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GENETIC DIVERSITY OF THE TALL-STILT MANGROVE (*Rhizophora apiculata* Blume) IN SOUTHERN CENTRAL COAST OF VIETNAM USING SSR MARKERS

Nhung Hong Nguyen[1], Trong Dinh Nguyen[1], Giang Huong Thi Tran[2], Huy Quoc Nguyen[4], Ngoc Bich Pham[1,3], Ha Hoang Chu[1,3], Duy Dinh Vu[5], Phat Tien Do[1,3,*]

[1]Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Ha Noi, Vietnam
[2]Institute of Genome Research, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Ha Noi, Vietnam
[3]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Ha Noi, Vietnam
[4]Institute of Ecology and Works Protection, Vietnam Academy for Water Resources, Vietnam
[5]Join Vietnam–Russia Tropical Science and Technology Research Center, 63 Nguyen Van Huyen, Ha Noi, Vietnam

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ABSTRACT

Rhizophora apiculata is one of the important mangrove species for coastal communities and the global ecosystem. However, it has been threatening due to land clearing and over-exploitation, particularly in the Southern Central Coast of Vietnam. The SSR-PCR analysis indicated low levels of genetic diversity in *R. apiculata* populations distributed in the Southern Central regions of Vietnam. The observed number of alleles (N_A) was 1.933 indicating a low polymorphism for surveyed overall loci in tested populations. The observed heterozygosity (H_O) was also less than 0.5 indicating low heterozygosity at the species level. The interpopulation genetic diversity for each locus ranged from 0.000 to 0.188 demonstrating a moderate genetic differentiation among tested populations. Results of AMOVA analysis indicated that genetic variation almost occurred within the population (88%). Moreover, genetic distances of *R. apiculata* populations were low (drifted less than 0.05) indicating the gene pool exchange. At the overall population level, two different groups were observed and confirmed from UPGMA analysis and genetic distance matrix data. To gain a comprehensive understanding of the genetic diversity of *R. apiculata* in the Southern Central Coast of Vietnam, it would be necessary to utilize more molecular markers and investigate the matter further in other coastline regions. This would provide recommendations for mangrove tree breeding and restoring the mangrove forests in Vietnam as well as support for coping with the change of global climate.

Keywords: Genetic diversity, mangrove, *Rhizophora apiculata*, SSR markers, Vietnam.

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[*]*Corresponding author email*: dtphat@ibt.ac.vn

INTRODUCTION

Mangrove consists of different plant species distributed along coastal wetlands around the world. It is very important not only for coastal communities but also for the global ecosystem (Simard et al., 2019). As one of the most diverse mangrove regions, South East Asia harbored up to forty-two true mangroves (FAO, 2007; Spalding, 2010). Vietnam has been reported to harbor approximately 2.1% of the total mangrove area in Southeast Asia with about 26 plant species of true mangroves. In Vietnam, the largest extent of mangrove is contributed in coastal regions in the south, while the smallest mangrove area occurs in the coastal areas of the central part (Giesen et al., 2007). Earlier studies of the distribution of mangroves throughout Vietnam was mainly reported on the Northeast coast, the Northern Delta coast, the Northeast Central coast, and the coast of Southern (Giri et al., 2011; Veettil et al., 2019). However, despite harboring a small area of mangrove forest, twenty-six species of true mangrove have been discovered in the Southern Central region of Vietnam. *Avicenia marina* and *Rhizophora apiculata* are widely distributed species in this area (Hoa et al., 2010).

In recent years, molecular markers have been developed and utilized for the analysis of genetic diversity and population structures of forestry plants. Different markers have shown significant support for mangrove forest ecosystem studies (Francisco et al., 2018). Random Amplified Polymorphic DNA (RAPD) was used to evaluate the genetic diversity of Rhizophoraceae in Bhitarkanika National Park, India (Sahoo et al., 2007). In addition, inter-simple sequence repeat (ISSR) and simple sequence repeat (SSR) methods were successfully utilized for the genetic analysis of Rhizophoraceae (Azman et al., 2020; Ge & Sun, 2001; Francisco et al., 2018; Yahya et al., 2014; Yan et al., 2016). The microsatellite maker, first described in 1981, was a simple sequence including a repeated motif of 1–6 base pairs and widely distributed in the DNA genome. This marker exhibits a high polymorphism and a codominant genotype, thus it is useful for evaluating the genetic diversity among individuals of a population and also between different populations (Stella, 2013; Vieira et al., 2016).

Although having substantially large mangrove areas, previous studies of mangroves in Vietnam have primarily focused on surveying ecological diversity (Giri et al., 2011; Hoa et al., 2010; Veettil et al., 2019). Genetic diversity and population structure studies are essential for developing strategies and policies to conserve and enhance coastal wetlands in Vietnam. In this study, we first evaluated the genetic diversity of *R. apiculata*, a dominant species in mangrove areas in Vietnam, by utilizing the SSR method. Our results showed the low genetic diversity of *R. apiculata* both within and among the three studied populations along the Southern Central coast of Vietnam.

MATERIALS AND METHODS

Sample collection

The 3rd and 4th leaves of the twigs of *R. apiculata* trees collected from three populations of *R. apiculata* in the southern central coast of Vietnam, which includes Quang Nam, Binh Dinh, and Ninh Thuan provinces (Table 1, Fig. 1), were individually placed in plastic bags and stored at -80 ^{o}C for DNA extraction.

Table 1. Collection localities of *Rhizophora apiculata*

Population	Sampling size	Collection locality	Latitude	Longitude
QN	16	An Hoa coastal lagoon in Quang Nam province	15^{o}28’27’’N	108^{o}39’20’’E
NT	20	Dam Nai coastal lagoon in Ninh Thuan province	11^{o}38’27’’N	109^{o}01’34’’E
BD	18	Thi Nai coastal lagoon in Binh Dinh province	13^{o}51’34’’N	109^{o}14’21E

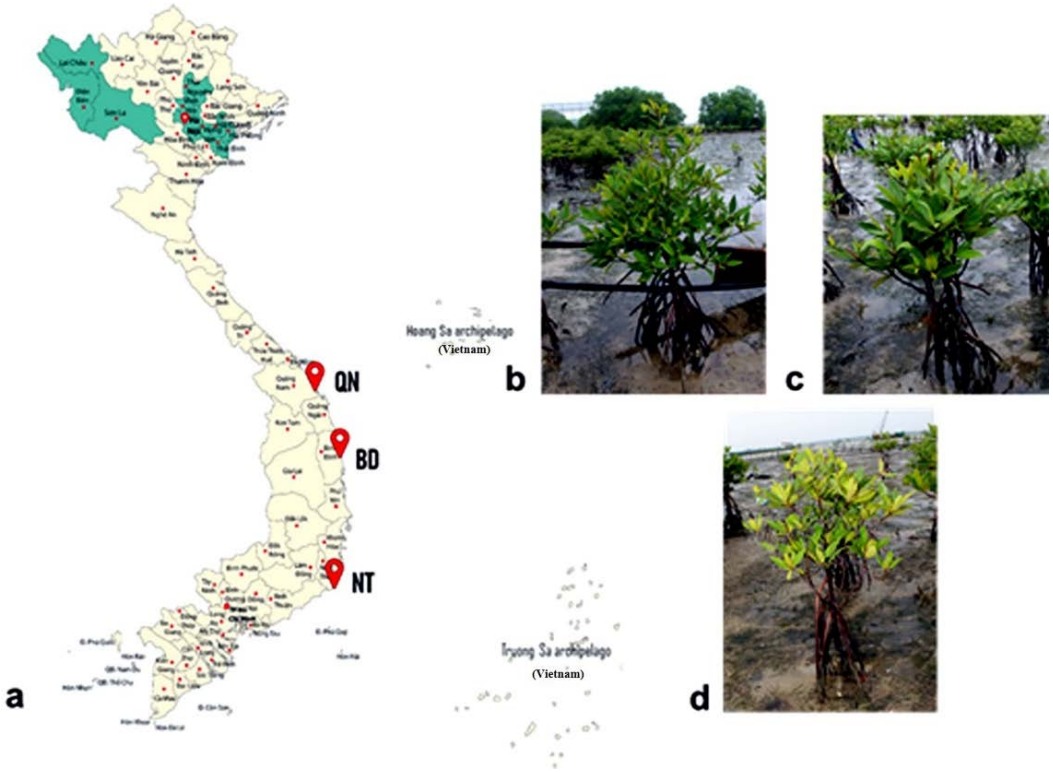

Figure 1. The position of three *Rhizophora apiculata* populations in the Southern Central Coast, Vietnam. a. The geographical location of three *Rhizophora apiculata* populations; QN, Quang Nam province; BD, Binh Dinh province; NT, Ninh Thuan province; b. *Rhizophora apiculata* plant at An Hoa coastal lagoon in Quang Nam province; c. *Rhizophora apiculata* plant at Thi Nai coastal lagoon in Binh Dinh province, d. *Rhizophora apiculata* plant at Dam Nai coastal lagoon in Ninh Thuan province

DNA extraction and SSR assay

The stored leaves were ground in liquid nitrogen and used for genomic DNA extraction utilizing a modified CTAB method. In brief, the leaf tissue powder was initially washed with a sorbitol buffer (50 mM Tris-HCl, 10 mM EDTA, and 0.35 M sorbitol), followed by the standard CTAB extraction procedure (Doyle & Doyle, 1987). The extracted genomic DNA was further cleaned by a purification column (GeneJET Gel Extraction Kit, Thermo Fisher Scientific, USA) to obtain high-quality genomic DNA.

DNA quality was analyzed by gel electrophoresis using 0.8% agarose and measured with the NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific, USA).

SSR-PCR was performed in 10 µL reactions containing 5 µL DreamTaq PCR Master Mix 2X (Thermo Fisher Scientific, USA), 5 pmol of each primer, 2 µL DNase-free water, and 2 µL template DNA (50 ng/µL) with the following PCR reaction cycles: 94 ^{o}C for 4 minutes, 35 cycles of 94 ^{o}C for 30 seconds with a variable annealing temperature (T_a), depending on the particular locus as indicated in Table 1, 72 ^{o}C for 1 min, and then a final extension of 72 ^{o}C for 10 mins. Amplicons were separated on 15% polyacrylamide gel for 4 hours at 100 volts of direct current and captured on a UV transilluminator (Cleaver Scientific, UK). Ten SSR microsatellite loci were preliminarily collected for this study (Table 2).

Table 2. SSR microsatellite loci in the genetic diversity analysis of *Rhizophora apiculata* (T_a, annealing temperature)

Locus	Primer sequences	Repeat type	T_a (°C)	Size (bp)
RM121 (Shinmura et al., 2012)	F:GGTTTTCCCAGTCACGACGTGGCCTATAGAGAAAGCGGA R: GTTTCCTTCAATCCCAAACAGC	$(ATC)_{12}$	55	174–183
RM106 (Shinmura et al., 2012)	F:GGTTTTCCCAGTCACGACGCCCTGGCTCTTACCGTTCTT R: GTTTAGAACCAAACTCCAAGGGTC	$(GA)_{13}$	55	192–200
RmBra18 (Ribeiro et al., 2013)	F: TCAAGGACAGGTCAACAGCA R: TGAATTAAGACGTCAAATCATCG	$(TC)_{18}$	54	156–174
RmBra19 (Ribeiro et al., 2013)	F: GAGGCAGAGTCAGGTCAGAA R: CACTGGTCCACTGACAGCAA	$(CT)_{14}$	54	90–103
RmBra45 (Ribeiro et al., 2013)	F: GAAAATGCAAGAGGGCTGAC R: CATGGGTCTCTCTCCTCTGC	$(AG)_{17}$ $(AGG)_3$	54	128–164
RmBra59 (Ribeiro et al., 2013)	F: GTGAACGGTCTGGACTGGAG R: TCACCGATCCCCTAGAACTG	$(AG)_{24}$	50	145–209
RmBra65 (Ribeiro et al., 2013)	F: CCATAGACAATACAGGATACCCAGA R: CAGCATGAACATCACCTTGG	$(AG)_{12}$	52	148–222
RmBra50 (Ribeiro et al., 2013)	F: ATCGTGGAAGAACGGGGTTT R: TCAAGAAGTCCAGGGTGCTT	$(AG)_{18}$	50	215–287
RhSt11 (Sugaya et al., 2002)	F: GTATGACTAATTGACTGTAGC R: ACTCCGACAGCATAGAGATC	$(GT)_{17}$ $(AT)_6$	54	143–177
Kcan034 (Sugaya et al., 2002)	F: CAGAAGCAGCAAGTAAGGAA R: GAAGAACGTGAAGACAGTGA	$(AG)_{26}$	50	178–214

Analysis of SSR data

Null alleles and other genotyping errors were detected using the MICRO-CHECKER v. 2.0 software with 1,000 bootstrap iterations over loci to generate the expected homozygote and heterozygote frequencies (Van Oosterhout et al., 2004). CERCUS was used to estimate the PIC (polymorphism information content) value for each locus (Kalinowski et al., 2007). Different parameters of genetic diversity such as observed number of alleles (N_A), effective number of alleles (N_E), observed proportion of heterozygote (H_O), expected proportion of heterozygotes (H_E), interpopulation gene diversity (D_{ST}), coefficient of gene differentiation (G_{ST}), and analysis of molecular variance (AMOVA) were calculated by the GenAlEx version 6.5 program using data generated from ten SSR markers (Peakall & Smouse, 2012). Polymorphic information content (PIC) for each locus was computed by the formula: $PIC_i = 1 - \sum p_{ij}^2$, where p was the frequency of the j^{th} allele at the i^{th} locus (Weir, 1996). The F-statistics (F_{ST}, F_{IT}, and F_{IS}) were conducted for each locus using the POPGENE version 1.32 software (Yeh et al., 1999). The F_{IS} values were corrected for null allele frequencies based on the individual inbreeding model (IIM) using INEst (Chybicki & Burczyk, 2009). Tests for the deviation from the Hardy-Weinberg equilibrium at each locus and the linkage disequilibrium for each locus pairwise combination in each population were analysed by CERCUS (Kalinowski et al., 2007). Three mutational/drift models, including IAM (the infinite allele model), SMM (stepwise mutation model), and TPM (the two-phased model of mutation) were performed using

BOTTLENECK v.1.2 (Piry et al., 1999). A two-phase model (TPM) was used and tested via the Wilcoxon signed-rank test. The proportion of the stepwise mutation model was set to 70% under the default settings. Bayesian clustering approach was implemented to analyze the population structure using STRUCTURE v. 2.3.4 (Pritchard et al., 2000). Setting the admixture model with correlated allele frequencies, ten separate runs of the number of groups in the dataset (K) were implemented for K between 1 and 10 at 500 000 Markov Chain Monte Carlo (MCMC) repetitions and 100 000 burn-in periods. To determine the optimal value of K, STRUCTURE HARVESTER was used to detect the number of groups that best fit the dataset based on the ΔK by Evanno et al. (2005) (Earl & vonHoldt, 2012; Evanno et al., 2005). After the best K value was inferred, the replicated results were aligned using CLUMPP v. 1.1.2, and the bar plots of the assigned cluster membership were drawn using DISTRUCT v. 1.1 (Jakobsson & Rosenberg, 2007; Rosenberg, 2004).

Nei's genetic distances and genetic distance matrix between individuals and between populations were also computed based on SSR data utilizing GenAlEx version 6.5 program followed by using MEGAX version 10.2.5 software for a UPGMA (unweighted pair group method with arithmetic mean) based phylogenetic tree estimation (Kumar et al., 2018).

RESULTS

Genetic diversity

The ten SSR markers produced a total of 23 different alleles, ranging in size from 90 to 287 bp, across 54 trees of three natural populations of *R. apiculata.* The mean observed number of alleles (N_A) was 1.933, indicating a low polymorphism for the overall loci. This also led to a poor value of the mean effective number of alleles (N_E), which was only 1.566. PIC (polymorphism information content) values ranged from 0.119 at one locus (RmBra45) to 0.909 at locus RM121, with an average of 0.361. Eight of the ten loci showed heterozygote deficits under the Hardy-Weinberg equilibrium indicating inbreeding or the presence of null alleles. Micro-Checker analysis detected null allele frequencies at one locus RhSt11.

Table 3. Profiles of microsatellite loci across all populations

Locus	PIC	N_A	N_E	H_O	H_E	Null allele	D_{ST}	G_{ST}	HW
RM121	0.909	2.000	1.377	0.000	0.261	no	-0.001	-0.001	ND
RM106	0.268	1.667	1.346	0.000	0.197	no	0.095	0.194	ND
RmBra18	0.249	1.667	1.328	0.000	0.218	no	0.025	0.053	ND
RmBra19	0.249	1.667	1.099	0.017	0.082	no	0.004	0.025	ND
RmBra45	0.119	3.333	2.787	0.900	0.641	no	0.049	0.017	ND
RmBra50	0.605	1.667	1.368	0.367	0.198	no	0.188	0.335	***
RmBra59	0.268	2.000	2.000	1.000	0.500	no	0.000	0.000	ND
RmBra65	0.402	2.000	1.722	0.619	0.410	no	0.035	0.032	***
Kcan034	0.365	1.333	1.200	0.019	0.125	no	0.040	0.150	NS
RhSt11	0.172	2.000	1.431	0.000	0.301	0.334	-0.025	-0.038	ND
Mean	0.361	1.933	1.566	0.292	0.293		0.044	0.063	

Note: Polymorphic information content (PIC); Observed number of alleles (N_A); Effective number of alleles (N_E); Observed heterozygosity (Ho); interpopulation genic diversity or Expected heterozygosity (H_E); interpopulation gene diversity (D_{ST}); coefficient of gene differentiation (G_{ST}); Hardy-Weinberg equilibrium (HW); ND: no determined; NS: no significance *P < 0.05, **P < 0.01, ***P < 0.001.

At the species level, the value of 0.000 showed the minimum observed heterozygosity (H_O) at locus RM121, RM106, RmBra18, and RhSt11, meanwhile, the value of 1.000 showed

the maximum observed heterozygosity (H_O) at locus RmBra59, and the mean of H_O was 0.292. The expected heterozygosity (H_E) for each locus ranged from 0.082 to 0.641 with an average of 0.293 (Table 3). Of all tested loci, only three microsatellites, including RmBra45, RmBra65, and RmBra59 showed high heterozygosity. The observed heterozygosity (H_O) values of these loci were 0.900, 0.619, and 1.000, respectively. When counted for all surveyed loci, the mean observed heterozygosity was less than 0.5, indicating a low heterozygosity at the species level. The interpopulation genetic diversity (D_{ST}) and the genetic differentiation (G_{ST}) for each locus were also computed to account for the genetic diversity among the population. The value of the interpopulation genetic diversity for each locus drifted from -0.001 to 0.188, with an average of 0.044. The low values of individual locus resulted from a relatively low value of interpopulation differentiation for the overall loci demonstrating a moderate genetic differentiation among populations. The coefficient of genetic differentiation (G_{ST}) was also quite low with 0.063 on average. This indicated a low level of genetic differentiation.

At population level, the observed number of alleles (N_A) per population ranged from 1.9 to 2.0, an average of 1.933. N_A values were not significantly different among tested populations. The average effective number of alleles (N_E) was 1.566 (1.488–1.673). Although the *R. apiculata* population from the BD region showed the highest value of the effective number of alleles, the observed heterozygosity (H_O) was only 0.289 while its expected heterozygosity (H_E) reached 0.357. The highest observed heterozygosity (H_O) was found in the *R. apiculata* population from the QN region. It was notable that the H_O of both *R. apiculata* populations from QN and NT was higher than its H_E demonstrating a deficit of heterozygosity. The mean value of H_O across three tested populations was only 0.292. (Table 4). On the other hand, negative values of the fixation index (F) were obtained in these regions while the *R. apiculata* population from the BD region presented a positive value for the fixation index. However, the fixation index (F) across the ten SSR loci was 0.214, indicating a high value of homozygosity and a low level of inbreeding overall. (Table 4). In addition, outcrossing rates (t) of *R. apiculata* population in QN, NT, and BD were 0.85, 0.78, and 0.64, respectively. The inbreeding corrected for null alleles based on the individual inbreeding model (F_{IS}IIM) varied from 0.043 in BD to 0.454 in QN, with an average of 0.186, also indicating homozygote excess. However, this value was low compared to the fixation index. BOTTLENECK analysis showed that a significant heterozygosity deficit was detected in BD and NT. The significant heterozygosity excess was found only in BD. This suggests that there is evidence of a recent bottleneck in the study populations.

Table 4. Descriptive statistics for all loci in three populations of *Rhizophora apiculata*

Pop	n	P (%)	N_A	N_E	H_O	H_E	F	F_{IS} IIM	t	*P* value of bottleneck		
										IAM	TPM	SMM
QN	16	70	1.9	1.488	0.313	0.248	-0.084	0.454	0.85	NS	NS	NS
NT	20	80	1.9	1.537	0.275	0.275	-0.123	0.061	0.78	*	NS	NS
BD	18	90	2.0	1.673	0.289	0.357	0.396	0.043	0.43	**	***	***
Mean	18	80	1.933	1.566	0.292	0.293	0.214	0.186	0.64			

Note: n: Number of individuals; P (%): Percentage of polymorphic loci; N_A: Observed number of alleles; N_E: Effective number of alleles; H_O: Observed heterozygosity; H_E: Expected heterozygosity; F: fixation index; outcrossing rate, estimated as $t = (1 - F)/(1 + F)$; F_{IS} IIM corrected inbreeding coefficient for null alleles; IAM: the infinite allele model, SMM: stepwise mutation model, TPM: two-phased model of mutation; NS: no significance; *$p < 0.05$, **$p < 0.01$.

Population genetic differentiation

F-statistics (also known as Wright's fixation indices) were computed to measure genetic distance within populations (Table 5) and resulted in the evaluation of different levels of population structure. The inbreeding coefficient (F_{IS}), which has known as a variance of allele frequencies within populations varied from -0.511 to 1.000, showing a large difference among loci in the average heterozygote. The overall inbreeding coefficient (F_{IT}), which represents the inbreeding coefficient of an individual relative to the total population, was 0.365, indicating a fairly high correlation between alleles of the total population. Moreover, the degree of genetic differentiation among populations (F_{ST}) for all alleles was 0.10 (Table 5). Indirect appraisals of gen flow for each locus resulted in a fairly high value on average (Nm = 22.865). In particular, almost locus had outcrossing rates greater than 1.00, except the locus RM106 and locus RmBra50 (Table 5). The highest value of migration rate was 198.11 for locus RhSt11.

Table 5. F-statistics for each locus and overall loci of all populations

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
RM121	1.000	1.000	0.037	6.537
RM106	1.000	1.000	0.227	0.849
RmBra18	1.000	1.000	0.090	2.518
RmBra19	0.797	0.809	0.059	3.987
RmBra45	-0.404	-0.364	0.029	8.453
RmBra50	-0.849	-0.224	0.338	0.490
RmBra59	-1.000	-1.000	0.000	ND
RmBra65	-0.511	-0.449	0.041	5.776
Kcan034	0.852	0.879	0.182	1.125
RhSt11	1.000	1.000	0.001	198.911
Mean	0.288	0.365	0.100	22.865

Note: F_{IS}: Inbreeding coefficient within individuals; F_{IT}: Overall inbreeding coefficient; F_{ST}: Fixation index; N_m: gene flow; ND: no determined.

Genetic structure

The genetic structure was tested by analyzing molecular variance (AMOVA). Most of the variation occurred within the populations (88%), while there was little variation among populations (9%) (Table 6).

An admixture model was performed to evaluate the population structure of the 54 accessions of *R. apiculata* based on Bayesian analysis using the STRUCTURE program. The results showed that the optimum number of genetic groups (K) was three, with the highest value of delta K (22.2) obtained from STRUCTURE HARVESTER (Fig. 2). At K = 3, the bar plot of the admixture assignment for each individual is exhibited in Figure 3. One group (Dark blue) was predominant in the BD populations, with strong ancestry values of 34.2% and two populations (NT and QN) with lower ancestry values of 4.6% and 4.1%, respectively. In the second group (orange), the NT population represented the largest group (36.8%), followed by two populations (QN and BD) with values of 19.8% and 24%, respectively. Similarly, last group (blue), the three populations (QN, NT, and BD) were 76.2%, 58.6%, and 41.8%, respectively.

UPGMA analysis for 54 *R. apiculata* accessions utilizing the genetic distance data showed a chaotic distribution (Fig. 4a). Each population is dispersed into groups consisting of several individuals that alternate with each other. While individuals from the Quang Nam region tended to form a closer genetic relationship, individuals from the Binh Dinh and Ninh Thuan regions were distributed

alternately with each other. Furthermore, we also used the genetic distance matrix data (not shown) (Nei, 1978) to construct a UPGMA tree for a population level. This analysis resulted in two groups, one group included the Binh Dinh and Ninh Thuan populations, and the other group comprised the Quang Nam population. (Fig. 4b).

Table 6. Analysis of molecular variance from the three *Rhizophora apiculata* populations

Source of variation	Degree of freedom	Sum of squares	Mean square	Percent of Variance	P value
Among Pops	2	13.597	6.798	9%	0.001
Among Indiv	51	80.431	1.577	4%	0.242
Within Indiv	54	78.500	1.454	88%	0.023
Total	107	172.528		100%	

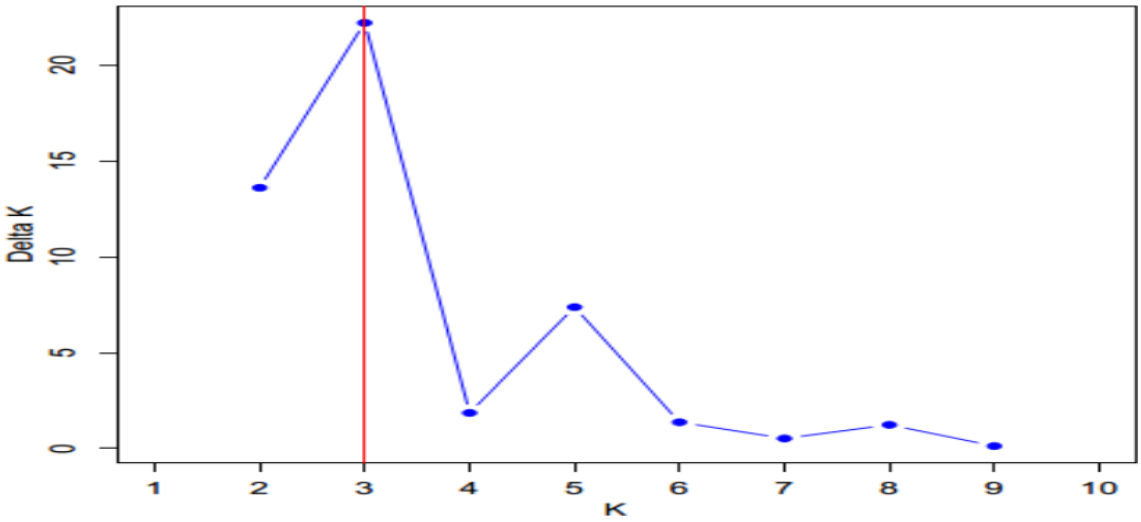

Figure 2. Delta*K* with cluster number *K* from one to ten

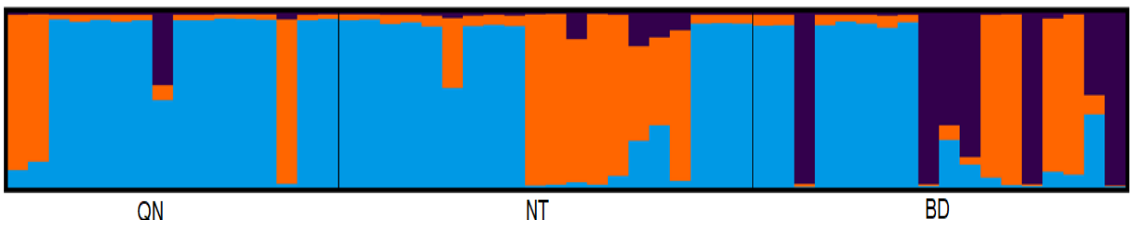

Figure 3. Barplot of admixture assignment for the 54 individuals of three populations with *K* = 3. QN, Quang Nam; NT, Ninh Thuan; BD, Binh Duong

DISCUSSION

Genetic diversity of Rhizophora apiculata populations in Vietnam

Genetic diversity and population structure of *R. apiculata* in various Asian coastal regions were analyzed and reported in several previous studies. These studies reported an alarming decline of *R. apiculata* in tropical Asia (Azman et al., 2020; Ruang- areerate et al., 2022; Yahya et al., 2014; Yan et al., 2016). In this study, we observed low levels

of genetic diversity in *R. apiculata* populations distributed in the Southern Central regions of Vietnam. In comparison with other regions in Southeast Asia such as the Philippines, Thailand, and Malaysia, the level of allelic diversity and heterozygosity of the *R. apiculata* population from Vietnam was relatively low (Yan et al., 2016). The lower values of effective numbers of alleles (on average, N_E = 1.566) for *R. apiculata* populations in Vietnam was recorded as compared to the populations in Indonesia (on average N_E = 2.0) (Yahya et al., 2014) and in Malaysia (on average N_E = 3.2) (Azman et al., 2020). In general, the low number of effective alleles reflected the bottleneck of populations as a consequence of the isolation of natural populations or the deficit of the effective population size (Nei, 1978). However, the process of forest-making might have suddenly caused the loss of alleles from initial parents. In Vietnam, Hoa and his colleagues reported the massive deforestation at Thi Nai Lagoon and the destruction of natural mangrove forest in the Ninh Thuan region (Hoa et al., 2010). The low heterozygosity was observed in *R. apiculata* populations from Vietnam (on average H_O = 0.292), however, it was higher compared to the *R. apiculata* populations in Indonesia (on average Ho = 0.299) and Malaysia (on average Ho = 0.339), respectively (Azman et al., 2020; Yahya et al., 2014). Similar to *R. apiculata* in Vietnam, lower genetic diversity of this species is also found in populations located in Australia (H_O = 0.23 on average) and Pacific islands (H_O = 0.156 on average). The tendency of low genetic diversity of *R.apiculata* might be attributable to natural events such as storms, waves, and other disasters (Veettil et al., 2019), the aftermath of wars in Vietnam, and the forest restoration activities performed by the Vietnamese government after that (Arnaud-Haond et al., 2009). In addition, the higher values of the observed heterozygosity (H_O) than the expected heterozygosity (H_E) of *R. apiculata* populations in Vietnam might be a result of breaking isolation or mixing of isolated populations in these regions. Therefore, other factors need to be considered for the genetic diversity analysis of *R. apiculata* in Vietnam.

Moreover, the study of Giang on *Avicennia marina*, which was the second most widespread species in Vietnam, revealed reduced levels of heterozygosity (Giang et al., 2003). Of these, the central region had the smallest diversity compared to the northern and southern regions. Notably, the observed heterozygosity of *R. apiculata* (H_O = 0.292) was higher than that of *A. marina* in the central region (H_O = 0.236). In addition, a moderately low observed heterozygosity of *Kandelia* species was reported (H_O = 0.116 on average) in the Northern region while a fairly high observed heterozygosity was noted in the Southern region of Vietnam (Giang et al., 2006).

Genetic variation and population differentiation

The observed positive F_{IS} values can be resulted from self-compatibility and/or wind pollination between closely located populations and/or genetic drift occurring in small population. Whereas, the negative F values indicate high outcrossing rates of tested populations (Azman et al., 2020). In current results, a positive fixation index (F) value was observed in the *R. apiculata* population from BD, while negative F values were found in the *R. apiculata* populations from QN and NT. This result aligns with previous ecological studies in the Southern Central regions of Vietnam, which reported that the decline in area and distribution of mangroves was due to not only shrimp farming (Hoa et al., 2010) but also resulted by the less favorable conditions for mangrove forests (Hong & San, 1993; Veettil et al., 2019). Therefore, the difference in F values of three tested *R. apiculata* populations might be explained by the low - estimate of genetic diversity for *R. apiculata* in Vietnam while their outcrossing rate was high. Nevertheless, the combination of ecological study in the Southern Central Coast region and the fairly high outcrossing rate of *R. apiculata* population across surveyed regions provided a promise for the acceleration of genetic diversity.

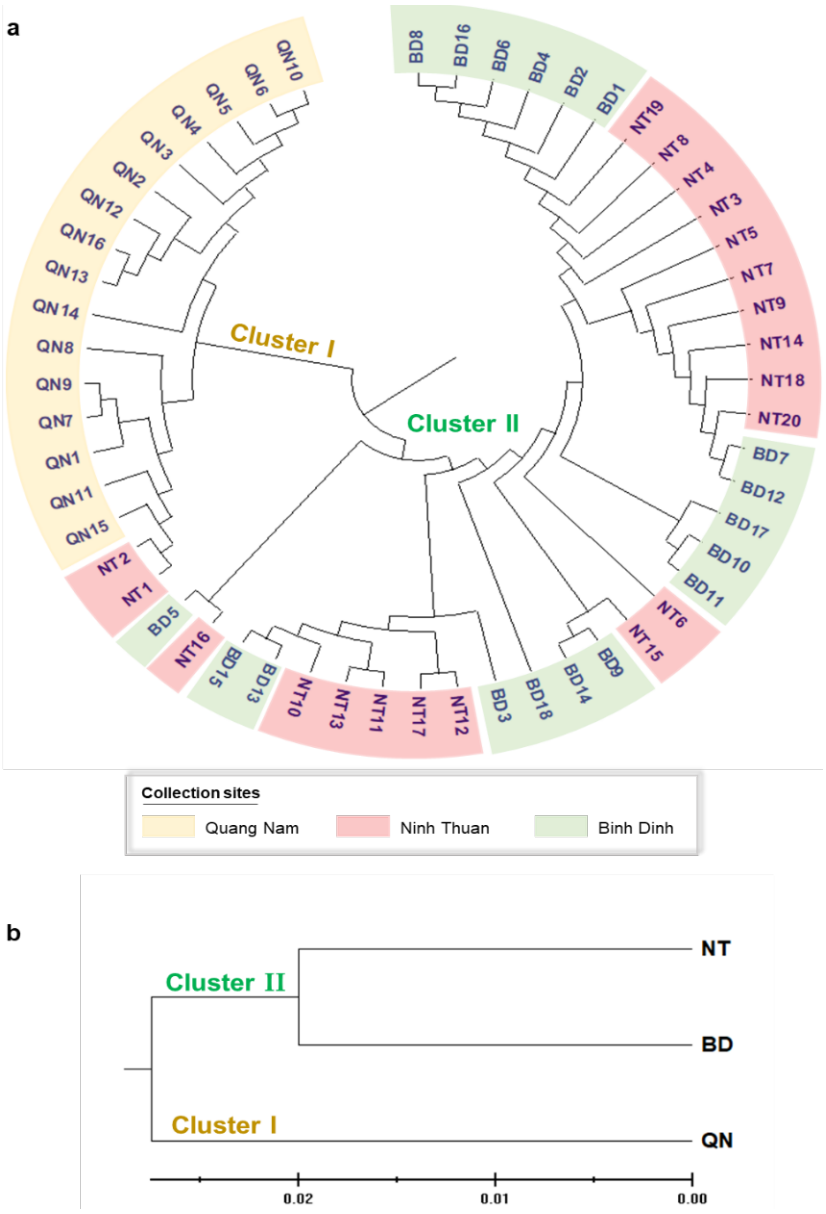

Figure 4. UPGMA dendrogram based Nei's genetic distance for *Rhizophora apiculata* populations; A, Phylogenetic tree of 54 *Rhizophora apiculata* accessions, yellow and green texts represent the first (cluster I) and second (cluster II) groups of *Rhizophora apiculata* according to unweighted pair group method, respectively; yellow, pink and green highlights represent collection sites in Quang Nam province, Ninh Thuan province and Binh Dinh province, respectively; B, Relationship of three surveyed regions

Genetic distances among *R. apiculata* populations ranged from 0.035 to 0.074, in which the genetic distance between *R. apiculata* from Quang Nam and Binh Dinh regions was D_{QN-BD} = 0.074, the genetic distance between *R. apiculata* from Quang Nam and Ninh Thuan regions was D_{QN-NT} = 0.043, and the genetic distance between

R. apiculata from Binh Dinh and Ninh Thuan regions was D_{BD-NT} = 0.035. For *A. marina*, the genetic distance among tested populations was approximately 0.0271 (Giang et al., 2003). For *Kandelia* species in Vietnam, genetic distances among surveyed regions were less than 0.05 (Giang et al., 2006). In comparison to these studies, genetic distances of *R. apiculata* distributed in the Southern Central Coastline were low, which presented an occurrence of the gene pool exchange. Further, the gene flow/migration rate also showed the level of genetic material transfer or the exchange of alleles between populations of a species. This might be affected by the isolation of geography and distances. In our study, the migration rate between three test *R. apiculata* populations drifted from 0.490 to 13.842. It was observed that no matter how far distances among surveyed populations were, there were fewer effects of human habitation continuously acting on at least a part of the mangrove forest areas. This explains why the genetic distance within *R. apiculata* populations was small while the geographical distance was large. It was suggested that *R. apiculata* might be widely distributed in the Southern Central coastline in the past, and the isolation (if it occurred) was primarily due to human activities such as construction, shrimp farming, deforestation, etc., thus the original distribution was broken and condensed as this day (Hoa et al., 2010).

CONCLUSIONS

This study preliminarily indicates the low levels of genetic diversity in *R. apiculata* populations distributed in the Southern Central regions of Vietnam. Compared to other regions in Southeast Asia, *R. apiculata* from the Southern Central coast of Vietnam had relatively low values of allelic diversity and heterozygosity. However, to gain a comprehensive understanding of this species in Vietnam, it would be necessary to further consider the genetic diversity of *R. apiculata* in the Northern coastline region and Southern coastline region. This provides valuable recommendations for mangrove tree breeding and the restoration of mangrove forests in Vietnam as well as support for coping with global climate change.

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