conservation of Alpinia SRAP

by Jamsari Jamsari

Submission date: 05-Aug-2020 09:54AM (UTC+0800)
Submission ID: 1366043934
File name: AJCB-Vol8-No1-Maulidah_et_al.pdf (1.4M)
Word count: 3527
Character count: 18748

Asian Journal of Conservation Biology, July 2019. Vol. 8 No. 1, pp. 72-78 AJCB: FP0105
ISSN 2278-7666 ©*TCRP* 2019

Genetic Diversity and Relationship of *Alpinia galanga* (L.) Willd. in Indonesia Using SRAP Markers for Genetic Conservation Strategy

Rayfiqa Maulidah[1], Maythesya Octavioni[2], Elma Nita Gozalia[2], Rahmi Hidayati[2], Bastian Nova[3], Dyah Subositi[3], Aniska Novita Sari[3], Yuli Widiyastuti[3], Jamsari Jamsari[2*]

[1] *Department of Biology, Faculty of Mathematics and Natural Science, Andalas University, Padang, West Sumatra, 25163, Indonesia.*
[2] *Biotechnology Laboratory, Department of Agrotechnology, Faculty of Agriculture, Andalas University, Padang, West Sumatra, 25163, Indonesia.*
[3] *Department of Crop Science, Andalas University, Padang, West Sumatra, 25171, Indonesia.*
[4] *Center for Research and Development of Medicinal Plants and Traditional Medicine (B2P2TOOT), Ministry of Health Republic of Indonesia, Karanganyar, Central Java, 57792, Indonesia.*

(Received: May 24, 2019; Revised: June 20, 2019; Accepted: July 02, 2019)

ABSTRACT

Genetic diversity and relationship analysis among *Alpinia galanga* (L.) Willd. populations in Indonesia was carried out using Sequence Related Amplified Polymorphism (SRAP) markers. A total of 120 individuals from 28 populations were screened using eight primer combinations, which resulted 75 polymorphic fragments. The dendrogram, distance matrix, and similarity coefficients were constructed using the Popgene (version 3.2) software package. The feature provided by the software package also applied to estimate the total genetic diversity (H_T), within-population diversity (H_S), genetic differentiation (G_{ST}), and gene flow (N_M). Total Nei's genetic diversity (h) among 28 populations ranged from 0.0000 to 0.1268 while Shanon's information index (I) ranged from 0.0000 to 0.1920. The genetic diversity of galangal in this study showed a fairly low level with random clustering among populations. Based on the results, we recommend a genetic conservation strategy for the species sustainability.

Key words: *Alpinia galanga*, genetic conservation, genetic diversity, greater galangal, , SRAP marker

INTRODUCTION

Alpinia galanga (L.) Willd. or known as greater galangal belongs to genus *Alpinia* and one of the species that is widely cultivated in East Bengal, South India, and South East Asia. Many authors believe that this species is native to Sumatra and Java (Roxburgh, 1812, Watt, 1883, Burkill, 1935, Singh, 1965, Schumann, 1904). However, many of the species can be found in the Malay Peninsula as cultivated crops or wild plants. Based on its wide distribution and its ability to survive in adverse conditions, Ridley (1899) believed that *A. galanga* was not a true wild plant from any region but it was was domesticated from cultivated plants. Compared with another genus in Zingiberaceae, only a few species of *Alpinia* are used in Indonesia. One of the most popular is *A. galanga* which is used as a spice in many traditional cuisine. Besides, the species is also used in many diverses traditional medication particularly in many several Asian regions (Nurainas dan Arbain, 2017, Namsa *et al.* 2009).

For that reasons, survey and exploration has been carried out by the RISTOJA team since 2012. During that activities, more than 100 individual plants were collected from 28 different populations (provinces). In order to asses their detail identity morphological characterization was prior carried out. However, in many cases morphological characterization fails to asses their detail identity, due to many environmental factors affecting their morphlogical performans. For that reason molecular-based marker is neceessary to be applied.

Since 2011, the SRAP (Sequence Related Amplified Polymorphism) marker system has become a popular marker for genetic diversity assessment (Li, Mcvetty, Quiros, 2013). Here we report, an assessment of *Alpinia galanga* genetic diversity using SRAP markers system combined with Bulked Segregant Analysis (BSA) developed by Michelmore, Paran, Kesseli (2011).

MATERIALS AND METHODS

Plant Material

A total of 120 samples from 28 *Alpinia galanga* populations were collected from various provinces and ethnicities in Indonesia. The dried and healthy tissues (mostly leaf) of the sample which is about 1 gram in weight was used as material for DNA isolation.

Genomic DNA Extraction

Total genomic DNA was extracted from silica-dried tissue using a minor modification of GeneJET Plant Genomic DNA Purification Mini Kit (ThermoScientific, USA) extraction protocol. The quality of DNA was assessed by electrophoresis using 1% agarose with 6X

*Corresponding Author's E-mail: ajamsari@yahoo.com

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	2		
	GACTGCGTACGAATTTGC	B	4	13	130-1,090
	GACTGCGTACGAATTGAC	C	2	12	50-1,000
	GACTGCGTACGAATTTG	D	3	9	50-700
TGAGTCCAAACCGGAGC	GACTGCGTACGAATTAAT	E	3	8	100-800
	GACTGCGTACGAATTTGC	F	1		
	GACTGCGTACGAATTGAC	G	1	12	100-1,050
	GACTGCGTACGAATTTG	H	0		
GACTGCGTACGAATTGAC	GACTGCGTACGAATTAAT	I	1		
	GACTGCGTACGAATTTGC	J	2	10	100-800
	GACTGCGTACGAATTGAC	K	3	8	150-1,000
	GACTGCGTACGAATTTG	L	2		
GACTGCGTACGAATTTG	GACTGCGTACGAATTAAT	M	2		
	GACTGCGTACGAATTTGC	N	0		
	GACTGCGTACGAATTGAC	O	1		
	GACTGCGTACGAATTTG	P	2	3	300-600
Total				**75**	

TriTrack DNA Loading Dye and GeneRuler 1Kb Plus DNA Ladder (ThermoScientific, USA). The DNA quantity was measured using NanoDrop Spectrophotometer (ThermoScientific, USA).

Primer Screening

Sixteen different SRAP primer combinations were tested using BSA (Bulked Segregant Analysis) strategy using five individual DNA sample. The bulked DNA was constructed by mixing each genomic DNA (50 ng/µl) from selected samples in one tube. The PCR mixture and amplification process is carried out using standard of SRAP protocolas described by Li *et al.* (2013). Data was scored as "1" for the present fragment and "0" for the absent fragment. The total number of amplified fragment was counted used for dendogram construction.

PCR Procedure

Eight SRAP primers were selected from BSA screening. Primer ID and their sequences is presented in Table 1.

DNA amplification was carried out using a modified protocol described by Li dan Quiros (2001). The PCR reaction was performeed in a total volume of 25 µL, containing 3 µL of genomic DNA (25 ng/µL), 13 µL of DreamTaq Green PCR Master Mix (2X) (ThermoScientific, USA), 2 µL of each primer, 7 µL of free nuclease water. The amplification process was carried out with the following condition: 5 min of initial denaturation at 94°C, 5 cycles of 1 min denaturation at 94°C, 1 min of annealing at 35°C and 1 min elongation at 72°C, followed by 35 cycles with an annealing temperature of 50°C, and a final elongation step of 8 min at 72°C.

Data Analysis

The SRAP fragments were scored for the presence or absence and converted into a spreadsheet as 0 and 1 code. The dendrogram, distance matrix, and similarity coefficients were constructed using the Popgene (version 3.2) (Yeh *et al*. 1997) software package. Total genetic diversity (H_T), within-population diversity (H_S), genetic differentiation (G_{ST}), and gene flow (N_M) were calculated using the feature provided by the software package.

RESULTS

SRAP Primer Screening

Screening of 16 primers had been carried out using the BSA method (Table 1, Figure 1). The combination of B primer produced four fragment as the most fragments, followed by D, E, K with three fragments, while the two combinations of primers (H and N) do not produce any fragments. Based on the number of fragments produced through this BSA method, eight primer combinations were chosen to be used in this study, namely B, C, D, E, G, J, K, P. All combinations of selected primers were successfully used in *A.galanga* amplification with a total of 75 fragments were produced in the range of 3-13 fragments for each individual. The range of fragment sizes produced was 50-1,090 bp for the whole individual. B primer produced the most fragments (13) followed by fragments C, G (12) and J (10), while P primer produced the least fragments (3).

PCR amplification on each individual plant sample (120 accessions) has been carried out using 8 selected SRAP primer combinations. Primer pairs C showed the clearest and the best DNA band

Table 2. Similarity coefficients and genetic distance among *Alpinia galanga* (L.) Willd collected from 28 populations

pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	****	0.8563	0.9417	0.9739	0.9494	0.9240	0.7394	0.8274	0.8627	0.9228	0.8735	0.9265	0.9281	0.9344	0.8947	0.8731	0.8903	0.8539	0.8517	0.9114	0.8296	0.8121	0.9275	0.8756	0.8282	0.8506	0.8624	0.8585
2	0.1552	****	0.7918	0.8758	0.9131	0.9110	0.7340	0.7935	0.8418	0.8013	0.7896	0.8283	0.8171	0.7878	0.8812	0.7995	0.8398	0.8580	0.8222	0.7854	0.7405	0.7500	0.8307	0.7696	0.7319	0.7379	0.7388	0.7623
3	0.0601	0.2335	****	0.9188	0.9109	0.8376	0.7039	0.8232	0.8697	0.9512	0.8336	0.9117	0.9259	0.9893	0.8981	0.8691	0.8832	0.8236	0.8311	0.9374	0.8262	0.7940	0.9095	0.8362	0.7936	0.8721	0.8627	0.8568
4	0.0264	0.1326	0.0847	****	0.9508	0.9274	0.7327	0.8460	0.8806	0.9210	0.8676	0.9149	0.9073	0.9135	0.9006	0.8731	0.8878	0.8685	0.8669	0.8967	0.8260	0.8245	0.9186	0.8730	0.8353	0.8441	0.8545	0.8555
5	0.0520	0.0909	0.0934	0.0505	****	0.9572	0.7643	0.8486	0.8934	0.9307	0.8751	0.9365	0.9343	0.9068	0.9505	0.8953	0.9145	0.8742	0.8748	0.8733	0.8641	0.8358	0.9210	0.8313	0.7820	0.8251	0.8223	0.8265
6	0.0790	0.0932	0.1772	0.0754	0.0437	****	0.7207	0.7951	0.8013	0.8620	0.8275	0.8759	0.8881	0.8372	0.9083	0.8694	0.8809	0.8322	0.8553	0.8409	0.8272	0.8259	0.9060	0.8058	0.7595	0.7906	0.7997	0.8212
7	0.3020	0.3093	0.3511	0.3110	0.2688	0.3275	****	0.8603	0.8511	0.7527	0.7957	0.8132	0.7914	0.7008	0.7894	0.7447	0.7842	0.7538	0.7180	0.6771	0.6748	0.6735	0.7189	0.7175	0.6564	0.6959	0.6910	0.6981
8	0.1894	0.2313	0.1945	0.1672	0.1642	0.2293	0.1505	****	0.9410	0.8717	0.9369	0.8957	0.8762	0.8217	0.8903	0.8578	0.8857	0.7980	0.7745	0.7966	0.7493	0.7201	0.8122	0.8219	0.7429	0.7954	0.7999	0.8202
9	0.1477	0.1722	0.1396	0.1271	0.1127	0.2215	0.1612	0.0609	****	0.9105	0.9048	0.9148	0.8955	0.8621	0.9028	0.8495	0.8946	0.8498	0.8156	0.8202	0.7825	0.7625	0.8371	0.8362	0.8006	0.8291	0.8315	0.8292
10	0.0803	0.2215	0.0501	0.0823	0.0718	0.1485	0.2841	0.1373	0.0938	****	0.9076	0.9673	0.9705	0.9647	0.9345	0.9377	0.9203	0.8647	0.8705	0.9220	0.8630	0.8306	0.9196	0.8180	0.7747	0.8508	0.8419	0.8411
11	0.1352	0.2362	0.1820	0.1421	0.1334	0.1894	0.2285	0.0652	0.1001	0.0969	****	0.9402	0.9029	0.8507	0.8920	0.9115	0.9076	0.8150	0.8142	0.8348	0.7918	0.7626	0.8607	0.7819	0.7147	0.7667	0.7649	0.7881
12	0.0763	0.1884	0.0925	0.0890	0.0657	0.1325	0.2068	0.1102	0.0890	0.0333	0.0617	****	0.9808	0.9228	0.9434	0.9432	0.9441	0.8912	0.8944	0.9055	0.8619	0.8358	0.9218	0.8313	0.7653	0.8417	0.8317	0.8398
13	0.0747	0.2020	0.0770	0.0973	0.0679	0.1187	0.2340	0.1322	0.1104	0.0300	0.1021	0.0193	****	0.9299	0.9414	0.9164	0.9154	0.8583	0.8622	0.8963	0.8534	0.8231	0.9081	0.8226	0.7591	0.8413	0.8307	0.8383
14	0.0678	0.2385	0.0107	0.0904	0.0978	0.1777	0.3556	0.1964	0.1483	0.0360	0.1617	0.0803	0.0726	****	0.8969	0.8932	0.8816	0.8450	0.8526	0.9538	0.8471	0.8152	0.9220	0.8332	0.7903	0.8669	0.8581	0.8537
15	0.1113	0.1264	0.1075	0.1047	0.0508	0.0962	0.2365	0.1162	0.1023	0.0677	0.1143	0.0583	0.0603	0.1088	****	0.9100	0.9558	0.8911	0.8854	0.8751	0.8539	0.8262	0.9052	0.8180	0.7730	0.8157	0.8128	0.8340
16	0.1357	0.2238	0.1403	0.1357	0.1106	0.1400	0.2948	0.1533	0.1631	0.0643	0.0926	0.0585	0.0873	0.1130	0.0943	****	0.9523	0.8448	0.8591	0.9013	0.8533	0.8220	0.9008	0.7907	0.7403	0.7974	0.7944	0.8029
17	0.1162	0.1746	0.1243	0.1190	0.0894	0.1268	0.2431	0.1214	0.1114	0.0830	0.0970	0.0575	0.0884	0.1260	0.0452	0.0489	****	0.8683	0.8787	0.8928	0.8571	0.8342	0.9155	0.8314	0.7917	0.8295	0.8278	0.8338
18	0.1580	0.1531	0.1941	0.1410	0.1344	0.1836	0.2826	0.2257	0.1627	0.1453	0.2045	0.1152	0.1529	0.1684	0.1153	0.1687	0.1412	****	0.9565	0.8814	0.8563	0.8747	0.8861	0.8000	0.7521	0.7835	0.7745	0.7702
19	0.1605	0.1957	0.1851	0.1428	0.1338	0.1564	0.3313	0.2555	0.2038	0.1387	0.2055	0.1116	0.1483	0.1595	0.1217	0.1518	0.1294	0.0445	****	0.9040	0.9308	0.9444	0.9140	0.7751	0.7364	0.7860	0.7758	0.7800
20	0.0927	0.2415	0.0646	0.1090	0.1355	0.1733	0.3899	0.2275	0.1983	0.0812	0.1805	0.0992	0.1095	0.0473	0.1334	0.1040	0.1134	0.1262	0.1010	****	0.8977	0.8705	0.9223	0.8044	0.7578	0.8159	0.8129	0.8215
21	0.1869	0.3005	0.1910	0.1912	0.1461	0.1897	0.3933	0.2886	0.2452	0.1474	0.2334	0.1487	0.1585	0.1659	0.1579	0.1586	0.1542	0.1551	0.0717	0.1080	****	0.9722	0.9220	0.7565	0.7269	0.7596	0.7669	0.7737
22	0.2081	0.2876	0.2307	0.1930	0.1794	0.1912	0.3953	0.3284	0.2711	0.1856	0.2711	0.1794	0.1947	0.2043	0.1909	0.1960	0.1812	0.1339	0.0572	0.1387	0.0282	****	0.9193	0.7607	0.7310	0.7742	0.7697	0.7672
23	0.0753	0.1855	0.0949	0.0849	0.0823	0.0987	0.3301	0.2080	0.1778	0.0838	0.1500	0.0814	0.0964	0.0813	0.0997	0.1045	0.0882	0.1209	0.0899	0.0809	0.0813	0.0842	****	0.9011	0.8554	0.9017	0.9062	0.9139
24	0.1329	0.2619	0.1789	0.1358	0.1848	0.2160	0.3319	0.1961	0.1789	0.2009	0.2460	0.1848	0.1953	0.1825	0.2009	0.2349	0.1847	0.2231	0.2548	0.2176	0.2790	0.2735	0.1041	****	0.9528	0.9499	0.9808	0.9701
25	0.1885	0.3122	0.2312	0.1800	0.2459	0.2751	0.4210	0.2972	0.2223	0.2552	0.3358	0.2675	0.2756	0.2354	0.2574	0.3006	0.2335	0.2849	0.3059	0.2773	0.3190	0.3134	0.1562	0.0484	****	0.8937	0.9418	0.9178
26	0.1618	0.3039	0.1368	0.1695	0.1922	0.2350	0.3626	0.2289	0.1875	0.1616	0.2656	0.1723	0.1728	0.1428	0.2037	0.2264	0.1869	0.2440	0.2408	0.2035	0.2749	0.2559	0.1035	0.0514	0.1124	****	0.9851	0.9644
27	0.1480	0.3027	0.1477	0.1572	0.1956	0.2235	0.3696	0.2232	0.1846	0.1720	0.2680	0.1843	0.1855	0.1530	0.2073	0.2302	0.1890	0.2556	0.2539	0.2071	0.2654	0.2618	0.0985	0.0194	0.0600	0.0150	****	0.9884
28	0.1526	0.2714	0.1546	0.1561	0.1906	0.1969	0.3594	0.1982	0.1873	0.1730	0.2382	0.1747	0.1764	0.1581	0.1816	0.2195	0.1818	0.2611	0.2485	0.1966	0.2566	0.2649	0.0900	0.0303	0.0858	0.0362	0.0117	****

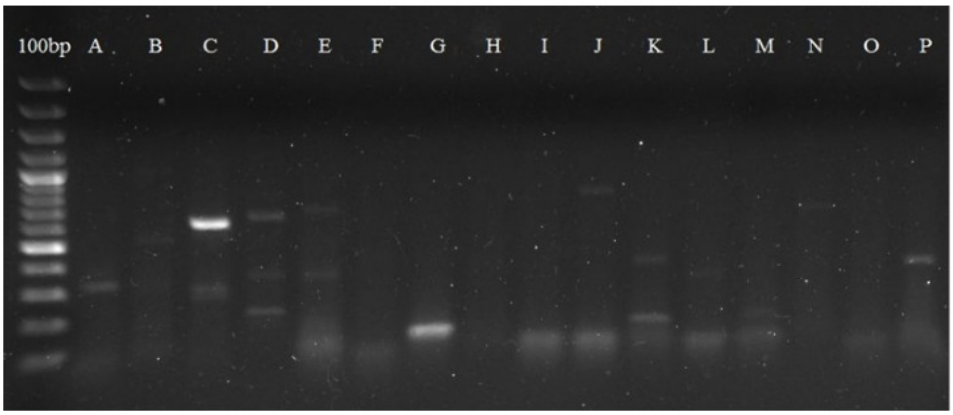

Figure 1. BSA-based screening of 16 SRAPS primer combinations using 5 selected individuals

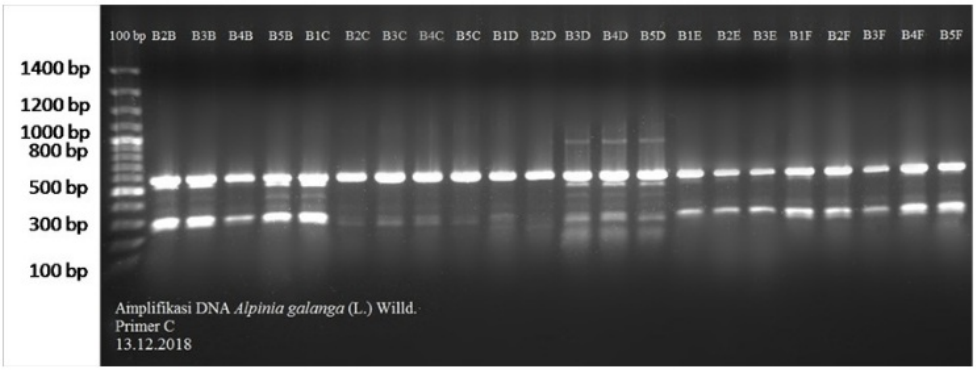

Figure 2. Electrophoregram of PCR product generated from by primer pairs C using DNA template of 5 *Alpinia galanga* (L.) Willd accession collected from B2B-B5B: East-North Kalimantan; B1C-B5C: Lampung; B1D-B5D: Papua; B1E-B3E: South Sulawesi; B1F-B5F: Central Sulawesi

profile (Figure 2). Two populations namely East Kalimantan and Papua showed a slight different fragment profile compared to the other three populations, Lampung, North and South Sulawesi.

Genetic Diversity

The similarity coefficients and genetic distances among 28 populations are presented in Table 2. The lowest similarity coefficients (0.6968) is exhibited from Maluku and North Sumatra populations, while the highest similarity (0.9882) is shown by Central Kalimantan and North Sumatra populations. The lowest genetic distance (0.0308) is shown by Central Sulawesi and South Sumatra populations, while the highest (0.4272) genetic distance could be seen from Maluku and South Sulawesi populations.

Genetic diversity, Shannon's information index, Number and Percentage of polymorphic loci can be seen in Table 3. The highest percentage of Genetic diversity (0.1268), Shanon information index (0.1920) and Polymorphic loci (37.33) is East Nusa Tenggara, while the lowest of Genetic diversity (0.0000), Shanon information index (0.0000) and Polymorphic loci (0.00) are Aceh_Gayo03 and South Sulawesi population.

The H_T and H_S values of *Alpinia galanga* was presented in Table 4. A total value of H_T obtained is 0.2032 and H_S is 0.0595. These results indicate that the total genetic diversity value is higher than the genetic diversity within populations. In other words, genetic variation remains among the population sample tested. The value of G_{ST} is 0.7073 which signifies an evolutionary process between populations since these differences reflect the divergence of populations from a common ancestor population. The speciation process between *Alpinia galanga* population is relatively low because of the value of N_M (0.2069), which indicates the level of gene flow between populations.

Dendrograms of relationships between *Alpinia galanga* populations in Indonesia have been constructed using the UPGMA method based on Nei's genetic distance (Fig. 3). These results show *A. galanga* which is clustered in cluster A (subcluster I and II) and cluster B. As for cluster B, there is only Maluku population.

Figure 3 showed that *A. galanga* accessions is clustered randomly between populations collected from different regions. Sumatra, as the largest population is seen in all groups, especially in sub-cluster I. Maluku as the only population in cluster B, had a different band profile from other populations in combination primer of G and K, which indicates genetic variation in the population can be clearly detected using this primer.

Table 3. Genetic diversity, Shanon information index and polymorphic loci of *Alpinia galanga*

No.	Population name	h	I	NPL	PPL
1.	Aceh_Gayo04	0.1124	0.1655	22	29.33
2.	West Java_Cirebon	0.0611	0.0902	12	16.00
3.	Central Kalimantan	0.0050	0.0090	2	2.67
4.	East Kalimantan	0.0775	0.1144	15	20.00
5.	North Kalimanta	0.0876	0.1253	15	20.00
6.	Lampung_Pedanum	0.0909	0.1353	19	25.33
7.	Maluku	0.0801	0.1151	14	18.67
8.	West Nusa Tenggara	0.0703	0.1056	15	20.00
9.	Papua_Demta	0.1066	0.1575	21	28.00
10.	Riau	0.0385	0.0599	9	12.00
11.	South Sulawesi_Duri	0.0630	0.0926	12	16.00
12.	Central Sulawesi_Dondo	0.1045	0.1577	22	29.33
13.	South Sumatra_Pegagan	0.0818	0.1224	17	22.67
14.	North Sumatra_Mandailing	0.0151	0.0270	6	8.00
15.	East Nusa Tenggara	0.1268	0.1920	28	37.33
16.	Aceh_Gayo03	0.0000	0.0000	0	0.00
17.	West Java_Bandung	0.0859	0.1256	16	21.3
18.	East-North Kalimantan_Lepo Tau	0.0469	0.0716	11	14.67
19.	Lampung_Abung Seputih	0.0546	0.0806	11	14.67
20.	Papua_Ngalum	0.0302	0.0445	6	8.00
21.	South Sulawesi	0.0000	0.0000	0	0.00
22.	Central Sulawesi_Tialo	0.0422	0.0630	9	12.00
23.	North Sumatra_Pakpak	0.0955	0.1447	21	28.00
24.	East-North Kalimantan_Bajau	0.0597	0.0932	15	20.00
25.	South Sulawesi_Padoe	0.0184	0.0278	4	5.33
26.	South Sumatra_Lauje	0.0275	0.0430	7	9.33
27.	Papua_Tobati	0.0531	0.0761	9	12.00
28.	South Sulawesi_Kalatoa	0.0300	0.0427	5	6.67
	Average	**0.0595**	**0.0887**	**12.25**	**16.33**

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

Tabel 4. Total genetic diversity of *Alpinia galanga* L. (Willd.) populations

H_T	H_S	G_{ST}	N_M^*
0.2032	0.0595	0.7073	0.2069

H_T = total genetic diversity; H_S = genetic diversity within populations; G_{ST} = coefficient of genetic differentiation; N_M* = gene flow among populations; [N_M* = 0.5 (1 - *GST*) / *GST* (McDermott and McDonald, 1993)].

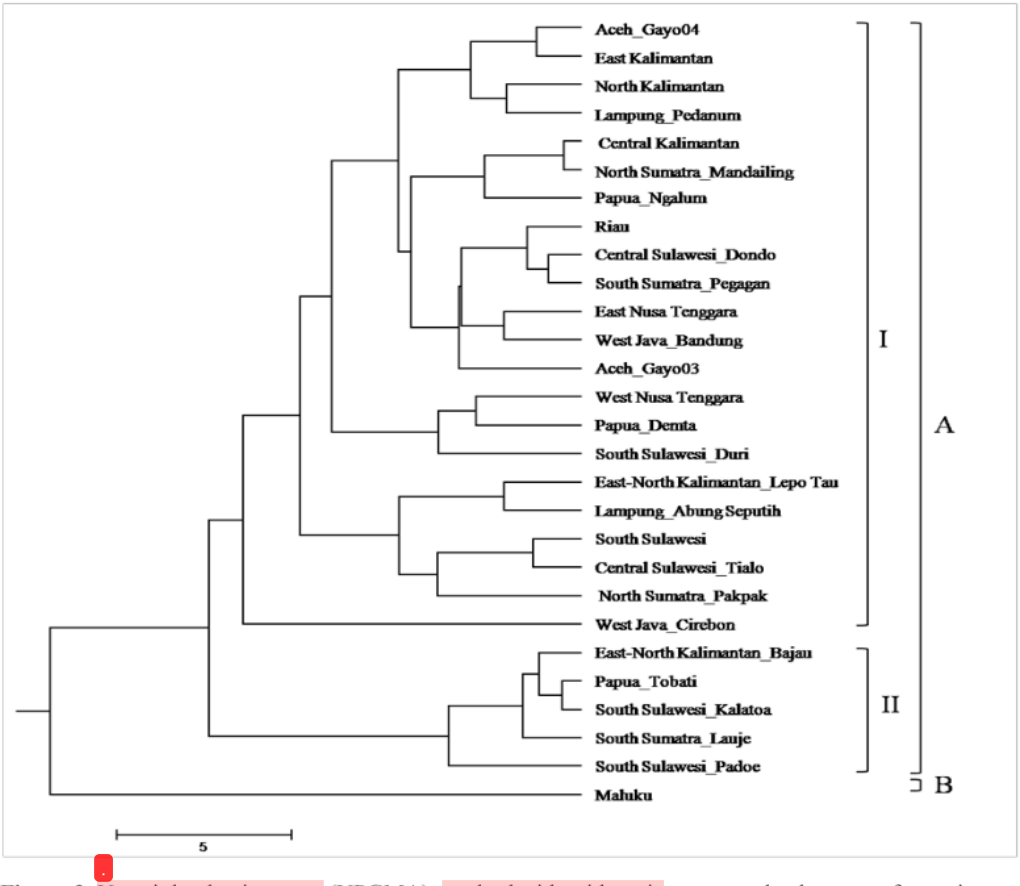

Figure 3. Unweighted pair–group (UPGMA) method with arithmetic averages dendrogram of genetic relationships among 28 population of *Alpinia galanga* in Indonesia based of SRAP markers.

DISCUSSION

In this study, PCR-based SRAP marker technique was successfully used to assess genetic differentiation among 28 populations of *Alpinia galanga* that spread in 16 provinces in Indonesia. Information related to genetic diversity and relationship between cultivated plant populations is important for conservation the plant genetic material. Based on the results, the value of genetic diversity, Shanon information index and the percentage of the *A. galanga* polymorphic loci in this study is relatively low. This condition is closely related to the high reproductive capacity of *A. galanga* vegetatively through rhizome.

Rajasekharan *et al.* (2016) also found the low genetic diversity values between 18 accessions were collected from different location in South India and conclude that the condition is mainly affected by its clonal propagation. Genetic variation which as an evolutionary basic material is very important in the survival of organisms. Genetic variation provide diverse survival mechanism in responding to the environmental changes and preventing species extinction. Clonal propagation species generally have low level of genetic variation within as well as among their population. This is closely related to the certain factors such as their evolution history, the reproductive system, genetic drift and natural selection. The loss of genetic diversity in *A. galanga* may be related to the history of its appearance and cultivation

Based on the theory of Nei (1973), genetic variation in the population can be reflected by the genetic diversity index, the results of this study show great variation within East Nusa Tenggara population (0.1268) while there is no variation within Aceh_Gayo03 and South Sulawesi population. The low similarity coefficients within or among populations indicate differences in the genotype of *A. galanga* collected from different regions. Genetic variations in this population may be related to genetic drift due to evolutionary history and differences in the environmental conditions of the two regions.

Random distribution from the entire population in this study indicates that *A. galanga* has experienced widespread distribution to various locations in Indonesia that have an impact on the occurrence of genetic differentiation. *A. galanga* has been cultivated for hundreds of years ago, wherein the 13th century Marco Polo examined that galangal traded in Europe originated from southern China as well as in Java. In 1563, Garcia Da Orta (Hanbury, 1876) became the first person to report galangal in two groups based on the morphological characteristics of the rhizome, they are the smaller rhizome and a strong scent (*Galanga minor* Rumph. or

Alpinia officinarum Hance) brought from China, and the thicker and less aromatic rhizome (*Galanga major* Rumph. or *Alpinia galanga* (L.) Willd.) cultivated in Java (Burkill, 1935). Various speculations were reported related to the origin of *A. galanga*. Many authors believe that this species is native to Sumatra and Java (Roxburgh, 1812, Watt, 1883, Burkill, 1935, Singh, 1965). Eventually, the results obtained cannot clearly explain the phenomenon in *A. galanga*, if anywhere, these species occurs as a native wild plant and completely cultivated from an unknown ancestor (Rangsiruji, Newman, Cronk, 2000).

Morphologically, *A.galanga* variety does not show distinctive character in its vegetative or generative organs. A little difference can be seen from the color of the rhizome, especially in the individuals who have been cultivated. The common color variations of rhizome are pink to red rhizomes and with yellow–white rhizomes. Many variations may appear as the species occurs naturally in many countries under varying agro-ecological situations; however the information concerning this topic is still lacking (Ravindran *et al.* 2012) (Scheffer and Janson, 1999 *In* Ravindran *et al.* 2012). This species chromosome number is 2n = 48 (Ravindran *et al.* 2007) and there has been no changes in the number of chromosomes reported. Meanwhile, the analysis with SRAP method used in this study showed the differences in the number of DNA fragments between and within populations. This condition indicates that the markers used in this study have the potential to be used to support morphological data especially in planning conservation strategies for this species.

CONCLUSION

PCR-based SRAP marker technique was successfully used to assess genetic differentiation among 28 populations of *Alpinia galanga.* The low value of genetic diversity obtained is an important thing for planning conservation strategies for this species.

ACKNOWLEDGEMENTS

This study was supported by The Health Ministry of Republic Indonesia through the collaboration of RISTOJA 2018 (Contract No.: HK.03.01/3/3000/2018 and 2031/UN.16.01.D/PP/2018). We would like to express sincere thanks to Dr. Nurainas for the discussion on this manuscript.

REFERENCES

Burkill, I. 1935. A dictionary of the economic products of the Malay Peninsula Vol. II. Univ. Press. Oxford–London.

Hanbury, D. 1876. Science Papers: Chiefly Pharmacological and Botanical, Macmillan.

Li, G., Mcvetty, P. B. and Quiros, C. F. 2013. SRAP molecular marker technology in plant science. Plant breeding from laboratories to fields. InTech.

Li, G. and Quiros, C. F. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theoretical and applied genetics 103**:** 455-461.

Michelmore R.W., Paran I. and Kesseli R.V. 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. 88: 9828–9832.

Namsa, N. D., Tag, H., Mandal, M., Kalita, P. and Das, A. 2009. An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit community of Arunachal Pradesh, India. Journal of Ethnopharmacology 125**:** 234-245.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70**:** 3321-3323.

Nurainas, N. and Arbain, D. 2017. A new species and a new record of Zingiberaceae from Sumatra, Indonesia. Taiwania 62**:** 294-298.

Rajasekharan, P., Kareem, V. A., Ravish, B. and Mini, S. 2016. Analysis of genetic diversity in Alpinia galanga using ISSR markers. Indian J. Plant Genet. Resour 29**:** 194-198.

Rangsiruji, A., Newman, M. and Cronk, Q. 2000. Origin and relationships of Alpinia galanga (Zingiberaceae) based on molecular data. Edinburgh Journal of Botany 57**:** 9-37.

Ravindran, P., Pillai, G., Balachandran, I. and Divakaran, M. 2012. Galangal. Handbook of herbs and spices. Woodhead Publishing Limited. India.

Ravindran, P., Pillai, G. S., Babu, K. N., Divakaran, M., Malhothra, S. K. 2007. Minor and under-Utilized Spices and Herbs, In Breeding of neglected and under-utilized crops, spices and herbs. (eds Ochatt, S. and Jain, S.M. Science Publisher, Enfield, NS, 299-373.

Roxburgh, W. 1812. Descriptions of Several of the Monandrous Plants of India: Belonging to the Natural Order, Called Scitamineae by Linnaeus, Cannæ by Jussieu, and Drimyrhizae by Ventenat, John Murray.

Scheffer, J.J.C and Jansen, P.C.M. 1999. *Alpinia galanga* (L.) Willd, in de Guzman CC and Siemonsma JS (eds), Plant Resources of South-East Asia No. 13, Spices. Backhyus Publishers, Leiden, 65–8, *In* Ravindran, P., Pillai, G., Balachandran, I. and Divakaran, M. 2012. Galangal. *Handbook of herbs and spices.* Woodhead Publishing Limited. India.

Schumann, K. 1904. Zingberaceae. Das Pflanzenreich 4**:** 1-458.

Singh, U. 1965. Dictionary of economic plants in India, Indian Council of Agricultural Research, New Delhi.

Watt, G. 1883. Economic Products of India Exhibited in the Economic Court, Calcutta International Exhibition, L883-84: Medicinal products, Nabu Press, South Carolina.

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

conservation of Alpinia SRAP

ORIGINALITY REPORT

6%	4%	5%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

2%

★ Chen, Zhen-Dong, Ru-Kui Huang, Qi-Qin Li, Jun-Li Wen, and Gao-Qing Yuan. "Development of Pathogenicity and AFLP to Characterize Fusarium oxysporum f.sp. momordicae Isolates from Bitter Gourd in China", Journal of Phytopathology, 2014.

Publication

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		