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Characterizing genetic variation of two popular durian (*Durio zibethinus* L.) varieties in southern Vietnam by using ISSR markers

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Molecular markers have been successfully used for genetic diversity characterization of durian (*Durio zibethinus* L.) worldwide. In this study, a total of 25 ISSR markers were used to characterize the genetic richness of 22 durian genotypes belonging to two most preferred varieties from different provinces in southern Vietnam consisting of Monthong and Ri6. High polymorphic levels were found with a total of 166 per 175 generated bands. ISSR marker revealed high polymorphism information content (PIC) up to 0.82, suggesting that this marker is effective for determining genetic variation of durian. Furthermore, the genetic composition of these two durian cultivars in Vietnam is highly variable. The dendrograms generated by clustering and PCoA analysis were able to distinguish the accessions genetically. The obtained results provide molecular biological information for classification, identification plant origins, breeding, and conservation programs; furthermore, utilization of molecular marker analysis could provide new insights to breeders for molecular-assisted selection of durian.

Keywords: Durian; Genetic diversity; ISSR; Monthong; Ri6.

INTRODUCTION

Durian is a famous and special tropical fruit tree in Southeast Asia. There are currently 30 species of *Durio* sp. identified, including nine edible species, but only *Durio zibethinus* L. species is marketed because of the high nutritional value and economic value (Amid et al. 2012). Traditionally, breeders and consumers classify durian mostly base on plant and fruit morphology such as taste; weigh; colour; and peel of fruit. Despite of ease and economic advantages, the morphological indicators do not bring high accuracy because the phenotypes are highly influenced by environmental conditions such as climate, weather, soil condition, regime of plant care and plant age (Sew et al. 2018; Angeliena et al.2019).

Molecular markers have been proven as a potential substitution for morphological identification with several advantages, such as unlimited in number, unaffected by environment and growing conditions, easy to interpret with reliable repeatable results (Antunes et al.1997). Numerous molecular markers have been developed such as isozyme, Restriction Fragment Length Polymorphism (RFLP), Random Amplified of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple sequence Repeat (SSR), Inter-Simple sequence Repeat (ISSR), and Single Nucleotide Polymorphisms (SNP). Among which, ISSR is highly preferred because it is PCR-based markers and possess several advantages such as simple, rapid, economic, require minimum laboratory skill, require small DNA quantity, high number of fragments in each reaction, and do not require prior knowledge of genetic genome of targeted plants. This marker is more reproducible marker

than RAPD (Wolfe and Liston, 1998) leading to expanded use in genetic diversity research, population genetic studies, genetic markers, crop identification, breeding in different plants such as potato (Bornet et al. 2002); sugar beet1 (Izzatullayeva et al. 2014); bitter gourd (Singh et al. 2015) and mango (Mansour et al. 2014). ISSR markers has also been used widely to study the genetic richness of durian in several countries in Southeast Asia such as Thailand (Vanijajiva et al. 2012); Indonesia (Handayani et al. 2017); and Malaysia (Husin et al. 2018).

In Vietnam, durian is grown mostly in southern region. As of 2015, the whole country has about 17,000 hectares. Among several durian cultivars being cultivated, but the most common are Chin Hoa, Monthong, Kho Qua Xanh, Chuong Bo, Ri6, Sau Huu. However, only Ri6 and Monthong are highly prepared and focused to develop due to their superior quality and economic value. To date, nevertheless, only a limited studies reported genetic composition of durian in this country. In 2014, Nguyen and colleagues reported the highly genetically variation of 10 durian accessions collected in Lam Dong province based on morphology and RAPD markers (Nguyen *et al.*, 2014). More recently, a broader study to compare the genetic relatedness of 11 durian genotypes from Vietnam with 6 genotypes from Thailand and Malaysia, the results reveal that durian collection are not grouped as geographical collection sites (Giang et al. 2016).

In present study, a total of 25 ISSR primers were used to evaluate the genetic diversity of 22 durian genotypes of Monthong and Ri6 cultivars collected from different regions in southern Vietnam. The obtained results could provide more scientific information for identification, classification, propagation and breeding purposes of these two favourable durian cultivars in Vietnam.

MATERIALS AND METHODS

A total of 22 durian accessions were collected from orchards, germplasms of research institutes, and seedling centres of different provinces in southern Vietnam (Figure 1 and Table 1). After sampling, leaf samples were dried in silica gel and stored in cool place until use. Total DNA was extracted from dried durian leaves using the cetyltrimethylammonium bromide (CTAB) method described by Doyle & Doyle (1987). DNA quality was then tested by electrophoresis on 1% agarose gel in TAE 1X buffer and stained with Gelred dye (Biotium, USA). The result was observed under ultraviolet light by Quantum - ST4 3000 gel reader (Montreal - Biotech, Canada). DNA concentrations were determined by spectrophotometer (Optima SP 3000 nano UV-VIS, Japan). DNA samples were stored at -20 °C until use.

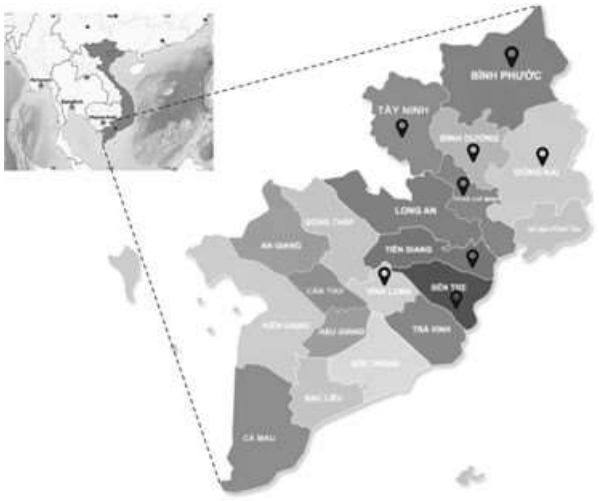

Figure 1: Targeted areas for collecting durian accessions in this study (📍: sample collection sites)

After gel electrophoresis of PCR products, only clear bands between the ranges 100 bp to 2000 bp were chosen and analysed; weak signal bands were excluded from final analysis. Since ISSR are dominant markers, at each locus, the presence of amplified band was interpreted as either a heterozygote or dominant homozygote and the absence of a band in corresponding position as recessive homozygote (Debnath *et al.,* 2007). Clearly visible ISSR amplified bands were scored as 1, whereas the absent band was scored as 0. The numbers of scored bands (SB), numbers of polymorphic bands (NPB) and percentage of polymorphic bands (PPB) were obtained. The quality information of the primers is determined by the PIC (Polymorphism Information Content) according to the formula of Chesnokov and Artemyeva (2015). Cluster analysis was performed by using Unweighted Pair Group Method with the Arithmetic mean (UPGMA). The SIMQUAL program was used to calculate the Jaccard's coefficients by using NTSYS-pc 2.1 (Rohlf, 2000). The Jaccard's coefficient was calculated as Rayar and colleagues (2015). The dendrogram was constructed on the algorithm with the SAHN module in NTSYS-pc 2.1.

Table 1: Durian samples collected for genetic characterization

No	Cultivar name	Collection site (ward- district-province)	Position	Sample code
1	Ri6	Hung Dinh-Thuan An- Binh Duong	10o56'1''N106o40'56''E	R6-HDBD
2	Ri6	Tay Hoa-Trang Bom- Dong Nai	10o56'10''N 107o2'38''E	R6-THDN
3	Monthong	Tay Hoa-Trang Bom- Dong Nai	10o56'11''N 107o2'39''E	MT-THDN
4	Ri6	Han Gon-Long Khanh- Dong Nai	10o52'38''N107o13'27''E	R6-HGDN
5	Ri6	Trung An-Cu Chi- Ho Chi Minh city	11o00'50''N 106o35'27''E	R6-TACC
6	Ri6	Phuoc Trach-Go Dau- Tay Ninh	10o19'33''N 106o5'24''E	R6-PTTN
7	Monthong	Phuoc Trach-Go Dau- Tay Ninh	10o19'29''N 106o5'29''E	MT-PTTN
8	Ri6	Phu Rieng-Phu Rieng- Binh Phuoc	11o39'53''N 106o53'58''E	R6-PRBP
9	Ri6	Thanh Binh-Vung Liem- Vinh Long	10o6'47''N106o13'39''E	R6-TBVL
10	Monthong	Thanh Binh-Vung Liem- Vinh Long	10o7'38''N 106o13'9''E	MT-TBVL
11	Ri6	Quoi Thien-Vung Liem- Vinh Long	10o7'57''N 106o12'53''E	R6-QTVL
12	Monthong	Quoi Thien- Vung Liem- Vinh Long	10o7'58''N 106o12'53''E	MT-QTVL
13	Ri6	Vinh Thanh- Cho Lach- Ben Tre	10o12'1''N 106o14'24''E	R6-VTBT
14	Monthong	Vinh Thanh- Cho Lach- Ben Tre	10o12'1''N 106o14'24''E	MT-VTBT
15	Ri6	Hung Khanh Trung B- Cho Lach- Ben Tre	10o20'44''N 106o7'56''E	R6-HKTBT
16	Monthong	Hung Khanh Trung- Cho Lach- Ben Tre	10o24'10''N 106o7'39''E	MT-HKTBT
17	Ri6	Tam Binh- Cai Lay- Tien Giang	10o18'39''N 106o7'9''E	R6-TBTG
18	Monthong	Tam Binh- Cai Lay- Tien Giang	10o18'33''N 106o7'12''E	MT-TBTG
19	Ri6	Long Trung- Cai Lay- Tien Giang	10o18'47''N 106o9'46''E	R6-LTTG
20	Monthong	Long Trung- Cai Lay- Tien Giang	10o18'47''N 106o9'46''E	MT-LTTG
21	Ri6	Vietnam Southern Fruit Research Institute (SOFRI)	10o23'50''N 106o16'47''E	R6-SOFRI
22	Monthong	Vietnam Southern Fruit Research Institute (SOFRI)	10o23'50''N 106o16'47''E	MT-SOFRI

Table 2:Sequences of 25 ISSR markers in this study

No	Primer	Sequence (5'-3')	Reference
1	UBC 880	(GGAG)4	Levi et al, 2004
2	UBC 825	(AC)8T	Levi et al, 2004
3	UBC 841	(GA)8YC	Levi et al, 2004
4	UBC 813	(CT)8T	Levi et al, 2004
5	UBC 810	(GA)8T	Levi et al, 2004
6	UBC 855	(AC)8YT	Shukla et al, 2017
7	UBC 866	(CTC)6	Shukla et al, 2017
8	UBC 888	DBD(CA)7	Shukla et al, 2017
9	UBC 834	(AG)8YT	Shukla et al, 2017
10	UBC 853	(TC)8RT	Shukla et al, 2017
11	UBC 809	(AG)8G	Shukla et al, 2017
12	UBC 814	(CT)8A	Shukla et al, 2017
13	UBC 811	(GA)8C	Shukla et al, 2017
14	UBC 829-11	(TG)8T	Kumar et al, 2015
15	UBC 814-11	(CT)7CAT	Kumar et al, 2015
16	UBC 820	(GT)8C	Kumar et al, 2015
17	UBC 854	(TC)8AGG	Singh et al, 2015
18	UBC 856	(AC)8YA	Singh et al, 2015
19	UBC 861	(ACC)6	Singh et al, 2015
20	UBC 840	(GA)8YT	Singh et al, 2015
21	UBC 890	VHV(GT)7	Singh et al, 2015
22	UBC 818	(CA)8G	Naik et al, 2017
23	UBC 826	(AC)8C	Naik et al, 2017
24	UBC 848	(CA)8RG	Naik et al, 2017
25	UBC 889	DBD(AC)7	Naik et al, 2017

Y = (C, T); B = (C, G, T); D = (A, G, T); H = (A, C, T); V = (A, C, G).
The numbers represent the repeated of corresponding motifs in parentheses.

The cut-off value of the dendrograms was determined based on method described by Jamshidi and Jamshidi (2011). Using similarity matrices, a Principal Coordinate Analysis (PCA) was carried out to construct a two-dimensional array of eigenvectors using DCENTER module of NTSYS-pc 2.1 program (Ibrahim et al. 2017).

RESULTS AND DISCUSSION

ISSR markers have been successfully used to study genetic composition of different plant species, such as potato (Bornet et al. 2002); strawberry (Debnath et al. 2007); sugar beet (Izzatullayeva et al. 2014); and bitter gourd (Singh et al. 2015). In this study, the potential ability of ISSR markers was used to examine the genetic relatedness of 22 genotypes belonging to two most popular durian varieties in southern Vietnam. After screening, only 13 of 25 ISSR primers showed clear and reproducible bands (Figure 2). The low effectiveness of using ISSR primers from different plant species for durian analysis is also supported by Maysian's group when they screened 25 ISSR primers but only 12 primers were found to be suitable for genetic study of durian (Siew et al.2018).

A total of 175 bands were generated in which 166 bands were polymorphic, accounting for 91,0% (Table 3). The size of amplified bands ranged from 100 to 2000 bp. The number of bands varied from 7 (primer UBC856, UBC 866, and UBC 889) to 18 (primer UBC 829-11). The large variation of band number among genotype suggests the diversity in genetic structure of analyzed durian genotypes (Cahyarini *et al*., 2004). The average of amplified bands and polymorphic bands per primer were 13.5 and 12.8, respectively. Polymorphic percentage ranged from 71 % to 100% with an average of 91.0%. Thus, the polymorphism of durian genotypes in this study is in agreement with research of Siew and colleagues (Siew et al. 2018) when they reported that the genetic variation of 27 durian genotypes from Vietnam and Malaysia is at 91.7%. However, the polymorphism in our study is slightly lower than study in Indonesia previously while Angeliena used 10 ISSR primers and found up to 93.25% of polymorphism among 55 durian genotypes (Angeliena et al., 2019). In addition, ISSR primers showed high PIC values from 0.52 to 0.94 with average of 0.82 meaning that all these primers are suitable for studying genetic diversity of durian according to Botstein and colleagues (1980) as following: highly informative if PIC > 0.5; reasonably informative if 0.5 > PIC > 0.25 and slightly informative if PIC < 0.25. The complex marker patterns of ISSR found in this study suggests the advantages for distinguishing closely related accessions. The high potential in detecting genetic variation of ISSR makers would be advantageous for future studies to identify the correlation between specific amplified bands and specific favourable traits of durian (Handayani and Rahayu, 2017).

Based on obtained ISSR data, the relatedness of 22 durian genotypes was analysed with NTSYSpc 2.1 to generate a similarity matrix and showed in Table 4. Overall, the genetic similarity varied from 0.13 to 0.62. The lowest similarity value (0.13) was observed between R6-QTVL and MT-SOFRI. This result is reasonable since R6-QTVL and MT-SOFRI were collected from Vinh Long and Tien Giang province, respectively. Whereas, the highest similarity value (0.62) was observed between R6-HKTBT and R6-VTBR. This high similarity is not surprised because both of them are from Ben Tre province suggesting these two accessions share a common ancestor.

The dendrogram was then constructed based on similarity matrix in Table 1. At cut-off value of 0.30, the 22 durian genotypes were divided into five groups (Figure 3). Group A consists of seven genotypes (R6-DHBD; R6-THDN; R6-HGDN, R6-TACC; R6-TBVL; R6-PRBP; and MT-THDN), group B consists of 11 genotypes (MT-TBVL; MT-QTVL; MT-VTBT; MT-HKTBT, R6-VTBT; R6-HKTBT; R6-TBTG; MT-TBTG; R6-LTTG; MT-LTTG; and R6-QTVL), group C consists two genotypes (R6-PTTN and MT-PTTN), whereas group D and E consists only one genotype for each group, namely R6-SOFRI and MT-SOFRI, respectively. The cluster analysis reveals that there is the significant complexity of genetic composition of durian genotypes in the South of Vietnam. It is surprised that different genotypes of Ri6 and Monthong varieties were not grouped in any separated group although majority durian genotypes in group A and B are Ri6 and Monthong variety, respectively. It is also strange that two samples collected from Vietnam Southern Fruit Research Institute (SOFRI) consist of Ri6 (R6-SOFRI) and Monthong (MT-SOFRI) are grouped separately in group D and E, respectively.

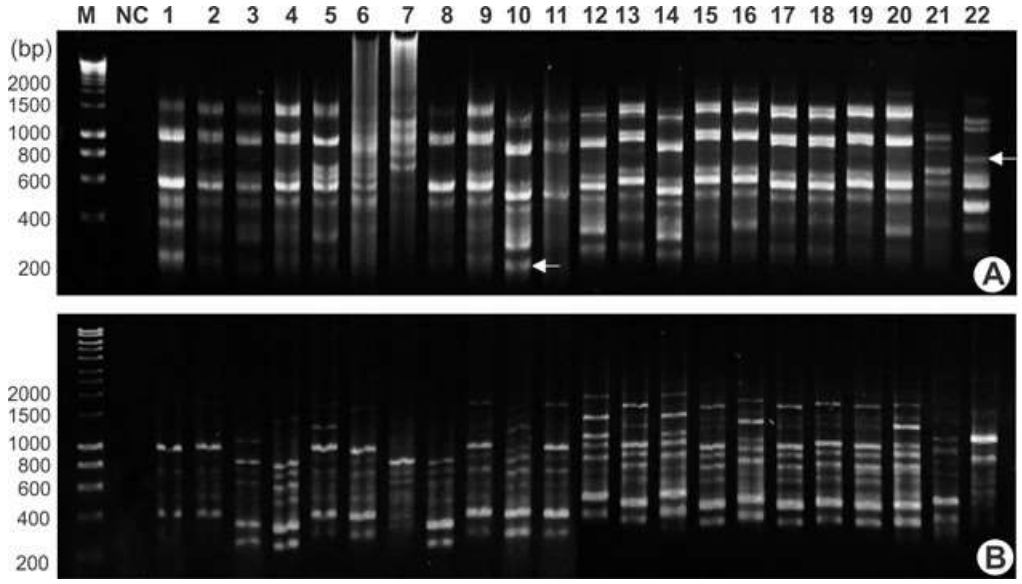

Figure 2. Representative ISSR results of 22 durian genotypes with UBC855 primer (A), and UBC888 primer (B). (The numbers are corresponding to sample codes in Table 1; M: DNA marker; NC: Negative control without DNA in reaction)

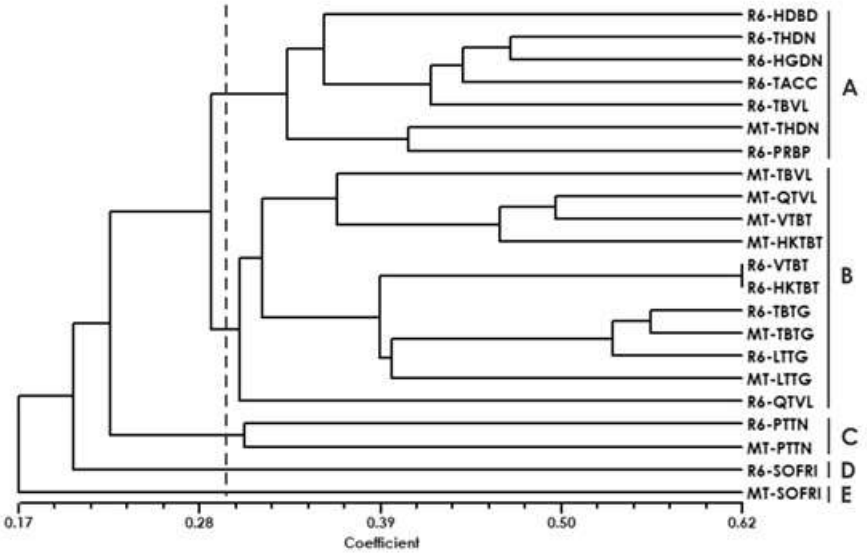

Figure 3: Dendrogram obtained from 22 durian genotypes collected in southern Vietnam with UPGMA based on Jaccard's coefficient by using 13 ISSR primers

Table 3: Characteristics of DNA profiles generated in 22 Durian genotypes by 13 ISSR primers

No	Primer	SB	NBP	PPB (%)	PIC
1	UBC 809	16	16	100	0.89
2	UBC 811	16	16	100	0.92
3	UBC 825	18	18	100	0.95
4	UBC 826	14	14	100	0.94
5	UBC 829-11	18	18	100	0.92
6	UBC 840	9	7	78	0.65
7	UBC 848	9	8	89	0.85
8	UBC 855	12	12	100	0.92
9	UBC 856	7	6	86	0.67
10	UBC 866	7	5	71	0.72
11	UBC 888	13	13	100	0.86
12	UBC 889	7	5	71	0.52
13	UBC 890	8	7	88	0.85
Total		175	166	-	-
Average		13.5	12.8	91,0	0.82

SB: Scored bands; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands; PIC: polymorphism information content.

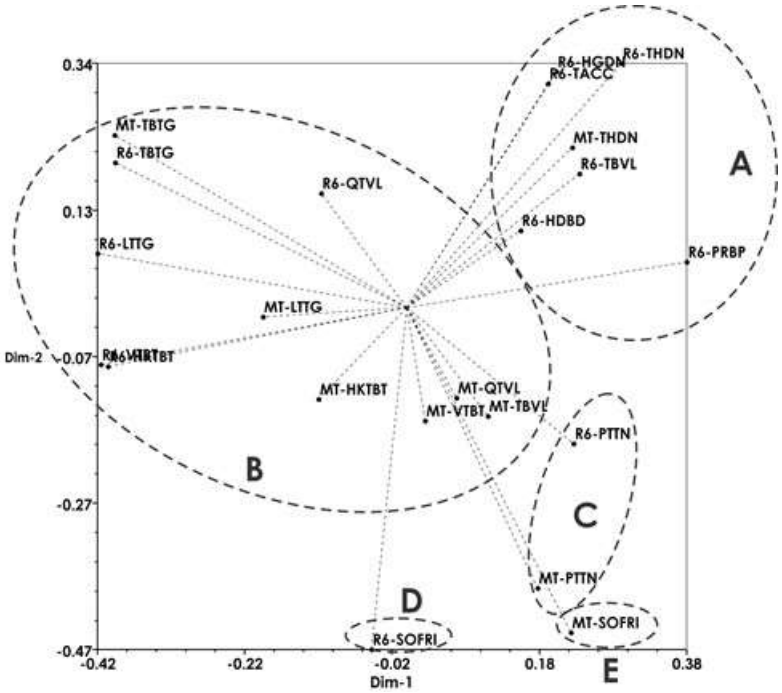

Figure 4: Principle coordinate plot for the first two principal coordinates estimated for 13 ISSR markers of the 22 durian genotypes

Table 4: Similarity coefficients among 22 durian genotypes with 13 ISSR markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1.00																					
2	0.45	1.00																				
3	0.36	0.38	1.00																			
4	0.30	0.53	0.41	1.00																		
5	0.34	0.45	0.45	0.47	1.00																	
6	0.28	0.39	0.23	0.36	0.33	1.00																
7	0.24	0.24	0.26	0.20	0.26	0.34	1.00															
8	0.24	0.42	0.44	0.36	0.32	0.33	0.24	1.00														
9	0.40	0.48	0.35	0.44	0.44	0.34	0.25	0.33	1.00													
10	0.36	0.29	0.34	0.30	0.26	0.26	0.19	0.25	0.33	1.00												
11	0.22	0.33	0.26	0.32	0.31	0.24	0.16	0.19	0.30	0.37	1.00											
12	0.35	0.34	0.37	0.34	0.33	0.31	0.22	0.30	0.43	0.38	0.27	1.00										
13	0.28	0.27	0.33	0.27	0.32	0.26	0.20	0.19	0.26	0.26	0.29	0.34	1.00									
14	0.28	0.28	0.37	0.27	0.35	0.31	0.21	0.30	0.27	0.40	0.30	0.50	0.31	1.00								
15	0.28	0.27	0.28	0.31	0.31	0.25	0.23	0.22	0.29	0.29	0.30	0.33	0.62	0.32	1.00							
16	0.34	0.32	0.34	0.34	0.37	0.35	0.19	0.29	0.34	0.37	0.24	0.48	0.42	0.45	0.43	1.00						
17	0.34	0.35	0.27	0.32	0.27	0.23	0.21	0.20	0.32	0.26	0.41	0.30	0.35	0.31	0.36	0.31	1.00					
18	0.28	0.37	0.31	0.36	0.34	0.26	0.18	0.23	0.33	0.21	0.31	0.32	0.43	0.26	0.45	0.36	0.57	1.00				
19	0.27	0.29	0.31	0.30	0.27	0.28	0.25	0.22	0.30	0.25	0.34	0.33	0.39	0.30	0.40	0.41	0.58	0.51	1.00			
20	0.28	0.30	0.40	0.33	0.36	0.24	0.21	0.24	0.30	0.28	0.27	0.36	0.41	0.36	0.34	0.40	0.37	0.42	0.41	1.00		
21	0.26	0.25	0.20	0.23	0.22	0.25	0.24	0.22	0.21	0.23	0.17	0.25	0.35	0.20	0.38	0.37	0.22	0.24	0.30	0.29	1.00	
22	0.17	0.23	0.20	0.23	0.14	0.23	0.15	0.22	0.27	0.24	0.13	0.32	0.15	0.24	0.14	0.19	0.16	0.19	0.16	0.26	0.17	1.00

The numbers represent the durian genotypes corresponding in Table

This means that the genetic composition of Ri6 and Monthong durian cultivars grown in different locations of southern Vietnam are not identical to the cultivars maintained in SOFRI which is a national research institute for fruit tree of Vietnam. Furthermore, the result of clustering analysis revealed that the studied Durian genotypes were not grouped based on geographical location where the samples were collected. This could be due to the exchanging of seedlings or breeding materials, resulting in the same genotype having different names in different location. In addition, the high and complex variation of durian samples in this study could be due to other reasons such as out-crossing system or the geographical difference of durian origin. This hypothesis could be supported by study of Giang and colleagues in 2016. This group found that Monthong, a Thailand origin cultivar, is more genetically similar to Vietnam durian cultivars such as Chi Hoa, La Keo than Thai durian cultivars, similar results also found in Ri6 cultivar, a Cambodia origin cultivar (Giang et al. 2016).

The relationship among 22 durian accession was also evaluated by PCoA, the first two coordinates were shown in Figure 4. The result of this analysis is relatively corresponding to UMPMA in Figure 3. Thus, PCoA can be used for further confirmation of genetic diversity by using UPGMA method as described previously by Johar et al. (2007).

CONCLUSION

This means that the genetic composition of Ri6 and Monthong durian cultivars grown in different locations of southern Vietnam are not identical to the cultivars maintained in SOFRI which is a national research institute for fruit tree of Vietnam. Furthermore, the result of clustering analysis revealed that the studied Durian genotypes were not grouped based on geographical location where the samples were collected. This could be due to the exchanging of seedlings or breeding materials, resulting in the same genotype having different names in different location. In addition, the high and complex variation of durian samples in this study could be due to other reasons such as out-crossing system or the geographical difference of durian origin. This hypothesis could be supported by study of Giang and colleagues in 2016. This group found that Monthong, a Thailand origin cultivar, is more genetically similar to Vietnam durian cultivars such as Chi Hoa, La Keo than Thai durian cultivars, similar results also found in Ri6 cultivar, a Cambodia origin cultivar (Giang et al. 2016).

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: VTH; Methodology: HVT; Project administration: VTH; Resources: VTH, MDH, TLT; Supervision: VTH; Validation; Laboratory work: HVT, MDH, TLT; Writing - original draft: VTH, MDH, TLT; Writing - review and editing: VTH, MDH, TLT. All authors read and approved the final manuscript.

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