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Genetic Characteristics and Nine Trial Species Growing Forests in The Coastal Alluvial Soil in The South-West of The Mekong River Delta in Vietnam

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Abstract

In this study, two research contents were identified, namely species through molecule and monitoring of adaptation to the first year growth of nine tree species of mangroves on the newly-accreted land. The research's goals are: (1) Determininggenetic characteristics through molecular markers in order todetermine the right species of indigenous plants to be tested and assessed the adaptive and grow characteristics for seedlings (native to on-site mother tree) on coastal mudflats in Kien Giang province (in the study area) and (2) Proposing potential forest tree species in the study area. The results showed that suspicious species have strong vitality andpotential prospects for highly adaptation. The conclusion contributes to the research theory of selecting forest tree species, adaptation of native species to newly reclaimed land in the context of unfavorable factors of new soil environmentand responding to the actual restoration of forests, creating artificial forests on new land, other practical significance is to response development requirements of local and national forestry.

Keywords: Genetic Characteristics; Trial Species Mangroves; The Coastal Alluvial Soil; Mekong Delta In Vietnam

Introduction

The major constraints for agricultural production in the Mekong delta involve multiple abiotic stresses, particularly salinity, flooding and other soil problems. Besides, the delta is vulnerable to numerous natural hazards such as typhoons and floods. Due to these high risks and low productivity, farmers follow the conserving strategies which minimize or no use agricultural inputs [1]. (Buu, *et al.* 2004) Mangrove forest is a buffer zone connecting land and sea, so it plays an important role in protecting sea dykes, sedimentation, mitigating waves and storms, preventing coastal erosion and limiting saline intrusion, protect the living environment for aquatic species that can reproduction, residence and development. Mangroves are woody trees or shrubs that grow in intertidal region along tropical and sub- tropical coasts. With favorable geomorphic conditions, mangroves commonly form extensive tidal forests in moist, humid equatorial climates [2]. On the other hand, mangroves are significant in ecosystem which are rich and diversive biological resources.Mangrove plants grow and develop on alluvial soils directly affected by tides, hot and humid climate and frequent flooding condition with high salinity. To ensure the optimal conservation of the mangrove forests in any region, it is nessesary to explore the genetic diversity of the populations. Population genetic studies of mangroves are also essential for evaluating afforestation, domestication and breeding programmes. Several species of mangroves at high risk of extinction may disappear before the next decade if existing protective measures are not enforced. In this context, it is a matter of urency to understand their geneticmaterials. Mangroves display a significant inter- and intra-specific variations as evidence by the molecular markers such as AFLP and RAPD. Microsatellite markers are the tools being used for understanding genetic variations. The forced fragmentation of this ecosystem affects the geneticdiversity distribution among natural populations. Moreover, some studies have analyzed how recent planted areas impact the original mangrove genetic diversity.Two mangroves species (Laguncularia racemosa and Avicenniaschaueriana) in three areas in Brazil are analized by using inter-simple sequence repeat (ISSR) marker [3]. Research on the adaptation and development of tree species to identify species adapted at the seedling stage; and the affection by harsh environmental factors impacting newly planted seedlings through species measuring with survival rate and first year growth. This result will provide more scientific basis for the study of forest plant species and the adaptive evaluation of young trees in the early stages. This study has it possible to recommend to promote regeneration of the lost and deforested jungle areas using the motherseed where the mother plant is sown to restore the forest which is a work of scientific and practical significance.

Material and Methods

Determine the characteristics of the study species

+ Scope and study area

Scope 1ha of new alluvial soil of coastal in Xeo Quao hamlet, Nam Thai a commune, An Bien district, Kien Giang province. From January 2018 to December 2018. There are 9 plant species for research objects.

+ Collecting sample and anlyzing method

Analyzing and classifying mangrove trees: samples were collected at the field, preserved in the cooling condition and returned to the laboratory for DNA analysis to determine the molecular index.

+ Collection list of leaves at the field in Kien Giang

Collecting the simple conducted two times. First time 2 days (January 27th - 28th 2018). The species' names were recorded from the field provided by the people. Secondtime, collect only samples of Coc trang, Cac do and Da quanh.

+ Process of DNA extraction

Based on the process of Molecular Biology Laboratory, Can Tho Biotechnology Research and Development Institute have improved (Clark, 1997). The steps have been taken as follows:

Weigh 0.2 g of washed and dried leaves, crushed in liquid nitrogen, into a 2.2ml tube. Add 1.2 ml of CTAB extract (heated at 65 °C in 10'), vortex thoroughly at low speed (600-800 rpm). Incubate overnight at 55 °C. Centrifugal 12,000 rounds in 15 minutes. Floating translation. Move the fluid into a new tube (800-1000 µl). Add 500 µl Chloroform: ISOamyl alcohol (24:1), turn gently. Centrifugal 12,000 rounds in 15 minutes. Floating translation. Repeat 2 times. Add 0.2 V 3M sodium acetate and 0.6 V cold Isopropanol. Stir well. Incubation at -20 °C for 90 minutes. Centrifugal 10,000 rounds in 10 minutes. Discard the clear fluid, collect precipitate. Wash the precipitate by adding 400 µl 100% ethanol and centrifuging 7,000 cycles for 5 minutes. Discard the fluid in (repeat 2 times).Dry the sample at 30 °C for 15 minutes or dry the precipitate dry at room temperature. Dissolve the precipitate in 100 µl TE 1X, incubate at 37 °C until the precipitate is completely dissolved (can be incubated overnight). Store samples at -20 °C.

The next step is to quantify the DNA concentration after extraction with a spectrophotometer. The results showed that the index value of OD 260 nm/280 nm=1.8-2, relatively high to meet the purification requirements of DNA used in experiments to perform PCR reaction. For qualification of DNA products uses agarose gel electrophoresis technique 0.8%. Use micropipette (Biorad) to absorb 10 µl of mixed DNA solution with about 2 µl loading prepared buffer on parafilm tape, mix well then pump into well.

+ Prepare PCR reaction components (Lang 2002)

Diluted DNA sample is 25 ng/µl concentration.Decay and reverse bait dilute to 100 pmol. And dTNP diluted to a concentration of 10 mM.Taq DNA polymerase (5U/µl). 2 times distilled water is sterilized by pressure autoclave at 121 °C for 15 minutes. Unused chemicals are stored at -20 °C

Micropipete is calibrated with distilled water to ensure accuracy before use to attract and mix reactive components. Perform the whole procedure on ice, completely dissolve and mix all ingredients before use (Table 1).

Water marble	13µl
Taq polymease (buffer, MgCl2, dTNP, Taq)	5µl
Forward	0.5µl
Reverse	0.5µl
DNA	1µl

Table 1: Process of DNA

Microsatellite analysis

The whole microsatellite analysis included PCR assay, agarose gel electrophoresis, and band detection and scoring. Microsatellite primers were used to survey polymorphism on the samples. These were randomly selected from the 6 microsatellite primer pairs currently available for plants. The PCR reaction was followed: reactions were overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 °C, 1 minute at 55 °C, and 2 minutes at 72 °C, with a final extension at 75 °C for 5 minutes. After amplification, 10 µl of stop solution was added to the PCR product, which was then denatured at 94 °C for 2 minutes. Eight microliters of each reaction were run on agarose gel [4].

Method of species trial research

Planting trial

Planting time and method: The experiment was planted in January 2018. Planted according to 9 experiments corresponding to 9 predetermined species, 1.5m away from trees, 1.5m away from each row, each design is repeated 4 times (PLOT corresponds to one repeat size 10x10m) for a tree species. Working on 3 experiments (TN) with lower terrain to the sea side, TN1 is located in the area adjacent to the forest edge, TN2 is located between TN1 and TN3, TN3 is located outside the sea.

+ Establish experiments

The experiment was established with the following experimental formulas: CT1: Determined for plant species 1, CT2: Specified for plant species 2; Identify 9 species of plants that are able to grow in the mudflats as a forest plantation of coastal protection forests in Kien Giang province.

+ Arrangement of propagation experiments

The selected tree species in the experiment are the species that are capable of growing in the mudflats, which are the forest planters of coastal protection forests. Including 9 species:

(1) Soneratia alba: Species 1 *(Sa)*
(2) Lumnitzera litorea: Species 2 *(Ll)*
(3) Ceriops decandra: Species 3 *(Cd)*
(4) Rhizophora apiculata: Species 4 (Ra)
(5) Avicennia marina: Species 5 (Am)
(6) Avicennia officinalis: Species 6 (Ao)
(7) Avicennia alba: Species 7 (Aa)
(8) Bruguiera cexangula) Species 8 (Bc)
(9) Xylocarpus grannatum: Species 9 (Xg)

Diagram of experimental design of adaptive tree species, selected in full block with 4 repetitions on site is homogeneous, experimental formulas are arranged in the direction from the mainland to the sea to ensure the natural impacts are the same (Table 2).

Expriment 1	Sa	Ll	Cd	Ra	Am	Ao	Aa	Rc	Xg
	Xg	Rc	Aa	Ao	Am	Ra	Cd	Ll	Sa
	Ll	Sa	Xg	Rc	Aa	Am	Ao	Ra	Cd
	Cd	Ra	Am	Aa	Ao	Rc	Sa	Xg	Ll
Expriment2	Ll	Sa	Xg	Bc	Aa	Am	Aa	Ra	Cd
	Cd	Ra	Am	Aa	Ao	Bc	Sa	Xg	Ll
	Sa	Ll	Cd	Ra	Am	Ao	Aa	Bc	Xg
	Xg	Bc	Aa	Ao	Am	Ra	Cd	Ll	Sa
Expriment 3	Cd	Ra	Am	Aa	Ao	Bc	Sa	Xg	Ll
	Sa	Ll	Cd	Ra	Am	Ao	Am	Bc	Xg
	Xg	Bc	Aa	Ao	Am	Ra	Cd	Ll	Sa
	Ll	Sa	Xg	Bc	Aa	Am	Ao	Ra	Cd

(1)Soneratia alba: Species 1 (Sa),Lumnitzera litorea: Species 2 (Ll),Ceriops decandra: Species 3 (Cd);Rhizophora apiculata: Species 4 (Ra);Avicennia marina: Species 5 (Am);Avicennia officinalis: Species 6 (Ao);Avicennia alba: Species 7 (Aa);Bruguiera cexangula) Species 8 (Bc);Xylocarpus grannatum: Species9 (Xg).

Table 2: Diagram of experimental experiments to select suitable plant species

Soil preparation: Clean up vegetation, dig holes of size 20x20x20cm and plant immediately after digging holes.

Planting techniques: Choose plants that are suitable for planting, before planting for 7-10 days, put the whole tree on the shore to make soil in the pot to drain, help to make it firm and stable. Use baskets, boards, boats to transport trees, avoid rotting, breaking roots. Peel the gourd, place the tree vertically, the surface of the gourd is 3-5cm lower than the hole, after filling the soil, use your hands and feet to press tightly to make the mud and soil compact around the gourd. The potting covers must be collected after being peeled to the garbage gathering place (septic pots do not need to be peeled).

- Plugging poles to hold trees: Using melaleuca poles, the length of piles from 90 -20 cm, diameter from l.5-3cm. Forcing one end of the string into the pile, the other end is tied to the stem (not tied to the stem, avoid rubbing), the length of the string between the pile and the plant is 5-7cm, the tie is 20cm from the original. Plug in 45° inclined pile, pile head facing to the sea (Figure1).

Figure 1: Techniques for planting seedlings

+ Measuring growth indicators

Tree growth indicators were monitored and recorded for a period of 12 months, once every 6 months.

Measures include tree height (Hm), growth status (He) according to 5 levels from 1-5, survival rate in the experimental plot.

+ Methods of evaluation

The statistical method used to evaluate analysis and calculate statistical characteristics is the average value. The calculation tool is Excel, the display tool is Excel and MapInfo.

+ Data processing methods

Use Microsoft Excel software to synthesize data and graph. Using SPSS statistical software to process data, use Duncan test at 95% confidence level to compare indicators between experiments and use ANOVA test to compare indicators between experiments

Results and Discussion

Determine characteristics of the studied species

Results of identification of varieties and species of mangroves based on molecular markers

Isolation and identification of species and plant varieties are important in the collection and preservation process as well as the exploitation of seed sources and species. Specifying species that can be based on plant morphology is a traditional method that has been used for a long time, but if based on morphology sometimes deviated by environmental factors, the development of biotechnology, molecular biology techniques (molecular markers) are an effective tool for effective and accurate identification of species, breeds, and genetic relationships. This content focuses mainly on the DNA markers of selected main mangrove species.

Leaf samples, as well as references to current mangrove trees in Kien Giang province consist mainly of 9 key species, species names as well as results of PCR amplification of ITS regions and SSR molecular markers are presented, in (Table 3).

TT	Local name	Science name	PCR-ITS	PCR-SSR
1	Ban chua	*Sonneratia alba*	x	x
2	Coc do	*Lumnitzera alba*	x	
3	Coc trang	*Lumnitzera racemosa Willd*	x	
4	Da quanh	*Ceriops decandra*	x	
5	Duoc	*Rhizophora apiculata*	x	
6	Mam bien	*Avicennia marina*	x	x
7	Mam den	*Avicennia officinalis*	x	x
8	Mam trang	*Avicennia alba*	x	x
9	Vet den	*Bruguiera cexangula*	x	
10	Vet trang	*Bruguier sp.*	x	
11	Xu oi	*Xylocarpus grannatum*	x	

Note: (x) is an amplification result and a molecular marker

Table3: Species identified for research

Results of extraction and measurement of DNA levels: Leaf samples processed leaf samples were analyzed to assess the purity of leaf samples. The OD measurement results to evaluate the purity of DNA extracted samples are presented in Table 2. In general, all samples give a high purity level (value A260/A208> 1.7).

PCR amplification results for ITS region: PCR reaction to amplify ITS region is carried out on 2 areas: ITS 1-2 and ITS 1-4. The DNA samples of the collected species (phase I) all clearly amplified the ice on the agarose gel. The results are shown in (Figures 2 and 3).

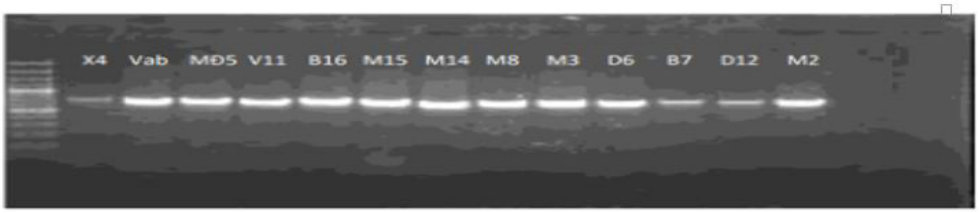


Figure 2: Results of PCR Rhizophora with ITS 1-2 primers

(Notes: X4: Xu oi; Vab: Vet du; MD5: Mam den; V11: Vet; B16: Ban chua; M15: Mam den; M14: Mam trang; M8: Mam moi; M3: Mam bien; D6: Duoc; B7: Ban chua; D12: Duoc moi; M2: Mam trang.)

Figure 3: Results of PCR Rhizophora with ITS 1-4 primers

PCR reaction to amplify ITS [1,2] region was performed on ITS 1-4. The DNA samples of the collected species (phase II) all clearly amplified the ice on the agarose gel. The results are shown in (Figure 4), below. PCR results with ITS 1-4 primers.

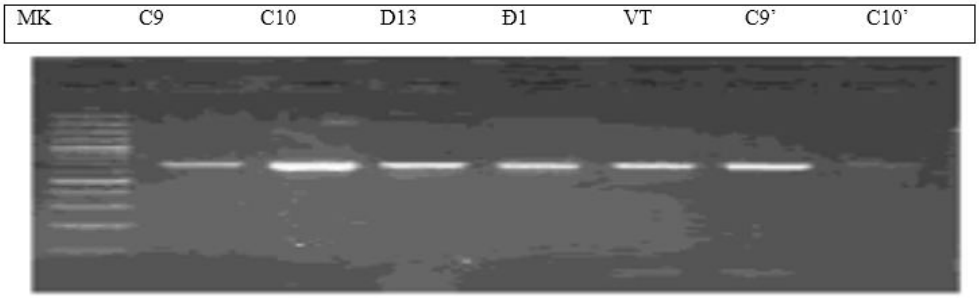


Figure 4: PCR results with ITS 1-4 primers.(PCR remaining primers do not give results.)

PCR-SSR

For primers that detect single repeaters, the results show only amplified some species such as: Avicennia (M3); Sonneratia (B7); New vinegar (M8); Black vine (MD5); and Sonneratia (B16). The size of the tape amplifier is the same as the previous authors.

In addition, there are still some species that have not yet amplified the ice for 10 primers, although DNA extraction with the concentration and quality is satisfactory through the expression of OD value (> 1.7) and PCR for the ITS region. (Table 4 and 5). The fruits were clearly amplified by the gel pattern (Figures 2,3 and 4), all showing the SSR primers that the author Yoshidaet, et al. used specifically for Sonneratia alba (belonging to Lythraceae family), the results were only obtained in Sonneratia with 2 pairs of primers SA102 and SA123, some in Avicennia, while others did not amplify bands (bands) due to the specialized SSR bait for these species except Sonneratia [5].

Species name	Sample collection symbols(Code)	Nucleic Acid(ng/uL)	A260/A280	A260	A280
Avicennia sp	M8	140.742	1.98	2.815	1.422
Avicennia sp	M8'	124.816	1.996	2.496	1.25
Soneratia alba	B7	148.975	1.738	2.98	1.714
Soneratia alba	B7'	201.643	1.753	4.033	2.301
Avicenniaofficin	M15	1 083.028	2.096	21.661	10.336
Avicenniaofficin	M15'	844.149	2.155	16.883	7.835
Soneratia alba	B16	355.072	1.962	7.101	3.62
Soneratia alba	B16'	318.744	1.954	6.375	3.262
Avicennia alba	M14	532.372	2.009	10.647	5.3
Avicennia alba	M14'	473.629	1.979	9.473	4.786
Xyclocarpus gra...	X4	182.763	1.837	3.655	1.989

Species name	Sample collection symbols(Code)	Nucleic Acid(ng/uL)	A260/A280	A260	A280
Xyclocarpus gra...	X4'	143.318	1.808	2.866	1.585
Xyclocarpus sp	C9	54.548	1.236	1.091	0.883
Xyclocarpus sp	C9'	88.22	1.025	1.764	1.721
B.g. (seedling)	V1-1'	266.833	1.94	5.337	2.75
B. g. (seedling)	V1-1"	2.008	2.677	0.04	0.015
B.g. (25 year)	V1-2'	63.671	1.82	1.273	0.7
B.g.(>25 year)	V1-2"	241.835	1.977	4.837	2.446
Rhizophora a.	D17'	429.415	1.882	8.588	4.564
Rhizophora a.	D17	931.888	1.389	18.638	13.419
Avicennia marine	M3	596.516	1.936	1.93	6.161
Avicennia marine	M3'	204.105	2.011	4.082	2.03
Avicennia alba	M2	352.156	1.942	4.957	2.552
Avicennia alba	M2	451.186	1.814	7.365	4.059
Avicenniaoff…	MD5	341.953	2.093	6.839	3.268
Avicennia off…	MD5'	360.323	2.107	7.206	3.42
*B.g.**	V1-3"	81.988	1.921	1.64	0.854
*B.g.**	V1-3'	168.434	1.812	3.369	1.86
Bruguiera sp	V11	692.776	1.865	13.856	7.429
Bruguiera sp	V11'	490.143	1.811	9.803	5.414
Lumnitzera lito…	C10	52.468	1.932	1.049	0.543
Lumnitzeralito…	C10'	28.713	1.646	0.574	0.349
Rhizophora a.	D6	368.324	1.272	7.366	5.789
Rhizophora a.	D6'	270.173	1.338	5.403	4.04
Rhizophora a. (n)	D12	145.56	1.437	2.911	2.026

(The second collection form (Divorce on July 2, 2018). Each sample extracted 2 tubes) (n: new)

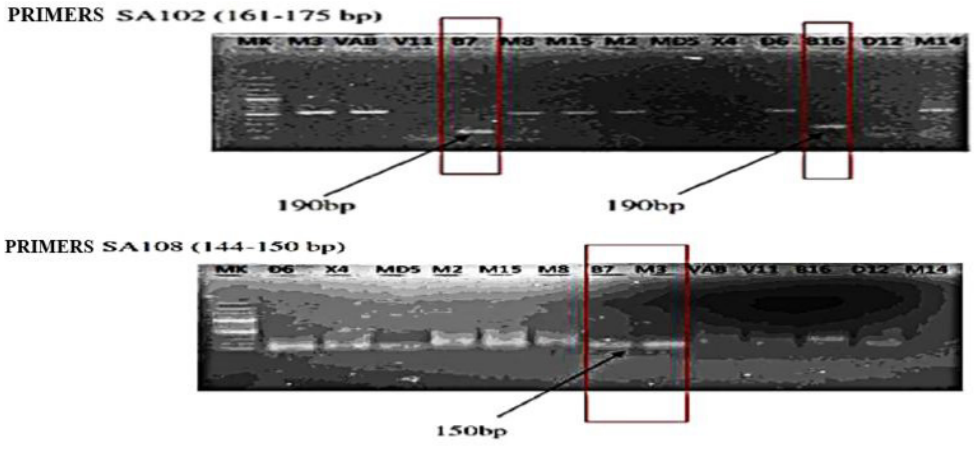
Note: (B. g.*: Bruguiera gymnorrhiza)

Table 4: Measure OD value of collected leaf samples

Symbol	Species name	OD measurement results		Results of PCR primers ITS 1-4
		Time 1	Time 2	
C9	*Lumnitzera racemosa*	*1,980*	1,901	x
C10	*Lummitzera ritorea*	*1,807*	1,870	x
D13	*Ceriops decandra*	*1,706*	1,754	x
Đ1	*Rhizophor apiculate*	*2,021*	2,131	x
VT	*Bruguiera sp*	*2,039*	2,073	x

Table 5: Measure OD value of leaf samples collected for the second time

According to research by Yoshida *et al.* 1995 showed that well-framed red wells have PCR products overlapping with the size of specialized primers (Figure 5) [5]. Markers in the framework are significant when using with specialized primers.



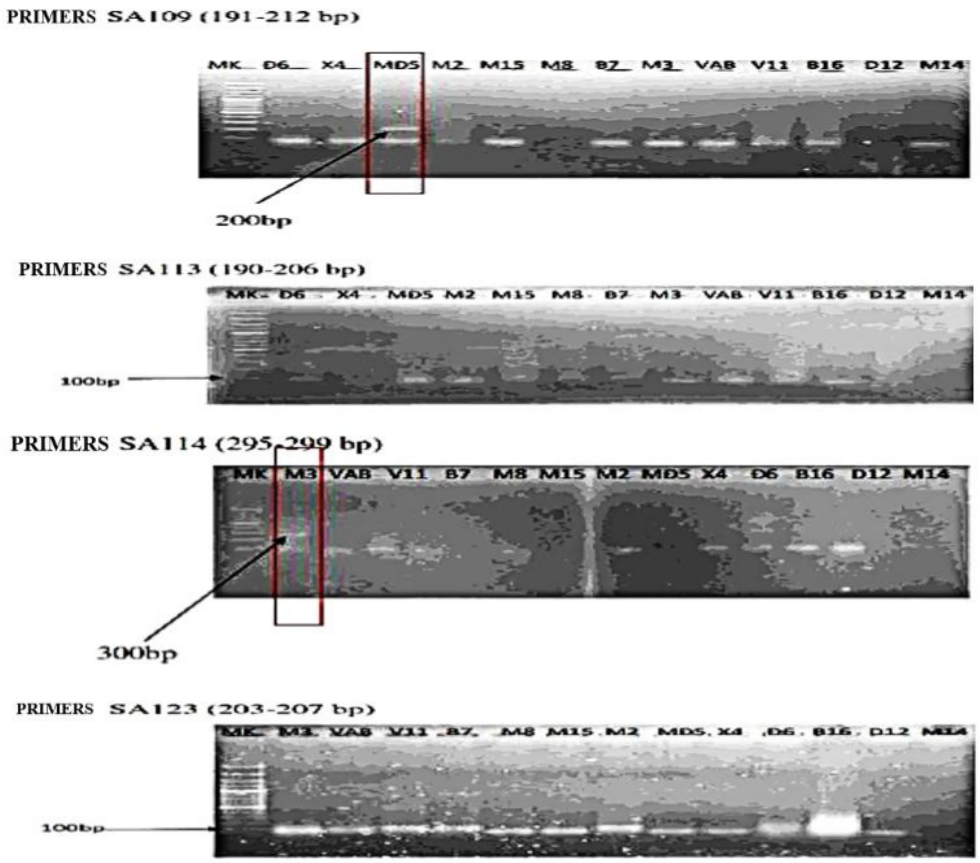


Figure 5: Treatment of Result PCR-SSR

About species trial

Location of test site: Some pictures of nursery area (Figure 6), location of field trials on the field: after implementing the trial plantation in January 2018 on alluvial soil, the model of experimental trees is gradually stabilized and young leaves develop (Figure 7). At the end of March 2018, the first tree was measured and the second one was in September, with the following results:

Figure 6: Area for sowing seedlings of 9 species

Figure 7: Measure the second tree in the trial area of 9 species

+ Comments and discussions

The research results show that there are differences among three experiments of each species. Specifically, the results of the first measurement of *Soneratia alba, Lumnitzera litorea,Rhizophora stylosa, Avicennia officinalis, Xylocarpus grannatum* in EX2 and EX3 were not statistically significantly different (P> 0.05), EX1 had statistical significance (p < 0.05). *Bruguiera cexangula* EX1, EX2 were not statistically significantly different (P> 0.05), EX3 had a statistically significant difference (P <0.05). *Avicennia alba* and *Avicennia marine* in 03 EX are not statistically significant (P> 0.05). Particularly, *Ceriops decandra* all three experiments were statistically significant differences (P <0.05) (Table 6). Second measurement, Soneratia albaEX1 and EX2 were not significantly different (P> 0.05), EX3 showed a statistically significant difference (P <0.05). *Lumnitera litorea* EX2 and EX3 were not statistically significantly different (P> 0.05), EX1 had a statistically significant difference (P <0.05). There is no statistically significant difference, *Ceriops decandra*, Avicennia marine, *Avicennia offcinalis, Avicennia alba* EX1 and EX3 (P> 0.05), EX2 has a statistically significant difference (P <0.05). Particularly for *Lumnitzera litorea, Avicennia officinalis*, all three experiments were not significantly different (P> 0.05) (Table 7) [6]. (Ngo Dinh Que, 2003) The research argues that the dominant factors such as climate, hydrology including heat regimes, rain regime and salinity of water affecting the distribution and growth of mangrove species in Vietnam. According to the author, the thermal regime affects the distribution and growth of mangrove species, but other criteria have effects such as soil properties, including particle size, soil type, maturity of soil, and organic matter in soil. The organic matter in the soil is too low (<1%) or too high (> 25%) that could lead the mangroves grow poorly.

Species name/EX	EX1	EX2	EX3
Sa	58,30±0,41^{b}	59,95±0,30^{a}	59,69±0,22^{a}
Ll	59,85±0,26^{a}	58,51±0,16^{b}	58,35±0,19^{b}
Cz	60,77±0,45^{a}	59,75±0,51^{b}	58,87±0,45^{c}
Rs	58,97±0,71^{b}	60,72±0,41^{a}	60,07±0,43^{a}
Am	58,85±0,66^{a}	58,95±0,52^{a}	59,70±0,89^{a}
Ao	59,95±0,93^{a}	57,87±0,76^{b}	58,35±0,77^{b}
Aa	59,35±0,55^{a}	58,82±0,73^{a}	59,12±0,50^{a}
Bc	57,92±0,53^{b}	57,80±0,69^{b}	59,87±0,29^{a}
Xg	58,35±0,46^{b}	59,12±0,60^{ab}	59,35±0,38^{a}

Note: In a row with at least one letter (a, b, c), there is no statistically significant difference of 5% through Duncan's test. Average value of using plus and minus SD. Unit of measurement (cm).

Table 6: Data for measuring trees for the first time in March 2018

Species/EX	EX1	EX2	EX3
Sa	161,30±3,01^{a}	164,75±2,80^{a}	155,27±3,05^{b}
Ll	70,95±0,95^{a}	68,22±0,42^{b}	69,07±0,30^{b}
Cz	66,00±0,43^{b}	67,35±0,42^{a}	65,00±0,94^{b}
As	69,82±1,31^{a}	70,82±0,88^{a}	69,27±1,35^{a}
Am	71,32±1,31^{b}	77,32±0,88^{a}	72,57±1,35^{b}
Ao	93,02±1,31^{b}	98,72±0,88^{a}	91,37±1,35^{b}
Aa	86,75±1,17^{a}	84,15±0,59^{b}	87,25±1,41^{a}
Bc	72,22±1,31^{a}	71,02±0,88^{a}	71,12±1,35^{a}
Xg	69,72±0,31^{a}	70,47±0,97^{a}	69,55±1,25^{a}

Note: In a row with at least one letter (a, b, c), there is no statistically significant difference of 5% through Duncan's test. In the same column with at least one identical letter (A, B), there is no statistically significant difference of 5% through Duncan test. Average value of using plus and minus SD. Unit of measurement (cm)

Table 7: Second measurement of tree data at the end of September 2018

Regarding to plant growth and development, all three experiments had growth after 6 months at an average of 10-30cm. In particular, Soneratia alba grows most strongly with a height of 101.13cm, followed by *Avicennia officinalis* 35.65cm, Avicennia alba is 26.95cm. The remaining species with low growth rate fluctuate at 10-14cm, particularly *Ceriops decandra* growth rate is only 6.31cm high (Table 8). According to Nguyen Duc Tuan, *et al.* 1994, research on growth and biomass of *Rhizophora stylosa*, *Rhizophora apiculata* and *Bruguiera gymnorhiza* at 1, 2, 3 and 4 years of age shows that on soft clay mud and coarse sand, plants grow well more than mud background mixed with coarse sand, hard high ground [7]. In 1995, the author continued to study the growth and biomass of *Rhizophora stylosa* and *Rhizophora apiculata* in Ha Tinh, Can Gio and Ho Chi Minh city. Compared to the substrate, there is a lot of coarse sand, high and hard sand (abbreviated EX1). On the surface of soft clay mud, there is less rough sand (abbreviated EX2) that the growth of *Rhizophora stylosa* is more pronounced clearly. With *Rhizophora apiculata* (in Can Gio - Ho Chi Minh city) 2 years old, the same results show similar trends.Thus, compared to the EX1 baseline, the EX2 matrix has

a more favorable effect on the growth process of the plant. The difference in clay glue content and dry sand has determined the difference in structure, physical, chemical properties, and nutrient content of the substrate, so that there is a different degree of influence on the plants growing on it. In the EX2 foundation with alluvial deposits deposited with much clay, less sand, more time for inundation has been a more favorable nutritional condition than EX1 for the more dominant development of planted trees.

Species symbol/TN	Time 1 (cm)	Time 2 (cm)	Average (cm)
Sa	59,31±0,81	160,44±4,97	101,13
Ll	58,90±0,72	69,41±1,31	10,51
Cz	59,80±0,91	66,11±1,16	6,31
Rs	59,92±0.89	69,97±1,28	10,05
Am	59,16±0,75	73,74±2,91	14,58
Ao	58,72±1,19	94,37±3,46	35,65
Aa	59,10±0,59	86,05±1,74	26,95
Bc	58,53±1,10	71,45±1,22	12,92
Xg	58,94±0,63	69,91±1,55	10,97

Note: Unit of measurement (cm) Average value of use plus and minus SD

Table 8: Results of average tree height growth in 2 measurements

The survival rate of planted trees tested in all 3 experiments with 9 species of trees is 100% in experiment 1, 99.07% in experiment 2, in experimental 3, survival rate is 97.22% or more (only 1 to 2 dead trees) (Table 9) [8]. Hoang Van Thoi, 2011 studied and experimented with some mangrove species on sand, rock, gravel, irregular tidal reefs in the southern coastal islands and the survival rate after 3 months experimental tree species: *Rhizophora stylosa* has the highest survival rate with 57%, followed by *Avicennia marine* 55%, *Bruguiera gymnorhiza* 54%, *Rhizophora apiculata* 52%. Species with very low survival rates such as *Ceriops tagal* 23%, especially *Aegiceras comiculatum* species give the worst rate with 5%.

Species symbol/EX	EX1	EX2	EX3
Sa	27,00a (100%)	26,75±0,25a (%)	26,50±0,28a
Ll	27,00a (100%)	27,00a (100%)	26,25±0,50b
Cz	27,00a (100%)	27,00a (100%)	26,75±0,50a
Rs	27,00a(100%)	27,00a (100%)	26,25±0,50b
Am	27,00(100%)	27,00 (100%)	27,00 (100%)
Ao	27,00(100%)	27,00 (100%)	27,00 (100%)
Aa	27,00a(100%)	27,00a (100%)	26,75±0,50a
Bc	27,00a(100%)	26,75±0,50a	26,50±0,57a
Xg	27,00a(100%)	26,75±0,50a	26,75±0,50a

Note: In a row with at least one letter (a, b, c), there is no statistically significant difference of 5% through Duncan's test. Average value of using plus and minus SD.

Table 9: Second measurement for density and survival rate at the end of September 2018

Thus, according to the assay model, we can see that *Avicennia officinalis, Avicennia alba* and *Soneratia alba* trees are able to grow and develop strongly, suitable to the new group of mudflats in Group I, shallow tidal regime, can be used for planting protective forests in coastal areas. However, considering the growth pattern of Sonneratia alba, requires their habitat conditions to grow in the estuarine and the sweet season in the year. Therefore, *Avicennia officinalis* and *Avicennia alba* is most suitable for selecting species for the large-scale afforestation model through the 9 species test [9,10].

Conclusion

Research has identified molecular markers of 9 species of mangrove trees, for the purpose of species classification and conservation of their biodiversity, 4 species have been identified as an important one, of which 3 species choose to be planted The forest in that area is *Avicennia offcinalis, Avicennia alba* has been identified to indicate the correct name of the species that can be planted on saline alluvial soils in the study area and the third species. Is *Sonneratia alba*, especially the conservation of rare species in red books that are in danger of extinction such as *Luminitzera alba*.

In terms of height growth in the first year of study, all species have a very high survival rate of over 90% of the trees that survive after the first year with the harsh conditions of weather and hydrology. Thinning of the soil substrate, but the species has the ability to adapt to very high survival rates. Regarding height growth, the species adapted in the first year of rapid growth are Ban chua 101.13cm, followed by *Avicennia offcinalis* at 35.65cm and *Avicennia albaat* 26.95cm, there are differences in height growth with fast growing species and slow growing species in the first year.

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