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The polymorphism and divergence of *cpDNA* in *Rhizophora*

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Abstract

This study was aimed to evaluate the DNA sequence polymorphism and divergence of *Rhizophora* species, this study studied nucleotide polymorphism in chloroplast intergenic space between trnL and trnF genes from samples of *Rhizophora taxa* collected from different locations in Peninsular Malaysia. High polymorphism was found within the population of *Rhizophora taxa,* and the nucleotide diversity (Pi) in *Rhizphora apiculata, Rhizophora mucronata, Rhizophora stylosa and Rhizophora* × *lamarckii* were 0.03, 0.04, 0.06 and 0.03, respectively. In addition, the relationship between *Rhizophora taxa* was estimated based on the level of polymorphism and divergence. The most significant relationship of the three *Rhizophora* species was between *R. apiculata and R. mucronata* which are closely related. The indel (insertion-deletion) in all *Rhizphora* species and hybrid was small and the number of polymorphic (segregating) sites was high, especially in *R. stylosa*. Intra-specific polymorphism was detected in *Rhizophora* according to the nucleotide diversity estimated by every population and the genetic differentiation (GST) by UPGMA method.

Keywords: Polymorphism, divergence, cpDNA and Rhizophora

Introduction

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Mangrove defined as plant (tree, shrubs, palm and ferns) widespread in the tropical and subtropical region, mangrove forests play an important role to avoid erosion (*1*). Mangrove has produces chaoal (*2*). The predominant and sympatric mangrove species of Rhizophoraceae in Southeast Asia are *Rhizophora mucronata* and *Rhizophora apiculata*. Rhizophoraceae includes 16 genara and 120 species of tree and shrubs (*3*). In peninsular Malaysia, *Rhizophora* consists of three species (*Rhizophora apiculata*, *Rhizophora mucronata* and *Rhizophora stylosa*) and two hybrids *R.* × *annamalai* and *R.* × *lamarckii* (*4*). Previous report studied the morphological characters of *Rhizophora* species. He found that *Rhizophora apiculata* and *Rhizophora mucronata* are the most closely related species (*3*). According to Inomata et al. who surveyed levels and patterns of genetic variation as well as population structure of two sympatric mangrove species, *R. apiculata* and *R. mucronata* in Thailand, using five nuclear genes and two cpDNA regions (*5*). In all investigated DNA regions, nucleotide variation within species was low, while nucleotide divergence between the two species was considerable (*5*). This study was aimed to evaluate the DNA sequence polymorphism and divergence of *Rhizophora* species.

Material and method

Leaf samples

Young leaves of *Rhizophora* species and hybrids was collected from different locations peninsular Malaysia. The locations for each site are listed in Table1 and showed in Figure 1**.**

Extraction of genomic DNA

A total 20 *trn*L-*trn*F regions of *Rhizophora* species successfully extracted by DNeasy Plant Mini Kit method protocol. The concentration of all genomic DNA of four *Rhizophora* species was determined by running the electrophoresis along with DNA markers lumda and 100 bp. A 1% agarose gel electrophoresis was used to determine the concentration of DNA. The presence of DNA can be seen on agarose gel under ultraviolet rays.

Polymerase Chain Reaction (PCR)

The *trn*L-*trn*F region was amplified by the polymerase chain reaction (PCR).The universal primers for the amplification of the *trn*L–*trn*F spacer were those of Taberlet et al.(*6*). The PCR reaction mixtures was 50 µl and the amplifications were carried out using 30 cycles of 1 min at 94 Co, 1 min at

annealing temperature. The annealing temperature was different in *Rhizophora* species (45C^{o} in *Rhizophora apiculata*, 48C^{o} in *Rhizophora mucronata* and *Rhizophora stylosa* and 50C^{o}*Rhizophoralamarchii*). And 2 min at 72C^{o}. the PCR products were excised from agarose gel under the long wave UV light.The fragment sized resulting from this amplification ranged between 400 bp to 450 bp.

Alignment of DNA sequences and data analysis

The obtained DNA sequences were aligned using MEGA5 program (*7*). In addition, the polymorphism within species in intergenic *trn*L-*trn*F chloroplast spacer of *Rhizophora* species was estimated using the DnaSP4software. To estimate nucleotide diversity and genetic divergence between populations were computed the average number of nucleotide substitutions per site (*Dxy*) (*8*) (Eq. 10.20) between populations and the number of net nucleotide substitutions per site between populations (*Da*) (*8*) (Eq. 10.21) using the DnaSP4 software (*9*).

Table 1 List of specimens and collection sites of each species

Species	Collection sites	trnL-trnF
R. apiculata	Johor, Mersing	AJ1
	Kedah, Pulau Langkawi	AL1
	Johor, Sedili, Anwar	AS1
	Melaka, Sungai linggi.	AM1
	Melaka, Port Dickson	AT1
R. mucronata	Kedah, PulauLangkawi	ML2
	Johor,Sedili	MS2
	Johor,Mersing	MO2
	Melaka, Sungai Linggi	MM2
	Selangor, Morib	MP2
R. stylosa	Kedah, PulauLangkawi.	SL3
	Melaka, Port Dickson	SM3
	N. Sembilan, Port Dickson	SP3
	Johor,Mersing	SJ3
	Johor, TanjungPiai,	ST3
R. × lamarckii	Kedah, PulauLangkawi	LL4
	N. Sembilan, Port Dickson	LS4
	Melaka, Port Dickson	LM4
	Johor, TanjungPiai	LT4
	Johor,Mersing	LJ4

Results

Intra-specific polymorphism was detected in *Rhizophora* according to the nucleotide diversity estimated from every population and the genetic differentiation (GST) by UPGMA method using MEGA5.

Rhizophora apiculate

The intergenic *trn*L-*trn*F spacer region in *R. apiculata* was sequenced from 5 individuals from five different localities. The length of the region was 240-245 bp. Twenty four distinct haplotypes sites were found. The sequences of these haplotypes are shown in Figure 4.4 as AL1, AJ1,AS1, AM1 and AT1. The AJ1, AL1, AS1, and AM1 haplotypes which differed by the length of a T repeat (see Figure 2 , sites 137-142). Furthermore, there are fifteen singleton variable sites (two variants) were estimated, their position sites (see Figure 2, 15 sites, 2, 5, 10, 49, 64, 72, 86, 89, 95, 105, 107, 111, 117, 120). Furthermore, eight parsimony informative sites (two variants) were estimated between the five sequences at 4, 18, 24, 37, 42, 88, 112, 124. In addition, one Singleton variable sites (three variants) was estimated at 55.

Rhizophora mucronata

The number of haplotype sites in *Rhizophora mucronata* was higher than in *Rhizophora apiculata*. The sequences of these haplotypes are shown in Figure 2 as ML2, MS2, MO2, MM2 and MP2. The length of the region was 379-397 bp. Thirty three distinct haplotypes sites were found. Whereas, ML2, MJ2, MO2, MM2 and MP2 haplotypes differ by the length of a T repeat (see 2 sites 89-100). Nineteen Singleton variable sites (two variants) were estimated their site positions are 4, 23, 25, 31, 36, 42, 47, 58, 63, 65, 69, 71, 98, 135,152,155, 158, 160 and 169. Furthermore, fourteen parsimony informative sites, their site positions are 1, 3, 22, 41, 46, 53, 54, 70, 97, 137, 145, 153, 171 and 72.

Rhizophora stylosa

In *Rhizophora stylosa* the length of the region was 333-335 bp. The sequences of these haplotypes are shown in Figure 2 as SM3, SL3, SS3, SJ3 and ST3. The number of distinct haplotypes sites was found in this species were higher than other *Rhizophora* species and it was forty one. They were distributed as follows, Singleton variable sites (two variants) was nineteen, their site positions were 83, 103, 105, 110, 128, 137, 141, 142, 143, 144 160, 181,190, 201, 203, 206, 212, 217 and 220. Parsimony informative sites

(two variants) were eighteen at sites 6, 15, 20, 32, 38, 44, 48, 54, 58, 77, 107, 119, 121,127, 133, 134, 139 and 150. Singleton variable sites (three variants) were two at sites 108 and 109. Parsimony informative sites (three variants) were their site positions was 106 and 151.

Rhizophora* × *lamarckii

The length of the region was 302-312 bp. The number of distinct haplotypes in *Rhizophora* × *lamarckii* was twenty three shown in table 1 . The sequences of these haplotypes are shown in Figure 2 as LL4. LS4, LM4, LT4 and LJ4. Their positions sites were as follows. Singleton variable sites (two variants) were zero. Parsimony informative sites (two variants) were twenty three at sites positions 17, 31, 42, 44, 49, 56, 62, 75, 79, 93, 94, 95,109, 114, 121, 137, 140, 299, 360, 362, 366, 369 and 371. On the other hand, Variable sites (three variants) and Variable sites (four variants) was zero.

Discussion

The levels of DNA polymorphism in *Rhizophora* species which collected from different localities in Peninsular Malaysia were estimated and the results summarized in Table 2. In all *Rhizophora* species the number of Indels (insertion-deletion) was small, it was estimated as follows; two sites in *Rhizophora apiculata* and *Rhizophora* × *lamarckii*, three sites *in Rhizophora mucronata* and four sites in *Rhizophora stylosa*. On other hand, the number of polymorphic (segregating) sites was high especially in *Rhizophora stylosa*, it was forty one sites. Furthermore, it was twenty four sites in *Rhizophora apiculata*, thirty sites in *Rhizophora mucronata* and twenty three in *Rhizophora* × *lamarckii*.The nucleotide diversity Pi in the four *Rhizophora* taxa was estimated. It was 0.03 in *Rhizophora apiculata*, 0.04 in *Rhizophora mucronata*, 0.06 in *Rhizophora stylosa* and 0.03 in *Rhizophora* ×*lamarckii*. In current finding, haplotype diversity (Hd) was high in all *Rhizophora* taxa as follows; 0.900, 1.000, 1.000 and 0.600 (shown in Table 2) in *R. apiculata, R. mucronata, R. stylosa and R.* × *lamarckii*, respectively. concurring with (*10, 11*) conclusions that the Malay Peninsula is the Centre of genetic diversity of *Ceriops tagal* (Rhizophoraceae) in Southeast Asia.

Figure 2 : Nucleotide sequence alignment of the intergenic spacer region of cpDNA between *trnL* and *trn*F *in Rhizophora* species and hybrid**.**

Table 2: Sequence polymorphism within taxa of *Rhizophora.*

Species	S	Indel	Hd	Pi(π)
Rhizophora apiculata	24	3	0.900	0.03
Rhizophora mucronata	30	2	1.000	0.04
Rhizophora stylosa	41	4	1.000	0.06
Rhizophora ×lamarckii	23	2	0.600	0.03

Indel Number of Indel haplotypes

S Number of polymorphic (segregating) sites

Pi (π) Nucleotide diversity

Hd Haplotype gene diversity

Conclusion

Nucleotide diversity is a measure of genetic variation. It is usually associated with other statistical measures of population diversity, and is similar to expected heterozygosity. This statistic may be used to monitor diversity within or between ecological populations, to examine the genetic variation in crops and related species, or to determine evolutionary relationships. In this study, nucleotide data analysis allowed to estimate the level of genetic polymorphism in intergenic spacer region between *trn*L-*trn*F in populations of *Rhizphora* taxa. Nucleotide diversity was high within all *Rhizophora* taxa.

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تعدد الأشكال وتحويل cpDNA في Rhizophora

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المستخلص العربي

هدفت هذه الدراسة إلى تقييم تعدد أشكال تسلسل الحمض النووي واختلاف أنواع Rhizophora ، ودرست تعدد أشكال في الفراغ بين جينات البلاستيدات الخضراء trnL و trnF من عينات أصناف *Rhizophora* التي تم جمعها من مواقع مختلفة في شبه جزيرة ماليزيا. تم العثور على تعدد أشكال مرتفع في مجموعة أصناف *Rhizophora* ، وكان تنوع النيوكليوتيدات (Pi) في *Rhizphora apiculata* و *Rhizophora mucronata* و *Rhizophora stylosa* و *Rhizophora* × lamarckii 0.03 و0.04 و 0.06 و 0.03 على التوالي. بالإضافة إلى ذلك ، تم تقدير العلاقة بين أصناف *Rhizophora* بناءً على مستوى تعدد الأشكال والتباعد. كانت العلاقة الأكثر دلالة بين أنواع *Rhizophora* الثلاثة هي العلاقة بين *R. apiculata* و *R. mucronata* والتي ترتبط ارتباطًا وثيقًا. كان indel (الإدراج والحذف) في جميع أنواع *Rhizphora* والهجين صغيرًا وكان عدد المواقع متعددة الأشكال (المنفصلة) مرتفعًا خاصة في *R. stylosa*. لقد تم اكتشاف تعدد الأشكال محدد بين *Rhizophora* وفقًا لتنوع النيوكليوتيدات المقدّر من كل مجموعة افراد والتمايز الجيني (GST) بواسطة طريقة UPGMA.

الكلمات المفتاحية: تعدد الأشكال ، الاختلاف ، cpDNA و Rhizophora