Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.6, No.16, 2016

Assessing Genetic Diversity of Asian-based Rubber Populations using SSR and Multivariate Statistics in the Philippines

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The research is financed by Department of Science and Technology, Philippines through Accelerated Science and Technology Human Resource Development Program

Abstract

Assessing genetic diversity of rubber populations is important for the effective utilization of rubber genetic resources. Diversity indices such as number of alleles (Na), observed heterozygosity (Ho), gene diversity (GD), polymorphism information content (PIC) and power of discrimination (PD) along with multivariate statistics such as principal component analysis (PCA) and clustering analysis were used in the study. Twenty-two SSR markers had means 5.09 Na, 0.579 Ho, 0.677 GD, 0.643 PIC and 0.785 PD for 63 rubber clones comprised of 34 Indonesian and 29 Malaysian clones. Malaysian subpopulation had 3.59 Na per clone greater than Indonesian subpopulation of 2.97 Na per clone. PCA detected 66.08% total variation for eight principal components (PCs). PC1, PC2 and PC3 contributed 13.24% variation (*v*) with 2.91 eigenvalue (*e*), 10.2% *v* with 2.24 *e* and 8.86% *v* with 1.95 *e*, respectively. Clustering analysis revealed 0.237 genetic similarity and ten clusters for all clones. Clusters will be the basis for making more genetically diverse hybrids while PC1 member clones will be the basis for considering genetically broad base parent. The high genetic diversity found in the Asian-based rubber populations and complementing results of multivariate statistics can optimize the selection and breeding of rubber genetic resources in the Philippines.

Keywords: Asian-based Rubber Populations, Genetic Diversity, Multivariate Statistics, SSR Diversity

1. Introduction

Rubber, *Hevea brasiliensis* (Willd. *ex* A. Juss) Muell. Arg produces natural rubber latex. Rubber latex is the raw material in making tires, shoes, slippers, condoms and especially aseptic gloves use in hospitals. China's economic progress added the demand of natural rubber (Beilen, 2006). The National Development Strategic Plan Council in the Philippines had allocated one million hectare for rubber to support its industry (DOLE, 2010). Most of high-yielding rubber cultivars however had narrow genetic basis (Yu *et al*., 2011; Perseguini *et al*., 2012) yet were intensively used as parent materials in Southeast Asia's *Hevea* breeding programs (Kinnarat and Rattanawong, 2002). There is a demand therefore to identify and develop rubber clones having broad genetic base. Breeding for rubber however is still difficult due to a long time period required to interspecific breeding between its related species. El-Kassaby *et al*. (2006) had presented a method called "breeding without breeding", it naturally pollinate rubber trees which produces full-sib (FS) and half-sib (HS) seeds. Molecular markers then assess seeds from that breeding site for hybrid authenticity, yield development and paternity testing (Priyadarshan, 2016). The cultivation and adaptation of rubber today grows wider to several continents of new environments which made the rubber clones to evolve over time.

DNA-based marker systems assess genetic variation of populations (Morgante and Olivieri, 1993) without environmental interaction and gives useful information on rubber genetics. Simple sequence repeats (SSR) among the DNA markers have been commonly used to measure the genetic diversity because it is multi-allelic, reproducible, codominantly inherit, abundantly high in number within the genome in many crops (Gupta and Varshney, 2000). SSR was used to analyze genetic variation (Feng *et al.,* 2012; Perseguini *et al.,* 2012) and population structure of rubber (Le Guen *et al.,* 2011; Cantila *et al.,* 2015).

This study aimed to assess genetic diversity of Asian-based rubber populations using SSR diversity and multivariate statistics in the Philippines.

2.0 Materials and Methods

2.1 Sample DNA Extraction

Sixty-three rubber clones (Table 1) were used as samples from the University of Southern Mindanao (USM), Kabacan, Cotabato, Mindanao, Philippines at 7° 6′ 54.86″ N, 124° 50′ 12.1″ E. Genomic DNA was extracted from young rubber leaves using the DArT protocol (Jaccoud *et al.*, 2001) with some changes. The samples were ground to degrade cells with the use of extraction buffer comprising 0.35 M sorbitol, 0.1 M TrisHCl pH 8.0, 5 mM EDTA pH 8.0, and water. The extracted DNA was preheated at 65 ºC and incubated at the same temperature for 30 minutes. The liquefied suspension was taken with volume equal to chloroform:isoamyl (24:1) mixture and was centrifuged for about 5-8 minutes at 13,000 rpm. The upper liquid phase was then transferred to a new tube. DNA was precipitated using 95% ethanol and was repeatedly centrifuged for two times. DNA pellets were washed using 70% ethanol. Two µl of RNAse was added to DNA to be incubated at 37 ºC for one hour after drying. The DNA pellets were dissolved in 1 x TE comprising 10mM TrisHCl pH 8.0, 1 mM EDTA pH 8.0.

The DNA concentration was finally viewed through agarose gel electrophoresis (0.8%) along with ethidium bromide staining.

Table 1. Asian-based rubber clones with their code name and corresponding country origin.

Rubber clones	Code name	Country origin
AV49, AV163, AV608, AV634, AV1153, AV1258, AV1301, AV1447, AV1581, AV1792 and AV1996	AV= AVROS, Algemene Vereniging Rubberplanters Oostkust Sumatra	Indonesia
BD5	BD= Bodjong Datar	Indonesia
GL1	GL= Glenshiel	Malaysia
GT1, GT127, GT161, GT252, GT446, GT532 and GT711	GT= Gondang Tapen	Indonesia
GyT19007	GyT=Goodyear T clones	Indonesia
GyX99, GyX101, GyX142, GyX157, GyX183, GyX232, GyX370, GyX19007, GyX20819 and GyX20896	GyX= Goodyear Cross	Indonesia
Mal1	Mal= Malaysia	Malaysia
PB5/51, PB86, PB217, PB235, PB255, PB260, PB275, PB310, PB311, PB330 and PB359	PB= Prang Besar	Malaysia
PR107 and PR261	PR= Proefstation voor Rubber	Indonesia
RRIM513, RRIM527, RRIM600, RRIM612, RRIM625, RRIM701, RRIM703, RRIM705, RRIM712, RRIM717, RRIM901, RRIM2001, RRIM2020 and RRIM2025	RRIM= Rubber Research Institute of Malaysia	Malaysia
Tjir1 and Tjir16	Tjir= Tjirandji	Indonesia
TK800	unknown	Malaysia
War4	War=Wariangiana	Malaysia

2.2 SSR Amplification

The 22 simple sequence repeats (SSR) rubber-based markers (Table 2) were purchased from SBS Genetech Co., Ltd, Beijing, China. These SSRs were amplified and completed on 10 µl PCR mix comprising 0.3 unit Taq DNA polymerase, 1.0 unit 10 mM dNTP, 1.0 10x PCR buffer, 0.8 unit SSR marker, 4.1 unit ddH2O, 2 µl of 10-ng/ul DNA from each 82 rubber DNA. PCR was completed under conditions following 30 cycles in 7 steps: 2 minutes at 94 ºC as step 1: denaturation for 30 seconds at 94 ºC as step 2: annealing for 1 minute at 56 ºC as step 3: extension for 1 minute at 72 ºC as step 4: 29 times repeating step 2 to step 4 as step 5: 5 minutes at 72 ºC as step 6; storage at 4 ºC as step 7. PCR amplification products were viewed using polyacrylamide gel electrophoresis (4.5%) along with silver stain.

Table 2. List of SSR markers' sequence with their corresponding annealing temperature and mean size used in the study.

Markers*	Marker sequence 5' to 3'	Annealing temp. Mean size
AF221699[1]	F-TTTGCAGTGTATGCGTTTGGAAGTTC	61.6 °C
	R-CTGCAGTTTTCTTTTTCAGTGCTAT	316 bp
AF221700[1]	F-TTTGGCATTGATGTTGA	53.2 °C
	R-CCAAATATGCTGTTTCAGGA	192 bp
AF221703[1]	F-GGTTATCAAAGAGAAGATGCCAAGA	59.7 °C
	R-TCCAAATGCTGGAATCAGATATTGC	200 bp
AF221705[1]	F-GCTAACCCTCTCTTCATTGATA	58.4 °C
	R-AGATTCGCCTTTTCTCAGACAG	254 bp
AF221706[1]	F-TGTGTCCTCTACTTGTCTTCATTTG	58.1 °C
	R-GCCTCTACTTTTCTTTCTCCTTTAT	236 bp
AF221711[1]	F-ACAAGAGATGCGAGAAGAAATACCC	61.3 °C
	R-CATAACAGCTGAATGAAAATAAAAC	417 bp
M124[2]	F-TCATTTCAAGTTCACCGTGCTTATT	61.3 °C
	R-AGCGCATGTATTTGCCTTATGTCTC	151 bp
M412[2]	F-CATTAGTTGGCTGCTCTTTCATTTC	59.7 °C
	R-ACTTATCTTATGTTCCATCTACCAC	181 bp
MnSod[2]	F-TGTGCTGCCTTTGTCTTAACATGCC	63 °C
	R-GCAAATAGCAATGAGTTTCTGACTC	204 bp
hmac5[3]	F-TCGGTTGGTTTACCATGACA	62.5 °C
	R-ACATCACATGAGTGTATCTGATCTC	274 bp
hmc4[3]	F-GTTTTCCTCCGCAGACTCAG	60.5 °C
	R-ATCCACCAAATAAGGCATGA	315 bp
hmct1[3]	F-AACCAGAAGGGTGTCATGCT	58.4 °C
	R-GGAATCCCATGACAATCCAC	225 bp
hmct5[3]	F-ATGTATGTGTGCGCAGGAAG	60.5 °C
	R-CTGTAGTCATGGCAGCAGGA	221 bp
Ma31[4]	F-TCCTGCCATCCTTATCCT	63 °C
	R-TTTTTGTATTGCCCCAGCCGTGAGT	254 bp
A2406[5]	F-GTCCACAGAAATAAAACTCA	51.2 °C
	R-AGCCATTTTCTCACCTC	119 bp
A2736[5]	F-GCAACCTGATGAATAAAGA	52 °C
	R-AAATGAGAAACAAGAAGACC	448 bp
AY486582[5]	F-CCTGTATGAAATCAAGAGAAGA	56.5 °C
	R-TAGAGGTAGAAGCCAATGAGTT	171 bp
AY486585[5]	F-GGCAGTAGCACAATCATTTTTAGTA	58.1 °C
	R-TTTCCTCACTGTTTTGTCATTCC	154 bp
AY486601[5]	F-CTTGACGTTCGCATTCCTT	59.3 °C
	R-CATACCCATTTACATACACACACC	152 bp
T2603[5]	F-TAGCAGAAGCAGTTATGG	52 °C
	R-TTATCTATTGGACTGAAGGA	300 bp
TA2163[5]	F-ATGCAACAGAGTAGGAGGAGA	52 °C
	R-TCAAGGCAAATGAAGTG	196 bp
TAs2172[5]	F-AGGAATGCTAAGGGTATG	52 °C
	R-AGGAGATTGTGGAAGAAA	117 bp

[1]Lespinasse *et al.*, 2000, [2]Seguin *et al.*, 2002, [3]Saha *et al.*, 2005, [4]Sales, 2010, [5]LeGuen *et al.*, 2011.

2.3 Data Analyses

Values one (1) as present and zero (0) as absent were used in identifying polymorphism in amplified SSR products. Number of alleles (Na), gene diversity (GD), observed heterozygosity (Ho) and polymorphism information content (PIC) by PowerMarker 3.0 (Liu and Muse 2005) and power of discrimination (PD) through Microsoft excel using the formula: $PD = (1 - \sum gi^2)$, where gi is the frequency of i^{th} genotype (Kloosterman *et al.*, 1993) diversity indices revealed the SSR diversity. Correlation based on Pearson's coefficient and multivariate statistics such as clustering analysis based on unweighted pair cluster method arithmetic average (UPGMA) with Jaccard coefficient and principal component analysis (PCA) by XLStat of Addinsoft (2010) showed the relationship of diversity indices and statistical-based clusters of the population, respectively.

3.0 Results and Discussion

3.1 SSR diversity

Twenty-two SSR markers derived 111 Na in all with 5.05 Na per marker (Table 3). TAs2172 had the highest with 9 Na. Ho was ranged from 0.125 (AF221699) to 0.968 (A2736) while GD was from 0.283 (AF221699) to 0.839 (TAs2172). PIC was ranged from 0.16 (AF221699) to 0.845 (TAs2172) with 0.643 PIC per marker (Table 3) while PD were from 0.23 (AF221699) to 0.92 (TAs2172) with 0.785 PD per marker (Table 3), indicative of ideal markers (Botstein et al., 1980). PIC evaluates marker capacity to detect polymorphism over a pool of genotypes (Anderson *et al., 1993*; Perseguini *et al.,* 2012) while PD measures marker efficiency to distinguish individuals (Tessier *et al.,* 1993 and Perseguini *et al.,* 2012). Correlation analysis however revealed highest correlation between PIC and PD (r=0.978) followed by GD and PIC (r=0.975) (Table 4). GD, PIC and PD were highly correlated to each other. PCA was also used to detect polymorphism. PCA revealed 8PCs with a range of 1.16 (PC8) to 2.91 (PC1) eigenvalues and 5.28% (PC8) to 13.24% (PC1) variation (Table 5). The variation detected PC was fairly distributed to 8PCs. A2376, AF221706, AF221711, TA2163 and TAs2172 on the other hand formed the PC1 and was considered the highest detector of variation. PC1 member markers had >0.65 GD, PIC and PD values. The resolving power of the 22 SSR markers comprising the Na, Ho, GD, PIC, PD and PCA were able to derive sufficient information of rubber evaluated in this study.

Table 3. Diversity indices such as allele number (Na), gene diversity (GD), observed heterozygosity (Ho), polymorphism information content (PIC) and power of discrimination (PD) with their corresponding mean and standard deviation (SD) explained the SSR diversity in the study.

Markers	Na	Ho	GD	PIC	PD
A2406	4	0.316	0.548	0.52	0.7
A2736	6	0.968	0.797	0.781	0.864
AF221699	4	0.125	0.283	0.16	0.23
AF221700	5	0.407	0.677	0.647	0.799
AF221703	6	0.621	0.697	0.649	0.831
AF221705	6	0.81	0.71	0.68	0.795
AF221706	5	0.729	0.748	0.748	0.915
AF221711	5	0.797	0.749	0.718	0.874
AY486582	5	0.597	0.736	0.724	0.882
AY486585	5	0.466	0.678	0.669	0.843
AY486601	3	0.426	0.501	0.486	0.648
hmac5	4	0.683	0.567	0.505	0.64
hmc4	5	0.29	0.588	0.414	0.507
hmct1	5	0.597	0.705	0.669	0.843
hmct5	5	0.806	0.743	0.737	0.889
M124	5	0.656	0.673	0.647	0.833
M412	5	0.455	0.743	0.686	0.833
Ma31	5	0.656	0.766	0.765	0.909
MnSod	5	0.517	0.709	0.696	0.848
T2603	4	0.49	0.735	0.729	0.858
TA2163	5	0.455	0.709	0.678	0.818
TAS2172	9	0.879	0.839	0.845	0.92
Total	111	-	-	-	-
Mean	5.05	0.579	0.677	0.643	0.785
SD	1.15	0.208	0.12	0.149	0.161

Table 4. Correlation analysis based on Pearson's coefficient explained the relationship of diversity indices.

Genetic diversity indices	Na	Ho	He	PIC	PD
Na	1				
Ho	**0.586**	1			
He	**0.634**	**0.741**	1		
PIC	**0.577**	**0.754**	**0.975**	1	
PD	**0.460**	**0.692**	**0.936**	**0.978**	1

Values in bold are different from 0 with a significance level alpha=0.05

Table 5. Twenty-two SSR markers with their squared cosine values formed the principal components (PCs) and the corresponding eigenvalue and variation derived by each PC.

Markers	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
A2406	0.18	**0.21**	0.06	0.00	0.01	0.15	0.03	0.02
A2736	**0.41**	0.03	0.03	0.01	0.02	0.17	0.02	0.00
AF221699	0.01	0.02	**0.38**	0.07	0.00	0.01	0.10	0.02
AF221700	0.01	0.02	0.05	0.04	0.11	**0.43**	0.02	0.03
AF221703	0.18	0.01	0.12	0.01	0.02	0.02	**0.19**	0.02
AF221705	0.18	0.00	0.01	0.02	**0.27**	0.01	0.03	0.05
AF221706	**0.26**	0.00	0.05	0.02	0.09	0.01	0.05	0.17
AF221711	**0.30**	0.00	0.08	0.13	0.04	0.03	0.03	0.06
AY486582	0.03	**0.39**	0.20	0.01	0.06	0.00	0.00	0.01
AY486585	0.08	**0.18**	0.01	0.13	0.02	0.18	0.10	0.00
AY486601	0.04	**0.27**	0.21	0.01	0.01	0.00	0.10	0.03
hmac4	0.10	0.03	0.00	**0.28**	0.08	0.00	0.02	0.15
hmac5	0.11	**0.18**	0.00	0.07	0.03	0.12	0.11	0.01
hmct1	0.11	0.03	0.02	0.13	**0.22**	0.00	0.06	0.03
hmct5	0.00	0.02	**0.35**	0.01	0.22	0.01	0.14	0.01
M124	0.06	**0.33**	0.03	0.00	0.01	0.07	0.15	0.03
M412	0.08	0.07	0.00	0.12	0.16	0.03	0.09	**0.27**
Ma31	0.00	0.00	0.08	**0.34**	0.24	0.00	0.01	0.01
MnSod	0.10	**0.17**	0.01	0.10	0.03	0.09	0.02	0.15
T2603	0.03	0.02	**0.16**	0.16	0.08	0.00	0.07	0.09
TA2163	**0.34**	0.12	0.08	0.00	0.01	0.02	0.02	0.00
TAS2172	**0.29**	0.14	0.01	0.12	0.01	0.00	0.00	0.00
Eigenvalue	2.91	2.24	1.95	1.79	1.75	1.38	1.36	1.16
Variability (%)	13.24	10.20	8.86	8.13	7.96	6.26	6.16	5.28
Cumulative %	13.24	23.44	32.29	40.42	48.38	54.64	60.80	66.08

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

3.2 Subpopulation diversity

Diversity within population is a source of genetic variability patterns must be accurately assessed in the germplasm (Smith, 1984; Cox *et al.*, 1986). Diversity can be variation in genotype form found within and among populations through molecular differences and expressed as phenotypes (Frankham *et al.*, 2002). Na was 104 for Malaysia and 101 for Indonesia with 102.5 Na per subpopulation (Table 6). Malaysian subpopulation (MS) had 3.59 Na per clone and 0.628 Ho which were greater than Indonesian subpopulation (IS) with 2.97 Na per clone and 0.604 Ho (Table 6). IS on the other hand had 0.97 GD greater than 0.965 GD in MS (Table 6).

Slight differences in the results on gene and heterozygotes were found in subpopulations but implications were the same. Two subpopulations had high GD values (0.968 GD on average), meaning high heterozygotes were expected but only moderate heterozygotes (0.616 Ho on average) were observed (Table 6). Reduction of heterozygotes was detected and could be a result of inbreeding. Reason can be due to Asian-based clones were originated from 22 seedlings of Wickham's original collection (Kinnarat and Rattanawong, 2002). Inbreeding however can easily be negated since rubber is a highly cross pollinated in nature (Venkatachalam *et al.*, 2007).

Table 6. Two subpopulations with diversity indices such as number of alleles (Na), Na per clone, observed heterozygosity (Ho) and gene diversity (GD) with their corresponding mean and standard deviation (SD) explained subpopulation diversity.

Subpopulation (N)	Na	Na per clone	Ho	GD
Indonesia (34)	101	2.97	0.604	0.970
Malaysia (29)	104	3.59	0.628	0.965
Mean	102.5	3.28	0.616	0.968
SD	2.121	0.438	0.017	0.004

3.3 Statistical-based clusters

Principal component and clustering analyses are commonly used tools in assessing genetic diversity of any crop. PCA makes each individual to group into one cluster (Mohammadi, 2002) while the clustering analysis is suitable for evaluating genetic relationships of individuals (Mellingers, 1972). PCA revealed eight groups of clones over the population in this study (Table 7). PC1 was comprised of AV1301, AV1447, AV1792, BD5, GL1, GT1, GT252, PB217, PB310, PB86, RRIM2001, RRIM600 and Tjir16 and contributed 13.24% of the total variation found (Figure 1; Table 7). These clones were the highest contributor in giving variation. PC2 (7 members) also contributed 10.2% variation, PC3 (9 members) with 8.86% variation and PC4 (10 members) with 8.13% variation (Table 7). Thirty-four Indonesian clones shared 0.275 genetic similarity (GS) and were distributed to nine subclusters (Figure 2) in the clustering analysis. The biggest cluster was subcluster I (15 members) which mostly comprised of Algemene Vereniging Rubberplanters Oostkust Sumatra (AV) clonal series with few mixtures from GT, PR and Tjir. Subcluster VI had five members while subcluster III had four members. Subcluster VI was comprised of clones from Good year company while subcluster III of clones from AV, GT and Gy. The rest of the subclusters had two or one member in their cluster. Twenty-nine Malaysian clones on the other hand shared 0.243 GS and were also distributed to nine subclusters (Figure 2). Subcluster I, biggest cluster, was dominated by PB clones while subcluster VI, the second biggest cluster, was dominated by RRIM clones. PB and RRIM clones separated each other by dominating their own cluster. Similar findings were found when using 12 Random Amplified Polymorphic DNA (RAPDs) markers in parent selection of rubber (Oktavia and Kuswanhadi, 2011) and 47 EST-SSRs genetic linkage map construction for rubber (Triwitayakorn *et al.*, 2011). The rest of the subclusters had two or one member in their cluster.

Sixty-three clones were distributed to ten clusters as one population (Figure 3). The biggest was cluster I with 40 members comprising 14 RRIM, 9 PB, 7 AV, 4 GT, 2 PR and 2 Tjir and sharing 0.37 GS (Table 8). Few mixture members were found such as GyX20896 and Mal1. GyX20896 had 0.436 GS to RRIM612 and RRIM513 while Mal1 to PB 5/51 had 0.583 GS. The next big clusters were II and VI with 6 members each. Cluster II was mostly comprised of AV clones while cluster VI of Gy clones. Clusters IV, V, VII and VIII had two members each sharing 0.382, 0.405, 0.42 and 0.4 GS, respectively. Clusters III (BD5), IX (PB359) and X (War4) with one member were considered farthest clones. The reason for clones of grouping the same cluster is parental relationship. PR261 for example is a progeny of PR107 and Tjir1 (Priyadarshan and Gonçalves, 2002), they grouped in the same cluster in this study. Nakannong *et al.* (2008) previously reported that institutions among and between Asian countries regularly exchange and share rubber parent materials.

Table 7. Grouping of clones based on PCA's squared cosine values. Enclosed values are variations explained by each PC.

PC1 (13.24%)	PC2 (10.2%)	PC3 (8.65%)	PC4 (8.13%)
AV1301 AV1447 AV1792 BD5 GL1 GT1 GT252 PB217 PB310 PB86 RRIM2001 RRIM600 Tjir16	AV1996 GT532 GyX157 PB359 RRIM513 RRIM701 RRIM2025	AV1581 GT711 GyX232 GyX370 GyX20819 GyX20896 PB275 RRIM612 RRIM2020	AV49 AV163 GT161 GyX101 GyX183 PR107 PR261 PB311 PB330 RRIM705
PC5 (7.96%)	**PC6 (6.26%)**	**PC7 (6.16%)**	**PC8 (5.28%)**
GT127 PB260 RRIM625 RRIM703 RRIM717 RRIM901 TK800	AV1258 GyX99 PB235 PB255 RRIM527 RRIM712 Tjir1 War4	AV1153 GT446 GyT19007 GyX142 GyX19007 Mal1	AV608 AV634 PB5/51

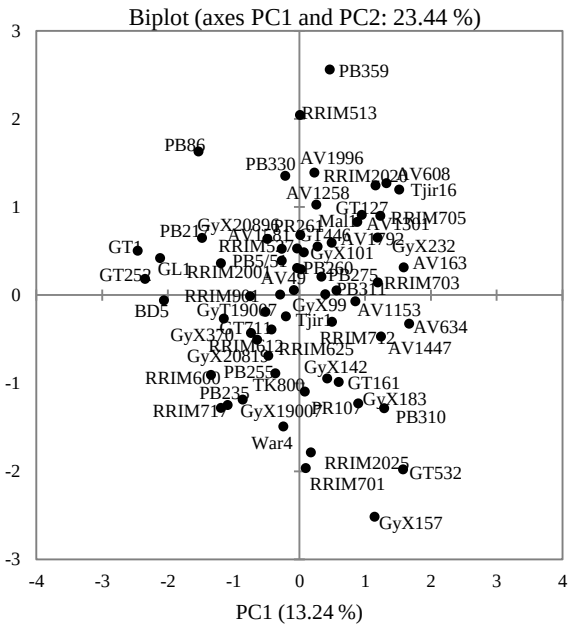

Figure 1. Sixty-three rubber clones were randomly distributed in the scatterplot of principal component analysis (PCA).

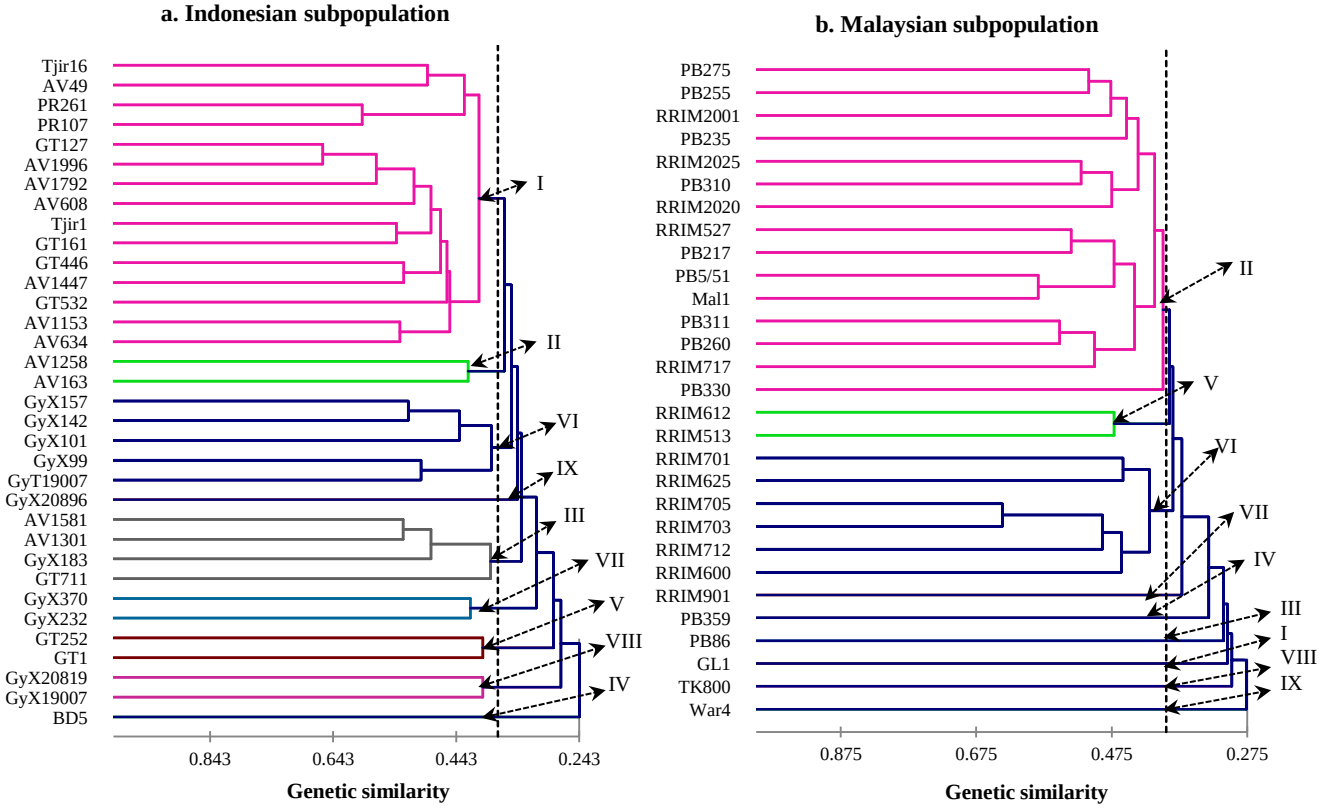

Figure 2. Clustering analyses of (a) 34 Indonesian clones and (b) 29 Malaysian clones derived nine subclusters for each subpopulation.

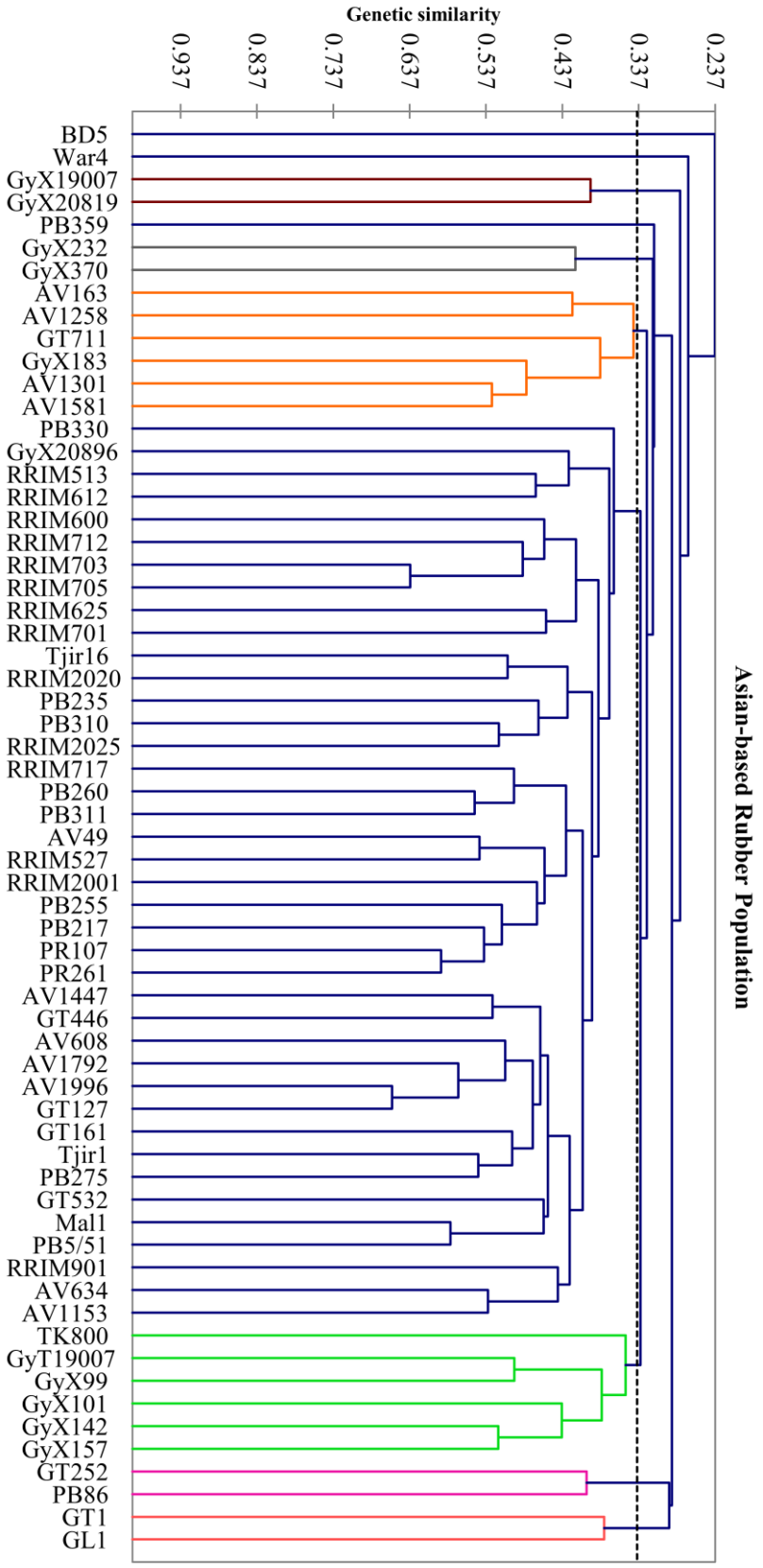

Figure 3. Clustering analysis of 63 rubber clones derived ten clusters in a population.

Table 8. Clusters with their corresponding clone member/s based on the clustering analysis.

Cluster (N)	Clones	Cluster (N)	Clones
I (40)	AV49, AV608, AV634, AV1153, AV1447, AV1792, AV1996, GT127, GT161, GT446, GT532, GyX20896, PR107, PR261, Tjir1, Tjir16, Mal1, PB5/51, PB217, PB235, PB255, PB260, PB275, PB310, PB311, PB330, RRIM513,RRIM527,RRIM600, RRIM612,RRIM625,RRIM701, RRIM703,RRIM705,RRIM712, RRIM717,RRIM901,RRIM2001, RRIM2020 and RRIM2025	II (6)	AV163, AV1258, GT711, GyX183, AV1301 and AV1581
		III (1)	BD5
		IV (2)	GT1 and GL1
		V (2)	GT252 and PB86
		VI (6)	TK800, GyT19007, GyX99, GyX101, GyX142 and GyX157
		VII (2)	GyX232 and GyX370
		VIII (2)	GyX19007 and GyX20819
		IX (1)	PB359
		X (1)	War4

4. Conclusion

SSR diversity was enough to explain the genetic diversity of Asian-based rubber populations in the Philippines. Not far difference was computed on diversity indices between two subpopulations. Implications from multivariate statistics on the other hand can be optimized by using principal component analysis in detecting clones that can contribute more genetic variation (PC1 member) and clustering analysis in detecting compatible clones for hybridization. For example, BD5 (PC1 and cluster III member) is best to be hybridized to a clone belonging to a different cluster such as RRIM600 (PC1 and cluster I member). There is better genetic variability will be derived on this cross where selection in progenies can be maximized.

References

Addinsoft. (2010). XLStat, data analysis and statistics software for Microsoft Excel. http://www.xlstat.com. Paris, France.

Anderson, J.A., Churchill, G.A., Autrique, J.E., Sorells, M.E., & Tanksley S. D. (1993). Optimizing parental selection for genetic-linkage maps. *Genome* 36: 181–186.

Beilen J. V. (2006). Alternative Sources of Natural Rubber Outputs from the EPOBIO project November 2006. CPL Press, Tall Gables, The Sydings, Speen, Newbury, Berks RG14 1RZ, UK. ISBN 10: 1-872691-08-0.

Botstein D., White R. L, Skolnick M. & Davis R. W. (1980). Construction of a genetic map in man using restriction fragment length polymorphism. *Am. J. Hum. Genet.* 32: 314-331.

Cantila A. Y., Espino R. R. E., & Sales E. K. (2015). Utilization of simple sequence repeats markers in the genetic characterization of Philippine rubber, *Hevea brasiliensis* (Willd. ex A. Juss) Muell. Arg., germplasm. *Philipp J Crop Sci* 40 (3): 33-39.

Cox T. S., Murphy J. P., & Rodgers D. M. (1986). Changes in genetic diversity in the red winter wheat regions of the United States. *Proc. Natl. Acad. Sci. (USA)* 83: 5583–5586.

DOLE. (2010). *Agro-Industrial Employment Framework for Rubber*. Department of Labor and Employment Retrieved February 3, 2011 from http://ro10.dole.gov.ph/default.php?retsamlakygee=44&resource=f5b15f58cabad73d1bf2de7bcb89db6c.

El-Kassaby Y. A., Lstibůrek M., Liewlaksaneeyanawin C., Slavov G. T., & Howe G. T. (2006). Breeding without breeding: approach, example, and proof of concept. In: *Proceedings of the IUFRO, low input breeding and genetic conservation of forest tree species, Antalya*. pp 43–54.

Feng S., Wu Y., Li W., Yu F., & Wang J. (2012). Analysis of Genetic Diversity and SSR Allelic Variation in Rubber Tree (*Hevea brasiliensis*). In: *The Molecular Basis of Plant Genetic Diversity*. Prof. Mahmut Caliskan (Ed.). pp. 136-150. ISBN: 978-953-51-0157-4.

Frankham R., Ballou J. D., & Briscoe D. A. (2002). Introduction to conservation genetics. Cambridge University Press. Cambridge, England.

Gupta P. K., & Varshney R. K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113: 163-185.

Jaccoud D., Peng K., Feinstein D., & Kilian A. (2001). Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Res.* 29 (4), e25. doi:10.1093/nar/29.4.e25. PMC 29632. PMID 11160945

Kinnarat P., & Rattanawong R. (2002). Improvement of rubber tree. *Academic Matter of Rubber Tree Conference (1st)*. Rubber Research Institute, Department of Agriculture, Nongkhai, Thailand. February 20-22, 2002.

Kloosterman A. D., Budowle B., & Daselaar P. (1993). PCR-amplification and detection of the human D1S80 VNTR locus. Amplification conditions, population genetics and application in forensic analysis. *Int J Leg Med* 105 (5): 257- 264. April 2005. ISSN: 0937-9827.

Le Guen V, Gay C, Xiong TC, Souza LM, Rodier-Goud M, & Seguin M (2011). Development and characterization of 296 new polymorphic microsatellite markers for rubber tree (*Hevea brasiliensis*). *Plant Breeding* 130: 294-296.

Lespinasse D., Rodier-Goud M., Grivet L., Leconte A., Legnate H., & Seguin M. (2000). A saturated genetic linkage map of rubber tree (*Hevea spp*.) based on RFLP, AFLP, microsatellite and isozyme markers. *Theor. Appl. Genet*. 100: 127-138.

Liu K., & Muse S. V. (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21: 2128–2129. doi: 10.1093/bioinformatics/bti282.

Mellingers J. S. (1972). Measures of genetic similarity and genetic distance studies in genetics. *VII Univ. Tex. Publ*. 27: 145- 153.

Mohammadi S. A. (2002). Statistical methods in genetics. Paper presented at the 63rd Int. Statistics Conf., University of Tarbiat modares, Iran, 26-28 August 2002.

Mohammadi S. A., & Prasanna B. M. (2003). Review & Interpretation: Analysis of genetic diversity in crop plants; salient statistical tools and considerations. *Crop Sci*. 43: 1235-1248.

Morgante M., & Olivieri A. M. (1993). PCR-amplified microsatellite as markers in plant genetics. *Plant J*. 3: 175-182.

Nakkanong K., Nualsri C., & Sdoodee S. (2008). Analysis of genetic diversity in early introduced clones of rubber tree (*Hevea brasiliensis*) using RAPD and microsatellite markers. *Songklanakarin J. Sci. Technol.* 30 (5): 553-560.

Oktavia F., & Kuswanhadi M. L. (2011). Selection of parent trees for Rubber (*Hevea brasiliensis*) breeding based on RAPD analysis. ISSN: 2087-3956. *NUSANTARA Bioscience* 3 (3): 124-129.

Perseguini J., Romão L., Briñez B., Scaloppi E. J. R., Gonçalves P., & Benchimol L. L. (2012). Genetic diversity of cultivated accessions and wild species of rubber tree using EST-SSR markers. *Pesq. agropec. bras., Brasília* 47 (8): 1087-1094.

Priyadarshan P. M., & Gonçalves P. (2002). Use of *Hevea* gene pool in rubber tree (*Hevea brasiliensis* Muell. Arg) breeding. *The Planter* 78: 123-138.

Priyadarshan P. M. (2016). Genetic Diversity and Erosion in *Hevea* Rubber. In: M.R. Ahuja and S.M. Jain (eds.), *Genetic Diversity and Erosion in Plants*—Sustainable Development and Biodiversity 8, © Springer International Publishing Switzerland. pp 233-267. DOI 10.1007/978-3-319-25954-3_6.

Saha T., Bindu C. R., & Nazeer M. A. (2005). Microsatellite variability and its use in the characterization of cultivated clones of *Hevea brasiliensis*. *Plant Breeding* 124: 86-92.

Sales E. K. (2010). SSR markers for profiling & authentication of rubber germplasm in the Philippines, presented at the IRRDA Annual Meeting & International Rubber Conference 18-22 October 2010. Department of Agriculture, University of Southern Mindanao, Kabacan, Cotabato, the Philippines.

Seguin M., Gay C., Chen X. T., & Rodier-Goud M. (2002). Microsatellite markers for genome analysis of rubber tree (*Hevea spp.*). The IRRDB Symposium 2001 Biotechnology & Rubber Tree, 25-28 September 2001. Montpellier, France. Retrieved from http://tropgenedb.cirad.fr/cgibin/generic/tree/rubbertree? name=4&class.

Smith J. S. C. (1984). Genetic variability within U.S. hybrid maize: Multivariate analysis of isozyme data. *Crop Sci.* 24:1041–1046.

Tessier C., David J., This P., Boursiquot J. M., & Charrier A. (1999). Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. *Theoretical and Applied Genetics* 98: 171-177.

Triwitayakorn K., Chatkulkawin P., Kanjanawattanawong S., Sraphet S., Yoocha T., Sangsrakru D., Chanprasert J,, Ngamphiw C., Jomchai N., Therawattanasuk K., & Tangphatsornruang S, (2011). Transcriptome sequencing of *Hevea brasiliensis* for development of microsatellite markers & construction of a genetic linkage map. *DNA Research* 18: 471-482.

Venkatachalam P., Jayasree P. K., Sushamakumari S., Jayashree R., Rekha K., Sobha S., Priya P., Kala R., & Thulaseedharan A. (2007). Current perspectives on application of biotechnology to assist the genetic improvement of rubber tree (*Hevea brasiliensis* Muell. Arg.): An Overview. *Functional Plant Science and Biotechnology* 1:1-17.

Yu F., Wang B. H., Feng S. P., Wang J. Y., Li W. G., & Wu Y. T. (2011). Development, characterization, and cross-species/genera transferability of SSR markers for rubber tree (*Hevea brasiliensis*). *Plant Cell Reports* 30: 335-344.