Application of ISSR markers to analyze the genetic diversity of the May nuoc mo species (*Calamus* sp.) in Quang Nam province, Central Vietnam

Hieu Huynh Kim[1], Loi Nguyen Van[1], Long Dang Thanh[2] and Minh Nguyen Van[1]*
1. University of Agriculture and Forestry, Hue University, 102 Phung Hung, Hue City, VIETNAM
2. Institute of Biotechnology, Hue University, Hue City, VIETNAM
*nvminhhuaf@hueuni.edu.vn

Abstract
The May nuoc mo species (Calamus sp.) is used as a raw material for the production of handicrafts and household wares. It is a species with high economic value that contributes to improving the livelihoods of planters. In this study, a total of 23 Calamus sp. individuals collected from two districts, Dong Giang and Nam Giang, in Quang Nam province were evaluated for genetic diversity using the molecular marker ISSR (Inter Simple Sequence Repeat). Out of 113 DNA bands, 91 were polymorphic DNA bands and 22 were monomorphic from fifteen ISSR primers. No private band DNA differences were detected among the studied Calamus sp. individuals.

The proportion of polymorphic DNA bands obtained from each primer ranged from 50 to 100% (mean = 79.918%). The level of genetic similarity between individuals in Calamus sp. populations ranges from 67% to 96%, indicating a high degree of genetic variation among the Calamus sp. individuals studied in different localities. The genetic tree showed that different Calamus sp. individuals in the same locality had high genetic similarity and were distributed on the same evolutionary branch. It is proposed to use Calamus sp. populations distributed in different localities in this study as seedlings to maintain genetic resources and develop planted forests according to forestry programs. This would contribute to improving production value and enhancing people's livelihoods.

Keywords: *Calamus* sp., Genetic, Diversity, Molecular marker, Phylogenetic, ISSR.

Introduction

Forests distributed in any locality around the world contain many helpful plant species which provide many beneficial services in the terms of society, economy and ecosystem diversity. Rattan is a climbing plant, naturally distributed in many different forests and soil types in alluvial plains to moist hill forests at altitudes up to 2000 m. They are considered the main climbing plant species in tropical forests in Asia[14]. Currently, in the world, there are about 650 rattan species reported belonging to 22 genera and about 400 species[5,28].

**** Author for Correspondence***

The local communities are harvested rattan for domestic or commercial use and contribute significantly to the rural economy[26]. As a result, the demand for local raw materials has increased[15]. Therefore, the conservation of available genetic resources needs to be given the highest priority[25]. Besides, the direct impact of climate change has affected biodiversity on a global scale for all plant and animal species, including rattan.

Therefore, selecting superior genotypes that are adaptable to climate change and capable of long-term survival by meeting new selective pressures based on natural variations is one of the requested problems and serves as a resource for breeding and crop improvement programs[4,22,25].

In Vietnam, rattan is the most important forest product after wood and bamboo. According to Peters et al[21], in Vietnam, Laos and Cambodia, there are about 65 species of rattan belonging to 6 different genera. Currently, Vietnam has over 1000 rattan and bamboo craft villages, accounting for 24% of the total number of craft villages around the country. According to data from the General Department of Customs, in the first five months of 2021, the products of bamboo and rattan were exported to more than 130 countries and territories around the world and export turnover reached 356.47 million USD, an increase of 76.8% over the same period in 2020. By the end of 2021, Vietnam's export turnover of rattan, bamboo, sedge and carpet products to other countries around the world reached 878.4 million USD (http://kinhtevn.com.vn).

Despite the increasing importance and pressure on natural rattan populations, including Vietnamese rattan, no serious attempt was made to characterize the genetic variation that exists between the natural populations. The base knowledge of genetic diversity, population structure and spatial patterns of genetic variation is useful in informing species conservation plans[15]. Therefore, an important issue today is an immediate need to generate baseline data on natural populations to scientifically manage and conserve this precious, dwindling resource in the future, along with breeding and planting species with important commercial value.

Currently, there are many different methods used to evaluate genetics and species diversity in plants. However, they did not obtain a complete account of the structure of complex genetic variation in wild plants. For rattan species, there have been many studies that applied molecular markers to

evaluate genetic diversity in different species in many localities around the world. A moderate level of genetic differentiation has been recorded for the species in the rattan populations[1-3,10,13,15,23,24].

However, the rattan in Vietnam has little data on genetic diversity to be published. Therefore, research is needed to apply molecular markers to evaluate the genetic diversity of rattan species in Vietnam in general and rattan species in Quang Nam in particular. The result is reference sources contribute to guiding their conservation and development.

In this study, ISSR marker was used to analyze the genetic diversity relationship among 23 samples of the May nuoc mo species (*Calamus* sp.)[8] collected in the districts of Dong Giang and Nam Giang, Quang Nam province. The aim of this study was to evaluate the genetic diversity of 23 May nuoc mo species (*Calamus* sp.) samples for studying future biodiversity conservation strategies and genetic improvement.

Material and Methods

Plant Material*:* A total of 23 individuals of May nuoc mo species (*Calamus* sp.). were collected from the different geographical locations in the districts of Dong Giang and Nam Giang of Quang Nam province, Vietnam. The origin of each individual is mentioned in table 1.

DNA Isolation: Total DNA from rattan leaf samples was extracted and purified according to the method described by Vaze et al[29] with modifications. That was a method based on the ability to form complexes between CTAB (Cetyl trimethyl ammonium bromide) and DNA present in the sample. Total DNA was then purified using the phenol: chloroform method[29]. Rattan leaves (100 mg) were cut into small pieces, ground in liquid nitrogen and after resuspended with 500 µL of CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl, 20 mM EDTA and 1.4 M NaCl, pH 8.0), supplemented with 2% β-mercaptoethanol containing 0.2 g PVP per gam of leaf powder). The mixture was then incubated at 65^{o}C for 60 minutes with shaking and transferred to room temperature.

The suspension was added with an equal volume of chloroform: isoamyl alcohol and gently stirred, centrifuged at 10,000 rpm for 15 minutes at 4°C and transferred the supernatant to a new tube (repeat a second time). The first DNA precipitates with an equal volume of propanol and 1/5th volumes of 5M NaCl at -40° overnight. Collect the DNA precipitate by centrifuging at 15,000 rpm for 20 minutes and wash twice with 70% ethanol, then dry at room temperature. The DNA precipitate was then redissolved with 1/10th volumes of TE (10 mM Tris HCl, 1mM EDTA, pH. 8.0) buffer and treated to remove RNA with RNase (10 µg/mL) at 37°C for 30 minutes.

Table 1
List of samples used in this study

Code sample	Sampling location	GPS
HMNGV01	Sub-region 223, Nam Giang, Quang Nam, Viet Nam	787654; 1743539
HMNGV02	Sub-region 223, Nam Giang, Quang Nam, Viet Nam	788353; 1745867
HMNGV03	Sub-region 223, Nam Giang, Quang Nam, Viet Nam	788585; 1746133
HMNGV04	Sub-region 294, Nam Giang, Quang Nam, Viet Nam	786012; 1735675
HMNGV05	Sub-region 294, Nam Giang, Quang Nam, Viet Nam	786008; 1735674
HMNGV06	Sub-region 294, Nam Giang, Quang Nam, Viet Nam	786005; 1735664
HMNGV07	Sub-region 286, Nam Giang, Quang Nam, Viet Nam	779146; 1736856
HMNGV08	Sub-region 286, Nam Giang, Quang Nam, Viet Nam	779142; 1736837
HMNGV09	Sub-region 286, Nam Giang, Quang Nam, Viet Nam	779169; 1736830
HMNGV10	Sub-region 299, Nam Giang, Quang Nam, Viet Nam	781292; 1730649
HMNGV11	Sub-region 299, Nam Giang, Quang Nam, Viet Nam	781260; 1730659
HMNGV12	Sub-region 299, Nam Giang, Quang Nam, Viet Nam	781307; 1730649
HMNGV13	Sub-region 154, Dong Giang, Quang Nam, Viet Nam	782639; 1751947
HMNGV14	Sub-region 154, Dong Giang, Quang Nam, Viet Nam	782639; 1751948
HMNGV15	Sub-region 154, Dong Giang, Quang Nam, Viet Nam	782557; 1751929
HMNGV16	Sub-region 159, Dong Giang, Quang Nam, Viet Nam	793648; 1751533
HMNGV17	Sub-region 159, Dong Giang, Quang Nam, Viet Nam	793310; 1752338
HMNGV18	Sub-region 166, Dong Giang, Quang Nam, Viet Nam	790879; 1748915
HMNGV19	Sub-region 166, Dong Giang, Quang Nam, Viet Nam	790225; 1748400
HMNGV20	Sub-region 166, Dong Giang, Quang Nam, Viet Nam	790363; 1747877
HMNGV21	Sub-region 158, Dong Giang, Quang Nam, Viet Nam	789331; 1751819
HMNGV22	Sub-region 158, Dong Giang, Quang Nam, Viet Nam	789798; 1751196
HMNGV23	Sub-region 158, Dong Giang, Quang Nam, Viet Nam	789123; 1752511

The DNA total was purified second time with an added aqueous phase of phenol (Tris-saturated phenol, pH 8.0) and mixed gently by inversion, then centrifuged at 10000 rpm for 5 minutes at room temperature. The aqueous phase above continued to be transferred to a new tube, washed with an equal volume of chloroform: isoamyl alcohol (24:1) (repeat a second time). The total DNA was precipitated with 1/10th volumes of 3M Na acetate pH 5.2 and 2.5 volumes of absolute ethanol. Centrifuge at 15000 rpm for 20 minutes collect the DNA pellet and wash twice with 70% ethanol. The DNA pellet was then dried and resuspended with 100 µL TE buffer. Total DNA quality was determined by 1% agarose gel electrophoresis in TAE buffer and then stored at -20°C to perform other experiments.

ISSR analysis: The PCR-ISSR amplification reaction was carried out with a total volume of 50 µL including the following components: 1 µL template DNA (50 ng/µL), 25 µL 2X Green GoTaq® Reaction Buffer (pH 8.5) (Promega, USA), 1 µL random primers (20 pmol/µL) and 23 µL sterile distilled water. PCR-ISSR program conditions consisted of 95°C for 5 min, then 35 cycles of 95°C for 45 seconds followed by 56°C for 45 seconds and 72°C for 1 min and one cycle at 72°C for 10 min. The DNA bands in the PCR-ISSR product were separated based on 1.6% agarose electrophoresis in 1x TAE buffer with Midori Green safe DNA stain. Electrophoretic images were captured using the gel documentation system and analyzed using the Quantity One program (Bio-rad, USA).

Data Analysis: The electrophoresis spectra of PCR-ISSR products of samples with primers were analyzed according to the identification of the presence or absence of DNA bands, numbered "1" if DNA bands appear and numbered "0" if no DNA band appears.

Genetic diversity parameters of rattan populations were analyzed based on PopGen 3.2 software as observed number of alleles (na), effective number of alleles (ne), gene diversity (H), Shannon's Information index (I) and calculated according to the following formula[30]:

$$I = -\sum pi \log 2 pi$$

where pi is the frequency of occurrence of the ith PCR-ISSR product in the population using PopGen 3.2 software.

Building a genealogical diagram according to the UPGMA algorithm of 23 rattan samples used in the study was carried out using the NTSYS 2.1 program (Exeter Software, USA) based on Jaccard's (1908) genetic similarity coefficient[9].

Results

Total DNA extraction results: The content and quality of total DNA extracted and purified from fresh *Calamus* sp. leaves species using the CTAB method showed that the purity obtained at the ratio of 280/260 nm ranged from 1.9 to 2.2. Total DNA recovery efficiency ranged from 30 to 120 µg/gram of fresh leaf sample. The quality of total DNA guaranteed to carry out further experiments (Figure 1).

Rattan cultivars genetic characterization based on PCR-ISSR marker: The product of PCR-ISSR was electrophoresed on agarose gel in 1X TAE buffer. The presence or absence of DNA segments of PCR products of individuals in the *Calamus* sp. helps us to determine the genetic similarity coefficient at the molecular level. The results obtained in this study after electrophoresis of PCR-ISSR products are summarized in tables 2 and 3.

All *Calamus* sp. individuals showed a PCR amplification rate of 100% for the 15 ISSR primers used in the study. A total of 113 DNA bands were generated from the PCR product of 15 primers ISSR (mean = 7.533 DNA bands/each ISSR primer). The number of polymorphic DNA bands formed is 91 loci (mean = 6.067 polymorphic DNA bands/each ISSR primer). The ratio of polymorphic DNA bands on primers in our study ranged from 50 to 100%, an average accounting for 79.918%. Primer UBC#827 gave the highest number of polymorphic DNA bands (10 DNA bands) and primer UBC#841 gave the lowest number of polymorphic DNA bands (3 DNA bands). The size of the obtained DNA bands ranges from 120 to 2000 bp. The amount of DNA obtained from different ISSR primers varies ranging from 5 to 12 DNA bands (Figure 2), in which primer UBC#827 captures the highest amount of DNA (12 DNA bands) and the lowest is primer UBC#811 (5 DNA bands) (Table 2).

The number of DNA bands obtained was different in *Calamus* sp. individuals with 15 ISSR primers, ranging from 58 to 82 DNA bands (Table 3). The *Calamus* sp. individual with the symbol HMNGV01 had the highest number of amplified DNA bands with 82 DNA bands (accounting for 5.040% of the total 1627 DNA bands created by all ISSR primers), followed by HMNGV02 (80 DNA bands, accounting for 4.917 %). The *Calamus* sp. individual with the least number of PCR amplification bands has the symbol HMNGV07 with 58 DNA bands, accounting for 3.565% (Table 3).

A study by Nei et al[18] shows that the greater is the number of amplified DNA bands, the greater is the ability to distinguish samples on the family tree, in which the minimum number of polymorphic bands is 50 to be able to build the tree Phylogenetic. In this study, we have obtained 91 polymorphic DNA bands (average accounting for 79.918%) from 23 different *Calamus* sp. individuals in 15 primers ISSR to serve genetic diversity research and build the tree Phylogenetic (Table 2). Therefore, the data obtained in this study are sufficient for the above problems to carry out further analysis.

Population genetic analysis: The genetic diversity analysis of individuals in the population of *Calamus* sp. based on Shannon's Information index (I) using the ISSR marker

shows that the "I" value ranges from about 0.324 to 0.551/each primer (mean = 0.417) (Table 4). Primer UBC#873 gave the highest coefficient of genetic diversity in study samples (I = 0.551) and primer UBC#855 showed the lowest coefficient of Shannon's Information index (I = 0.324).

The number of observed alleles (na) in the rattan population ranged from 1.5 to 2 (mean = 1.805). Primers UBC#811, UBC#844 and UBC#864 show the highest values (na = 2) and primer UBC#841 gave the lowest values (na = 1.5). Besides, the number of effective alleles (ne) of the population of *Calamus* sp. ranged from 1.144 to 1.751 (mean = 1.473). Primer UBC#810 gave the highest number of effective alleles (ne = 1.751) and primer UBC#807 showed the lowest values (ne = 1.144). The "na" and "ne" values collected the highest values obtained from primers UBC#811, UBC#844, UBC#864 and UBC#810, suggesting that these primers resulted in greater genetic diversity compared to other primers used in research among individuals of the *Calamus* sp. population in this study (Table 4).

Besides, the Nei's gene diversity (H) among ISSR primers among individuals in the study population of *Calamus* sp. ranged from 0.116 to 0.386 (average ± StDv = 0.277 ± ?) for each ISSR primer. Primer UBC#873 gave the highest value (H = 0.386) and primer UBC#807 showed the lowest value H (0.116) (Table 4).

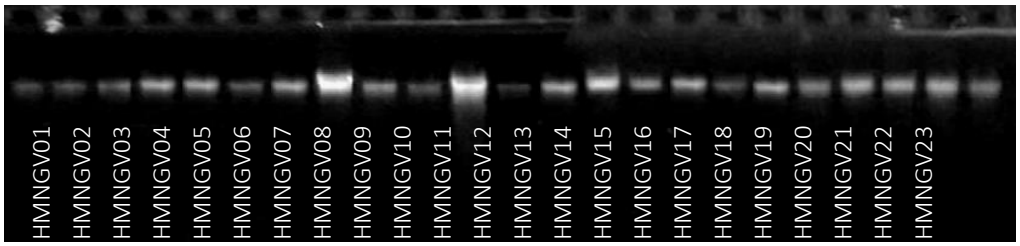

Figure 1: Electrophoresis of total DNA from fresh leaf samples of 23 different *Calamus* sp. individuals

Table 2
Number of cultivars and number of amplified fragments in each primer ISSR

Primers	Sequences 5' to 3'	Ref.	Number of amplified cultivars	The rate of amplified cultivars (%)	Number of total amplified fragments	Number of polymorphic fragments	Size range (bp)	Polymorphism (%)
UBC#807	AGAGAGAGAGAGAGAGT	Digvender Pal et al., 2013	23	100	6	4	120-620	66.667
UBC#808	AGAGAGAGAGAGAGAGG		23	100	8	5	300-1400	62.500
UBC#810	GAGAGAGAGAGAGAGAT		23	100	7	6	350-1000	85.714
UBC#811	GAGAGAGAGAGAGAGAC		23	100	5	5	100-900	100
UBC#812	GAGAGAGAGAGAGAGAA		23	100	6	4	200-700	66.667
UBC#825	ACACACACACACACACT		23	100	6	5	100-1500	83.333
UBC#827	ACACACACACACACACG		23	100	12	10	150-1800	83.333
UBC#841	GAGAGAGAGAGAGAGAYC		23	100	6	3	100-1400	50
UBC#855	TGTGTGTGTGTGTGTGRT		23	100	10	7	200-1600	70
UBC#858	ACACACACACACACACYT		23	100	8	7	220-2000	87.500
UBC#864	ATGATGATGATGATGATG		23	100	8	8	460-1500	100
UBC#888	BDBCACACACACAGACA	Munankarmi et al., 2018	23	100	9	8	120-2000	88.889
UBC#873	GACAGACAGACAGACA		23	100	8	7	100-1700	87.500
UBC#835	AGAGAGAGAGAGAGAGYC		23	100	6	4	250-1500	66.667
UBC#844	CTCTCTCTCTCTCTCTAC		23	100	8	8	100-1000	100
Mean				**100**	**7.533**	**6.067**	**100-2000**	**79.918**
Total				**-**	**113**	**91**	**-**	**-**

Table 3
Number of amplified fragments of cultivars in each primer ISSR

Code sample	Primers															
	UBC # 807	UBC # 808	UBC# 810	UBC # 811	UBC# 812	UBC # 825	UBC # 827	UBC# 841	UBC# 855	UBC# 858	UBC # 864	UBC # 888	UBC # 873	UBC# 835	UBC# 844	Total number of DNA fragments/ sample
HMNGV01	5	8	4	3	3	4	7	3	9	7	5	7	5	5	7	82
HMNGV02	5	7	3	3	4	5	7	3	7	7	5	7	5	5	7	80
HMNGV03	5	5	3	3	5	5	7	3	8	7	5	7	5	5	6	79
HMNGV04	4	6	3	3	4	4	7	6	10	1	5	1	3	6	4	67
HMNGV05	4	6	3	2	4	5	6	5	10	1	4	1	8	6	5	70
HMNGV06	5	4	3	3	4	4	5	4	7	2	4	3	4	6	4	62
HMNGV07	5	7	3	1	4	4	4	4	4	4	0	4	4	4	6	58
HMNGV08	3	7	5	3	4	3	4	4	7	4	5	4	3	4	6	66
HMNGV09	5	7	1	4	3	4	6	4	7	4	6	4	3	4	3	65
HMNGV10	5	7	4	3	4	4	7	5	8	6	6	6	5	5	1	76
HMNGV11	3	5	4	4	4	4	6	5	10	4	5	5	5	5	3	72
HMNGV12	5	4	4	4	5	3	8	5	5	4	5	5	5	6	2	70
HMNGV13	5	4	5	1	3	5	7	4	8	6	4	6	4	2	4	67
HMNGV14	5	4	5	1	3	5	8	4	8	6	1	6	4	2	4	65
HMNGV15	5	4	5	4	2	5	6	4	8	6	4	6	4	2	4	69
HMNGV16	4	8	1	3	3	5	5	3	8	4	5	4	3	3	4	63
HMNGV17	5	7	5	3	4	5	7	4	8	4	4	4	3	3	4	70
HMNGV18	5	4	5	4	4	3	6	3	8	6	5	6	7	5	6	77
HMNGV19	5	4	5	3	4	4	5	3	8	6	5	6	7	5	6	76
HMNGV20	5	7	2	2	3	5	6	4	9	7	5	7	4	3	5	74
HMNGV21	5	7	4	3	3	6	5	4	9	7	5	8	4	3	4	77
HMNGV22	5	7	3	2	3	3	4	4	9	7	5	7	1	3	4	67
HMNGV23	5	7	5	3	3	3	5	6	9	7	5	7	1	5	4	75
Total DNA bands/primer	**108**	**136**	**85**	**63**	**83**	**98**	**138**	**94**	**184**	**117**	**103**	**121**	**97**	**97**	**103**	**1627**
Mean/ primer	**4. 70**	**5. 91**	**3. 70**	**2. 74**	**3. 61**	**4. 26**	**6. 00**	**4. 09**	**8. 00**	**5. 09**	**4. 48**	**5. 26**	**4. 22**	**4. 22**	**4. 48**	**70. 74**

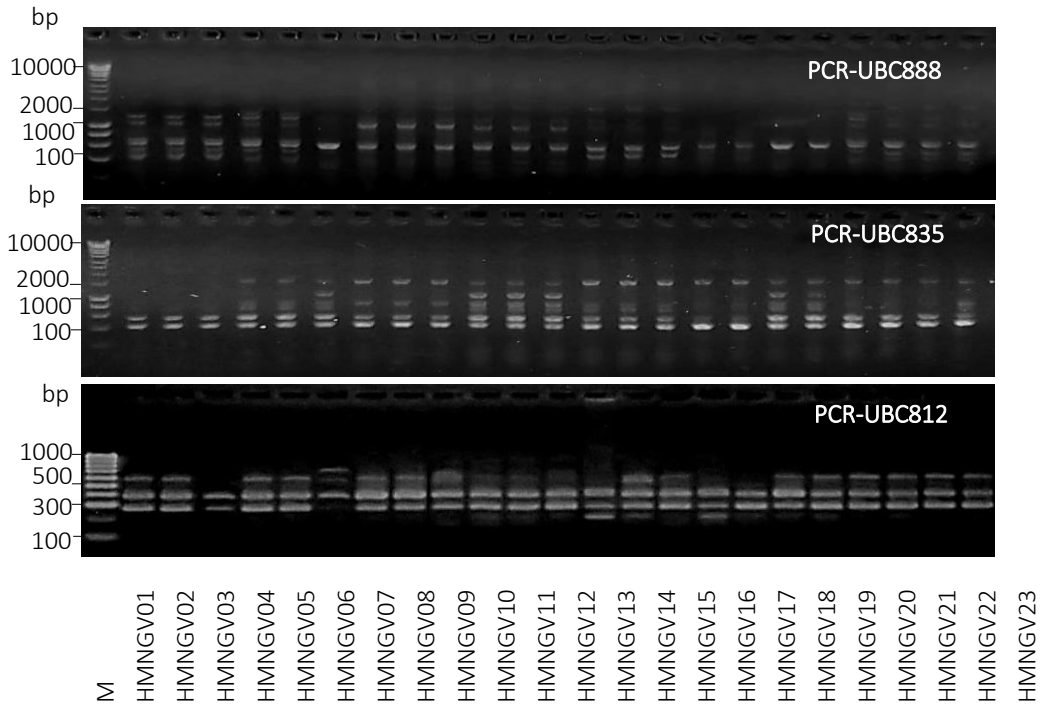

Figure 2: Dendrogram of PCR- ISSR amplified products.
M: DNA Standard Marker (100 -10.000 bp, Bioline và 100-1000 bp, Biobase)

Table 4
Diversity indices in each primer

Locus	Sample Size	na	ne	h	I
UBC#844	23	2.000	1.435	0.283	0.445
UBC#811	23	2.000	1.560	0.343	0.522
UBC#825	23	1.833	1.520	0.290	0.429
UBC#835	23	1.667	1.453	0.260	0.382
UBC#841	23	1.500	1.393	0.218	0.313
UBC#873	23	1.875	1.715	0.386	0.551
UBC#888	23	1.889	1.580	0.336	0.498
UBC#858	23	1.875	1.602	0.342	0.502
UBC#807	23	1.667	1.144	0.116	0.208
UBC#812	23	1.667	1.363	0.224	0.343
UBC#808	23	1.625	1.459	0.252	0.366
UBC#810	23	1.857	1.751	0.399	0.563
UBC#827	23	1.833	1.400	0.249	0.387
UBC#855	23	1.700	1.343	0.209	0.324
UBC#864	23	2.000	1.369	0.245	0.398
Mean	**23**	**1.805**	**1.473**	**0.277**	**0.417**
St. Dev		**0.398**	**0.362**	**0.183**	**0.251**

Note: na = Observed number of alleles; ne = Effective number of alleles (Kimura and Crow, 1964); h = Nei's (1973) gene diversity; I = Shannon's Information index (Lewontin, 1972); St.Dev: Standard deviation

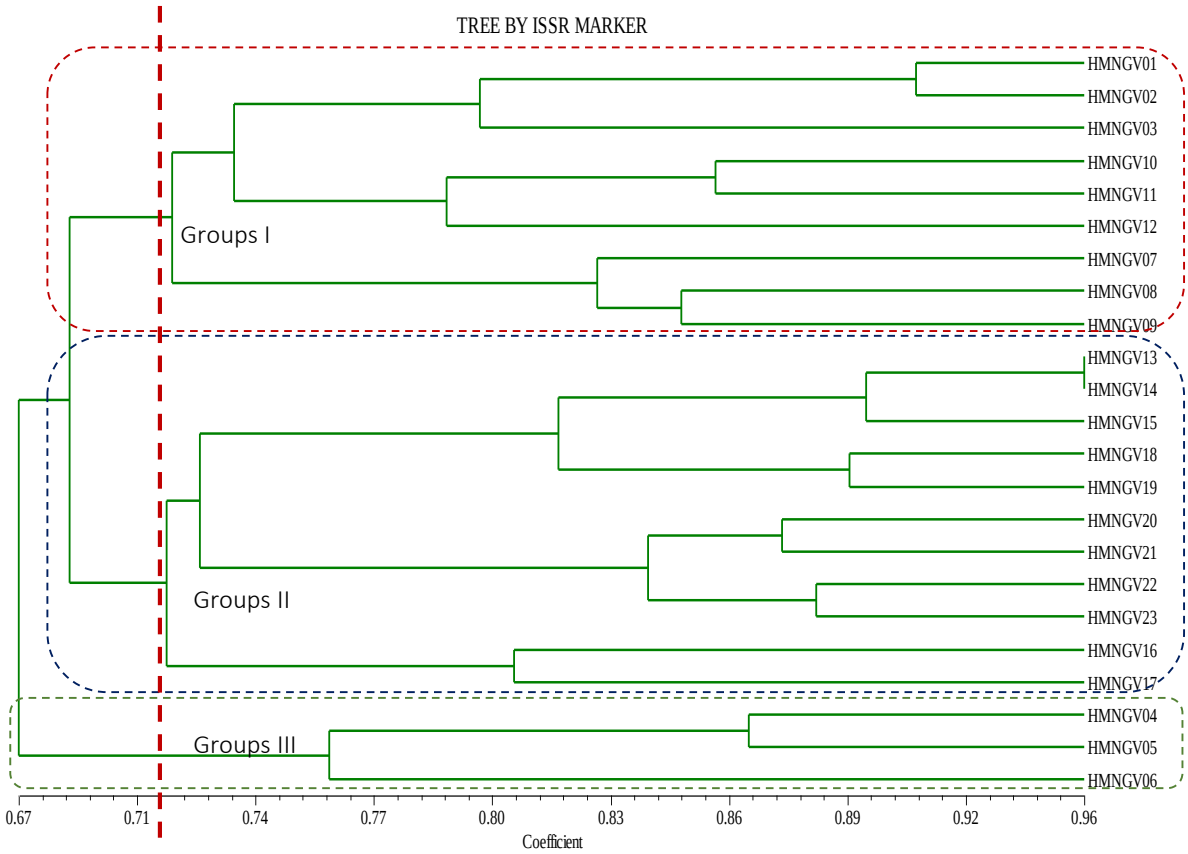

Figure 3: UPGMA tree showing the relationship base on marker ISSR

Phylogenetic Analysis: The phylogenetic tree built based on the UPGMA method using NISYSpc21 software shows that 23 *Calamus* sp. individuals were collected from different sub-regions in Quang Nam province, Viet Nam. Based on the genetic similarity, at the cutting point of 71.6% genetic similarity, the *Calamus* sp. individuals are distributed into three groups. Group I was created by nine *Calamus* sp. individuals collected in sub-regions 223, 286

and 299 in Nam Giang district, Quang Nam. Group II was formed by eleven *Calamus* sp. individuals distributed in the sub-regions 154, 159, 166 and 158 in Dong Giang district, Quang Nam.

The group III was created by three individuals of *Calamus* sp. whose origin was sampling in sub-region 294, Nam Giang district, Quang Nam. Three individuals of *Calamus* sp. presented the greatest genetic distance compared to all the other individuals. Based on the similarity matrixes and the dendrogram of the molecular marker under study show that the ISSR markers were efficient in studying the genetic diversity of population *Calamus* sp. The individuals of *Calamus* sp. in the same sub-region and between sub-regions in the same local have the same level of genetic diversity, distributed on the same evolutionary branch when analyzed based on 15 markers ISSR.

Discussion

Molecular characteristics of 23 individuals in May nuoc mo species (*Calamus* sp.) populations collected from two districts in Quang Nam province are rattan species with high commercial value in Vietnam. Based on the characteristic genetic diversity and population structure of each *Calamus* sp., it serves the work of selecting and creating varieties as a source of raw materials for the exploitation and development of this plant variety in a sustainable direction, contributing to improving the lives of local people. The level of genetic diversity is evaluated through several indices such as the percentage of polymorphic bands (PPB), Nei's genetic diversity index (h) and Shannon's diversity index (I)[31] between different rattan individuals in many localities showing that high levels of polymorphism have been recorded[2,3,15,23].

In this study, the results showed that the level of genetic diversity among individuals in the recorded population (H = 0.277, I = 0.417) (Table 4) and the percentage of polymorphic bands (PPB) ranged from 50 to 100 %. The highest polymorphic DNA obtained (100%) was for primers UBC-844, UBC-811 and UBC-864; the average percentage of polymorphic DNA bands accounted for 79.918% (Table 2). These results were higher than some studies on some *Calamus* sp. species in different localities that have been published previously such as *A. Calamus* (PPB = 51.5%) in South and Northeast India[1], *Acorus calamus* L. (PPB = 63.7%) in India[3] and 12 rattan genotypes of the genera *Calamus*, *Korthalsia* and *Daemonorops* (PPB = 58.92%) in Andaman and Nicobar Islands[10].

The other genetic diversity information obtained from this study was also compared with rattan species in other localities, showing that Nei's gene diversity index (h) is equivalent to some rattan species in some regions. Other localities include *C. flagellum* Griff.ex Mart. (h = 0.201) in Arunachal Pradesh[13], *C. thwaitesii* Becc. (h = 0.272 - 0.333) in the central Western Ghats[24], *A. Calamus* (h = 0.2005) in South and Northeast India[1], *Calamus guruba* (h = 0.216) in North East India[15]. Besides, Shannon's diversity index (I) is higher than that of rattan species distributed in some other localities such as *A. Calamus* (I = 0.2946) in South and Northeast India[1], *Calamus guruba* (I = 0.341) in North East India[15].

Low genetic diversity with very high genetic differentiation (I = 0.146) was reported in the Indonesian populations of *Daemonorops draco* due to geographic isolation and apomictic behavior of the species[2]. In general, many research results show that in different geographical regions, the level of genetic diversity of some rattan species distributed is also different. Rattan species with wide distribution ranges tend to maintain higher levels of genetic diversity than species distributed in the same locality[6]. Similarly, species formed by outcrossing tend to maintain higher levels of genetic diversity than self-pollinated species[7].

Based on this assumption, our research results for rattan individuals collected from two different localities (Dong Giang and Nam Giang) in Quang Nam province are considered comparable with species with wide geographical distribution[19] corresponding to the genetic diversity index I = 0.417 (Table 4). The clustering pattern shows that the 23 *Calamus* sp. individual wilds fall into three main groups comprising the entire variation and most genotypes were clustered following along the same with their geographical distribution and have differences in genetic diversity that differ significantly.

Therefore, it can infer that the individuals in the population of rattan wilds obtained in Quang Nam are a single wild population of rattan and share a common genetic ancestor. The genetic diversity of the wild population of rattan was adopted as changes of rattan individuals in response to habitat fragmentation, genetic drift, and/or barriers to gene flow. Thus, the conservation measures are needed to protect rattan biodiversity in its natural habitat by preserving rare alleles. The basic but extremely useful information generated in our research will be very effective in informing a conservation, management and genetic improvement program for the depleted rattan gene pool.

Conclusion

In this study, the result revealed that the ISSR marker showed the level of high genetic diversity and polymorphism DNA bands among individuals in the recorded population (PPB = 79.918%, H = 0.277, I = 0.417) among 23 individuals of May nuoc mo species (*Calamus* sp.) distributed in Dong Giang and Nam Giang Quang Nam. The clustering pattern shows that the 23 *Calamus* sp. individual wilds fall into three main groups comprising the entire variation and most genotypes were clustered following along the same with their geographical distribution and have differences in genetic diversity that differ significantly.

From this study, it can be concluded that the utility of the

ISSR marker in elucidating patterns of genetic variation between genotypes of 23 *Calamus* sp. individuals collected in different localities in Quang Nam, Viet Nam and in identifying genotyped water cloud individuals, can serve as a potential source of unique genetic materials for genetic improvement and conservation.

Acknowledgement

This study was funded by Hue University under grant number DHH.2025-02-195 and Hue University under the Core Research Program grant number NCTB.DHH.2024.10. This study is also a part of the collaborative activities within WWF (World Wide Fund for Nature) and IKEA Partnership in the Greater Mekong region. We are grateful to the administration of the Nam Giang and Dong Giang watershed protection forest management boards for providing us with an opportunity to carry out the field surveys.

References

1. Abdul Kareem V.K., Rajasekharan P.E., Ravish B.S., Mini S., Sane A. and Kumar T.V., Analysis of genetic diversity in *Acorus calamus* populations in South and North East India using ISSR marker, *Biochemical Systematic and Ecology*, **40**, 156-161 **(2012)**

2. Asra R. et al, The study of genetic diversity of *Daemonorops draco* (Palmae) using ISSR markers, *Biodiversitas*, **15(2)**, 109-114 **(2014)**

3. Avani K., Krishnamurthy R. and Rajkumar K., Genetic variation in the endangered Indian sweet flag (*Acorus calamus* L.) estimated using ISSR and RAPD markers, *Journal of Applied Research on Medicinal and Aromatic Plants*, **3(3)**, 112-119 **(2016)**

4. Frankham R., Genetic adaptation to captivity in species conservation programs, *Molecular Ecology*, **17(1)**, 325-333 **(2008)**

5. Govaerts R., Dransfield J., Zona S., Hodel D.R. and Henderson A., World Checklist of Arecaceae, The Royal Botanic Gardens, Kew, Available from: http://apps.kew.org/wcsp/ **(2014)**

6. Hamrick J.L. and Godt M.J.W., Allozyme Diversity in Plant Species, In Brown A.H.D., Clegg M.T., Kahler A.L. and Weir B.S., eds., Plant population genetics, breeding and genetic resources, Sinauer Associates Inc., Sunderland, MA, USA, 43-63 **(1989)**

7. Haridharan Neelamegan, SET-LRP - A Convenient Polymerization Method to Produce Rapid and Controlled Polymers, *Res. J. Chem. Environ.*, **28(4)**, 102-106 **(2024)**

8. Hieu H.K., Minh N.V., Loi N.V. and Ha H.T., Application of DNA barcode for the genetic analysis and identifying a May nuoc mo species in Quang Nam Province, Central Vietnam, *Biodiversitas*, **25(8)**, 3300-3309 **(2024)**

9. Jaccard A., Nouvelles recherches sur la distribution florale, *Bulletin de la Societe Vaudoise des Sciences Naturelles*, **44(163)**, 223-70 **(1908)**

10. Jaisankar I., Subramani T., Velmurugan A. and Singh A.K., Genetic diversity revealed among rattan genotypes from Andaman and Nicobar Islands based on RAPD and ISSR markers, *Forest Res Eng Int J.*, **1(2)**, 45-50 **(2017)**

11. Kimura M. and Crow J.F., The Number of Alleles That Can Be Maintained in a Finite Population, *Genetics*, **49**, 725-738 **(1964)**

12. Lewontin R.C., The Apportionment of Human Diversity, In Dobzhansky T., Hecht M.K. and Steere W.C., eds., Evolutionary Biology, New York, Springer, 381-398 **(1972)**

13. Lyngdoh N., Santosh S.H., Ramesha B.T., Rao M.N., Ravikanth G., Narayani B., Ganeshaiah K.N. and Uma Shaanker U., Rattan species richness and population genetic structure of *Calamusflagellum* in North-eastern Himalaya, India, *Journal of Bamboo and Rattan*, **4**, 293-307 **(2005)**

14. Meena R.K., Raj H., Sharma P., Bhandari M.S. and Ginwal H.S., Low diversity and high genetic divergence depicted the threat of genetic erosion in *Calamus nambariensis* and *Calamus tenuis*, *Indian Journal of Biotechnology*, **20**, 273-283 **(2021)**

15. Meena R.K., Raj H., Sharma P., Yadav S., Kant R. and Bhandari M.S., Assessment of genetic diversity in natural populations of *Calamus guruba* Buch.-Ham. ex Mart. using ISSR marker, *Tropical Plant Research*, **5(2)**, 250-259 **(2018)**

16. Munankarmi N.N., Rana N., Bhattarai T., Shrestha R.L., Joshi B.K., Baral B. and Shrestha S., Characterization of the Genetic Diversity of Acid Lime (*Citrus aurantifolia* (Christm.) Swingle) Cultivars of Eastern Nepal Using Inter-Simple Sequence Repeat Markers, *Plants*, **7**, 46 **(2018)**

17. Nei M., Analysis of gene diversity in subdivided populations, Proc. Natl. Acad. Sci. USA, **70(12)**, 3321-3 **(1973)**

18. Nei M., Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals, *Genetics*, **89**, 583-590 **(1978)**

19. Nybom H. and Bartish I.V., Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants, *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 293-114 **(2000)**

20. Pal D., Malik S.K., Kumar S., Choudhary R., Sharma K.C. and Chaudhury R., Genetic Variability and Relationship Studies of Mandarin (*Citrus reticulata* Blanco) Using Morphological and Molecular Markers, *Agric Res.*, **2**, 236-245 **(2013)**

21. Peters C.M. and Henderson A., System, ecology and management of rattans in Cambodia, Laos and Vietnam, The biological bases of sustainable use **(2014)**

22. Porth I. and El-Kassaby Y.A., Assessment of the Genetic Diversity in Forest Tree Populations Using Molecular Markers, *Diversity*, **6**, 283-295 **(2014)**

23. Priya K., Indira E., Sreekumar V.B. and Renuka C., Assessment of genetic diversity in *Calamus vattayila* Renuka (Arecaceae) using ISSR markers, *Journal of Bamboo and Rattan*, **15(1-4)**, 61-69 **(2016)**

24. Ramesha B.T., Ravikanth G., Rao M.N., Ganeshaiah K.N. and Umashaanker R., Genetic structure of the rattan *Calamus thwaitesii* in core, buffer and peripheral regions of three protected areas in central Western Ghats, India: do protected areas serve as refugia for genetic resources of economically important plants, *Journal of Genetics*, **86**, 9-18 **(2007)**

25. Rao A.N. and Rao V.R., eds., Bamboo conservation, diversity, ecogeography, germplasm, resource utilization and taxonomy, Proceedings of training cum workshop (Plant genetic resources conservation and use in China), 10-17 May, 1998, Kunming and Xishuangbanna Yunnan, China, 275 **(2000)**

26. Ravikanth G., Ganeshaiah K.N. and Uma Shaanker R., Mapping genetic diversity of rattans in Central Western Ghats: identification of hot-spots of variability for *in situ* conservation, In Uma Shaanker R., Ganeshaiah K.N. and Bawa K.S., eds., Forest genetic Resources: Status, Threats and Conservation Strategies, Oxford and IBH Publishing Co. Pvt. Ltd., 69-83 **(2001)**

27. Renuka C., Indira E.P. and Muralidharan E.M., Genetic diversity and conservation of certain species of rattan in Andaman and Nicobar Islands and southern India, KFRI Research Report, 157 **(1998)**

28. Renuka C., Status of rattan resources and uses in South Asia, In Dransfield J., Tesoro F.O. and Manokaran N., eds., Rattan current research issues and prospects and sustainable development, FAO, Rome, Italy, 101-114 **(2002)**

29. Vaze A., Nerkar G., Pagariya M., Devarumath R.M. and Theertha P.D., Isolation and pcr amplification of genomic DNA from dry leaf samples of Sugarcane, *International Journal of Pharma and Bio Sciences*, **1(2)**, 1-6 **(2010)**

30. Verma S., Karihaloo J.L., Tiwari S.K., Magotra R. and Koul A.K., Genetic diversity in Eremostachys superba Royle ex Benth. (Lamiaceae), an endangered Himalayan species, as assessed by RAPD, *Genet Resour Crop Evol*, **54**, 221-229 **(2007)**

31. Yeh F.C., Population genetics, In Forest Conservation Genetics, Principles and Practice, Young A., Boshier D. and Boyle T., ed., CSIRO Publishing, United Kingdom **(2000)**.

(Received 29th July 2025, accepted 03rd October 2025)