DEVELOPMENT OF CHLOROPLAST MICROSATELLITE MARKERS FOR *Bruguiera hainesii* C.G.ROGERS (RHIZOPHORACEAE) IN THE CON DAO NATIONAL PARK, BA RIA - VUNG TAU PROVINCE, VIETNAM

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1. INTRODUCTION

Mangrove plays important roles in coastal protection, erosion control, raw material, herbal, tourist, and study places [1]. Still, in recent decades, mangroves in Vietnam have decreased for many reasons mainly is economic development purposes [2]. The Con Dao mangrove ecosystem on the China sea coastline of Vietnam is a unique place that has primary forest remaining in the country. There are 45 mangrove species in Con Dao National Park, of which 35 tree species, shrub, and climber are 5 species for each. There are 26 true mangrove species belonging to 11 families; and 19 associated mangrove species in 15 families [2]. *Bruguiera hainesii* C.G.Rogers, is a true mangrove species classified as ‘‘Critically Endangered (CR)’’ within the IUCN Red List of threatened species [3]. It is a species discovered in the Dam Quoc area, Hon Ba islands, Con Dao National Park with very small populations (only 7 individuals). The species is a tree that can grow to a height of 15 metres. The flowers are observed from January to March and are concentrated in clusters with 2-3 flowers on stalks [4].

Information regarding the ecological and genetic diversity of populations is necessary for the conservation and management of a species [5]. Powerful biological techniques are necessary to acquire such information, particularly a more comprehensive understanding of genetic processes. The profuse expression of phenotypes and genotypes in plants is known as genetic diversity. The polymorphic genes lead to the presence of heterozygosity genotypes in the population. When confronted with environmental changes, populations are capable of adapting to other populations due to the diverse genotypes of their members. Heterosis is established through genetic diversity.

Molecular marker technology has been recognised as a highly effective method of genetic analysis for some mangrove species [6-8]. It has also substantially facilitated the evaluation of the genetic resources of plants and the level of genetic diversity in tree species [6]. Chloroplast simple sequence repeats (cpSSRs) are a molecular marker technology that has been developed in recent years and is relatively new and effective [9]. Due to their codominant nature, high polymorphism, uniparental inheritance through chloroplast DNA (cpDNA), and absence of sexual recombination, cpSSRs are the optimal markers for population genetic diversity evaluation, population structure analysis, and phylogenetics. To date, the genetic diversity and population structure in *B. hainesii* have been largely unexamined. Previous research has detected the sequences chloroplast genome of *B. hainesii* and phylogenetic analysis with associated species [10-13]. Numerous

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published studies have attempted to identify the genetic diversity of some mangrove species using molecular markers, including RAPD (Random Amplified Polymorphic DNA) for genetic differentiation between *B. gymnorhiza* and *B. sexangula* in Sri Lanka [7], microsatellite (SSRs) for genetic diversity and structure of *B. gymnorrhiza* and *Kandelia obovate* [6, 8], and chloroplast microsatellite (cpSSR) markers for *B. gymnorrhiza*, *Kandelia candel*, and *Rhizophora stylosa*, as well as other mangrove species [14]. It is surprising that no research has surveyed the genetic diversity and population structure of *B. hainesii* using cpSSR markers. The protection and utilisation of the valuable genetic resources concealed in *B. hainesii* have been impeded by this dearth of research.

In the current study, we identify and develop novel chloroplast microsatellite markers (cpSSR) in *B. hainesii* and assess its level of genetic diversity. Microsatellite markers have been widely used for genetic diversity analyses because of their co-dominant inheritance and high degree of polymorphism. Following this, the study's findings are incorporated into this species' management decisions, conservation efforts and restoration efforts.

2. MATERIALS AND METHODS

2.1. Plant Material

Sampling was carried out along the coasts of Hon Ba islands, Con Dao National Park, where *B. hainesii* grow abundantly in saltwater regions. We collected seven samples (VH1 to VH7) from natural populations of *B. hainesii* in 2023 and 2024 (Fig. 1). Leaf samples were collected randomly from individual trees, cleaned, and then transferred to the Laboratory of the Joint Vietnam-Russia Tropical Science and Technology Research Center, stored at -30°C for DNA extraction.

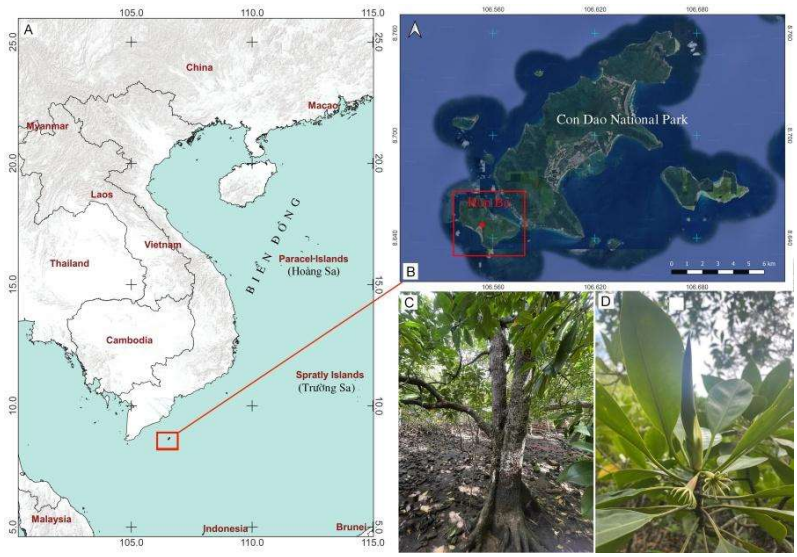

Figure 1. Map of field survey locations and geographic distributions of *B. hainesii* in the study. Map showing the collection sites (A, B); adult plant (C); leaves, and flower (D)

2.2. Chloroplast microsatellite marker development

We searched the complete chloroplast genome of *B. hainesii* [11] (GenBank: OR086085) for microsatellite loci. cpSSR markers were identified using MISA software [15]. We applied a threshold based on minimum length criteria (unit size/minimum repeat time): six for mononucleotide and dinucleotide, five for trinucleotide, and four for tetranucleotide, pentanucleotide, and hexanucleotide repeats, respectively. Based on the flanking regions of the repeat regions, primers for 68 cpSSR loci were designed using Primer v.7.0 software [16]. The parameters for designing PCR primers were as follows: (1) primer length (18 to 22 bp); (2) PCR product size (100-300 bp); (3) melting temperature between 50°C and 70°C, with 55°C as the optimum annealing temperature; and (4) GC content (40-60%), with an optimum of 50%. After primer design, amplification efficiency and polymorphism were evaluated using seven *B. hainesii* samples.

2.3. DNA extraction

Leaf samples were rinsed with deionized water and ethanol (70%), and the total genomic DNA was extracted from the plant DNA Kit according to the manufacturer's instructions (BioTeke, Beijing, China). The DNA purity and integrity were tested by Nanodrop ND-2000 spectrophotometer (NanoDrop Technologies, DE, USA) and then diluted to 20 $ng \cdot \mu l^{-1}$.

2.4. cpSSR analysis

The SSR-PCR was performed in a 25 µl reaction volume, comprising 2.5 µl of template DNA, 12.5 µl of 2X Taq Master mix, 1 µl of each primer, and 8 µl deionized water. All reactions were performed in a MasterCycler (Eppendorf, Hamburg, Germany) with the following settings: 3 min of initial denaturation at 95°C; 35 cycles of denaturation for 45 s at 95°C, annealing for 45 s at 54°C - 56°C, and extension for 45 s at 72°C; followed by a final extension for 10 min at 72°C. The amplification products were separated using a Sequi-Gen®GT DNA electrophoresis system in 8% (w/v) polyacrylamide gels in TAE buffer and then stained by ethidium bromide for 10 min. The banding patterns were visualized under UV light and photographed using a UV Transilluminator camera (CLEAVER sci. ltd). A 100 bp DNA ladder (Invitrogen) was used as the standard.

2.5. Data analysis

The genetic diversity was estimated based on the SSR allele frequencies, including the number of alleles (Na), number of effective alleles (Ne), Shannon's information index (I), the observed heterozygosities (Ho), and the expected heterozygosities (He) were calculated with GenAlEx 6.5 [17]. Additionally, the genetic distance matrix between individuals was also computed based on cpSSR data utilizing GenAlEx 6.5. Unweighted Pair Group Method with Arithmetic mean (UPGMA) phylogenetic trees were generated using MEGA 11.0 [18].

3. RESULTS AND DISCUSSION

3.1. Frequency and distribution of cpSSR

We used the recently sequenced chloroplast (cp) genome of *B. hainesii* [11]. All 164,305 bp were examined to discover microsatellite markers in the cp genome of *B. hainesii* (Table 1). A total of 133 potential cpSSRs were identified. A total of 1 and 133 sequences had one and more than one microsatellite locus, respectively and the number of cpSSR involved in compound formation (26).

Table 1. Summary cpSSR in the cp genome of *B. hainesii*

Item	Parameters	Number
Chloroplast microsatellite markers (cpSSR)	Total size of examined sequences (bp)	164,305
	Total number of identified SSRs	133
	Number of SSR-containing sequences	1
	Number of sequences containing more than 1 SSR	133
	Number of SSRs present in compound formation	26

Of the 133 potential cpSSR, distribution to different repeat type classes comprising mononucleotide repeats was the most abundant (121; 91%), followed by dinucleotide repeats (11; 8.3%), and trinucleotide repeats (1; 0.7%) (Table 2). It can be seen that the main repeat types of microsatellite sites in the cp genome of *B. hainesii* mononucleotide repeats, followed by dinucleotide repeats and trinucleotide repeats.

Table 2. Frequencies of SSR repeat types in the cp genome of *B. hainesii*

Number of repeats	Repeat type			Total	Percentage(%)
	Mono-	Di-	Tri-		
5	0	0	1	1	0.8
6	0	6	0	6	4.5
7	0	3	0	3	2.3
8	0	1	0	1	0.8
9	0	1	0	1	0.8
10	43	0	0	43	32.3
11	34	0	0	34	25.6
12	11	0	0	11	8.3
13	15	0	0	15	11.3
14	6	0	0	6	4.5
15	6	0	0	6	4.5
16	4	0	0	4	3.0
17	2	0	0	2	1.5
Total	**121**	**11**	**1**	**133**	
Percentage (%)	91	8.3	0.8		

Through statistical analysis of cpSSR based on motif types in the cp genome of *B. hainesii*, the results are shown in Table 3. In the mononucleotide repeats, A/T had a large proportion (88% of all SSRs), followed by C/G (3%). In the dinucleotide, the dominant nucleotide repeats were AT/AT (7.5%), followed by AG/CT (0.8%). Only 1 (0.8%) trinucleotide AAT/ATT was observed in the species.

Table 3. Frequencies of cpSSR motif types in the cp genome of *B. hainesii*

Microsatellite motif	Number of repeats							Total	Percentage (%)
	5	6	7	8	9	10	> 10		
A/T	-	-	-	-	-	42	75	117	88.0
C/G	-	-	-	-	-	1	3	4	3.0
AG/CT	-	1					0	1	0.8
AT/AT	-	5	3	1	1		0	10	7.5
AAT/ATT	1							1	0.8

3.2. Genetic diversity

The 68 pairs of cpSSR primers were designed by Primer 7.0 based on 133 cpSSR (Table 4). Of the 20 pairs randomly tested 9 pairs produced PCR products of the expected size and revealed allelic polymorphism, which were used to investigate the genetic diversity of *B. hainesii* (Table 5, Fig. 2). In the present study, a total of 21 alleles were detected across nine cpSSR loci from 7 adult trees of *B. hainesii.* The number of observed alleles (Na) ranged from 2 to 3, effective number of alleles (Ne) ranged from 1.2 to 2.0, Shannon's information index ranged from 0.14 and 0.71. The mean values of the observed (Ho) and expected heterozygosity (He) were 0.25 and 0.27, respectively (Table 5). This result showed the natural populations of *B. hainesii* maintain a low genetic diversity level. The results of this study are similar to previously reported low genetic diversity among populations of *R. apiculata* (Rhizophoraceae) (He = 0.352) using microsatellite markers [6]. *B. gymnorrhiza* (H = 0.027-0.475) and *Kandelia candel* (H = 0.175-0.370) by cpSSR [14]. Genetic diversity is important because it gives species a better chance of survival. However, genetic diversity can be lost when populations get smaller and isolated, which decreases a species' ability to adapt and survive. If genetic diversity gets too low, species can go extinct and be lost forever. This is due to the combined effects of inbreeding depression and failure to adapt to change. In such cases, the introduction of new alleles can save a population. During field surveys in 2023 and 2024, we found the population size of *B. hainesii* very small with only 7 individuals with more than 20 cm diameter (dbh). UPGMA tree for 7 *B. hainesii* accessions was constructed based on genetic distance data showed that all individuals had a close

genetic relationship and were divided into two groups (Figure 3). The first group included three individuals (VH1, VH4, and VH7) and the second group included four individuals (VH2, VH3, VH5, and VH6). Notably, *B. hainesii* is considered a hybrid between *B. gymnorhiza* and *B. cylindrica* [12, 13, 19] rare hybrid individuals can reproduce successfully. Thus, these trees should be protected as an important genetic resource bank which makes it necessary to continue with asexual reproduction experiments (propagation of *B. hainesii* by Air-layering, grafting, stem cuttings, micropropagation).

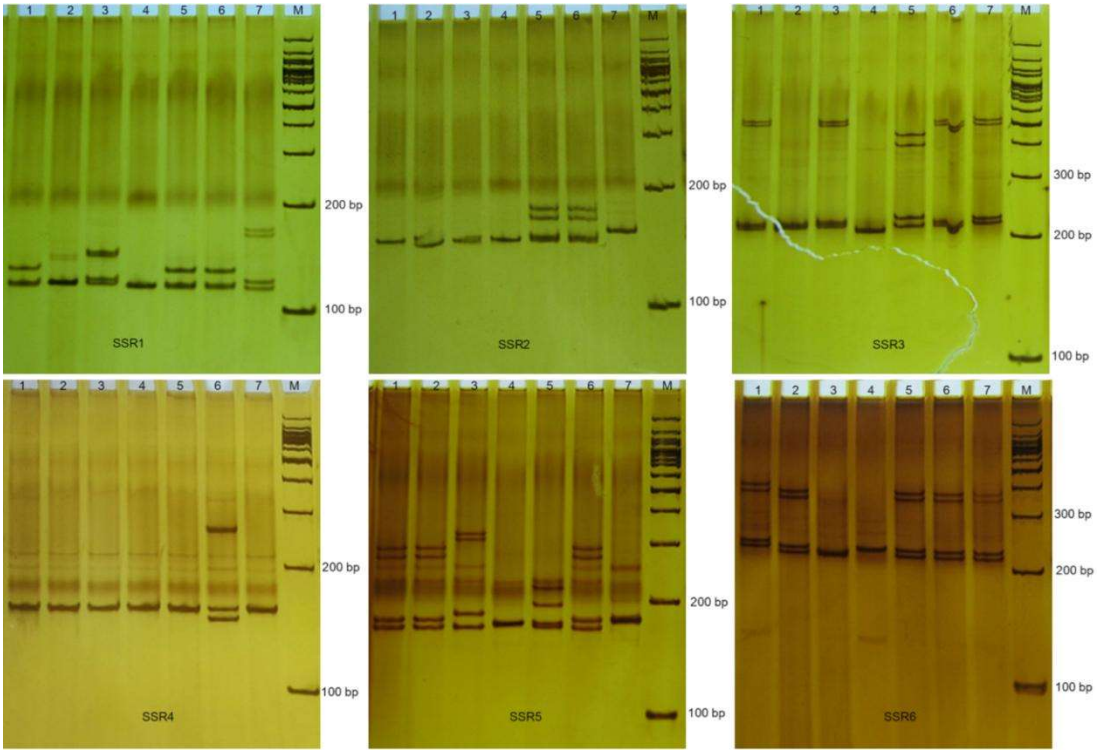

Figure 2. Gel pictures of seven *B. hainesii* genotypes produced with some primer pairs SSR (lane M is a 100bp ladder and lanes 1 to 7 represent different)

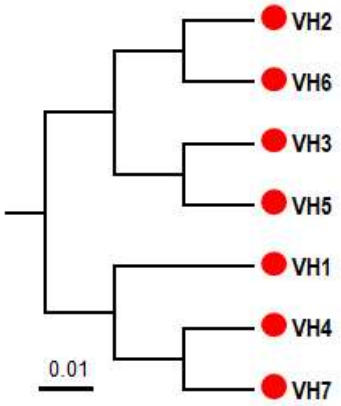

Figure 3. UPGMA phylogenetic tree of 7 individuals *B. hainesii*

Table 4. List of primers pairs designed for *B. hainesii*

Loci	Primer sequence (5'-3')	Repeat sequence	Start (bp)	End (bp)	Product size (bp)	Loci	Primer sequence	Repeat sequence	Start (bp)	End (bp)	Product size
SSR1	AGCTTGGTATTGCTCCCCT	(T)17	706	722	112	SSR35	TGCCAAACTTAGTTCAGCCT	(T)11	53,601	53,611	275
	AGACCTAGCTGCTGTCGA						CCTCGATGCTACAACTCTCGA				
SSR2	CAACCAAACCCCACCCCA	(AT)6	2,115	2,127	266	SSR36	TGCCAAACTTAGTTCAGCCT	(T)15	53,612	53,626	225
	GTTCCGGGTTCGAGTCCC						CCTCGATGCTACAACTCTCGA				
SSR3	GGGACTCGAACCCGGAAC	(A)16	2,229	22,44	122	SSR37	GGTACCATAGAGAAGCGGCC	(T)14	55,064	55,077	230
	AGGGAAAGCCGTGTGCAA						GCATGGCGAAAAGGAGCA				
SSR4	CCAAGTCAATCGCTCTTGTGA	(A)14	4,311	4,324	170	SSR38	GCTAGTGTTCTCCGGTTCCC	(A)12	57,645	57,656	136
	GCCTGATGCGGGAAAGGT						TTTACACGCGCGCCAATG				
SSR5	AATGATCCGGGGCGCAAT	(A)13	8,173	8,185	276	SSR39	TTGAAGCGTGGAACCCCC	(A)11	60,201	60,211	184
	GGGAGAGATGGCTGAGTGG						GGTACCAACGAAATCGAGTGC				
SSR6	GAGCAATGCCGTCGCCTA	(C)12	12,322	12,333	184	SSR40	TTGTTCGATAGCACAACCTCA	(A)12	66,825	66,836	245
	CCGATTAGTCCGTTGTAGGCA						CGAAGCAATTCCCTCTTTTCC				
SSR7	GAGCAATGCCGTCGCCTA	(A)15	12,334	12,348	184	SSR41	TTGTTCGATAGCACAACCTCA	(T)14	66,857	66,870	245
	CCGATTAGTCCGTTGTAGGCA						CGAAGCAATTCCCTCTTTTCC				
SSR8	TCTTCCCGAACCAAACACGA	(A)11	12,957	12,967	203	SSR42	GGGCTAACCAGTGACTCACA	(A)12	67,203	67,214	221
	TGGTCAGAAGAGTCCTCCGA						ACGGGGATCCACCATAGCA				

Loci	Primer sequence (5'-3')	Repeat sequence	Start (bp)	End (bp)	Product size (bp)	Loci	Primer sequence	Repeat sequence	Start (bp)	End (bp)	Product size
SSR9	TCGGTACCAGGTCTTGTGT	(A)12	14,112	14,123	167	SSR43	CGGACTCATGTGAGGATAGGT	(T)12	72,142	72,153	199
	CCCACGGGAAGGGTTGAT						AAAGCACTTCCCTAAGTTCCA				
SSR10	GGGGGAAGGAAGAAAGCGA	(A)13	14,273	14,285	202	SSR44	CAAGCTGCGCTACATCCCT	(A)13	73,391	73,403	257
	ACCTTGGATTTGCTGCTTGC						TCCCTTTCCGCCCGAGTA				
SSR11	GCAAGCAGCAAATCCAAGGT	(A)11	14,358	14,368	205	SSR45	TCCAGATTTGACTGTCGTGTC	(A)14	75,127	75,140	195
	GCGGGCCAAGCTGTAGAA						TGAGGTTCCCCGGAAAACG				
SSR12	CCTAGTTTGATCCCACTCTCC	(T)12	15,246	15,257	101	SSR46	TGGTGGTTCGTTGCTTT	(T)14	77,304	77,317	192
	TCCGTGTGGGAAAACGATAA						GGGAGCCCATTTCAGCGT				
SSR13	TCCCGGAAATTGGACGTTGA	(A)16	17,827	17,842	198	SSR47	TGGTGGTTCGGTTGCTTT	(A)11	77,322	77,332	192
	TCGAACTATTGGTGGTCCTGT						GGGAGCCCATTTCAGCGT				
SSR14	TCCGTTGGGCATAGATCCA	(T)12	19,912	19,923	195	SSR48	TGTGTAGCTTCCAGCCCTC	(T)11	78,075	78,085	114
	GTCGAGTAGGTGGATTGGTCC						GGCAAACGGGTCAAAACTCA				
SSR15	CCGGCTCAAGTAGTTACACCA	(C)12	24,016	24,027	279	SSR49	TGCTCCCAATACCGCACC	(T)12	78,392	78,403	228
	GCAAAGCACTGGGAATTCATC						GGCTCCGTTTAGAAAAACCCA				
SSR16	CCGGCTCAAGTAGTTACACCA	(T)15	24,092	24,106	280	SSR50	TCCCCGTCGACGTATCAGT	(T)11	87,095	87,105	202
	TGCAAAGCACTGGGAATTCA						CCGCAACAAAGATTCGAATGA				
SSR17	CCGGCTCAAGTAGTTACACCA	(T)13	24,109	24,121	280	SSR51	TGGAGGATACCGCGGGAA	(T)11	87,431	87,441	278
	TGCAAAGCACTGGGAATTCA						CGGGGTATAATGACAGACCGA				

Loci	Primer sequence (5'-3')	Repeat sequence	Start (bp)	End (bp)	Product size (bp)	Loci	Primer sequence	Repeat sequence	Start (bp)	End (bp)	Product size
SSR18	AGTTTGGTGCCCCCTAGA	(A)11	29,573	29,583	142	SSR52	GGCTCCGCTGTTATCTGCT	(A)17	88,569	88,585	235
	AGGCGACACCCGGATTTG						GCAGCGTCCAAAATGCCT				
SSR19	CCAGGAGTGCCGTTACGT	(T)11	30,610	30,620	173	SSR53	GGCTCCGCTGTTATCTGCT	(A)18	88,601	88,618	125
	AGAATCACTAGCGCGGAGTC						GCAGCGTCCAAAATGCCT				
SSR20	CCGCCTTGACACTTACATTGG	(A)12	32,771	32,782	212	SSR54	GGGAATCTTTGAGATTTGCCC	(T)15	89,391	89,405	257
	TCATTCCCGCCATATTCCGG						AGAGTAAATATTCGCCCGCG				
SSR21	TGGTTTGCCAGAAAGCCAGA	(T)11	34,412	34,422	241	SSR55	GATGCTCGGGACCACGTT	(T)11	91,937	91,947	265
	TCGAATCACGGATGAACGGA						CGCAACCCCTTGGGGTTA				
SSR22	AGGCTCTTTATTTTGGGGGCA	(G)11	38,557	38,567	219	SSR56	CGAGTCGGACATCCAATTGC	(TA)6	101,420	101,432	223
	TGCCAGATTCCGCCAAGT						GGCGTAATCGGACCTGCT				
SSR23	GCGAGAAAGGGGCGGTAA	(AT)8	40,007	40,023	156	SSR57	CATTCTGGGGCGACGGAG	(T)11	110,293	110,303	155
	ACTCGACCAACCATCAGGAG						CTGGTCCCTGCGGAAAGG				
SSR24	TGATCCAGACATTGCGTATCA	(A)11	41,310	41,320	108	SSR58	TGTAGGGGAGGTCCTGCG	(A)15	115,367	115,381	171
	GCGGATAGCGGGAATCGA						GTGCGTTCCGAGGTGTGA				
SSR25	GATTCCCGCTATCCGCCC	(T)12	41,482	41,493	245	SSR59	TGTCTTTTTCCACGAAGTCCT	(T)11	118,844	118,854	254
	TCCCGTCTCCGCAACATT						TGTATTCGCCGATTTTCGCA				
SSR26	CTTTCCTCCCGTTCCAAAAGA	(A)11	41,594	41,604	195	SSR60	TGCGGTTATATGCTATGCCCA	(T)12	123,015	123,026	199
	TTTCAGCACGCGGGGTAG						GGTCTGACTTCAATCCATGGA				

Loci	Primer sequence (5'-3')	Repeat sequence	Start (bp)	End (bp)	Product size (bp)	Loci	Primer sequence	Repeat sequence	Start (bp)	End (bp)	Product size
SSR27	ACGGGGCTTCCTCTATCAA	(T)11	48,334	48,344	280	SSR61	TTCGGGAGCAGTTTGGGC	(A)11	124,534	124,544	274
	TCGGGCACTAGAACGAAACC						TCGTCGGACTCTTGCTTCA				
SSR28	GAGGCTGCATTAATCGGGGA	(A)14	49,332	49,345	208	SSR62	CGGTCTCGGTCTCGTATTCT	(T)11	130,670	130,680	276
	TCCGACAACCTTGGGAGA						TCTCTACGTGCGATTCGTTAA				
SSR29	CGCTCGTGAGAAAACAATCCC	(A)12	49,598	49,609	196	SSR63	ATTGAGGGGTGGTGGGGA	(A)14	132,727	132,740	275
	TCATGGTTGCGAAGGCGG						AACGCCAACGACTGCTGT				
SSR30	CCGCCTTCGCAACCATGA	(A)13	49,794	49,806	249	SSR64	GCGGTTTTGCGTCTTTGC	(T)11	137,248	137,258	209
	GCCCCGAAACCGAGTGAA						GCAGCAAATCCTGGGATTCG				
SSR31	CCGCCTTCGCAACCATGA	(T)12	49,862	49,873	249	SSR65	GTGCGTTCCGAGGTGTGA	(T)15	140,648	140,662	171
	GCCCCGAAACCGAGTGAA						TGTAGGGGAGGTCCTGCG				
SSR32	TCACAAGCATATCCGGGCT	(T)14	52,698	52,711	230	SSR66	GATCGGGGGCGTTCGTAG	(A)11	145,726	145,736	248
	ACCATTCCCAACGCGTCA						CATTCTGGGGCGACGGAG				
SSR33	TCACAAGCATATCCGGGCT	(T)13	52,836	52,848	230	SSR67	GGCGTAATCGGACCTGCT	(AT)6	154,596	154,608	150
	ACCATTCCCAACGCGTCA						CGAGTCGGACATCCAATTGC				
SSR34	TCTGGTTCCTGGCACATGA	(T)14	53,100	53,113	249	SSR68	CGCAACCCCTTGGGGTTA	(A)11	164,082	164,092	265
	ACGCGAGGAAACACATTGTG						GATGCTCGGGACCACGTT				

Table 5. The characteristics of nine polymorphic chloroplast microsatellite loci developed for *B. hainesii*

Loci	Primer sequence (5'-3')	Repeat sequence	Start (bp)	End (bp)	Allele range (bp)	Tm (°C)	Na	Ne	I	Ho	He
SSR53	PF: GGCTCCGCTGTTATCTGCT PR: GCAGCGTCCAAAATGCCT	(A)18	88,601	88,618	124-130	56	3.0	1.3	0.29	0.24	0.41
SSR23	PF: GCGAGAAAGGGGCGGTAA PR: ACTCGACCAACCATCAGGAG	(AT)8	40,007	40,023	150-170	56	3.0	1.3	0.29	0.24	0.41
SSR56	PF: CGAGTCGGACATCCAATTGC PR: GGCGTAATCGGACCTGCT	(TA)6	101,420	101,432	220-228	54	2.0	2.0	0.14	0.50	0.69
SSR67	PF: GGCGTAATCGGACCTGCT PR: CGAGTCGGACATCCAATTGC	(AT)6	154,596	154,608	150-160	56	2.0	1.2	0.14	0.13	0.26
SSR7	PF: GAGCAATGCCGTCGCCTA PR: CCGATTAGTCCGTTGTAGGCA	(A)15	12,334	12,348	180-192	55	2.0	1.2	0.14	0.13	0.26
SSR36	PF: TGCCAAACTTAGTTCAGCCT PR: CCTCGATGCTACAACTCTCGA	(T)15	53,612	53,626	220-235	55	3.0	1.2	0.14	0.13	0.26
SSR1	PF: AGCTTGGTATTGCTCCCCT PR: AGACCTAGCTGCTGTCGA	(T)17	706	722	112-120	55	2.0	1.7	0.29	0.41	0.60
SSR3	PF: GGGACTCGAACCCGGAAC PR: AGGGAAAGCCGTGTGCAA	(A)16	2,229	2,244	122-130	55	2.0	1.8	0.71	0.46	0.65
SSR15	PF: CCGGCTCAAGTAGTTACACCA PR: GCAAAGCACTGGGAATTCATC	(C)12	24,016	24,027	276-282	55	2.0	1.2	0.14	0.13	0.26
Mean							**2.3**	**1.42**	**0.42**	**0.25**	**0.27**

Note: Na = No. of different alleles; Ne = No. of effective alleles; Ho = observed heterozygosity; He = expected heterozygosity; I = Shannon's information index.

4. CONCLUSIONS

We developed and characterised nine chloroplast microsatellite markers using the recently sequenced cp genome of *B. hainesii*. These cpSSR markers were subsequently employed to identify low genetic diversity of *B. hainesii*. The polymorphic cpSSR primers are expected to be beneficial in intra- or interspecific genetic studies of the genus *Bruguiera*.

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SUMMARY

Mangroves consist of various tropical trees or woody shrubs like plants growing at the interface between sea and land zones and form an ecologically important ecosystem. *Bruguiera hainesii* C. G. Rogers belonging to the family (Rhizophoraceae) is a true mangrove tree. This species was discovered in the Dam Quoc area, Hon Ba islands, Con Dao National Park, Ba Ria-Vung Tau Province. However, studies on the genetic diversity of *B. hainesii* are limited. Hence, this study was initiated to develop chloroplast microsatellite (cpSSR) markers and applied them to evaluate the genetic diversity of *B. hainesii*. A total of 133 cpSSRs markers were identified from the chloroplast genome of *B. hainesii*. Among all cpSSR loci, mononucleotide markers were the most abundant (91%), followed by dinucleotide (8.3%), and trinucleotide (0.7%). In total, 9 newly developed cpSSR markers with polymorphism and good stability were selected for genetic diversity analyses of 7 *B. hainesii* samples. These cpSSRs amplified a total of 21 alleles. We report low levels of genetic diversity of *B. hainesii* with Na = 2.3, Ne = 1.42, I = 0.42, Ho = 0.25, and He = 0.27. Our study not only provided a batch of efficient genetic markers for research on *B. hainesii* but also laid an important foundation for the breeding and conservation of *B. hainesii*.

Keywords: *Bruguiera hainesii C. G. Rogers, mangrove species, chloroplast microsatellite markers (cpSSR), genetics diversity, đa dạng di truyền, loài ngập mặn, microsatellite lục lạp, Vẹt hainesii.*

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