	Population/ Origin	Group	Acces-sions	Population/ Origin
I.A	PN 323	PN'81/RO/A	II.A	PN 702	PN'81/RO/C	II.B	PN 235	PN'81/MT/IT
I.A	PN 328	PN'81/RO/A	I.D	PN 717	PN'81/RO/C	II.A	PN 295	PN'81/MT/IT
I.A	PN 412	PN'81/RO/A	II.A	PN 177	PN'81/RO/J	II.B	PN 406	PN'81/MT/IT
I.A	PN 441	PN'81/RO/A	I.D	PN 502	PN'81/RO/J	III	PN 494	PN'81/MT/IT
I.B	PN 71	PN'81/RO/C	I.C	PN 120	PN'81/RO/JP	II.D	PN 534	PN'81/MT/IT
I.B	PN 88	PN'81/RO/C	II.A	PN 361	PN'81/RO/JP	II.D	PN 621	PN'81/MT/IT
I.B	PN 93	PN'81/RO/C	III.B	PN 365	PN'81/RO/JP	II.E	PN 666	PN'81/MT/IT
II.A	PN 99	PN'81/RO/C	II.A	PN 491	PN'81/RO/JP	II.E	PN 667	PN'81/MT/IT
I.B	PN 138	PN'81/RO/C	II.B	PN 545	PN'81/RO/JP	II.E	PN 725	PN'81/MT/IT
II.E	PN 223	PN'81/RO/C	II.A	PN 680	PN'81/RO/JP	II.E	PN 261	PN'81/MT/VB
I.D	PN 232	PN'81/RO/C	II.A	PN687	PN'81/RO/JP	I.D	PN 373	PN'81/AC/F
II.E	PN 229	PN'81/RO/C	II.C	PN 305	PN'81/RO/OP	I.D	PN 604	PN'81/AC/S
I.C	PN 262	PN'81/RO/C	II.B	PN 316	PN'81/RO/PB	III	BPM 24	W/Indonesia
II.C	PN 265	PN'81/RO/C	II.A	PN 519	PN'81/RO/PB	III	BPM 1	W/Indonesia
II.E	PN 379	PN'81/RO/C	II.C	PN 142	PN'81/MT/C	III	GT 1	W/Indonesia
II.E	PN 386	PN'81/RO/C	II.C	PN 171	PN'81/MT/C	III	RRIC 100	W/Sri Lanka
II.A	PN 451	PN'81/RO/C	II.C	PN 309	PN'81/MT/C	III	PB 260	W/Malaysia
I.C.	PN 452	PN'81/RO/C	II.C	PN 22	PN'81/MT/IT	III	RRIM 600	W/Malaysia
III	PN 560	PN'81/RO/C	II.B	PN 186	PN'81/MT/IT			

Remarks: The location name of the original accessions -Rondonia (RO) / Ariquemes (A), Calama (C), Jaru (J), Jiparana (JP), Ouro Preto (OP), and Pimenta Bueno (PB); Mato Grosso (MT) / Cartriquacu (C), Itauba (IT), and Vila Bela (VB); Acre (AC) / Feijo (F), and Sena Madureira (S); and Wickham (W).Icae tam quo nonsum prae confectum

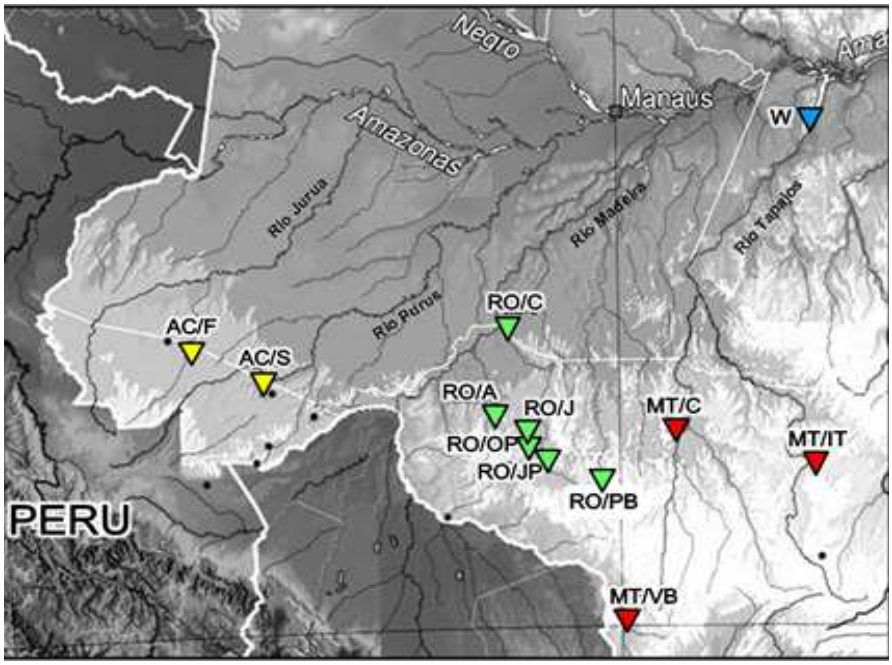

Fig. 1. Map of Amazone basin and the location of the original rubber germplasm collection. The site of Wickham (W) and PN'81 accessions of rubber germplasm. The PN'81 accessions consisted of samples from (▽) : Acre (AC)/Feijo (F) and Sena (S); (▼) : Rondonia (RO)/Calama (C), Ariquemes (A), Jaru (J), Ouro Preto (OP), Jiparana (JP), and Pimenta Bueno (PB); and (▼) : Mato Grosso (MT)/Cartriquacu (C), Itauba (IT) and Vila Bela (VB)

Table 2. List of primer names and their sequences, the expected allele sizes, number of alleles (N), polymorphic information content (PIC), observed (Ho) and expected (He) heterozygosity for the evaluated rubber population generated by 15 EST-SSR marker loci

Names of primers	Sequences of primers	Allele sizes (bp)	N	PIC	Ho	He
gSSR 213[1]	F: CCTTCCCCACTGATTCTTCA R: CTCTGCCTGGTCCTACTTGC	480-520	6	0.8	0.3	0.8
HB-52[2]	F: ACCCTACTATCCTATCGTCTTG R: AAAATCGTAGCTTCTTCATCAG	180-210	6	0.7	0.7	0.8
HBE 280[3]	F: GGACACCTGGAGCAAAATAG R: TATGCTTCGATGTATATTCACAGT	280	2	0.1	0.0	0.1
EHBc 34[2]	F: ATTCTGGTGGAAATCGAACG R: AAGGCGAGCAAGAAAACTGT	234-270	8	0.8	0.3	0.9
SSRH 103[4]	F: TCCTCTCCTCGTCAACATCC R: TGTCATTCGAACTCCGTCAA	251	6	0.7	0.5	0.7
gSSR 268[1]	F: TGGCATGATCGTTTAAGAAAAA R: CGGTTTCCTACCTCAGCTTG	230-280	13	0.9	0.7	0.9
gSSR 194[1]	F: GGGCCTCATTGTTCGTTTTA R: GTAGGGTGCCCATAAAGCAC	470-530	10	0.8	0.5	0.9
HB-152[2]	F: TATTTTGGAGCTTTGGGTGTTC R: CTGAGAGCGTTGTATGGGTGTG	170-240	6	0.7	0.3	0.8
EHBc 32[2]	F: TTGGCTACCTACCCAGATGC R: ATGTTCCTTGTGCTCCCAAC	225-258	8	0.7	0.6	0.8
EHB 61[5]	F: CCACAGCAACACCACCATTA R: TCATCCATCCAATGAAGCAA	150-200	8	0.7	0.5	0.8
EHB 33[5]	F: ATACCCAGACCTATGTGGCG R: AATGGGCTCGGAGATTCTTT	225-240	3	0.6	0.4	0.7
EHB 178[5]	F: TCGTGACCCAACAGAATAAAGA R: GGAAATTCTGCTGGCACTGT	190-215	5	0.4	0.4	0.5
HB 17[2]	F: AGGGCTTCGGGACAATCA R: GACATATGCCCCAACAAGTG	200-280	6	0.5	0.3	0.6
EHB 25[5]	F: ACCGTCCACCATAACCACAT R: AAAGGCCATGCCTACATTTG	245-250	3	0.4	0.1	0.5
EHB 168[5]	F: TCAAGCGCATCACAGGTATC R: TGGTCACCGAACAACAACAT	118-120	3	0.6	0.7	0.6
	Averages		6.2	0.6	0.4	0.7

Remarks: The primers were from [1]Pootakham et al., 2012, [2]Mantello, Suzuki, Souza, L. M., Gonçalves, & Souza, A. P., 2012, [3]Feng, Li, Huang, Wang, & Wu, 2009, [4]García-R, González-S, Montoya-C, & Aristizabal, 2011 and [5]Triwitayakorn et al., 2011

SSR Analysis

Fresh rubber leaf samples were collected from the rubber nursery at the Indonesian Rubber Research Institute and directly used for total DNA isolation. Total DNA as templates for the SSR analysis was isolated according to the procedure as described by Orozco-Castillo, Chalmers, Waugh, & Powell (1994). The isolated total DNA stocks were either dissolved in TE for storage in -20° C freezer or diluted in ddH_2O for working solution. The previously reported fifteen highly polymorphic SSR primer pairs (Table 2), developed by Feng, Li, Huang, Wang, & Wu (2009); García, González, Montoya, & Aristizabal (2011); Mantello, Suzuki, Souza, Gonçalves, & Souza (2012); Pootakham et al. (2012) and Triwitayakorn et al. (2011) were used to genotype all rubber accessions.

PCR amplification in a total volume of 12.5 µl was carried out using each of the evaluated primer pairs to obtain the SSR markers. The PCR reaction mixes consisted of 2 µl of approximately 25 ng $µl^{-1}$ DNA template, 0.75 µl (10 nM) each of the forward and the reverse primer, 2.75 µl MQ water, and 6.25 µl ready to use PCR Mix (Kapa Biosystem Inc. USA). The research performed amplifications in a DNA thermal cycler (Model T-100 Thermal Cycler, Bio-Rad, USA). The amplification steps were as follow: one cycle of pre-denaturation at 95° C for 3 minutes, followed by 35 cycles of denaturation at 95° C for 15 seconds, primer annealing at 53-

56° C for 15 seconds, and primer extension at 72° C for 30 seconds, and terminated by one cycle of final primer extension at 72° C for 3 minutes. It preliminarily evaluated the amplified PCR products in 1 % agarose gel electrophoresis and separated positively produced PCR amplified products in a vertical denaturing SDS-polyacrylamide gel electrophoresis (SDS-PAGE) containing 7 M urea, using a single gel dedicated manual sequencer (Cole-Parmer®). A visual observation of allelic patterns was conducted for the accessions by staining the gel using silver nitrate following procedures developed by Creste, Tulmann Neto, & Figueira (2001) and routinely utilized in the lab for SSR analysis of coconuts (Maskromo et al., 2015) and oil palm (Tinche, Asmono, Dinarti, & Sudarsono, 2014).

Allele Scoring and Data Analysis

Allele diversity was determined based on the appearing DNA banding pattern of each SSR locus. They were manually determined based on their fragment sizes (Fig. 2) and used to compose the genotype of each locus for the evaluated populations. It subsequently used the genotype data of all accessions based on SSR markers for further genetic analysis.

Dissimilarity matrix was calculated based on allelic data for two ploidy levels and simple matching dissimilarity index. Some bootstrap analysis at 10,000 iterations was set. Factorial analysis on dissimilarity was set using the option of 5 axes to edit, and the default axis as determined after the factorial analysis was selected. Tree construction was done by weighted Neighbour Joining approach and using the previously calculated dissimilarity matrix. All steps for dissimilarity matrix, bootstrap, factorial analysis and tree construction for the rubber accessions were done using Dissimilarity Analysis and Representation for WINDOWS (DARwin) software version 6.05 (http://darwin.cirad.fr).

A calculation of population genetic parameters was conducted, such as allele numbers (N), observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content (PIC) by using CERVUS software version 3.0 (Kalinowski, Taper, & Marshall, 2007) and GENALEX software version 6.501 (Peakall & Smouse, 2012). The analysis of population structure was done using STRUCTURE software version 2.3.4 (http://pritch.bsd.uchicago.edu/structure.html). Ad-hoc statistics were evaluated to rate changes in the log probability of data according to the K value as suggested by Evanno, Regnaut, & Goudet (2005), whereas the ideal number of population clusters were determined based on the highest K value estimated using STRUCTURE HARVESTER at http://taylor0.biology.ucla.edu/struct_harvest/ (Earl & vonHoldt, 2012).

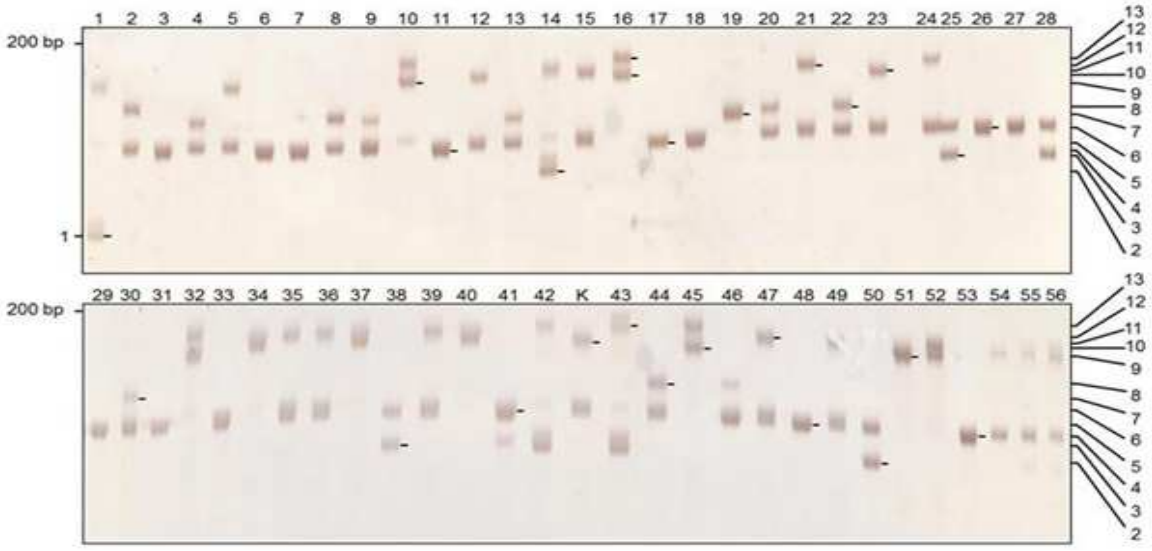

Fig. 2. Allele variabilities in the 56 accessions of rubber germplasm evaluated using gSSR 268 marker locus. 1-56 in black: the number of evaluated accessions.1-13: the number of alleles presence for each accession. 200 bp: position of the 200 bp size of the DNA marker

RESULTS AND DISCUSSION

The Origin of Rubber Tree Accessions

The rubber tree accessions analyzed in this study consisted of the Wickham accession and PN'81 populations (Table 1). Fig. 1 indicated the original locations in the Amazon Basin of Wickham and PN'81 accessions.

Allelic and Population Genetic Diversity

One of the major problems in the future rubber breeding program is the availability of germplasm with the wide genetic variation. Currently, continuously used of the same superior clones as a parent in rubber trees improvement have caused the genetic drift of the characters, such as genetic drift toward high latex yield and adaptability. However, the strategy at the same time also increased inbreeding depression level. Therefore, availability of data for either the genetic diversity or the genetic similarity among breeding materials will assist the selection of parent clones for hybridization and improve the efficiency of the rubber breeding program. Moreover, one shall only use parent clones carrying commercial characters and exhibiting high genetic distances for hybridization to prevent inbreeding depression. Determining various population genetic parameters may assist the decisions for selecting parent clones.

Amplification of 56 rubber accessions using the SSR primers indicated that the 15 primer sets (100 %) generated polymorphic markers. Fig. 2 presented the example of amplification profiles for rubber accessions generated with gSSR 268 primers. The total number of generated alleles were 93, ranged from 2 to 13 allele per locus (Table 2). The gSSR 268 primer pairs generated the highest number allele per locus (N), polymorphic information content (PIC), observed heterozygosity (Ho) and expected heterozygosity (He) while HBE 280 primers generated the lowest for most of the genetic parameters (Table 2). The average number of allele per locus, PIC, Ho and He obtained in this study were higher than those in previously reported results (Feng, Li, Huang, Wang, & Wu, 2009; Triwitayakorn et al., 2011; Perseguini et al., 2012). The differences may be due to the type and the number of populations analyzed in this study from the previous ones.

Most of the population used in this study came from PN'81, the newly introduced rubber genetic materials, while some previous studies using mostly of Wickham associated clones (Lekawipat et al., 2003; Oktavia & Kuswanhadi, 2011). However, the estimated population parameters were lower than that observed by de Souza et al. (2015). The de Souza et al. (2015) studies, they used more samples (ca.1,117 accessions) from many geographical origins in their study. The types and the number of population samples and markers used to affect the values of estimated population parameters.

Based on the genetic parameters of population, the rubber accessions originated from Rondonia showed the highest values for all parameters, while those from Acre showed the lowest ones, indicating the genetic diversity of the Rondonia accessions was greater than others. In the PN'81 populations, the observed heterozygosity (Ho) was less than the expected heterozygosity (He) values (Table 3), indicated the lower frequency of the heterozygous genotypes in the population based on the 15 EST-SSR loci analysis. On the other hand, the He and Ho values of the Wickham accessions were similar (Table 3). Moreover, the He values of the Wickham is lower than the PN'81 populations (Table 3), indicating the genetic diversity of the PN'81 population was higher than that of the Wickham. These finding demonstrated that PN'81 accessions might be used to increase the genetic basis of rubber tree accessions for breeding of rubber in the future.

Table 3. Estimated means of the population parameters estimated in the rubber populations genotyped by EST-SSR. Na and Ne referred to the number of different alleles and the effective alleles, Ho and He were the observed and the expected heterozygosity, and F is fixation index.

Population	Sub population	Number of accession	Na	Ne	Ho	He	F	Common allele	Specific allele
	Rondonia	33	6.1	3.9	0.4	0.7	0.37	1.9	1.5
PN'81	Mato Grosso	15	4.1	2.9	0.4	0.6	0.29	1.5	0.1
	Acre	2	1.8	1.7	0.2	0.3	0.35	0.5	0.0
Wickham	Wickham	6	2.4	1.9	0.4	0.4	0.16	0.4	0.0

The present study also indicated that the Rondonia and the Mato Grosso accessions had a few numbers of specific alleles occurring only in these rubber tree populations. The positive estimated fixation index value (F) with values ranging from 0.29-0.37 (Table 3) indicated the allelic frequency heterogeneity among in PN'81 populations were also responsible for the overall heterozygous deficiency. These ideas were previously proposed by de Souza et al. (2015) who indicated the Amazonian rubber germplasm showed the highest number of specific alleles. The F value also indicated the presence of specific genetic background among PN'81 that did not exist in other rubber populations. Scientists have reported the usefulness of PN'81 accessions to enlarge genetic background of rubber trees since they may potentially carry the sources of tolerance genes to abiotic stresses (Mercy, 2001), resistance to South America Leaf Blight (SALB) (Le Guen, Garcia, Mattos, & Clément-Demange, 2002), *Colletotrichum* sp. (Le Guen, Doaré, Weber, & Seguin, 2009) and the source of high quality wood characters (Mydin, Reju, Narayanan, & Abraham, 2012; Reghu, Mercy, & Lakshmanan, 2012).

Cluster Analysis of Genetic Relationship

Fig. 3 presented the resulting phylogenetic tree of 56 rubber germplasm constructed using Neighbour Joining method based on Simple Matching Dissimilarity Matrix. Based on the cluster analysis, the evaluated rubber tree accessions were grouped into three main groups. The first major cluster consisted of the Rondonia accessions and the admixture of accessions derived from the Acre. The second major cluster consisted of the admixture genotypes from Rondonia and Mato Grosso, and the third one consisted of the Wickham clones and one genotype from Rondonia and Mato Grosso. This results demonstrated that the newly introduced rubber tree accessions were differed and separated from cultivated rubber clones. These results are also similar to the genetic diversity estimates of the rubber tree using SSR markers reported by Lekawipat et al. (2003) and those using RAPD (Oktavia & Kuswanhadi, 2011). The cluster analysis also showed that some accessions belonged to the sub-populations of PN'81 did not exactly reflect their geographical origins. However, those collected from the same location tended to group in the same cluster.

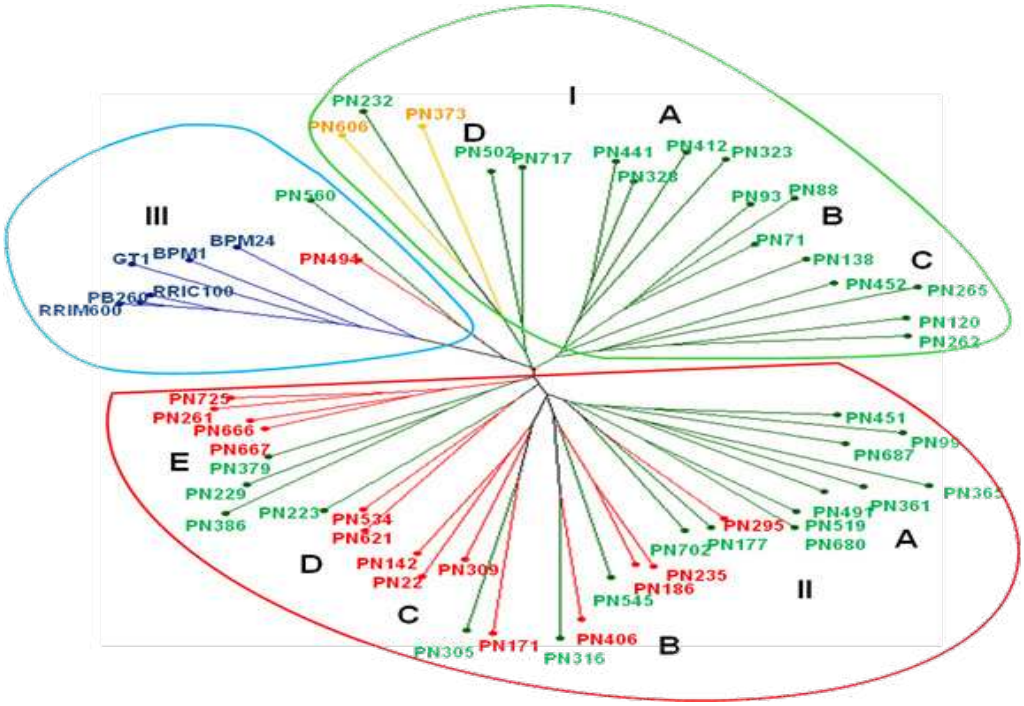

Fig. 3. Clustering of 56 genotypes of rubber germplasm based on matrix Dissimilarity Simple Matching. The evaluated rubber accessions consisted of the PN'81 samples from the Acre (▽), the Mato Grosso (▽), and the Rondonia (▽) populations, and the Wickham (▽) one.

Similarly, the germplasm divergence analyzed using PCoA (Fig. 4) showed that the PN'81 accessions were distantly related to Wickham clones. Moreover, Fig. 4 also indicated the genotypes of the PN'81 accessions were genetically more diverse than the Wickam clones. This research proposed based on the geographical origin (Fig. 1) and the genetic information revealed in this study (Fig. 3 and Fig. 4) that PN'81 rubber germplasm from the Acre, the Rondonia, and the Mato Grosso might be part of a single large natural population of rubber trees. According to a position in the Amazon Basin of each of the rubber population, the Acre location is in the Rio Purus, the Rondonia is in the Rio Madeira, and the Mato Grosso is in the Rio Tapajos watersheds (Fig. 1), respectively. Although it was also in the Rio Tapajos river, the Wickham rubber clones originated from further downstream location than the Mato Grosso (Fig. 1). Therefore, this research explained the presence of a close genetic association between two samples of PN'81 rubber accessions (PN560 and PN494) to the Wickham (Fig. 3) might be due to previous seed dispersal through the river stream.

Genetic Structure of Population

In the finding, when the results evaluated the rubber population structure analysis using Structure Harvester (Earl & vonHoldt, 2012), it has ΔK with the K value = 3. Therefore, there was three genetic backgrounds existed in the evaluated rubber accessions, represented by blue, green, and red primary colors in Fig. 5. Moreover, there are a few admixture accessions, indicated by bars with at least two primary colors (Fig. 5). The individuals from PN'81 populations were classified as either having the first (green color), the second (red color), or a mixture of green and red color (admixtures). The third color (blue color) consisted of the Wickham clones. The two individuals (PN560 and PN494) previously identified as closely related to the Wickham (Fig. 3) showed admixture between blue and green color bars (Fig 5). In previous studies, Le Guen, Doaré, Weber, & Seguin (2009) separated the IRRDB 1981 germplasm into three population groups, i.e. the Acre, the Rondonia and the Matto Grosso. Le Guen, Doaré, Weber, & Seguin (2009) also identified the existence of a few admixture of genotypes in each population. Phumichai, T., Teerawattanasuk, Kongsiri, Sansing, & Phumichai, C. (2011) grouped the Wickham clones of rubber into two groups, and Perseguini et al. (2012) identify eight groups of the Wickham clones. Therefore, the results confirmed those previous findings.

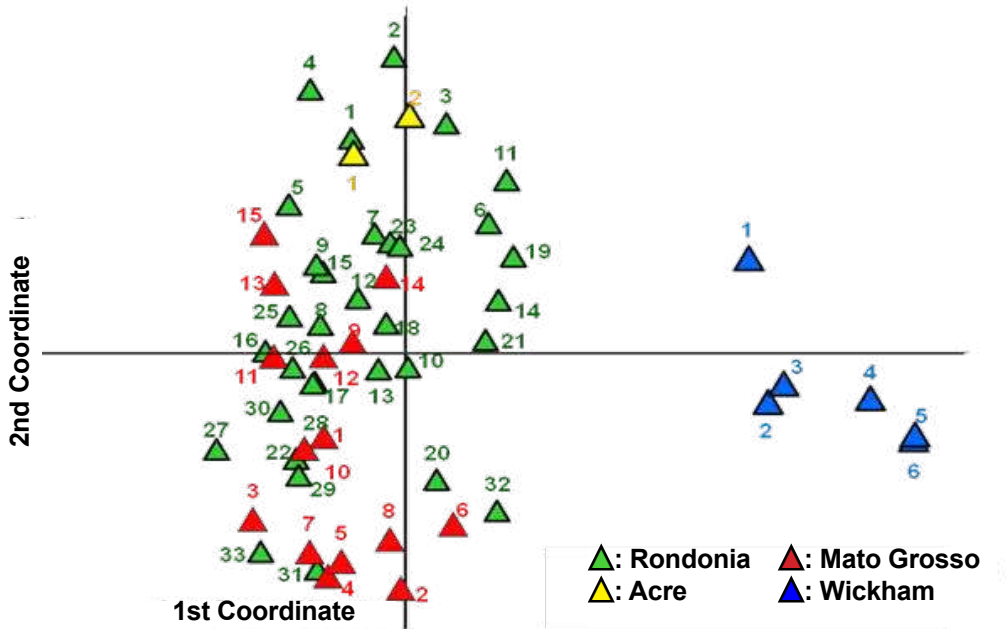

Fig. 4. Principal Coordinate Analysis (PCoA) distribution of 56 genotypes rubber germplasm. The evaluated rubber accessions consisted of the PN'81 samples from the Acre (▽), the Mato Grosso (▽), and the Rondonia (▽) populations, and the Wickham (▽) one.

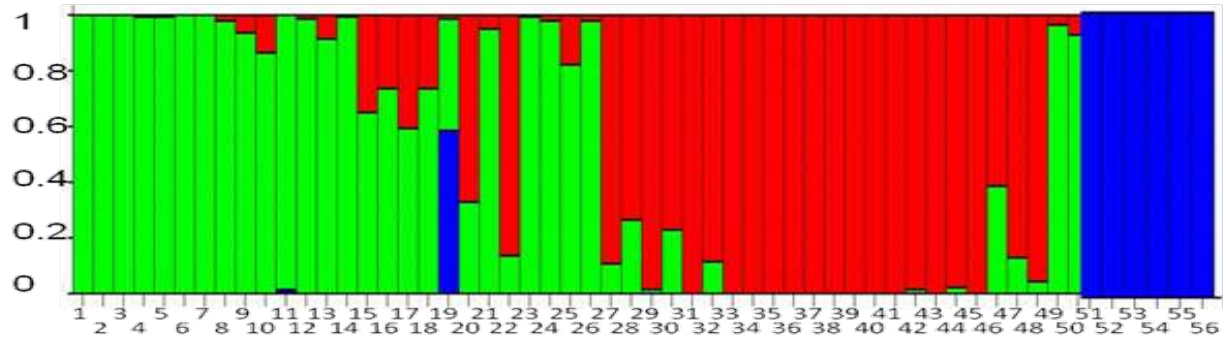

Fig. 5. Estimation of population structure of 56 rubber genotypes based on Structure analysis (K=3). The vertical bars represented the genotypes, and each color indicated a different cluster (K). The height of bar represented the probability of each genetic background for each of the accession.

Clustering of the evaluated rubber tree accessions based on the phylogenetic tree is correlated with that based on the population genetic structure as presented in Fig. 3 and Fig. 5. In general, this study concluded there were three major genetic backgrounds and admixtures among them. In a previous and more comprehensive evaluation using 1,117 accessions of rubber tree germplasm collections in Brazil, de Souza et al. (2015) proposed there be only two groups of rubber trees. Group I consisted of the Wickham clones and accessions from Mato Grosso and Group II consisted of accessions from Acre, Rondonia, Amazonas, and Para (de Souza et al., 2015).

Based on the results of this study, it proposed that there be a single large natural population of rubber trees in the Acre, the Mato Grosso, and the Rondonia regions and another population representing the Wickham clones. The hypothesized single natural population was supported by the presence of individuals with a mixture of genetic background in Fig. 5, indicating the presence of preferential gene flow between populations. Insect assisted natural pollination in rubber trees, and pollens travel reaching for up to 1.1 km in artificially planted population. Although rubber tree seeds would be difficult to disperse widely because of their weight and dispersal by animals was unlikely, seed dispersal through the associated seasonal flooding in the Amazon basin should be possible. Both long distance pollen travel and flood associated seed dispersal should create gene flow between populations and further support the proposed hypothesis.

Results of the previous genetic analysis of rubber tree germplasm revealed the presence of correlation among population clustering to the geographical origin of the populations. Le Guen, Doaré, Weber, & Seguin (2009) suggested that hydrographical network condition of Amazon basin were the main structuring trait for the natural rubber tree population differentiation and the main factor affecting their genetic diversity. Rubber seed dispersal through the river flows in the Amazon basin, such as Purus river (Rio Purus) flowing through Acre, Madeira river through Rondonia, and Tapajos river through Mato Grosso might have something to do with gene flow among populations located in the same Amazon basin (Fig. 1). Purus river flowing through Acre may have spread rubber seeds from Rondonia to Acre. The hypothesized gene flow may result in the close genetic relationship among rubber trees collected from Acre to Rondonia populations.

Based on their genetic data, Asian clones of Wickham population were genetically close to Mato Grosso population. Therefore, both populations are often found in the same group in some of the previous reports (Le Guen, Doaré, Weber, & Seguin, 2009; de Souza et al., 2015). One explanation for this is because of Boim area, the location where one collected the majority of Wickham clones, geographically closer to Mato Grosso and in the upstream of Tapajos River flowing through the Boim and Mato Grosso areas. Therefore, it should be possible for rubber seeds to drift from one region to the others through river flow, such as from Boim to Mato Grosso through Tapajos River. In the Amazon basin, the hydrogeographic condition affected the genetic diversity of rubber trees, such as accessions from Vila Bela (VB) district of Mato Grosso were genetically more closely related to those of Rondonia. On the other hand, ones from Pimenta Bueno (PB) district of Rondonia were closely related to those of Mato Grosso (Seguin, Gay, Xiong, & Rodier-Goud, 2001).

The ability to identify genetic resources having wide genetic distances would be beneficial in the identification of parents for hybridization programs since crossing among those parents' result in in more diverse progenies and prevent the occurrence of the inbreeding depression. Inbreeding depression was the major problem in the current rubber trees breeding program (Lopes & Marques, 2015). Taken together, based on the genetic diversity and the population analysis results using EST-SSR primers, we could summarize that the genetic structure of the evaluated PN'81 rubber accessions was more widely and diverse than that of the Wickham clones. The findings would be useful to support future rubber tree breeding programs in Indonesia. The available information on the genetic structure of rubber tree population was also important in rubber tree germplasm preservation.

CONCLUSION

Among the evaluated 15 EST-SSR loci, the SSR 268 primer pairs yielded in the most informative markers, and HBE 280 was the least ones. Results of the genetic diversity and population structure analysis supported that the PN'81 population belonged to a single large natural population of rubber trees while the Wickham clones belonged to a different group than that of PN'81. The analysis results also indicated that PN'81 populations would be useful for future rubber breeding in Indonesia, especially as the sources of parent clones for rubber tree hybridization programs and rubber tree genetic resource conservation.

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