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Genetic Diversity Analysis Based on Retrotransposon Microsatellite Amplification Polymorphisms (REMAP) for Distinguishing the Ginger Chemotype of Thua Thien Hue (*Zingiber Officinale* Roscoe) from other Vietnamese Ginger Types

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Abstract

The ginger of Thua Thien Hue ("Hue" for short) is considered by local people as a chemotype that is different from other Vietnamese ginger populations. However, there is no molecular evidence supporting this statement. Thus, our purpose is to find whether there is a genetic difference between Hue's ginger and other ginger types by using Retrotransposon Microsatellite Amplification Polymorphisms (REMAP). The results of our two cluster analyses (Unweighted Pair Group Method with Arithmetic mean (UPGMA) and Principal Coordinates Analysis (PCoA)) proved that they could separate Hue's ginger samples (in Thuy Bieu Ward, Hue City) from other populations. Therefore, according to these genetic analyses, Hue's ginger is markedly different from other ginger types. Based on our results, future studies could be conducted with more Vietnamese ginger DNA samples to provide stronger evidence about the unique genomic features of Hue's ginger.

Keywords: genetic diversity, Hue's ginger, Retrotransposon Microsatellite Amplification Polymorphisms, *Zingiber officinale* Roscoe
Abbreviations: **%P** (percentage of polymorphic band), **AMOVA** (Analysis of molecular variances), **BSA** (bovine serum albumin), **df** (degree of freedom), **EP** (Eppendorf), **Fis** (inbreeding coefficient), $\mathbf{H_e}$ (expected heterozygosity), **I** (Shanon's diversity index), **IRAP** (inter-retrotransposon amplified polymorphism), **ISSR** (Inter-Simple Sequence Repeat), **LTR** (Long Terminal Repeat), **MI** (Marker Index), **MS** (Mean of Squares), **N** (number of bands), $\mathbf{N_a}$ (observed number of allele), $\mathbf{N_e}$ (effective number of allele), $\mathbf{N_p}$ (number of polymorphic bands), **PCI** (phenol, chloroform, isoamyl alcohol), **PCoA** (Principal Coordinates Analysis), **PCR** (Polymerase Chain Reaction), **PIC** (Polymorphism Information Content), **RAPD** (Randomly amplified polymorphic DNA), **REMAP** (Retrotransposon Microsatellite Amplification Polymorphisms), **Rp** (Resolving power), **SM** (simple matching), **SS** (Sum of Squares), **S-SAP** (Sequence-Specific Amplified Polymorphism), **SSR** (Simple Sequence Repeat), **UPGMA** (Unweighted Pair Group Method with Arithmetic mean)

1. Introduction

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) has many important bioactive compounds (gingerol, paradol, shogaol, zingerone, zerumbone, terpenoids, and ginger flavonoids) proved to have remarkable medical effects, including antioxidant activity, antiemetic action, reducing inflammation, antimicrobial activity, hepato-protection, and antitumor activity (Alqasoumi *et al.*, 2011; Chung *et al.*, 2009; Chung *et al.*, 2001; Galal, 1996; Kim *et al.*, 2011; Kirana *et al.*, 2003; Ling *et al.*, 2010; Liu *et al.*, 2012; Manjunatha *et al.*, 2013; Masuda *et al.*, 2004; Park *et al.*, 2008; Rahmani *et al.,* 2014; Shin *et al.*, 2005).

People in Thua Thien Hue (central Vietnam) have long believed that the ginger population in this province ("Hue's ginger" for short) is a unique chemotype. This belief is in line with the study results of Hien *et al.* (2018) and Stoyanova *et al.* (2006). In all nine comparable substance concentrations, six substances in Hue's ginger have higher amounts than the ones in the unknown ginger type. Specifically, the concentrations of α-pinene, camphene, α-zingiberene, copaene, β-bisabolene, and β-sesquiphellandrene in Hue's ginger and the ginger with unknown origin are 3.81 % - 0.70 %, 11.52 % - 2.50 %, 32.52 % - 10.30 %, 2.10 % - 0.80 %, 5.54 % - 4.10 %, and 11.37 % - 7.40 %, respectively (Hien *et al.*, 2018; Stoyanova *et al.*, 2006). However, Hue's ginger identification is widely based on the rhizome size. This morphological marker and the mentioned chemical markers make selecting the chemotype for cultivation and oil extraction extremely confusing since they depend on environmental factors (Beleke and Beleke, 2014; Liu *et al.*, 2016). Therefore, today, only the people in Thuy Bieu Ward (Hue City) can declare that their ginger rhizomes are Hue's ginger. Molecular markers can reliably point out an individual's genomic features (Paterson *et al.*, 1991). Thus, they can overcome the mentioned detrimental drawback of morphological and chemical markers and can be used for correctly selecting the ginger chemotype. Unfortunately, there has been no research on determining Hue's ginger at the molecular scale.

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Retrotransposon microsatellite amplification polymorphism (REMAP) is an efficient technique involving the polymerase chain reaction (PCR) amplification, with a long terminal repeat (LTR) primer and a simple sequence repeat (SSR) or inter-simple sequence repeat (ISSR) primer (Kalendar and Schulman, 2006; Pandotra *et al.,* 2014). REMAP has been efficiently used for cultivar characterizations of many species, including barley (*Hordeum vulgare*), grapevine (*Vitis vinifera* L.), olive (*Olea europaea* L.), and rice (*Oryza sativa* L.) (Branco *et al.,* 2007; Kalendar *et al.*, 1999; Kaya and Yilmaz-Gokdogan, 2016; Strioto *et al.,* 2019; Šimon *et al.*, 2010). In terms of distinguishing ginger cultivars, REMAP can discriminate all the 92 ginger landraces (with a high variation rate of 96.5 %) in the study of Pandotra *et al.* (2014). The authors also demonstrated that REMAP was one of the best markers in all examined ones (ISSR, randomly amplified polymorphic DNA - RAPD, inter-retrotransposon amplified polymorphism - IRAP, and REMAP) (Pandotra *et al.*, 2015).

Considering the lack of scientific evidence for identifying Hue's ginger and the competence of REMAP, this research was conducted to find whether Hue's ginger chemotype is genetically different from the kinds of ginger grown in other regions of Vietnam.

2. Materials and methods

2.1. Plant materials

Thirty-eight (38) leaf samples, collected from Thua Thien Hue and other provinces in Vietnam, were plant materials for the research. The inner young leaves of ginger shoots, obtained from ginger fields of local people one month after sprouting time (depending on the regions), were separated for DNA extraction. Of all the samples, the local people only consider ginger samples from Thuy Bieu ward as Hue's ginger. The samples were divided into three subpopulations based on the places of sample collection (**Table 1**), namely P1 (samples in Thuy Bieu), P2 (samples in other regions of Thua Thien Hue), and P3 (samples in several other provinces in Vietnam). The subpopulations were only for genetic diversity analyses to find if P1 could be separated from P2 and P3. P1, P2, and P3 were not real subpopulations in population genetics. The regions of collecting samples are illustrated in **Figure 1**.

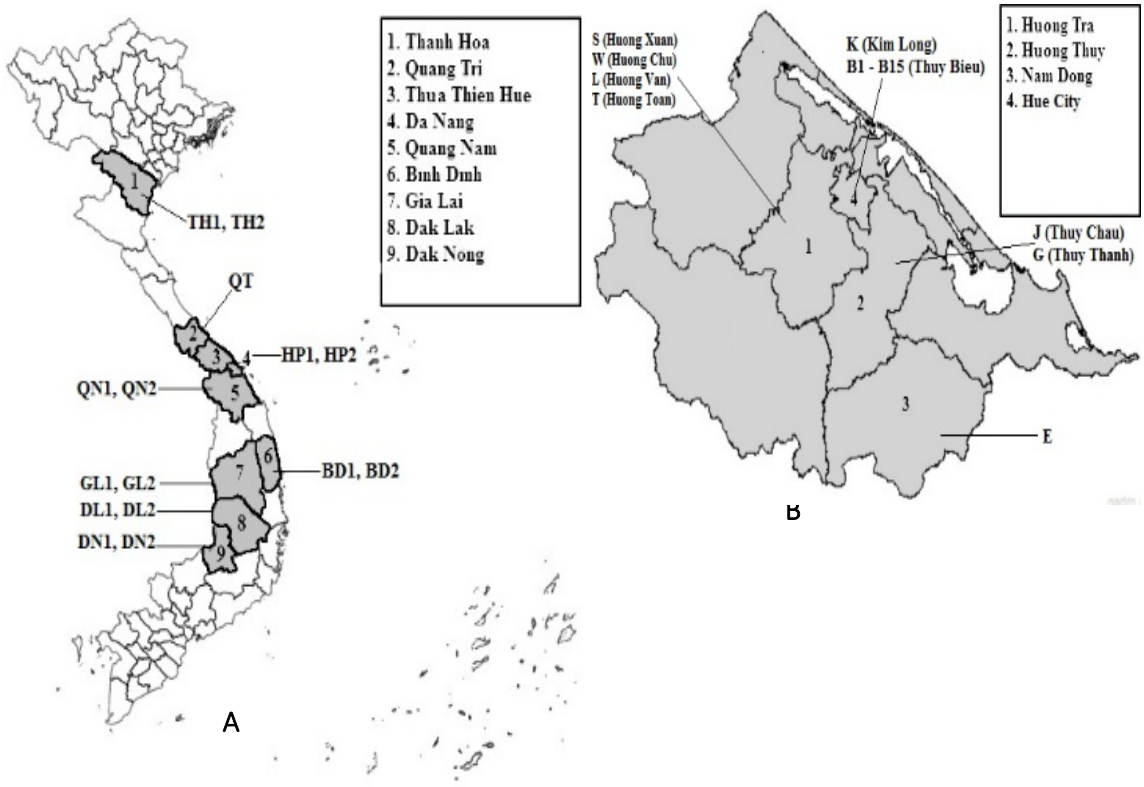

Figure 1. Provinces in Vietnam - Thanh Hoa, Quang Tri, Thua Thien Hue, Da Nang, Quang Nam, Binh Dinh, Gia Lai, Dak Lak, and Dak Nong (**A**) and regions in Thua Thien Hue - Huong Tra, Huong Thuy, Nam Dong, and Hue City (**B**) from which ginger samples were collected (the number of samples and sample codes are illustrated in the figure).

Table 1. The list of ginger leaf samples (only the samples from Thuy Bieu were the unique chemotype of Thua Thien Hue)

Province	District/City	Ward	Number of sample(s)	Sample codes	Sub-population	Coordinates
Thua Thien Hue	Hue City	Thuy Bieu	15	From B1 to B15	P1	16.4446° N, 107.5511° E
		Kim Long	1	K	P2	16.4652° N, 107.5605° E
	Huong Tra	Huong Xuan	1	S		16.4770° N, 107.4766° E
		Huong Van	1	L		16.4972° N, 107.4736° E
		Huong Toan	1	T		16.5128° N, 107.5348° E
		Huong Chu	1	W		16.4931° N, 107.5209° E
	Huong Thuy	Thuy Chau	1	J		16.4355° N, 107.6630° E
		Thuy Thanh	1	G		16.4652° N, 107.6416° E
	Nam Dong	Thuong Long	1	E		16.0755° N, 107.6275° E
Gia Lai	Mang Yang	Lo Pang	2	GL1, GL2	P3	13.9367° N, 108.2637° E
Thanh Hoa	Thuong Xuan	Luan Thanh	2	TH1, TH2		19.8015° N, 105.4127° E
Quang Nam	Tien Phuoc	Tien Son	2	QN1, QN2		15.5804° N, 108.2674° E
Dak Lak	Krong Nang	Tam Giang	2	DL1, DL2		12.9749° N, 108.3920° E
Dak Nong	Dak Song	Dak N'Drung	2	DN1, DN2		12.2160° N, 107.5624° E
Da Nang	Cam Le	Hoa Phat	2	HP1, HP2		16.0287° N, 108.1725° E
Quang Tri	Cam Lo	Cam Tuyen	1	QT		16.8229° N, 106.9241° E
Binh Dinh	An Lao	An Tan	2	BD1, BD2		14.5748° N, 108.8849° E

2.2. Genomic DNA extraction

Total genomic DNA of the samples was extracted by using the protocol of Doyle and Doyle (1987) (modified). RNA in the genomic DNA solutions was digested by 1 μL of RNase (100 μg/μL).

200 mg of washed and cut ginger leaves was homogenized by using liquid nitrogen. Then, the leaf powder was transferred into a 1.5 mL Eppendorf (EP) tube.

Next, 1 mL of extraction buffer (200 nM Tris-HCl, pH 8.0; 50 mN ethylenediaminetetraacetic acid; 2 % hexadecyltrimethylammonium bromide, 2.5 % polyvinylpyrrolidone; 1.4 M NaCl) and 20 μL of 100 % 2-mercaptoethanol were added into each EP tube. After that, tubes were vortexed for two minutes before being incubated at 65°C for 3 hours.

After the incubation, 700 μL of the supernatant liquid, obtained by centrifugation (13000 rpm, 4°C, 15 minutes), was mixed with an equal volume of PCI (25 phenol : 24 chloroform : 1 isoamyl alcohol). The mixture was then centrifuged under the same condition. 500 μL of the transparent top liquid was collected after the centrifugation.

The DNA precipitation process was then done by mixing 500 μL of 100 % isopropanol with the above-mentioned transparent liquid. After that, the mixture was incubated overnight at - 40°C.

The precipitated total genomic DNA would be collected on the next day by centrifugation (13000 rpm, 4°C, 15 minutes). DNA pellets were then washed two times by using 500 μL of cold 70% ethanol.

30 μL of autoclaved double distilled water was used to dissolve the DNA, which had been dried at ambient temperature.

2.3. Primer selection

Ten REMAP primer pairs (Baruah *et al.,* 2019; Pandotra *et al.*, 2014) were screened (**Table 2**). Each tested primer pair was used to amplify seven randomly chosen DNA samples from distinct regions. The PCR steps were as follows:

Initial denaturation at 94°C for two minutes,

Forty-four (44) amplification cycles: one minute at 94°C, one minute at 45 – 64°C (depending on primers' melting points), and two minutes at 72°C,

Final extension at 72°C for ten minutes.

Each PCR tube contained the following compositions:

1 μL of genomic DNA (35 ng),

1 μL of RT-6 primer (10 μM),

1 μL of ISSR/SSR primer (10 μM),

12.5 μL of GoTaq Green Master Mix 2X (Promega, USA),

9.5 μL of bovine serum albumin (BSA) (0.421 mg/mL).

PCR products were observed on a 1.6 % agarose gel containing SafeView™ DNA stain (Applied Biological Materials Inc., USA). Primer pairs with the highest indices, namely polymorphism information content (*PIC*), resolving power (*Rp*), marker index (*MI*), number of bands (*N*), and number of polymorphic bands (N_p) (Chesnokov and Artemyeva, 2015; Kumar *et al.,* 2014), were chosen for REMAP analysis.

Table 2. The list of primers used for the screening process

Primer codes	5' – 3' sequences	References
RT-6/RM-2	GATAGGGTCGCATCTTGGGCGTGAC/ TCAGCTTCTGGCCGGCCTCCTC	Baruah *et al.*, 2019 ; Pandotra *et al.*, 2014
RT-6/RM-3	GATAGGGTCGCATCTTGGGCGTGAC/ GCCTCGAGCATCATCATCAG	
RT-6/RM-4	GATAGGGTCGCATCTTGGGCGTGAC/ ATCAACCTGCACTTGCCTGG	
RT-6/RM-125	GATAGGGTCGCATCTTGGGCGTGAC/ AGGGGATCATGTGCCGAAGGCC	
RT-6/RM-130	GATAGGGTCGCATCTTGGGCGTGAC/TTCCCTGTTAAGAGAGAATC	
RT-6/ISSR-2	GATAGGGTCGCATCTTGGGCGTGAC/$(AG)_8$T	
RT-6/ISSR-4	GATAGGGTCGCATCTTGGGCGTGAC/$(GT)_8$CTC	
RT-6/ISSR-11	GATAGGGTCGCATCTTGGGCGTGAC/AGG$(TC)_7$	
RT-6/ISSR-42	GATAGGGTCGCATCTTGGGCGTGAC/$(AG)_8$CA	
RT-6/ISSR-72	GATAGGGTCGCATCTTGGGCGTGAC/$(GGAGA)_3$	

2.4. REMAP analysis

The selected primer pairs were utilized for the amplification of all 38 genomic DNA samples, with PCR conducted similarly to the primer screening process. The amplified products were then also be viewed on a 1.6 % agarose gel containing SafeView^{TM} DNA stain.

2.5. Data analysis

2.5.1. Band scoring

Amplified products of 38 samples were scored to create a binary matrix. Obvious bands were considered as "1" while absent bands were scored as "0".

2.5.2. Analysis of molecular variances (AMOVA)

The AMOVA (999 permutations) was done by using GenAlEX 6.51 software (Peakall and Smouse, 2006), with the binary matrix as input data, to examine genetic diversity within and among the subpopulations.

2.5.3. Parameters Calculation

The indices for primer pair assessments were manually calculated by using the following formulas (Chesnokov and Artemyeva, 2015; Kumar *et al.*, 2014):

$$PIC = \frac{1}{N} \times \sum_{i=1}^{N} 2f_i \times (1 - f_i) \quad (1)$$

$$Rp = \sum_{i=1}^{N} I_i; I_i = 1 - 2 \times \left| \frac{1}{2} - f_i \right| \quad (2)$$

$$MI = \frac{N_p^2}{N} \times PIC \quad (3)$$

Where f_i is the frequency of the i^{th} allele, N_p is the number of polymorphic bands, N is the total number of bands.

The mentioned binary matrix was also used as the input data for POPGENE software (version 1.32) (Yeh *et al.*, 2000) to calculate diversity parameters, namely expected heterozygosity (H_e), Shanon's diversity index (I), the observed number of allele (N_a), and the effective number of allele (N_e). Genetic diversity parameters were calculated on the Hardy-Weinberg disequilibrium assumption, with an inbreeding coefficient of 1 (F_{is} = 1) estimated based on the method of Dasmahapatra *et al.* (2008).

2.5.4. Cluster analysis

NTSYS version 2.1 (Rohlf, 2000) and GenAlEX 6.51 (Peakall and Smouse, 2006) software packages were used for building an UPGMA dendrogram, with simple matching (SM) genetic distance coefficient (Sokal and Michener, 1958), and PCoA graphs from the distance matrix, generated from the raw binary data.

3. Results

3.1. Primer selections

Of all ten REMAP primer pairs, only four pairs (RT-6/RM-2, RT-6/RM-125, RT-6/RM-130, and RT-6/ISSR-2) for which *PIC*, *Rp*, *MI*, *N*, and N_p were the highest and ranged from 0.35 – 0.43, 2.86 – 5.14, 1.88 – 3.51, 5 – 10, and 5 – 10, respectively, were selected. (**Table 3**).

Table 3. Indices for primer pair assessments

Primer codes	*PIC*[a]	*Rp*	*MI*	*N*	N_p	Percentage of polymorphic bands (%)
RT-6/RM-2	**0.38**	**2.86**	**1.88**	**5**	**5**	**100**
RT-6/RM-3	0.00	0.00	0.00	3	0	0.00
RT-6/RM-4	0.15	0.67	0.05	3	1	33.33
RT-6/RM-125	**0.43**	**4.86**	**3.02**	**7**	**7**	**100**
RT-6/RM-130	**0.35**	**5.14**	**3.51**	**10**	**10**	**100**
RT-6/ISSR-2	**0.40**	**4.29**	**2.78**	**7**	**7**	**100**
RT-6/ISSR-4	0.33	2.00	0.75	4	3	75.00
RT-6/ISSR-11	0.15	0.67	0.05	3	1	33.33
RT-6/ISSR-42	0.22	1.33	0.22	4	2	50.00
RT-6/ISSR-72	0.33	2.00	0.75	4	3	75.00

[a]*Note: PIC (Polymorphism Information Content), Rp (Resolving power), MI (Marker Index), N (total number of bands), N_p (number of polymorphic bands).*

3.2. REMAP analysis

The total number of amplicons from REMAP analysis was 29. Additionally, the selected REMAP primer pairs produced a high proportion of polymorphic loci (100 %). There were six region-specific bands generated, namely RT-6/ISSR-2-1134 and RT-6/RM-130-238 of Thuy Bieu samples; RT-6/RM-125-621, RT-6/RM-130-889, and RT-6/RM-130-765 of HP2 (Da Nang); and RT-6/RM-130-250 of QT (Quang Tri) (**Table 4**).

Table 4. Results of REMAP analysis using the four selected primer pairs to amplify 38 samples.

Primers' codes	N^a	N_p	%P	Amplicon lengths (bp)	Region-specific bands (bp)	Samples containing the region-specific bands
RT-6/ISSR-2	7	7	100	250 – 1134	1134	B1, B3-B6, B10-B14
RT-6/RM-2	5	5	100	409 – 941	-	-
RT-6/RM-125	7	7	100	229 – 1446	621	HP2
RT-6/RM-130	10	10	100	238 – 1461	889	HP2
					765	HP2
					250	QT
					238	B9, B11
Total:	**29**	**29**	**100**			

[a]*Note: N (total number of bands), N_p (number of polymorphic bands), %P (percentage of polymorphic bands)*

3.3. Analysis of molecular variances (AMOVA)

As is highlighted in **Table 5**, the proportion of variance among populations was 35 %, while the diversity within populations accounted for 65 % of the total variation ($P < 0.01$).

Table 5. Analysis of Molecular Variance (AMOVA) of REMAP

Source	*df* [a]	*SS*	*MS*	Variance component	Percentage of total variance (%)	*Phi*PT	*P*
Among populations	2	57.04	28.52	2.06	35	0.355	0.001**
Within populations	35	131.12	3.75	3.75	65		
Total	37	188.16		5.81	100		

[a]*Note: df: degree of freedom; SS: sum of squares; MS: mean of squares*

3.4. Genetic diversity parameters

It is clarified in **Table 6** that the diversity indices of P1, P2, and P3 were low, with H_e, *I*, N_a, N_e, and *%P* of 0.22 – 0.29, 0.35 – 0.43, 1.76 – 1.79, 1.36 – 1.49, and 75.86 % – 79.31 %, respectively. Moreover, several parameters reached the minimum values when data from P3 were used to calculate them (H_e of 0.22, *I* of 0.35, and N_e of 1.36).

Table 6. Genetic diversity parameters of the three ginger subpopulations, calculated on the Hardy – Weinberg disequilibrium assumption ($F_{is} = 1$)

Parameters	P1	P2	P3
H_e [a]	0.25 (0.19)	0.29 (0.17)	0.22 (0.18)
I	0.38 (0.26)	0.43 (0.25)	0.35 (0.24)
N_a	1.76 (0.44)	1.79 (0.41)	1.79 (0.41)
N_e	1.42 (0.37)	1.49 (0.33)	1.36 (0.34)
N_p	22	23	23
%P	75.86 %	79.31 %	79.31 %

[a]*Note: H_e: expected heterozygosity; I: Shanon's information index; N_a: observed number of alleles; N_e: effective number of alleles; N_p: number of polymorphic loci; %P: percentage of polymorphic loci; The values in the table are mean (standard deviation).*

3.5. Cluster analysis

3.5.1. UPGMA dendrogram

There were two clusters (A and B) obtained from building the UPGMA dendrogram. Almost the ginger samples from P1 were clustered in A (B1, B3, B4, B5, B6, B11 in A1-1; B10, B12, B13, B14, B15 in A1-2; and B2, B9 in A2). All samples from P2 and P3 (except K, S, E, and BD1) were grouped in B. The genetic distance between A and B was 47.00 % (**Figure 2**).

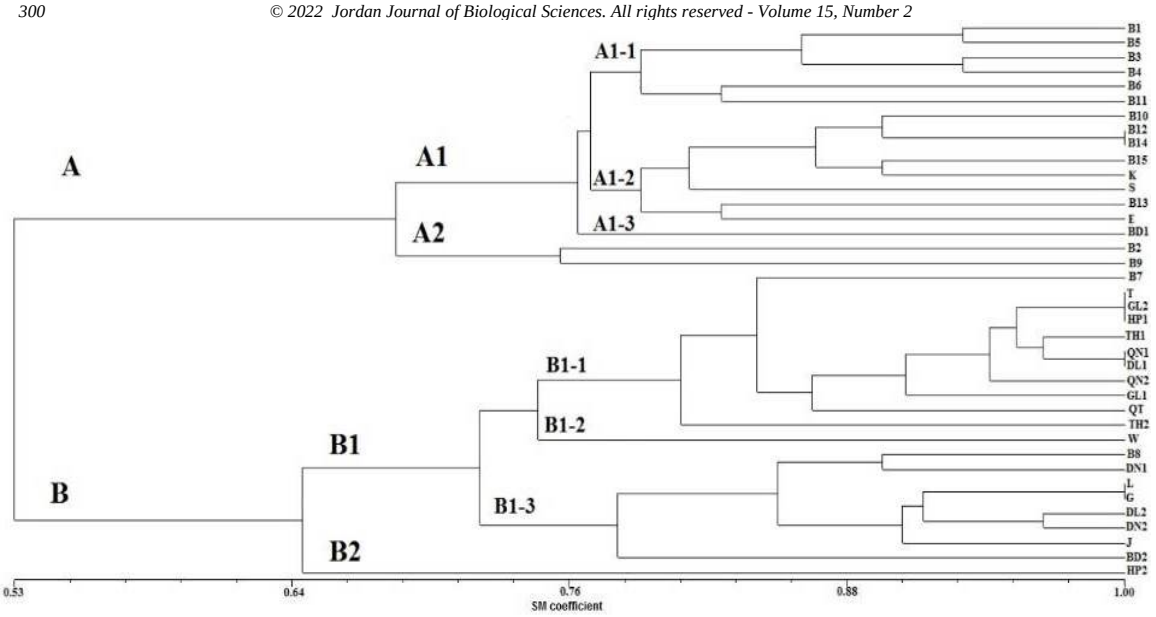

Figure 2. Clusters formed by UPGMA analysis. Cluster A was divided into A1 (containing A1-1, A1-2, and A1-3) and A2. Cluster B also has two groups, namely B1 (with three subclusters – B1-1, B1-2, and B1-3) and B2. Ginger samples from P1 (Hue's ginger) were in cluster A except for B7 and B8

3.5.2. Principal coordinates analysis (PCoA)

Samples in PCoA graphs were divided into three distinct clusters (I, II, and III), with almost P1's samples in cluster I, except B2, B7, and B8. This result demonstrates that the samples from Thuy Bieu have unique genomic features separating this subpopulation from other clusters. Cluster II contained ten samples from all subpopulations (P1, P2, and P3), while only samples from P3 were grouped in cluster III (**Figure 3**).

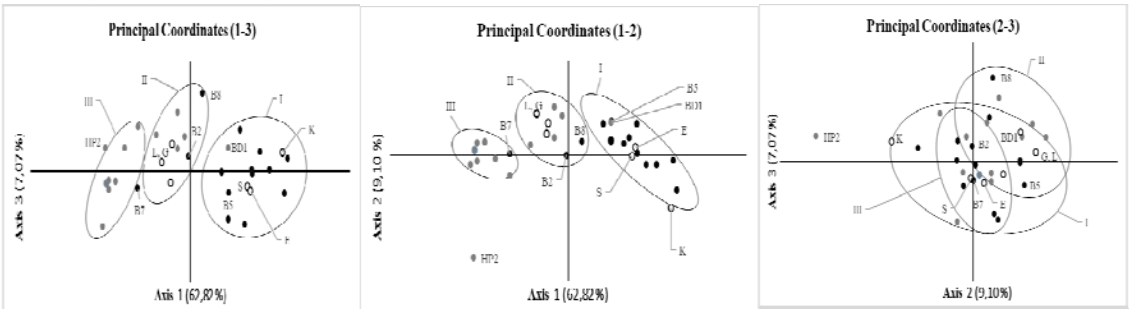

Figure 3. PCoA graphs with three separate clusters (I, II, and III). Samples from P1 were grouped in cluster I. This indicates that ginger from Thuy Bieu was genetically different from ginger grown in other regions. The first three planes of PCoA (1-2, 1-3, and 2-3) reveal 71.92 %, 69.89 %, and 16.17 % of the variations of the three subpopulations, which are P1 (black points), P2 (white points) and P3 (gray points).

4. Discussion

The UPGMA dendrogram supported the conclusion that the ginger samples from P1 were genetically different from the samples of P2 and P3. Specifically, 13 in 15 samples of P1 were grouped in a separated cluster (A), while most of the other samples were in cluster B, with a significant genetic distance between them (47.00 %) (**Figure 2**). The PCoA graphs well supported this result. Samples of P1 formed cluster I separated from cluster II and cluster III, containing other samples (**Figure 3**). However, three P1 samples (B2, B7, and B8) were not in cluster I. Lovell *et al.* (2017) reported that individuals in asexual populations could harbor DNA mutations. Therefore, the mutations accumulated in P1 might cause the difference between those three samples and the other P1 samples.

Choosing the suitable primer pairs played a key role in achieving the above-mentioned positive results.

First, we selected the RT-6 primer (also known as sukkula or sukkula 9900 primer) for our analyses. RT-6 was one of the best retrotransposon primers for producing the highest number of bands in the studies of Leigh *et al.* (2003) and Muhammad and Othman (2005). Muhammed and Othman (2005) observed the maximum number of 13 PCR products when the authors used RT-6 as an IRAP marker in characterizing *Fusarium* wilt-resistant and *Fusarium* wilt-susceptible *Musa* AAB soma clones. Moreover, Leigh *et al.* (2003) obtained one of the highest numbers of PCR products (28) by using RT-6 in the research on sequence-specific amplified polymorphism (S-SAP) markers. The maximum number of bands produced by the primer pairs containing RT-6 in the present study was only ten (**Table 4**). However, all bands generated were polymorphic, proving the prominence of RT-6 primer.

Second, our SSR and ISSR primers, selected from the studies of Pandotra *et al.* (2014) and Baruah *et al.* (2019),

were also proved as efficient primers for ginger genetic diversity analysis. Specifically, Pandotra *et al.* (2014) concluded that RT-6/RM-2, RT-6/RM-3, RT-6/RM-4, RT-6/RM-125, and RT-6/RM-130 were among the best primer pairs, with *PIC*, *Rp*, *MI*, N_p, and *%P* of 0.17 – 0.31, 7.84 – 10.92, 3.06 – 4.14, 17 – 23, and 89.47 – 100 %, respectively. Additionally, ISSR-2, ISSR-4, ISSR-11, ISSR-42, and ISSR-72 were also the optimal ISSR primers in the study of Baruah *et al.* (2019), with the *Np*, *%P*, *MI*, *Rp*, and *PIC* values ranging from 5 – 9, 81.8 – 100 %, 2.56 – 4.15, 3.57 – 4.9, and 0.51 – 0.68, respectively. However, our primer screening process revealed only four primer pairs (RT-6/ISSR-2, RT-6/RM-2, RT-6/RM-125, and RT-6/RM-130), which were efficient enough for genetic analyses of Vietnamese ginger (**Table 3**). Additionally, using the same primers/primer pairs, the number of polymorphic bands in our study (29) was lower than in the studies of Baruah *et al.* (2019) (32) and Pandotra *et al.* (2014) (61). The genetic variations between the Vietnamese ginger samples and the ginger samples used in the mentioned authors' studies might cause those differences in the results. Though the number of bands in our study was low, all the four selected primer pairs produced the absolute polymorphic rate (100 %). Moreover, the region-specific band (1134 bp), produced by the primer pair RT-6/ISSR-2, was one of the main factors discriminating Thuy Bieu samples from other samples (**Table 4**). Thus, the selected primer pairs satisfactorily fulfilled the expectations of our study.

Because of using the effective primer pairs, our AMOVA results could clearly show the genetic variations among the three subpopulations (**Table 5**). This suggests that REMAP is appropriate for distinguishing the three ginger subpopulations. Specifically, 35 % of the total variations occurred among subpopulations. However, REMAP's ability to reveal differences among populations depends on the species. Noormohammadi *et al.* (2016) found that 33 % of the variations took place among *Gossypium* spp. populations. In contrast, Tanhuanpaa *et al.* (2016) reported that REMAP could only point out a low proportion of variation arising among *Phleum pratense* L. populations (10 %).

From the supportive results of PCR amplification and AMOVA, we conducted the cluster analyses (UPGMA and PCoA) and came to the expected conclusion as described at the beginning of the section.

In parallel, genetic diversity parameters (H_e, *I*, N_a, N_e) of the subpopulations were also calculated. The values in our study were low. Ginger's asexual reproduction might be the cause of the low diversity indices. Barrett *et al.* (2008) and Qiu *et al.* (2005) proved the effect of lowering diversity parameters of this kind of reproduction in studies on other species, namely *Melampsora lini and Dysosma versipellis* (Berberidaceae) (**Table 6**).

The diversity parameters observed in P1 and P2 were higher than in P3. This finding demonstrates that REMAP might be effective for genetic variation assessments of samples collected from regions adjacent to one another (**Table 6**). In contrast, Das *et al.* (2017), Kavyashree (2008), and Kizhakkayil and Sasikumar (2010) concluded that there was a correlation between geographical distance and genetic diversity (populations being geographically far from one another would have high genetic diversity among them).

5. Conclusions

Using REMAP markers, we concluded that the ginger population grown in Thuy Bieu, which has long been known as the special ginger chemotype of Thua Thien Hue, is genetically different from populations in other regions of Thua Thien Hue and Vietnam.

The positive results of our research are precursors for future studies, with more ginger DNA samples. Specifically, samples should be collected from a higher number of regions in Thua Thien Hue and other Vietnamese provinces. Moreover, the number of leaf samples in each region should also be increased. Those increments will provide stronger evidence about the unique genomic features of Hue's ginger in Thuy Bieu after a genetic diversity analysis.

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