Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(3):2897-2904. **OPEN ACCESS**

Sequence-related amplified polymorphism (SRAP) marker system for identification of Indonesian ginger (*Zingiber* sp.) genetic diversity

Maythesya Oktavioni[1], Rayfiqa Maulidah[2], Elma Nita Gozalia[3], Rahmi Hidayati[3], Bastian Nova[4], Dyah Subositi[5], Anshary Maruzy [5], Slamet Wahyono[5], and Jamsari Jamsari[1*]

[1]Department of Agrotechnology, Universitas Andalas, Padang, West Sumatera, 25136, **Indonesia**
[2]Postgraduate Biological Program, Universitas Andalas, Padang, West Sumatera, 25136, **Indonesia**
[3]Postgraduate Biotechnology Program, Universitas Andalas, Padang, West Sumatera, 25136, **Indonesia**
[4]Department of Crop Science, Universitas Andalas, Padang, West Sumatera, 25171, **Indonesia**
[5]Center for Research and Development of Medicinal Plants and Traditional Medicine, Ministry of Health Republic of Indonesia, Tawangmangu, Karanganyar, Central Java, 57792, **Indonesia.**

***Correspondence:** ajamsari@yahoo.com **Accepted: 26** June2019 **Published online:** 27 August 2019

Genome diversity of Indonesian ginger is an important data for their breeding program and utilization. In order to precisely dissect their genome characteristic a powerful marker system is necessary. Morphological characteristic so far failed to fulfill such necessity, for that reason a molecular marker system which is free from environmental bias should be available. The SRAP marker system is chosen, since it has been reported to fit with such need. A total of 51 ginger accessions, collected from the various regions across Indonesian archipelago was subjected for this study. Eight SRAP primer combinations were selected from the total of 16 SRAPs primer combinations. The Popgene (Ver. 3.2) software package was applied to estimate the value genetic diversity (HT) of the total population as well as genetic diversity within-population (HS), genetic differentiation (GST) and gene flow (NM). Data obtained from the software calculation showed that the average of gene diversity (h) in the 51 germplasms ranged from 0.0000 to 0.1262, while Shannon's Information Index (I) ranged from 0.0000 to 0.1901. The number of polymorphic loci average ranged from 0 to 38 with percentage range from 0.00 to 36.19%. Clustering analysis exhibited two main clusters (cluster A and B) where the cluster A could be separated into three subgroups. In conclusion, this finding showed incolinearity between traditional classification which is mainly based on the rhizome characteristic and SRAP marker system.

Keywords: genetic diversity, geographical distribution, germplasm, SRAP marker system, *Zingiber officinale*.

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is an important aromatic perennial herb belonging to Zingiberaceae family. The species is probably originated from the Indo-Malayan and South-East Asian region (Kizhakkayil and Sasikumar, 2011; Dhanik et al., 2017). In Indonesia, ginger is commonly used as spice, food additive, beverage, herbal medicine, pharmaceutical, and cosmetics industry. For that reasons many local varieties of the species are grown over the world which is mostly for commercial purposes but some are still underutilized. More than 400 accessions of ginger are maintained at the Indian Institute for Spice Research in Calicut, Kerala, India while only 45 accessions are conserved at the Research

Institute for Spices and Medicinal Crops in Bogor, Indonesia (Wahyuni et al., 2003).

Three well-known varieties ie.: giant ginger (*Zingiber officinale* Roscoe var. officinale), small ginger (*Zingiber officinale* Roscoe var. amarum) and red ginger (*Zingiber officinale* Roscoe var. rubrum) are cultivated around the Indonesian archipelago (Daryono et al.,2012; Rafi et al., 2013). The three cultivated varieties contain different active compounds which is mainly determined by their genetic basis and their habitat (Wu et al. 2013). For that reason in term of medicinal aspect, understanding of their genetic variation has a crucial impact.

Some molecular techniques have been widely applied to asses genetic variation in ginger species for instance Amplified Fragment Length Polimorphism (AFLP) (Wahyuni et al. 2003). They used 22 accessions of Indonesian ginger germplasm and differentiated the investigated population into three main groups of Indonesian ginger. However the marker system failed to clearly differentiate between small (*Zingiber officinale* Roscoe var. amarum) and giant ginger(*Zingiber officinale* Roscoe var. officinale). For that reason, an appropriate molecular system should be applied in order to visualize a representative genetic variation among the ginger accession. This is particular important, since the species is vegetatively propagated, where genetic variation is commonly narrow.

In this article a relative new marker system designated Sequence-Related Amplified Polymorphism (SRAP) developed by Robarts and Wolfe, (2014) has been applied. The system is a combination of an ease of RAPD technique and the high accuracy of AFLP system to detect polymorphism in most coding sequences commonly found in the genome of cultivatedspecies and have relatively low mutation rates (Keyfi and Beiki, 2012; Zeng et al., 2012).

MATERIALS AND METHODS

Plant Material and Genomic DNA Isolation

Dry leaves of 51 Indonesian ginger germplasms were collected from various ethnic in several regions across the Indonesian archipelago composed of 7 samples from Sumatera Island (Aceh, South Sumatera, and North Sumatera province), 7 samples from Java Island (West Java and Central Java province), 9 samples from Kalimantan Island (West Kalimantan, Central Kalimantan, and East-North Kalimantan province), 6 samples from Moluccas Island (Moluccas and North Moluccas province), 7 samples from Papua Island (Papua and West Papua province), 13 samples from Sulawesi Island (South Sulawesi and Central Sulawesi province), and 2 samples from East Nusa Tenggara.

Genomic DNA was isolated from dry leaves of each samples using GeneJET Plant Genomic DNA Purification Mini Kit (ThermoScientific, USA) protocol with minor modification. The DNA quantity and quality was determined by applying 1% agarose gel for electrophoresis and verified usingananodrop (ThermoScientific, USA)based on spectrophotometrically measurement by applying a wavelengths A260 and A280.

Primer Screening and PCR Amplification

Primer screening was performed using bulked segregant analysis (BSA) methods (Michelmore et al., 1991) using 16 SRAP primer combinations. The genomic DNA from 5 different single plants was used for pool construction. The selected primer combination producing clear and polymorphic bands were selected for single plant analysis. All primer combination used along BSA analysis dan their expected product are listed in the Table 1.

The PCR reaction mostly was carried out in a total volume of 25 µL consisting of 13 µL DreamTaq Green PCR Master Mix (2x) (Thermo Scientific, USA), 2.5 µL genomic DNA (25 ng/µL), 1.7 µL of each primer (20 ng/µL), and 8.1 µL nuclease-free water. The condition of PCR amplification was performed as follows: pre-denaturation at 94°C for 5 min, followed by 5 cycles of 94°C for 1 min and 35°C for 1 min. The 35 cycles used 94°C for 1 min, 50°C for1 min and 72°C for 1 min. The final extension was run at 72°C for 8 min. The PCR product was separated using 1% agarose gel on 100 volts for 30 min and then was visualized with UV-transilluminator and gel documentation system (Biometra-Germany).

Data Collection and Statistical Analysis

Clear and scorable bands produced from the gel images were scored in a binary data, where“1” represent presence band and “0” representing absence band. The dendogram, distance matrix, and similarity coefficients were constructed using the Popgene (Ver. 1.32) software package (Yeh et al., 1997). The total genetic diversity (HT), within-population diversity (HS), genetic differentiation (GST), and gene flow (NM) were calculated using the Popgene Genetic Analysis (version 1.32) software package (Yeh et al., 1997).

RESULTS

Primer Screening

A total of 16 primer combinations were screened using BSA method (Table 1).Two primer combinations (A and D) produced no fragment, while M produced up to 7 fragment. Based on number of scorable fragments produced during BSA-based secreening, 8 primer combinations designated E, G, H, J, K, L, M, and P were selected. The 8 primer combinations successfully produced 105 distinct scorable fragments (50 to 1,280 bp in size) or 13.5 fragments in average ranging from 11 to 18 fragments from each single plant. Primer H produced the highest number of fragments (18) while primer G, K, M and P produced only 11 fragments.

Genetic Diversity Among Indonesian Ginger Germplasms

Fragments produced from 51 ginger accessions were scored as binary data and subjected to multivariate analysis using Popgene(1.32). Germplasms collected from East Kalimantan-North Kalimantan ethnic Putuk showed the highest score on all parameters associated with genetic diversity index (Table 2). The average gene diversity (h) in the 51 germplasm ranged from 0.0000 to 0.1262, while Shannon's Information Index (I) ranged from 0.0000 to 0.1901. The average number of polymorphic loci ranged from 0 to 38 (0.00-36.19%) while the total genetic diversity value is about 0.2185 (Table 4).

To assess the clustering pattern, UPGMA based on Nei's algorithm (1972) was applied. Genetic distance based on the dendrogram classified all the 51 germplasms into two major clusters (A and B) (see Fig. 3). The dendrogram also represented a diverse genetic profile where Siang02 ethnic collected from Central Kalimantan (cluster A) is clustered separately with cluster in B (consisting of three sub-group I, II and III). This germplasm have different fragment profile with another germplasms in amplification using primer combination G (see C1D to C5D in Fig. 2).

Table 1; Nucleotide sequence, primer ID, number of fragments produced along BSA and individual selection and range of fragment size produced during selection.

Forward (5′-3′)	Reverse (5′-3′)	ID	Fragment no in BSA	Fragment no in individual plant	Fragment size range (bp)
TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAT	A	0		
	GACTGCGTACGAATTTGC	B	2		
	GACTGCGTACGAATTGAC	C	1		
	GACTGCGTACGAATTTG	D	0		
TGAGTCCAAACCGGAGC	GACTGCGTACGAATTAAT	E	4	12	50-1,200
	GACTGCGTACGAATTTGC	F	2		
	GACTGCGTACGAATTGAC	G	4	11	100-1,050
	GACTGCGTACGAATTTG	H	3	18	100-1,200
GACTGCGTACGAATTGAC	GACTGCGTACGAATTAAT	I	1		
	GACTGCGTACGAATTTGC	J	4	16	100-1,280
	GACTGCGTACGAATTGAC	K	3	11	40-1,000
	GACTGCGTACGAATTTG	L	2	15	100-1,100
GACTGCGTACGAATTTG	GACTGCGTACGAATTAAT	M	7	11	50-900
	GACTGCGTACGAATTTGC	N	2		
	GACTGCGTACGAATTGAC	O	2		
	GACTGCGTACGAATTTG	P	3	11	150-800
Total				105	

Table 2; Genetic diversity index of 51 Indonesian ginger germplasms

No	Population Name (Province and ethnic source)	h	I	NPL	PPL
1	Aceh_GayoSerbajadi	0.0257	0.0377	7	6.67 %
2	Aceh_Singkil01	0.1063	0.1611	33	31.43 %
3	Aceh_Singkil02	0.0685	0.1010	19	18.10 %
4	Aceh_Singkil03	0.0483	0.0681	11	10.48 %
5	Aceh_Singkil04	0.0478	0.0707	13	12.38%
6	West Java_Bandung	0.0569	0.0804	13	12.38 %
7	West Java_Baduy	0.0634	0.0932	17	16.19 %
8	Central Java_Solo01	0.0563	0.0808	14	13.33 %
9	Central Java_Solo02	0.0723	0.1044	18	17.14 %
10	Central Java_Solo03	0.0607	0.0876	15	14.29%
11	Central Java_Solo04	0.0000	0.0000	0	0.00%
12	Central Java_Banyumasan	0.0000	0.0000	0	0.00%
13	West Kalimantan_Galik	0.0738	0.1118	23	21.90 %
14	West Kalimantan_Sambas01	0.0000	0.0000	0	0.00 %
15	West Kalimantan_Sambas02	0.0678	0.1025	20	19.05 %
16	West Kalimantan_Ngabang	0.0329	0.0485	9	8.57 %
17	Central Kalimantan_Siang01	0.0450	0.0672	13	12.38 %
18	Central Kalimantan_Tomum	0.0544	0.0814	16	15.24 %
19	Central Kalimantan_Siang02	0.0706	0.1071	22	20.95 %
20	East Kalimantan-North Kalimantan_Putuk	0.1262	0.1901	38	36.19 %
21	East Kalimantan-North Kalimantan_Lepo Tau	0.1036	0.1519	28	26.67 %
22	Moluccas_Alfuru01	0.0276	0.0403	7	6.67 %
23	Moluccas_Alfuru02	0.0532	0.0765	13	12.38 %
24	Moluccas_Alfuru03	0.0443	0.0668	13	12.38 %
25	Moluccas_Wahai	0.0649	0.0972	19	18.10%
26	North Moluccas_Makian	0.0857	0.1280	25	23.81 %
27	North Moluccas_Galela	0.0995	0.1475	28	26.67%
28	East Nusa Tenggara_Ende	0.0123	0.0190	4	3.81 %
29	East Nusa Tenggara_Kabola	0.0728	0.1103	22	20.95 %
30	Papua_Asmat	0.0449	0.0642	11	10.48 %
31	Papua_Dani	0.0498	0.0710	12	11.43 %
32	Papua_Ngalum	0.0057	0.0091	2	1.90 %
33	Papua_Komoro	0.0357	0.0532	10	9.52 %
34	Papua_Amungme	0.0581	0.0869	17	16.19 %
35	Papua_Sawi	0.0450	0.0662	12	11.43 %
36	West Papua_Tehit	0.0612	0.0904	17	16.19 %
37	South Sulawesi_Duri01	0.1429	0.2127	40	38.10 %
38	South Sulawesi_Duri02	0.0272	0.0396	7	6.67 %
39	South Sulawesi_Duri03	0.0355	0.0518	9	8.57 %
40	South Sulawesi_Rongkong	0.0221	0.0370	10	9.52%
41	South Sulawesi_Patinjo	0.0450	0.0662	12	11.43 %
42	Central Sulawesi_Balantak	0.0225	0.0331	6	5.71%
43	Central Sulawesi_ToBadaya	0.0393	0.0571	10	9.52 %
44	Central Sulawesi_Bangai	0.0000	0.0000	0	0.00%
45	Central Sulawesi_Mia Sea-Sea	0.0266	0.0386	7	6.67%
46	Central Sulawesi_Bare'e	0.0705	0.1030	19	18.10%
47	Central Sulawesi_Tolage	0.0597	0.0886	17	16.19 %
48	Central Sulawesi_Tialo	0.0284	0.0407	7	6.67 %
49	Central Sulawesi_Bungku	0.0687	0.1019	20	19.05 %
50	South Sumatera_Pegagan	0.0197	0.0288	5	4.76 %
51	North Sumatera_Simalungun	0.0995	0.1475	28	26.67%

h: Gene Diversity; I: Shannon's Information Index; NPL: Number Of Polymorphic Loci; PPL: Percentage Of Polymorphic Loci.

Table 3: Total population genetic diversity of *Zingiber officinale*

HT	HS	GST	NM*
0.2185	0.0507	0.7680	0.1510

HT = total genetic diversity; HS = genetic diversity within populations; GST = coefficient of genetic differentiation; NM* = gene flow among populations; [NM* = 0.5 (1 - GST) / GST (McDermott and McDonald, 1993)].

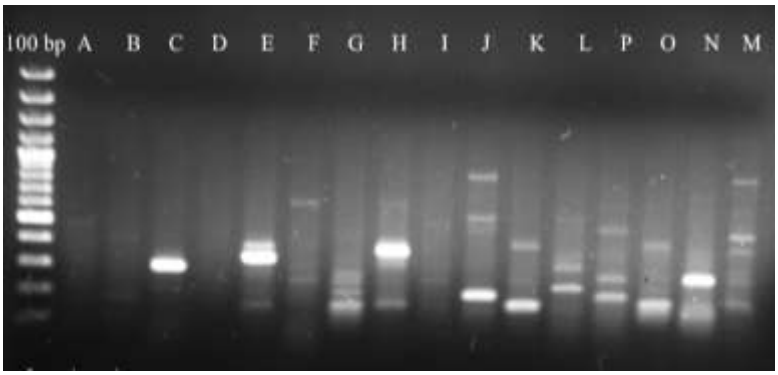

Figure 1: BSA-based screening of 16 SRAPS primer combinations using Indonesian ginger accessions.

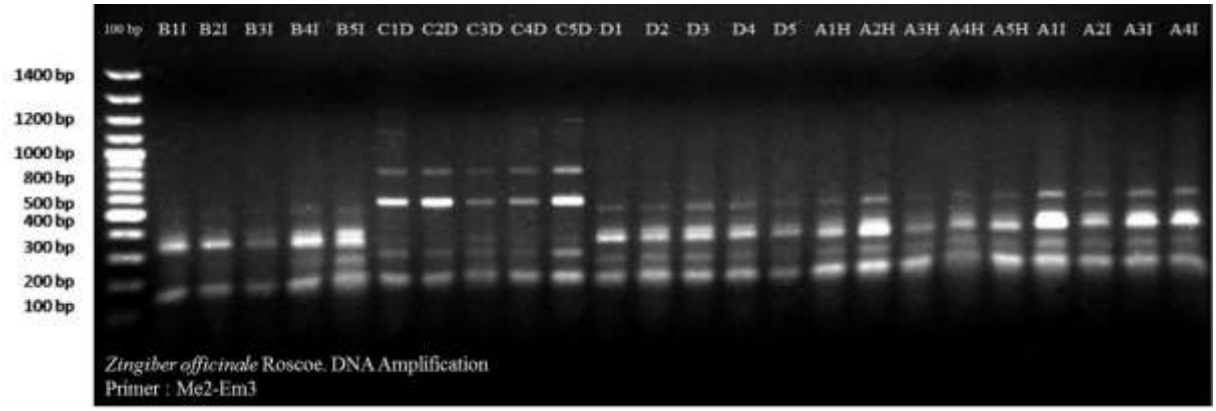

Figure 2: Visualization of ginger DNA amplification using primer combination G. B1I-B5I: Central Kalimantan_Tomum; C1D-C5D: Central Kalimantan_Siang02; D1-D5: West Kalimantan_Ngabang; A1H-A5H: Central Kalimantan_Siang01; A1I-A4I: East Nusa Tenggara_Ende.

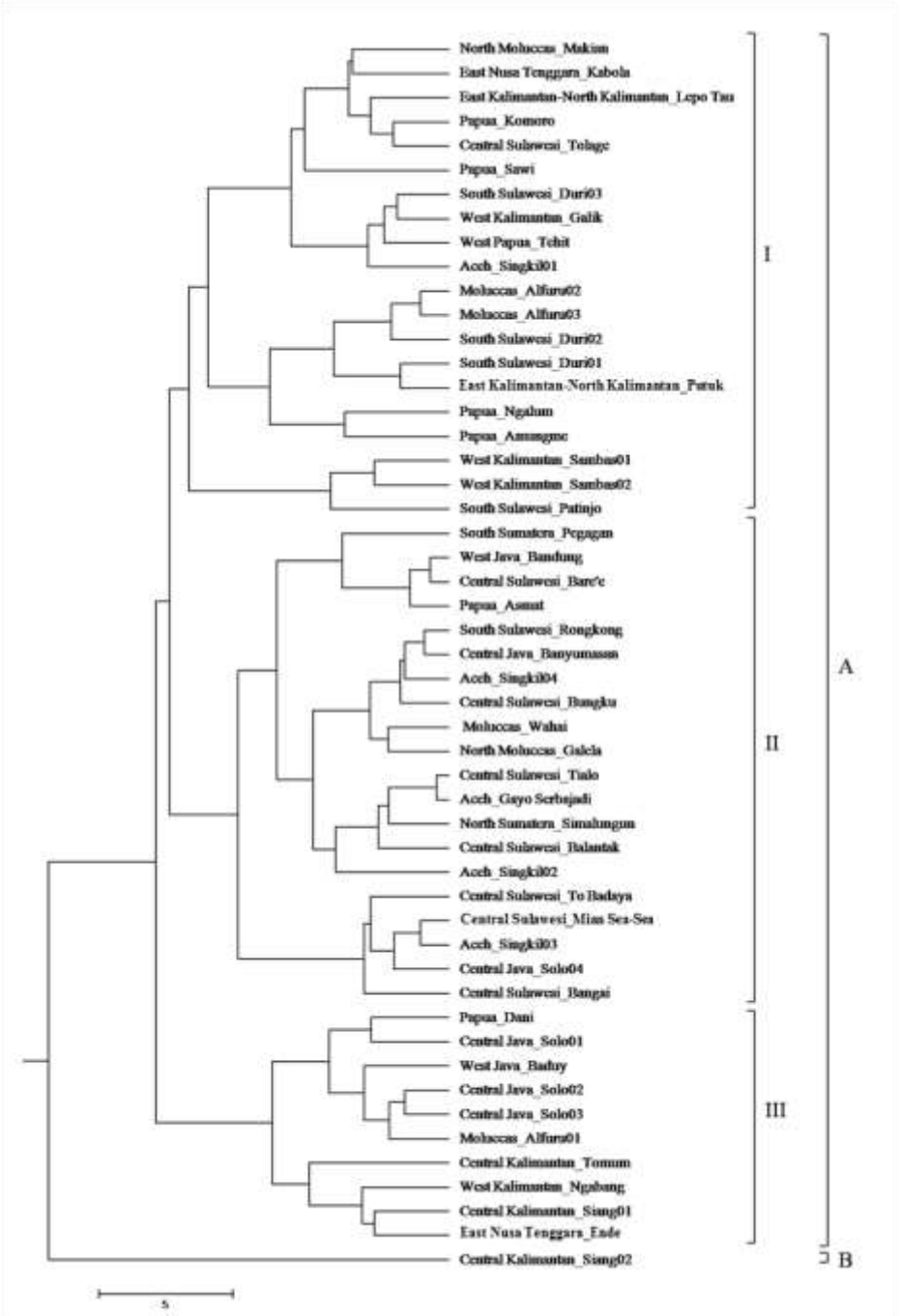

Figure 3; Dendrogram depicting genetic relationships among the 51 Indonesian ginger germplasms.

DISCUSSION

Genetic diversity of 51 ginger germplasms collected from several ethnics in 16 provinces in Indonesia were successfully assessed with SRAP marker system. The data presented here could serve as a baseline in use of *Zingiber* sp. as an active compound source for pharmaceutical and cosmetics. The data is also necessary for their conservation for breeding program and also for food and beverage utilization.

Genetic diversity of 51 ginger accessions presented here showed low value, indicating that our ginger germplasms have narrow genetic background eventhough they were collected from across of Indonesian archipelago. Ssimilar result

was also reported by Kizhakkayil and Sasikumar (2011) investigating 34 *Zingiber* accessions collected from India with others 16 accessions collected from China, Nigeria, Nepal, Brazil, Oman, Pakistan, Japan, and Queensland. They found a geographical bias phenomenone. However, the genetic distance among those accession was also low.

The Indonesia ginger germplasms is clusterred into two different main cluster, despite they collected from similar ethnic location (province). The cluster A consists of three sub-groups: I, II and III. Those clustering involve all the three main groups which is traditionally based on the rhizome types (giant ginger, small ginger and red ginger). The prove of our finding can be shown by a red ginger group: Aceh_Singkil01, Aceh_Singkil03, Central Java_Solo01, and Central Java_Solo02, which is in fact, they are clustered into separate group (see Fig.3). Interestingly, Siang02 ethnic germplasm collected from Central Kalimantan is clustered very distinctly into a separate singly cluster B. Hence our finding here is not in line with the previous classification based on the morphological character. However, our study show similar pattern as described by Pandotra et al., (2013) using ISSR and SSR for accessing their Himachal Pradesh's germplasm collection. They assumed that the possible reason for the discrepancy could be caused by insufficiency of the used markers for genome dissection.

Study reported by Wang et al.,(2014) concluded that ginger genetic diversity in China is not linear with their geographical distribution, but more associated with the ploidy levels. They found that 74.6% of 63 ginger acessions were diploid (2x) and 25.4% were mixploid (a mixture between diploid and tetraploid). In the diploid cells, they found 22 chromosomes while in the mixploid cells they found 44 chromosomes. Previous studies regarding chromosome number of ginger also reported different results. Etikawati and Setyawan (2000) reported that 10 varieties of Indonesian *Zingiber* sp. have similar chromosome number, 2n=32. But a study reported by Daryono et al., (2012) described that only the giant ginger and small ginger have similar chromosome number, where 2n=2x=30, while the red ginger has 2n=2x=22. The later chromosome number is similar to Thailand ginger accession reported by Eksomtramage *et al.* (2002), Saensuk and Saensouk (2004) and Sanpote (2004).

CONCLUSION

Based on this study the Indonesian ginger could be grouped in two main clusters (cluster A and B) where the cluster A could be separated into three subgroups. This finding also showed the incolinearity between traditional classification which is mainly based on the rhizome characteristic and the SRAP marker system

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This research was fully funded by The center for research and development of medicinal plants and traditional medicine (B2P2TO-OT), Ministry of Health Republic of Indonesia, contract number: HK.03.01/3/3000/2018 and 2031/UN.16.01.D/PP-/2018

AUTHOR CONTRIBUTIONS

MO performed DNA isolation, primer selection, data analysis and manuscript preparation, RM performed DNA isolation, BSA analysis, control of DNA quality and clustering, ENG conducted primer selection, fragment scoring and manuscript preparation, RH controlled scoring process and clustering analysis, BN performed bioinformatic analysis, dendogram construction, DS maintained samples dan data collection, AM controlled sample collection, SW managed data administration and data collection integrity, JJ managed and controlled overall experiment, controlled data acquisition, funding acquisition, reviewed final manuscript. All authors already read and approved the final version.

REFERENCES

Daryono, BS., Rahmah, SNAF, Purnomo, and Sudarsono. 2012. Chromosome Characterization of Three Varieties of Ginger

(*Zingiber officinale* Rosc.). *Indonesian Journal Pharmacy,* 23 (1), pp. 54-59.

Dhanik, J., Arya, N., and Nand, V. 2017.A review on *Zingiber officinale*.*Journal of Pharmacognosy and Phytochemistry,* 6 (3), pp. 174-184.

Eksomtramage, L., Sirirugsa, P., Jivanit, P., and Maknoi, C., 2002, Chromosome Counts of Some Zingiberaceous Species from Thailand. *Songklanakarin Journal of Science and Technology* 24 (2), pp. 311-319.

Etikawati, N. and Setyawan, AD. 2000. StudiSitotaksonomipada Genus Zingiber.*JurnalBiodiversitas,* 1 (1), pp. 8-13.

Food and Agriculture Organization of the United Nations. 2002. GINGER: Post-Production Management for Improved Market Access. 20 pages.

Keyfi, F., and Beiki, AH. 2012. Exploitation of random amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) markers for genetic diversity of saffron collection. *Journal of Medicinal Plants Research*, 6(14), pp. 2761-2768.

Kizhakkayil, J. and Sasikumar, B. 2011. Diversity, Characterization, and Utilization of Ginger: A Review. *Plant Genetic Resources*, 9 (3), pp. 464-477.

MichelmoreRW.,Paran I. and Kesseli RV. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations.Proc. Natl Acad. Sci. USA,88, pp. 9828–9832

Pandotra, P., Gupta, AP., Husain, MK., Gandhiram and Gupta S. 2013.Evaluation of genetic diversity and chemical profile of ginger cultivars in north-western Himalayas.*Biochemical Systematics and Ecology,* 48, pp. 281-287.

Rafi, M., Lim, LW., Takeuchi, T., and Darusman, LK. 2013. Simultaneous determination of gingerols and shogaol using capillary liquid chromatography and its application in discrimination of three ginger varieties from Indonesia. *Talanta*, 103, Pp. 28-32.

Robarts, D. W., & Wolfe, A. D. (2014). Sequence-related amplified polymorphism (SRAP) markers: A potential resource for studies in plant molecular biology(1.).*Applications in plant sciences*,*2*(7), apps.1400017. doi:10.3732/apps.1400017

Saensouk, S. and Saensuk, P. 2004. Chromosome Numbers of Zingiberaceae in Thailand.*KKU Res Journal*, 9 (1), pp. 3-9.

Sanpote, P. 2004. Species Diversity and Chromosome Number of Some Zingiberaceae in the Region of Thailand.*Thailand Journal of Genetics,* 20 (3). pp. 3-9

Wahyuni, S., Xu, D.H., Bermawie, N., Tsunemats, H., Ban, T., 2003.Genetic relationships among Ginger accessions based on AFLP marker.*JurnalBioteknologiPertanian*, 8, pp. 60-68.

Wang, L., Gao, F. ,Xu, K., and Li, X. 2014. Natural occurrence of mixploid ginger (*Zingiber officinale*Rosc.) in China and its morphological variations. *ScientiaHorticulturae,* 172, pp. 54-60.

Wu, Y.B., Zheng, L.J., Yi, J., Wu, J.G., Chen, T.W., and Wu, J.Z., 2013. Quantitative and Chemical Fingerprint Analysis for the Quality Evaluation of *Receptaculumnelumbinis* by Rp-HPLC Coupled with Hierarchical Clustering Analysis. *International Journal of Molecular Science,* 14(1), pp. 1999-2010.

Yeh, F. C., Yang, R., Boyle, T. B. J., Ye, Z. H, Mao, J. X. 1997.POPGENE, the user friendly shareware for population genetic analysis.Molecular Biology and Biotechnology Centre, University of Alberta.

Zeng, B., Wang, G. Z., Zuo, F. Y., Chen, Z. H., and Zhang, X. Q. 2012. Genetic diversity analysis of cocksfoot (*Dactylisglomerata*L.) accessions with sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeat (ISSR) markers. *African Journal of Biotechnology* 11(67), pp. 13075-13084.