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Genetic Diversity *Hopea celebica* an Indonesian endemic species by ISSR Marker

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Abstract

Indonesia is a mega-diversity country with numerous endemic plants distributed throughout its regions. An Indonesias' island with the unique and the highest endemic plant species due to being located in the Wallace area is Sulawesi Island. *Hopea celebica*, an endemic species to Sulawesi Island, is currently categorized as endangered by IUCN. Here, we selected the ISSR primers suitable for the genetic study of *H. celebica* from Luwu and Konawe provenances and investigated their genetic diversity. Ten ISSR primers were employed in primer screening, and fifty *H. celebica* individuals were genetically analyzed for their genetic diversity. The selected ISSR primers for genetic diversity analysis were UBC 810, UBC 813, UBC 814, UBC 820, UBC 822, UBC 823, and UBC 827. The evaluated

H. celebica individuals have high genetic diversity, and this information will be beneficial for designing *H. celebica* breeding and conservation strategies

Keywords: dominant marker, endangered plant, genetic variation

A. Introduction

Indonesia is a mega-diversity archipelago country with high genetic diversity distributed throughout its area. An Indonesias' island with high and unique endemic species flora, and Fauna, is Sulawesi Island, located in a transitional area between Asia and Australia continents (Wallace area) (Sutrisna, T., Umar, M. R., Suhadiyah, S. & Santosa, S., 2018). IUCN states *Hopea celebica* is endemic species to Sulawesi, which is classified as endangered due to fragmentation of population and habitat destruction. This species has been utilized for construction materials, cosmetics, and chocolate (Sudarmonowati. E., Yulita, K. S., Partomihardjo, T., & Wardani, W., 2020).

Propagation of *H. celebica* is commonly performed by seed (generative). The seeds are harvested from the trees; meanwhile, the seedlings are obtained around the adult trees. They must be planted immediately after being harvested because the seeds' viability will significantly decrease if stored long term (Wulandari, A. S., Subiakto, A. & Novan, R., 2015).

Tree breeding is an effort that generates genetic improvements to increase yields, both in quality and quantity, from generation to generation. These programs can be well directed by implementing appropriate breeding strategies based on morphological and genetic diversity to gain improved trees (Gusmiaty, Restu, M., & Ira, P., 2012). Genetic diversity is an influential factor in establishing tree breeding strategies. The genetic characteristics of a tree species grown in an area or different provenances can be distinct, indicated by the nature and peculiarities of a stand. Stands or provenances with good genetic characteristics can be a valuable source for tree breeding activities (Langga, I. F., Restu, M., & Kuswinanti, T., 2012).

Molecular analysis is one of the strategies that can be employed to reduce the breeding cycles (Langga et al., 2012). DNA-based molecular techniques have been widely developed and utilized as practical tools for analyzing plant genomes. Inter Simple Sequence Repeat (ISSR) is a most common molecular marker, which is usually mononucleotide, dinucleotide, or trinucleotide, and the part of the microsatellite that does not code for proteins (non-coding region) (Widiastuti, A., Sobir. & Suhartanto, M. R., 2013). ISSR marker compensates for the limitations of Random Amplified Polymorphic DNA (RAPD) technique, as ISSR is more sensitive in detecting genetic diversity at low level and as simple and cost-effective as RAPD (Bradford, 2008). ISSR markers have been extensively utilized in several forestry species, including *Gmelina arborea*, *Pinus gerardiana* and *Aquilaria* spp (Azhari, H., Azhar, M., & Othman, R., 2015). Studying genetic diversity in *H. celebica* using ISSR markers will be beneficial in the breeding process; thus, it is necessary to determine the genetic diversity of this species in Luwu and Konawe Provenances.

B. Methodology

1. Plant Materials

The samples used were 50 leaves of *H. celebica*. from Luwu (33) and Konawe (17). The samples were a collection from Biotechnology and Tree Breeding Lab, Faculty of Forestry, Universitas Hasanuddin.

2. DNA Extraction

DNA isolation was performed using CTAB (*Cetyl Trimethyl Ammonium Bromide*) method. The DNA extraction were used CTAB method. As many as 0.2 g of each leaf sample without leaf venation were ground until powder and stored in 1.5 ml of microtube. The 700 µl CTAB extraction buffer was added into each microtube to separate DNA from other components and vortexed for 15 seconds. Microtubes were incubated in a water bath at 65°C for 90 minutes. Each sample that had been incubated was added 100 µl of isoamyl alcohol: chloroform (24:1), mixed slowly and then centrifuged at 10,000 rpm for 5 minutes. The supernatant was transferred to a new microtube and 800 µl of isopropanol was added to remove the remaining salt content. The solution was then centrifuged at 10,000 rpm for 10 minutes and the DNA precipitate was dried overnight. The DNA precipitate obtained was purified by adding 500 µl of 1x TE buffer, then centrifuged for

10 minutes at 10,000 rpm. The supernatant was transferred into a new 2 ml microtube and 100 µl chloroform was added. The supernatant solution was then centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected, then added 100 µl sodium acetate and 800 µ1 isopropanol, then centrifuged for 10 minutes at 10,000 rpm. The solution was discarded, and the precipitate was dried overnight, then added 100 µl Buffer TE and stored in a freezer at - 20°C.

3. Amplification of ISSR markers and Primer screening

The selected primers are polymorphic primers that produce clear bands. Different primers and DNA samples in the same condition were required to acquire the optimal condition and variation level of the generated bands from each primer. As much as 10 primers were screened to analyze genetic diversity of *H. celebica* (Table 1).

Table 1. ISSR Primer Name and The Nucleotide Sequence

No.	Primer Name	Nucleotide sequence (5'-3')	Melting Temperature (°C)
1	UBC 810	CTC TCT CTC TCT CTC TT	45,4
2	UBC 813	CTC TCT CTC TCT CTC TA	45,7
3	UBC 814	GTG TGT GTG TGT GTG TC	44,7
4	UBC 820	TCT CTC TCT CTC TCT CA	51,0
5	UBC 822	TCT CTC TCT CTC TCT CC	47,0
6	UBC 823	TCT CTC TCT CTC TCT CG	48,1
7	UBC 824	ACA CAC ACA CAC ACA CG	48,5
8	UBC 827	TGT GTG TGT GTG TGT GG	53,0
9	UBC 830	GAA GAA GAA GAA GAA GAA	52,7
10	UBC 868	GAG AGA GAG AGA GAG AT	43,2

Source: (Kalpana *et al.,* 2012)

The primer screening was conducted by randomly selecting 12 DNA samples and 12 gradient temperatures to determine the accurate primer temperature for DNA amplification. A PCR reaction consisted of 3 µl of working DNA, 1.25 µl primer, 6.25 µl PCR Mix Kappa, and 3 µl DDH2O with 13.50 µl of total reaction. DNA amplification was performed using a PCR machine with the following process: a cycle of initial denaturation at 95°C for 180 seconds, 35 cycles of denaturation at 95°C for 30 seconds, specific primer annealing (the annealing temperature was adjusted to each primer) for 50 seconds, primer elongation at 72°C for 60 seconds, and final elongation at 72°C for 300 seconds, and storage at 4°C.

4. Electrophoresis

The procedure of electrophoresis was: 3.6 grams of agarose was added 180 ml of 1 x TAE buffer and heated at the microwave for 5 minutes, the dissolved agarose was added 1.5 µl gelred and then left until cool down, the solution was poured into the agarose mold and set a comb on it until the agarose became harden, the comb was removed, and then the agarose was put into a tank containing 1 x TAE buffer solution, the DNA samples were inserted into the agarose wells with the ladder at each edge well, the electrophoresis was performed for 60 minutes at 120 volts, and the agarose was visualized in the gel documentation system.

5. Data Analysis

The results were displayed on the agarose in the form of DNA bands. The bands represent alleles at a locus, and each primer used represent a locus. The appeared band is scored as 1 based on its band length, and the absent band is given 0 by manually observing the bands on agarose. The data were then tabulated and analyzed using Darwin 6.5 software to determine the relationship and genetic variation level of the individuals. Heterozygosity was calculated by:

Heterozygosity: $qi = \left(\frac{The\ individuals\ with\ absent\ allele}{total\ sample}\right)^{1}/_{2}$

$Pi = 1 - qi$

$He = 1 - (pi^2 - qi^{2)}$

Notes: qi = Frequency of absent allele

Pi = frequency of dominant allele

*Polymorphic information content (*PIC) was calculated by (Guo, 2014):

$$PIC = 2\ fi\ (1 - fi)$$

Notes: PIC = *polymorphic information content*

Fi = Frequency of the allele

C. Findings and Discussion

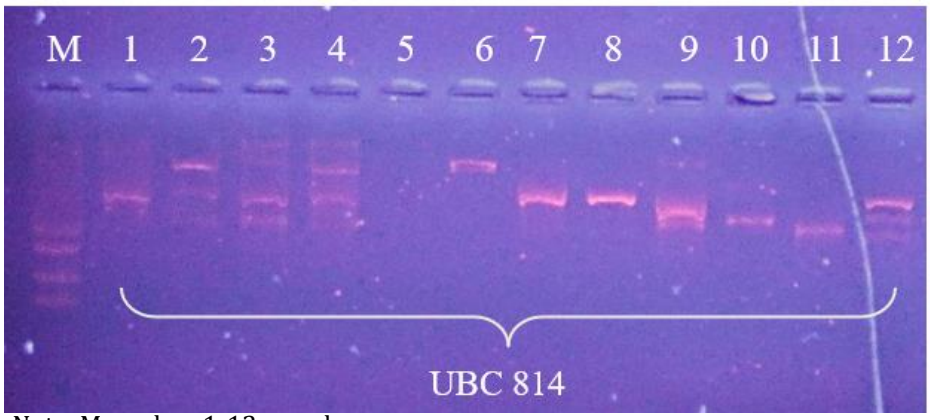
1. Primer screening

Primer screening of 10 ISSR primers on 12 evaluated DNA samples obtained seven polymorphic primers, which generated clear bands, UBC 810, UBC 813, UBC 814, UBC 820, UBC 822, UBC 823, and UBC 827 (Figure 1).

One of the primers that produced clear and polymorphic bands was UBC 814. In order to study genetic diversity, polymorphic primers which generate clear, bright, and polymorphic bands are required (Larekeng, S. H., Restu, M., & Gusmiaty. 2015). Polymorphic bands are not present in all samples (Sinaga, A., Lollie, A. P. P. & Mbue, K. B. 2017). A high percentage of polymorphic bands suggests that each sample examined has high variation, and the number of amplified polymorphic bands is varied. The more polymorphic bands produced, the simpler it is to observe the variation (Larekeng, S. H., Paelongan, R., Cahyaningsih, Y. F., Nurhidayatullah, & Restu, M., 2020a).

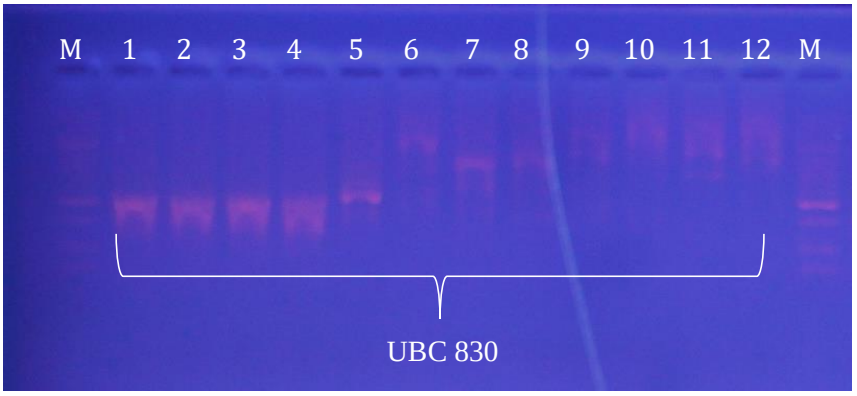
The smear/unclear bands with UBC 824, UBC 830, and UBC 868 are presented in Figure 2. UBC 830, as shown in Figure 2, is a primer that produced less clear and smear bands which caused the primer could not be used for further analysis. (Larekeng *et al.*, 2020a) reported low quality of the PCR amplification might be affected by the unmatched primer to the DNA samples, PCR efficiency, and process.

The distribution of nucleotide base positions in the genome where the primer attached causes variation in the size of DNA bands or polymorphisms in amplified DNA bands. Differences in the profile of amplified DNA bands or variation, particularly the number and size of the bands, are crucial in identifying population diversity (Gusmiaty, Restu, M., & Ira, P., 2012).



Note: M marker, 1-12 sample

Figure 1. Electroforegram of PCR amplification using ISSR UBC 814 primer



Note: M marker (100 bp), 1-12 sample

Figure 2. Electroforegram of PCR amplification using ISSR UBC 830 primer

The suitable ISSR primer will also create higher and more informative polymorphic DNA bands, which is notably valuable for genetic diversity studies (Rohela, G.K., Phanikanth, J., Mir, M.Y., Aftab, A.S., Pawan, S., Sadanandam, A., Kamili, A.N., 2020). Table 2 displays ISSR primer screening and Polymorphic Information Content (PIC) in *H. celebica*. The PIC was calculated manually from the overall PCR amplification scoring and tabulated into Polymorphic Information Content (PIC) formula.

Table 1. ISSR primer name, number of amplified bands and PIC of *Hopea celebica*

No.	Primer Name	*Annealing* Temperature (°C)	Number of Amplified Bands	Note	PIC	
					Luwu	Konawe
1	UBC 810	50.4	12	Polymorphic	0.4	0.5
2	UBC 813	47.4	11	Polymorphic	0.3	0.3
3	UBC 814	41.9	11	Polymorphic	0.2	0.3
4	UBC 820	55.5	12	Polymorphic	0.4	0.4
5	UBC 822	48.7	12	Polymorphic	0.4	0.4
6	UBC 823	45.3	11	Polymorphic	0.3	0.4
7	UBC 827	56.7	12	Polymorphic	0.5	0.5

Table 2 shows that seven ISSR primers had polymorphic bands with 41 to 57 °C of annealing temperatures. The primer attached to DNA template is called annealing, and the number and nucleotide sequence of the primer determine the annealing temperature of the primer (Suryanto 2003). The annealing temperature generally ranges from 36°C to 72°C with a 30-45 second annealing duration. By either deducting or adding 5 °C to the Tm, the annealing temperature of a primer can be optimized (Handoyo dan Rudiretna. 2000).

The success of DNA amplification is controlled not only by the primer sequence but also by the PCR conditions, including the primer annealing temperature. Gusmiaty et al. (2012) explained that the annealing temperature in the PCR process has a significant impact on the primer attachment process, with even a one-degree change in the temperature causing it fails to anneal.

Table 2 shows the PIC range from 0.2 to 0.5. The highest PIC observed in Luwu provenance (0.5) using UBC 827, indicating that the primer has a high polymorphism level. UBC 814 had the lowest PIC (0.2), which proved to have a low polymorphism level. UBC 827 and UBC 810 had the highest PIC of 0.5 in Konawe Province. In contrast, UBC 813 and UBC 814 primers had the lowest ones (Missio, R., Caixeta, E., Zambolin, E., Zambolin, L., Cruz. C., & Sakiyama, N., 2010) stated the higher PIC of the primer, the better the primer is employed as a molecular marker.

The PIC is a parameter that reflects the informative level of the primer. PICs are classified as PIC≤0.25 (low polymorphism or having a low informative level), 0.25≤ PIC ≤ 0.5 (moderate polymorphism), and PIC ≥ 0.5 (high polymorphism or the primer is very informative) (Hidayat, R. A., Depamede, S. N., & Maskur., 2015).

2. Analysis of Genetic Diversity

The heterozygosity and number of bands are genetic diversity parameters. These parameters were manually calculated from the total scoring results of PCR band amplification and tabulated into the Expected Heterozygosity (He) formula. Table 3 depicts the number of bands and He of all evaluated samples.

Table 3. Number of bands and He

No	Primer	Total of Band	Number of Bands		He	
			Luwu	Konawe	Luwu	Konawe
1	UBC 810	19	10	9	0.5	0.4
2	UBC 813	7	5	2	0.5	0.5
3	UBC 814	12	4	8	0.5	0.5
4	UBC 820	15	7	8	0.5	0.5
5	UBC 822	8	4	4	0.5	0.5
6	UBC 823	12	7	5	0.5	0.5
7	UBC 827	10	5	5	0.4	0.4

Table 3 displays the band number produced by seven ISSR primers. The highest number of bands was by UBC 810 (19 bands). The primer with the lowest bands was UBC 813, which generated seven bands. The number of bands in a genetic diversity analysis considerably influences diversity in a population. Genetic variety reflects a plant population. A population with high genetic diversity indicates that the population's adaptability level is higher (Mis'al., 2017).

Every sample used with ISSR primers is treated as a character unit. and the produced ISSR band is scored as 1 (present band) or 0 (absent band), which is set into a binary matrix (Ardiyani, M., Sulistiyaningsi, L. D., & Esthi, Y. N., 2014). The genetic diversity and relationship with molecular markers are stable and not affected by the environment (Tar'an, B., Zhang, B., Warkentin, T., Tullu. A., & Vandenberg, A., 2005).

He of *H. celebica* from Luwu and Konawe Provenances ranged from 0.4 to 0.5 (Table 3). Only UBC 827 had the lowest He of 0.4 in Luwu provenance, whereas only UBC 810 and UBC 827 had the lowest He of 0.4 in Konawe provenance. These findings imply that *H. celebica* in both provenances has a high level of variation and resistance. Only 2 of 7 primers had the lowest He. Weising (2005) reported ISSR marker is a dominant marker that can only detect two homozygous alleles at each locus and is not sensitive to heterozygous alleles. resulting in a maximum He value of 0.5.

3. Genetic relationship in the populations

High genetic diversity is an essential factor in developing new superior varieties, and increasing genetic diversity can be accomplished by hybridization. Therefore,improving plant genetics necessitates germplasm with high genetic diversity (Rameshkumar R, Pandian S, Rathinapriya P, Selvi CT, Satish L, Gowrishankar S., 2019).

The genetic distance of *H. celebica* in Table 4 ranges from 0.27 to 1.00. The farthest genetic distance was 1.00 between individuals 3 and 31. The closest genetic distance was 0.27 between individuals 26 and 28 compared to others. The closer the genetic distance between individuals in a population, the lesser variation in the population. Contrastly. the higher the genetic distance of individuals in a population, the more diverse the individuals (Pandin, 2009).

Genetic distance describes the variance in a population induced by genetic mixing derived from crossbreeding. Decreasing individuals number in population might also contribute to inbreeding (Gusmiaty et al., 2016). A factor that can maintain the gene and genotype frequencies (genetic stability) in natural populations is random mating (Widyatmoko, A. Y. P. B. C., Lejo, E. S., Prasetyaningsih, A. & Rimbawanto, A. 2010).

The relationship between individuals depicted by the dendrogram is correlated to the genetic distance of the individuals. Close relatives have a low genetic distance, whereas distant relatives have a high genetic distance (Lucic, I., Rakonjan., Mataruga., Babic., Ristic., & Drinic, 2011). A dendrogram was created utilizing the similarity coefficient of each random primer used for clustering analysis (Figure 4). A dendrogram

classifies populations that are geographically isolated but genetically related. The genetic relationship illustrated in Figure 4 shows that the 50 individuals were clustered into three main clusters with a different individual numbers. Cluster I had two sub-clusters, with the first and the second sub-clusters consisting of 13 and 11 individuals, respectively. Cluster II was divided into two sub-clusters. The first sub-cluster had 11 individuals, and the second one had eight individuals. Cluster III consisted of 2 sub-clusters, four individuals (sub-cluster 1) and three individuals (sub-cluster 2).

Understanding genetic diversity and relationships are critical for genetic conservation and tree breeding. This information is required for ex-situ conservation to determine the number of populations and individuals within the population that must be collected to maintain the basics of genetic diversity established. Diversity is vital in breeding programs because it can form specific genetic characteristics for gene selection of the desired traits, some case in Duabanga moluccana (Larekeng, S.H., Yusniar, Restu, M., Rismawati, Cahyaningsih, Y.F., Arsyad, M.A., Nirsatmanto, Arif. 2020b). Meanwhile. it is required for in-situ conservation to determine the number of individuals and locations (Sharma, R; Santosh S. and Sushil K., 2018).

Table 2. Genetic distance of Hopea celebica in all provenaces

Units	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	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
2	0,48																																																	
3	0,60	0,58																																															
4	0,64	0,62	0,67																																														
5	0,33	0,54	0,65	0,52																																													
6	0,71	0,60	0,76	0,58	0,67																																											
7	0,64	0,71	0,67	0,60	0,60	0,68																																										
8	0,60	0,58	0,50	0,67	0,74	0,53	0,56																																									
9	0,43	0,40	0,53	0,58	0,50	0,67	0,47	0,65																																								
10	0,68	0,42	0,71	0,65	0,79	0,73	0,57	0,52	0,64																																							
11	0,42	0,65	0,60	0,55	0,63	0,71	0,45	0,70	0,43	0,60																																						
12	0,74	0,55	0,68	0,43	0,77	0,40	0,71	0,58	0,70	0,58	0,57																																				
13	0,33	0,48	0,50	0,64	0,41	0,71	0,64	0,80	0,43	0,68	0,50	0,74																																			
14	0,36	0,63	0,67	0,62	0,35	0,68	0,46	0,75	0,44	0,72	0,50	0,78	0,21																																		
15	0,39	0,45	0,58	0,62	0,31	0,80	0,71	0,68	0,60	0,83	0,74	0,82	0,48	0,48																																	
16	0,68	0,67	0,73	0,65	0,55	0,88	0,88	0,73	0,75	0,80	0,68	0,78	0,79	0,74	0,56																																
17	0,67	0,53	0,57	0,50	0,62	0,73	0,63	0,71	0,47	0,79	0,78	0,76	0,56	0,55	0,41	0,54																															
18	0,70	0,79	0,88	0,67	0,74	0,76	0,67	1,00	0,65	0,81	0,50	0,79	0,70	0,67	0,68	0,60	0,57																														
19	0,50	0,57	0,70	0,55	0,56	0,52	0,55	0,60	0,43	0,60	0,50	0,74	0,67	0,50	0,57	0,68	0,44	0,30																													
20	0,52	0,50	0,71	0,39	0,50	0,45	0,48	0,52	0,55	0,62	0,52	0,58	0,68	0,59	0,67	0,70	0,68	0,52	0,36																												
21	0,58	0,56	0,73	0,41	0,73	0,50	0,76	0,73	0,38	0,80	0,58	0,67	0,68	0,65	0,78	0,71	0,54	0,47	0,37	0,30																											
22	0,36	0,67	0,62	0,83	0,50	0,73	0,57	0,71	0,55	0,77	0,44	0,75	0,36	0,38	0,58	0,70	0,79	0,71	0,68	0,62	0,80																										
23	0,44	0,58	0,62	0,74	0,57	0,64	0,57	0,71	0,55	0,69	0,44	0,75	0,36	0,38	0,50	0,80	0,68	0,62	0,52	0,54	0,70	0,15																									
24	0,56	0,65	0,71	0,50	0,52	0,60	0,75	0,71	0,47	0,79	0,67	0,76	0,78	0,64	0,76	0,69	0,67	0,57	0,33	0,47	0,38	0,79	0,79																								
25	0,73	0,62	0,78	0,70	0,76	0,58	0,70	0,56	0,68	0,74	0,64	0,71	0,73	0,62	0,71	0,88	0,63	0,67	0,45	0,57	0,65	0,83	0,65	0,63																								
26	0,52	0,60	0,65	0,58	0,58	0,56	0,79	0,65	0,56	0,82	0,62	0,70	0,43	0,52	0,40	0,75	0,47	0,53	0,52	0,64	0,63	0,55	0,36	0,73	0,47																							
27	0,69	0,76	0,73	0,67	0,79	0,65	0,67	0,55	0,74	0,70	0,62	0,60	0,69	0,67	0,68	0,81	0,60	0,64	0,54	0,63	0,71	0,63	0,63	0,90	0,50	0,57																						
28	0,44	0,42	0,52	0,65	0,57	0,73	0,74	0,62	0,55	0,62	0,60	0,67	0,36	0,52	0,33	0,80	0,47	0,62	0,52	0,69	0,70	0,54	0,38	0,79	0,57	0,27	0,56																				
29	0,55	0,53	0,48	0,59	0,53	0,57	0,59	0,63	0,57	0,50	0,55	0,60	0,42	0,49	0,47	0,62	0,52	0,63	0,61	0,63	0,69	0,44	0,44	0,84	0,72	0,50	0,52	0,38																		
30	0,57	0,55	0,47	0,71	0,54	0,70	0,52	0,37	0,60	0,58	0,57	0,73	0,74	0,70	0,55	0,56	0,76	0,89	0,65	0,50	0,78	0,67	0,67	0,76	0,62	0,80	0,68	0,67	0,53																
31	0,76	0,88	1,00	0,73	0,90	0,71	0,87	0,69	0,86	0,67	0,76	0,88	1,00	0,90	1,00	0,83	1,00	0,85	0,65	0,56	0,50	0,89	0,89	0,82	0,87	1,00	0,79	1,00	0,83	0,75															
32	0,60	0,79	0,63	0,67	0,74	0,76	0,78	0,63	0,76	0,81	0,70	0,79	0,70	0,58	0,68	0,87	0,71	0,88	0,70	0,62	0,73	0,71	0,71	0,71	0,67	0,65	0,55	0,71	0,70	0,68	0,69														
33	0,62	0,68	0,55	0,75	0,52	0,74	0,75	0,73	0,65	0,85	0,62	0,92	0,62	0,67	0,52	0,62	0,70	0,73	0,69	0,70	0,71	0,56	0,56	0,80	0,67	0,57	0,79	0,56	0,45	0,60	0,89	0,82													
34	0,67	0,79	0,69	0,71	0,70	0,78	0,64	0,77	0,63	0,68	0,53	0,72	0,73	0,65	0,72	0,84	0,75	0,54	0,47	0,55	0,68	0,55	0,61	0,67	0,71	0,70	0,63	0,68	0,51	0,79	0,74	0,69	0,56												
35	0,64	0,78	0,83	0,85	0,74	0,60	0,54	0,67	0,68	0,66	0,57	0,78	0,71	0,50	0,70	1,00	0,82	0,67	0,50	0,66	0,74	0,59	0,52	0,82	0,62	0,60	0,60	0,45	0,54	0,70	0,71	0,67	0,60	0,47											
36	0,64	0,63	0,67	0,77	0,74	0,68	0,54	0,67	0,68	0,52	0,43	0,70	0,64	0,56	0,70	0,83	0,82	0,75	0,57	0,66	0,83	0,45	0,38	0,82	0,69	0,68	0,60	0,52	0,54	0,63	0,90	0,75	0,60	0,53	0,31										
37	0,66	0,71	0,68	0,93	0,75	0,77	0,56	0,76	0,69	0,67	0,45	0,79	0,66	0,64	0,71	0,83	0,91	0,68	0,66	0,67	0,83	0,33	0,40	0,91	0,78	0,77	0,61	0,60	0,50	0,57	0,73	0,76	0,55	0,37	0,33	0,33									
38	0,64	0,62	0,56	0,70	0,76	0,68	0,70	0,67	0,68	0,74	0,64	0,81	0,55	0,69	0,62	0,76	0,50	0,56	0,55	0,65	0,65	0,74	0,65	0,88	0,70	0,47	0,50	0,39	0,52	0,71	0,87	0,78	0,58	0,71	0,62	0,69	0,70								
39	0,52	0,75	0,71	0,83	0,71	0,64	0,57	0,71	0,64	0,69	0,44	0,75	0,60	0,59	0,92	0,90	1,00	0,81	0,68	0,62	0,70	0,38	0,46	0,79	0,91	0,82	0,70	0,69	0,63	0,67	0,67	0,71	0,63	0,55	0,45	0,45	0,27	0,74							
40	0,70	0,62	0,65	0,84	0,73	0,92	0,68	0,83	0,67	0,64	0,48	0,85	0,63	0,68	0,69	0,82	0,81	0,83	0,70	0,79	0,91	0,50	0,50	0,90	0,76	0,75	0,79	0,57	0,59	0,62	0,90	0,83	0,52	0,45	0,55	0,35	0,31	0,76	0,43						
41	0,66	0,64	0,84	0,70	0,69	0,77	0,70	0,84	0,54	0,73	0,52	0,79	0,72	0,70	0,64	0,75	0,74	0,68	0,59	0,67	0,67	0,67	0,60	0,74	0,78	0,69	0,68	0,60	0,61	0,64	0,91	0,76	0,61	0,49	0,52	0,39	0,53	0,78	0,53	0,38					
42	0,69	0,60	0,64	0,83	0,72	0,74	0,67	0,55	0,65	0,56	0,62	0,68	0,77	0,73	0,76	0,52	0,70	0,73	0,62	0,70	0,81	0,63	0,70	0,80	0,75	0,74	0,64	0,70	0,52	0,44	0,79	0,73	0,71	0,69	0,73	0,60	0,61	0,67	0,63	0,52	0,68					
43	0,76	0,75	0,71	0,91	0,71	0,82	0,65	0,71	0,82	0,77	0,60	0,75	0,76	0,72	0,58	0,80	0,89	0,81	0,76	0,77	1,00	0,46	0,46	1,00	0,83	0,73	0,70	0,54	0,56	0,50	0,89	0,81	0,63	0,55	0,45	0,45	0,27	0,83	0,46	0,29	0,53	0,48				
44	0,57	0,82	0,58	0,81	0,54	0,70	0,62	0,58	0,60	0,75	0,65	0,82	0,65	0,70	0,64	0,67	0,76	0,89	0,74	0,83	0,89	0,58	0,67	0,88	1,00	0,70	0,76	0,67	0,53	0,45	0,88	0,89	0,52	0,72	0,63	0,63	0,64	0,52	0,58	0,69	0,64	0,44	0,58			
45	0,64	0,56	0,67	0,54	0,48	0,60	0,69	0,67	0,52	0,66	0,57	0,63	0,64	0,69	0,56	0,57	0,55	0,50	0,43	0,52	0,57	0,59	0,59	0,64	0,69	0,52	0,47	0,52	0,37	0,56	0,81	0,83	0,47	0,53	0,63	0,69	0,64	0,54	0,72	0,61	0,45	0,53	0,59	0,56			
46	0,74	0,55	0,79	0,62	0,77	0,50	0,81	0,58	0,60	0,75	0,57	0,55	0,74	0,78	0,73	0,67	0,65	0,58	0,57	0,58	0,56	0,75	0,67	0,65	0,43	0,40	0,52	0,58	0,60	0,73	0,88	0,58	0,60	0,66	0,70	0,70	0,79	0,71	0,75	0,77	0,50	0,52	0,75	0,82	0,48			
47	0,60	0,58	0,75	0,33	0,57	0,65	0,78	0,75	0,53	0,71	0,70	0,68	0,70	0,67	0,68	0,73	0,57	0,63	0,50	0,43	0,33	0,90	0,81	0,43	0,67	0,65	0,82	0,62	0,70	0,68	0,69	0,63	0,73	0,77	0,75	0,92	1,00	0,78	0,90	0,91	0,68	0,82	0,90	0,79	0,50	0,58		
48	0,50	0,58	0,50	0,56	0,74	0,65	0,67	0,50	0,65	0,71	0,50	0,68	0,50	0,58	0,68	0,87	0,57	0,75	0,60	0,52	0,60	0,62	0,52	0,71	0,44	0,41	0,45	0,43	0,63	0,68	0,85	0,38	0,64	0,77	0,67	0,58	0,76	0,44	0,71	0,74	0,76	0,73	0,81	0,89	0,67	0,47	0,63		
49	0,57	0,82	0,68	0,71	0,69	0,70	0,71	0,58	0,70	0,67	0,65	0,73	0,83	0,78	0,64	0,67	0,76	0,79	0,65	0,75	0,78	0,67	0,67	0,76	1,00	0,70	0,60	0,67	0,53	0,55	0,88	0,79	0,76	0,72	0,63	0,56	0,71	0,62	0,58	0,77	0,50	0,52	0,67	0,27	0,56	0,73	0,79	0,89	
50	0,60	0,67	0,62	0,83	0,57	0,73	0,65	0,62	0,73	0,77	0,68	0,75	0,68	0,66	0,42	0,80	0,68	0,81	0,60	0,69	0,90	0,46	0,38	0,89	0,74	0,55	0,56	0,46	0,56	0,50	0,89	0,62	0,56	0,68	0,52	0,45	0,47	0,74	0,62	0,43	0,53	0,48	0,31	0,50	0,52	0,67	0,81	0,62	0,58

Notes:

Closest genetic distance
Furthest genetic distance

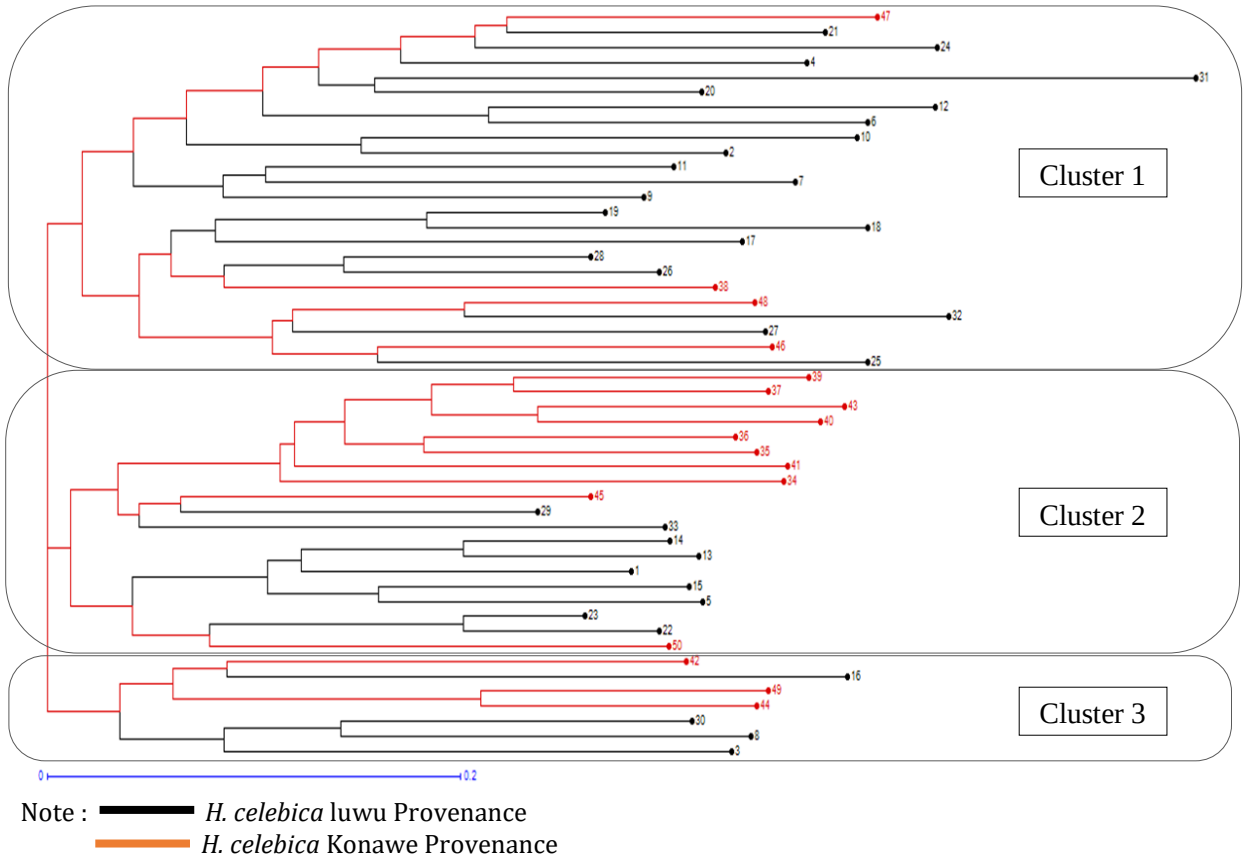


Figure 4. Dendogram of genetic relationship in Hopea celebica from 2 provenances

Planning for future tree breeding activities needs to be continued. These efforts are carried out by developing seed orchards from different populations and the selected parent trees to maintain genetic variation and produce genetically superior plants. High genetic variations allow the populations to be more adaptable to the environmental changes, and eventually, the biodiversity will be protected (Mulyadiana, 2010).

D. Conclusion

There were seven ISSR primers used for genetic diversity analysis of *H. celebica* were UBC 810, UBC 813, UBC 814, UBC 820, UBC 822, UBC 823, and UBC 827. The genetic diversity of 50 samples *H. celebica* from Luwu and Konawe was high.

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