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GENETIC DIVERSITY AMONG PASSION FRUIT (*Passiflora edulis*) ACCESSIONS OF SOUTHERN VIETNAM USING RAPD AND ISSR MARKERS

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SUMMARY

Passion fruit (*Passiflora edulis*) is becoming an increasingly important fruit crop in Vietnam; however, its genetic diversity has not been properly investigated. In this study, two set of molecular markers consisted of 10 RAPD and 10 ISSR markers were used to characterize the genetic relatedness of 20 passion fruit accessions, collected from different provinces in southern Vietnam. High levels of polymorphismwere found in both RAPD (97.9%) and ISSR (100%) markers; in which a total of 130 and 118 amplified-bands were generated, respectively. Both markers revealed high polymorphism information content (PIC) (0.85 for RAPD and 0.88 for ISSR). Cluster analysis and Principal Coordinate Analysis (PCA) of both markers generated similar grouping patterns. The Mantel test also showed a high correlation between these two marker methods. However, the relation among genetic data and geographical location of samples was not detected. Our results could provide molecular information for classification, plant identification, breeding, and conservation of passion fruit in Vietnam.

Keywords: Dendrogram, diversity, ISSR, passion fruit, RAPD

Key findings: Detection of genetic variation of 20 passion fruit accessions collected from Southern Vietnam by RAPD and ISSR molecular markers. The obtained results can be used for conservation, breeding and development of this fruit.

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INTRODUCTION

Passion fruit belongs to the Passifloraceae family which is native in South America and grown mainly in the tropic and sub-tropic regions. The fruit is distinguished not only by its delicious taste and pleasant aroma, but also for its high nutritional value as well as the ability to cure a number of diseases, such as diabetes, sedation, convulsion, cardiovascular diseases, osteoarthritis, and asthma (Zas and John, 2016). A high amount of vitamin A and C and small quantities of

calcium, phosphorus and iron are also found in fruit juice. In the early 20th century, passion fruit was imported to Vietnam and grown widely in southern provinces for daily consumption and beverage industries (Thuy, 2020). Due to its economic value, the Vietnam government has considered passion fruit as a tree to eradicate poverty, so the cultivation area has increased rapidly in recent times (Le and Pham, 2010). Previously, passion fruit was only a plant used as a beverage at household scale, now it has gradually become a potential crop in Vietnam. By 2019, the fruit was produced in more than 10,000 hectares and it is expected that by 2020-2021, it will be produced in between 12 to 15 thousand hectares (Manh, 2020). In spite of several efforts to increase the economic value, improper cultivar identification results heterogenicity of fruit quality which cause difficulty in industrial processing. At present, the classification of passion fruit is largely based on morphological characteristics such as locality, leaf-flower-flesh color, growth habit and other characteristics of plants. Although morphological classification is commonly performed due to its low cost and easy to do, there are numerous limitations for this method, such as complex inheritance pattern, dependency to plant growth stage development, and vulnerability to environmental changes. These have made morphological identification not effective and sometimes inaccurate (Ahmedand and Mohamed, 2014).

As an alternative, molecular marker utilizations for plant identification have been widely accepted due to several advantages, such as unlimited number, unaffected by environment and growing conditions, easy interpretation, and reliable repeatable results (Antunes *et al*., 1997). Several molecular markers have been developed, for example amplified fragment length polymorphism (AFLP), single nucleotide polymorphisms (SNP), restriction fragment length polymorphism (RFLP), random amplified of polymorphic DNA (RAPD), simple sequence repeat (SSR), inter-simple sequence repeat (ISSR), and DNA barcode. Among these, RAPD and ISSR methods are preferred to use on plants with limited prior genetic knowledge, since they possess several practical advantages. These methods are simple and rapid, relatively cheap, require minimum laboratory skills, require small DNA quantity, and generate high number of fragments in each reaction. RAPD for evaluating passion fruit diversity has been used in many countries around the world such Colombia (Fajardo *et al*., 1998); Brazil (Cerqueira-Silva *et al*., 2010); and Indonesia (Wulandari *et al*., 2017). Nevertheless, this marker also reveals numerous drawbacks consisting of low reproducibility due to reactive conditions such as DNA concentrations, the concentration of PCR components, and the number of cycles of the reaction (Williams *et al*., 1990; Mbwana *et al*., 2006). In order to overcome these RAPD limitations, ISSR is considered as suitable alternative as a highly variable, more reproducible marker leading to expanded use in genetic diversity of passion fruit by several research groups (dos-Santos *et al*., 2011; Costa *et al*., 2012; Vianna *et al*., 2019).

To our best knowledge, no genetic study has been conducted in Vietnam for passion fruit to date. In this study, a total of 10 RAPD and 10 ISSR primers were used to evaluate the genetic diversity of 20 passion fruit accessions harvested from different regions of southern Vietnam. The results will provide scientific information for identification, classification, propagation and breeding purposes of passion fruit in passion fruit growing areas of Vietnam.

MATERIALS AND METHODS

Plant material

Leaf samples of twenty passion fruit accessions were collected from farmers' orchards, university nurseries, and seedling centers of different provinces of southern Vietnam (Figure 1 and Table 1)

Table 1. Locations of the twenty collected passion fruit samples for genetic characterization.

Sample number	Collected location	Sample abbreviation
1	Trung An- Cu Chi- Ho Chi Minh City	CC
2	Lan Nam- Cho Lach- Ben Tre province	BT
3	Pham Van Hai- Binh Chanh- Ho Chi Minh City	BC
4	Binh Tri Dong- Binh Tan- Ho Chi Minh City	TB
5	Bom Bo- Bu Dang- Binh Phuoc province	BP
6	Iabang- Chuprong- Gia Lai province	GL
7	Cu Bong- Dak Lak province	DK1
8	Cu Jang- Dak Lak province	DK2
9	Eapan- Dak Lak province	DK3
10	My Yen- Ben Luc- Long An province	LA1
11	My Yen- Ben Luc- Long An province	LA2
12	My Yen- Ben Luc- Long An province	LA3
13	My Yen- Ben Luc- Long An province	LA4
14	My Yen- Ben Luc- Long An province	LA5
15	Yen Luong- Go Cong Tay- Tien Giang province	TG1
16	Binh Dong- Go Cong- Tien Giang province	TG2
17	Dao Nghia- Dak R'Lap- Dak Nong province	DN1
18	Quang Son- Dak G'Long- Dak Nong province	DN2
19	Quang Son- Dak G'Long- Dak Nong province	DN3
20	Vung Liem- Vinh Long province	VL

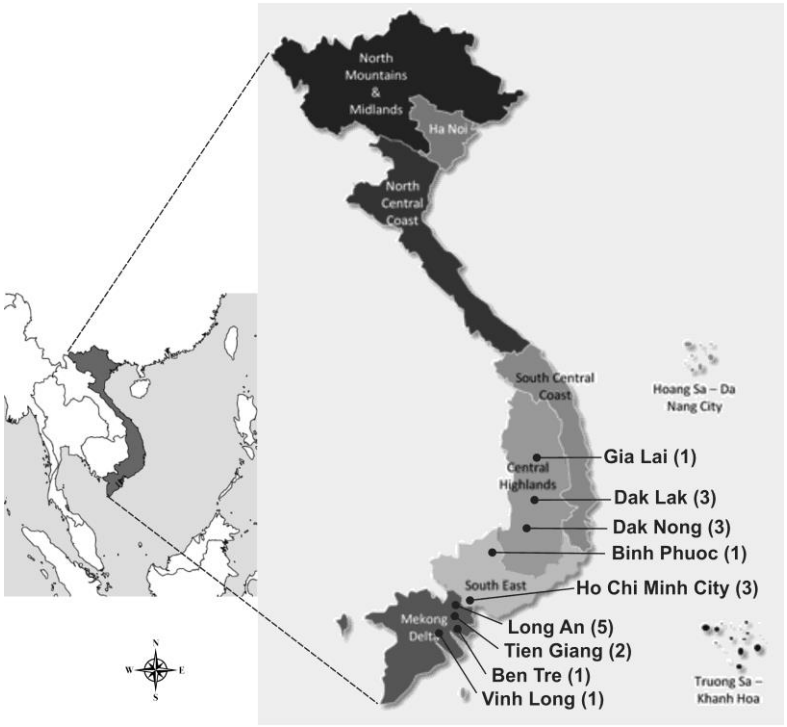

Figure 1. Map of sample collection sites of passion fruit accessions in this study. (Numbers in parentheses is corresponding to samples collected in each location).

from 2018 to 2019. The harvested samples were then dried in silica gel and stored at room temperature until use.

DNA extraction

Total DNA was extracted from dried passion fruit leaves using the cetyltrimethyl ammonium bromide (CTAB) method described by Doyle and Doyle (1990). The 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). The DNA concentrations were determined by spectrophotometer (Optima SP 3000 nano UV-VIS, Japan) and stored at -20 °C until use.

RAPD and ISSR amplification

A total of 10 RAPD and 10 ISSR primers were used in PCR (Table 2). The PCR was performed using reaction mix containing 7.5 µL 2X Mytaq Red Mix (Bioline, UK), 20 ng DNA, 0.2 µM primer and PCR water for a final volume of 15 µl. The RAPD reaction conditions were modified based on Ho and Tu (2019) as follows: initial denaturation at 95 °C for 2 minutes; then 35 cycles of 30 seconds denaturation at 95 ^{o}C, 30 seconds annealing at 35 ^{o}C, and 1 minute extension at 72 ^{o}C. Final extension was added for 5 minutes at 72 ^{o}C to complete the reaction. The ISSR reaction conditions were conducted as follows: initial denaturation at 95 °C for 2 minutes; then 35 cycles of 30 seconds denaturation at 95 ^{o}C, 30 seconds annealing at 54 ^{o}C, and 1 minute extension at 72 ^{o}C. Final extension for 5 minutes at 72 ^{o}C was performed to finish the reaction. All reactions were carried out with the SureCycler 8800 Thermal Cycler (Agilent, USA). PCR product was then separated by electrophoresis in 1.5% agarose gel in 1X TAE buffer, and stained with 0.5 µg/ml Gelred dye loading buffer then visualized under Quantum - ST4 3000 gel reader.

Data analysis

Only clear bands resulted from gel electrophoresis were used in analysis. Clearly visible amplified bands were scored as "1", whereas the absence of corresponding band was scored as "0". The numbers of scored bands (SB), numbers of polymorphic bands (NPB) and percentage of polymorphic bands (PPB) were recorded. The quality information of the primers is determined by the PIC (Polymorphism Information Content) according to the formula of Chesnokov and Artemyeva (2015). The ability of primers to differentiate between genotypes was evaluated by their resolving power (RP) values as described by Prevost and Wilkinson (1999). 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 dendrograms were constructed using the algorithm with the SAHN module. The cut-off values of dendrograms were then determined based on calculation method described by Jamshidi and Jamshidi (2011). Using similarity matrices, principal coordinate analysis (PCA) was carried out to construct a three-dimensional array of eigenvectors using DCENTER module (Ibrahim *et al*., 2017). The correlation between the RAPD and ISSR similarity matrices, between either single RAPD; ISSR data or pooled data of the two markers and the geographical locality of collected samples were computed by Mantel test at a significant level of 5% in 1000 simulations by using the Mantel test in Microsoft Excel 2010 (Mantel, 1967).

Table 2. RAPD and ISSR primers for genetic diversity analysis of 20 passion fruit accessions.

RAPD Primers	Sequence (5'-3')	ISSR Primer	Sequence (5'-3')
OPB-04	GGACTGGAGT	UBC 826	ACACACACACACACACC
OPB-07	GGTGACGCAG	UBC 829	TGTGTGTGTGTGTGTGT
OPM-18	CACCATCCGT	UBC 848	CACACACACACACACARG
OPA-05	AGGGGTCTTG	UBC 866	CTCCTCCTCCTCCTCCTC
OPF-06	GGGAATTCGG	UBC 814	CTCTCTCTCTCTCTCAT
RAPD-09	GACCGCTTGT	UBC 818	CACACACACACACACAG
OPB-18	GGGAATTCGG	UBC 826	ACACACACACACACACC
OPN-18	GGTGAGGTCA	UBC 834	AGAGAGAGAGAGAGACYT
RAPD-01	TCCTACGCAC	UBC 840	GAGAGAGAGAGAGAGACTT
OPM-06	CTGGGCAACT	UBC 854	TCTCTCTCTCTCTCTCAGG

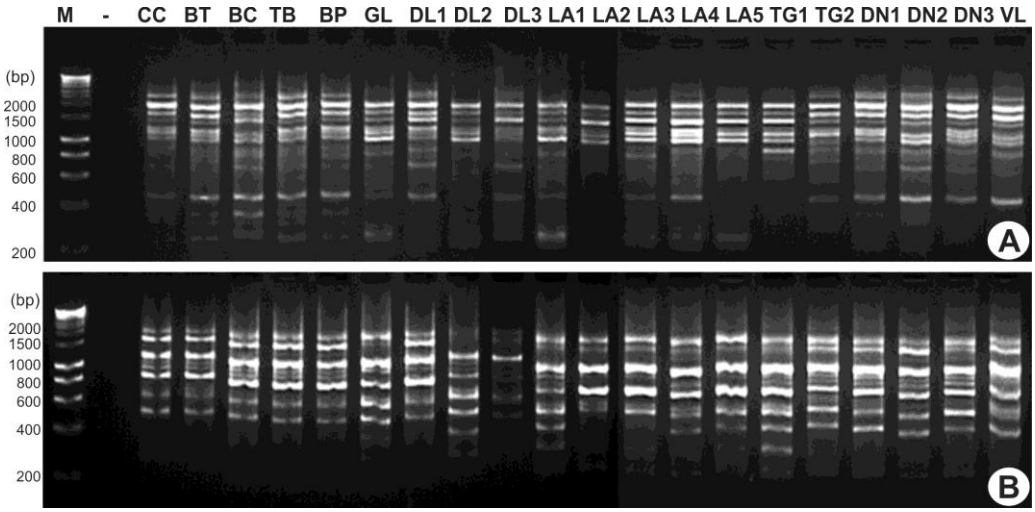

Figure 2. Representative RAPD results with OPN-18 primer (A) and OPA-05 primer (B). (M: DNA marker; "-": Negative control without DNA).

RESULTS

RAPD analysis

The collected DNA samples were characterized with 10 RAPD primers. The obtained results showed that the bands appeared clearly on 1.5% agarose gel (Figures 2A and B). The primers generated 8 to 21 amplifications per reaction and the length of amplifications varied approximately from 200 to 2,500 bp. The average number of bands and polymorphic bands per primer were 13.0 and 12.8, respectively. Polymorphism ranged from 88.9% to 100%, with an average of 97.9% across all analyzed passion fruit accessions. Polymorphism information content (PIC) value varied from 0.65 to 0.96 with the average of 0.85. The resolving power (RP) value varied from 3.9 to 12.8 with average of 7.78 as shown in Table 3.

The matrix of Jaccard's coefficient showed similarity across accessions (above diagonal of Table 4). The lowest similarity coefficient is 0.47, which is found between BT and LA5, whereas the highest similarity coefficient is 0.83, between DN1 and DK2 samples. Based on this similarity matrix, the phylogenetic tree was constructed and shown in Figure 3A. At the cut-off value of 0.64, the dendrogram was divided into two major

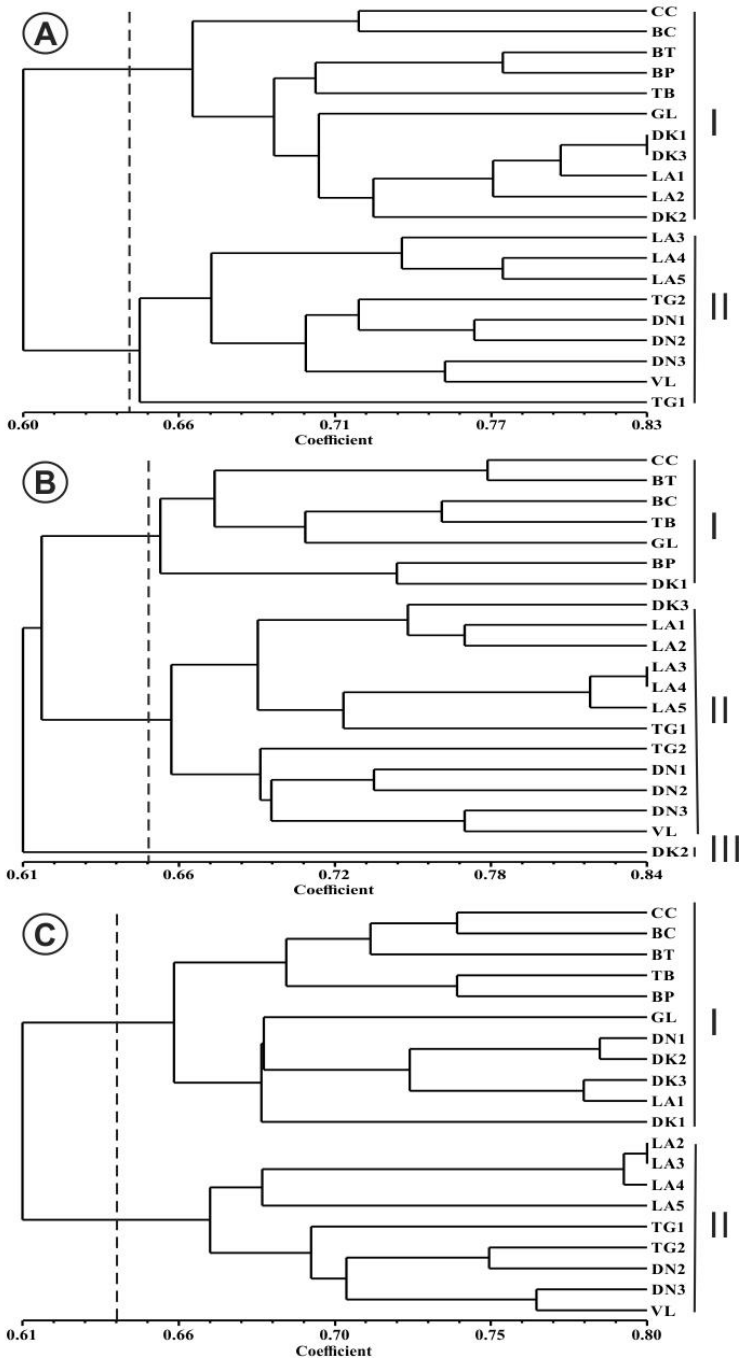

Figure 3. Dendrogram for 20 accessions of passion fruit accessions collected in Vietnam with UPGMA based on Jaccard's coefficient by using 10 RAPD primers (A); 10 ISSR primers (B) and the combination of 10 RAPD + 10 ISSR primers (C). The vertical lines indicate the cut-off values of each dendrogram. The scale shown at the bottom is the measure of genetic similarity through Jaccard's coefficient.

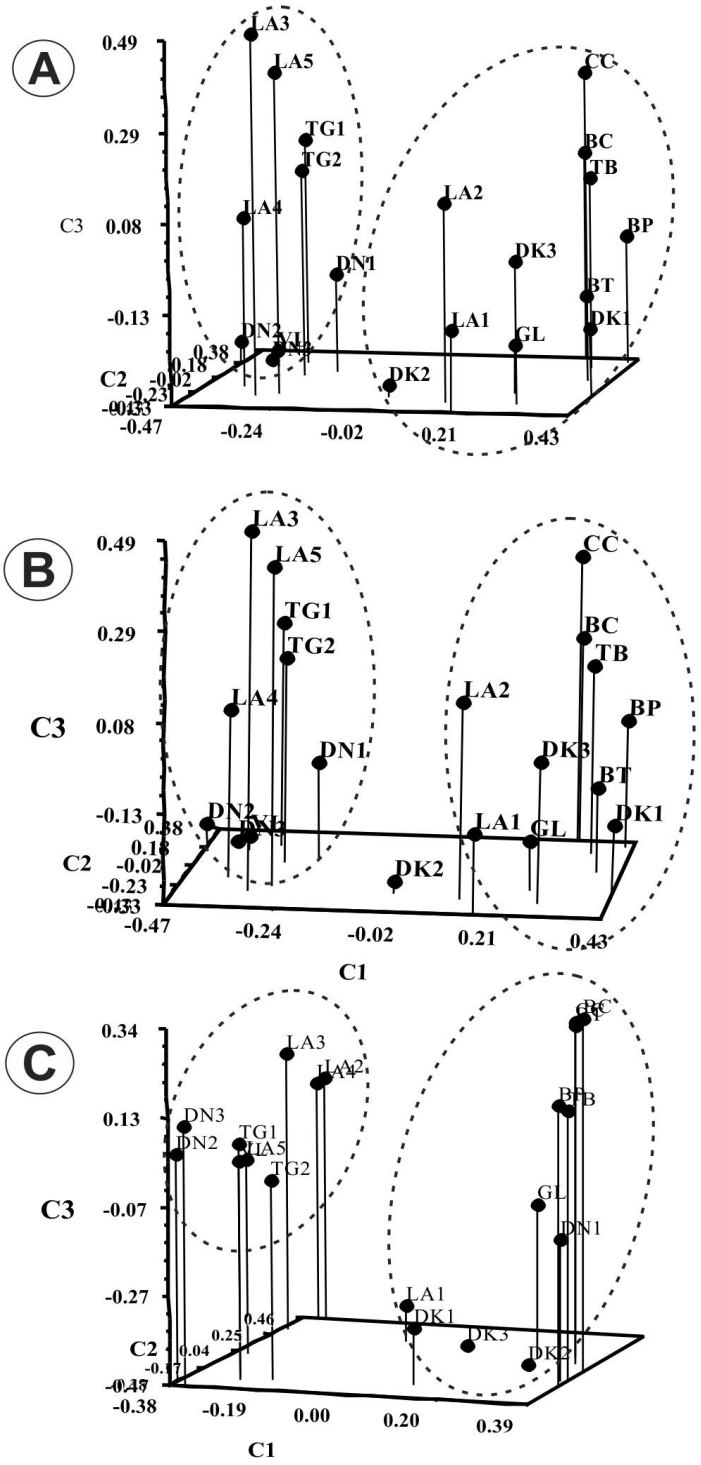

Figure 4. Three-dimensional plot of the principal coordinate analysis (PCA) of genetic distance among 20 passion fruit by using 10 RAPD primers (A) 10 ISSR primers (B) and the combination of 10 RAPD + 10 ISSR primers (C).

Table 3. Characteristics of DNA profiles generated in 20 passion fruit accessions by using 10 RAPD primers and 10 ISSR primers.

RAPD Primer	SB	NPB	PPB (%)	PIC	RP	ISSR Primer	SB	NPB	PPB (%)	PIC	RP
OPB-04	9	9	100	0.94	3.90	UBC 826	14	14	100	0.96	4.9
OPB-07	8	8	100	0.82	5.40	UBC 829	12	12	100	0.88	7.1
OPM-18	13	13	100	0.93	6.20	UBC 848	10	10	100	0.82	8.3
OPA-05	15	15	100	0.86	9.90	UBC 866	11	11	100	0.90	6.1
OPF-06	14	14	100	0.88	9.40	UBC 814	10	10	100	0.91	5.1
RAPD-09	9	8	88. 9	0.77	6.20	UBC 818	13	13	100	0.84	6.5
OPB-18	12	12	100	0,96	4.10	UBC 826	8	8	100	0.86	5.3
OPN-18	11	10	90.9	0.65	10.90	UBC 834	15	15	100	0.95	6.2
RAPD-01	18	18	100	0.89	12.80	UBC 840	14	14	100	0.93	6.7
OPM-06	21	21	100	0.94	9.00	UBC 854	11	11	100	0.78	6.9
Sum	130	128	-	-	-	-	118	118	-	-	-
Average	13.0	12.8	97.9	0.85	7.78	-	11.8	11.8	100	0.88	6.3

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

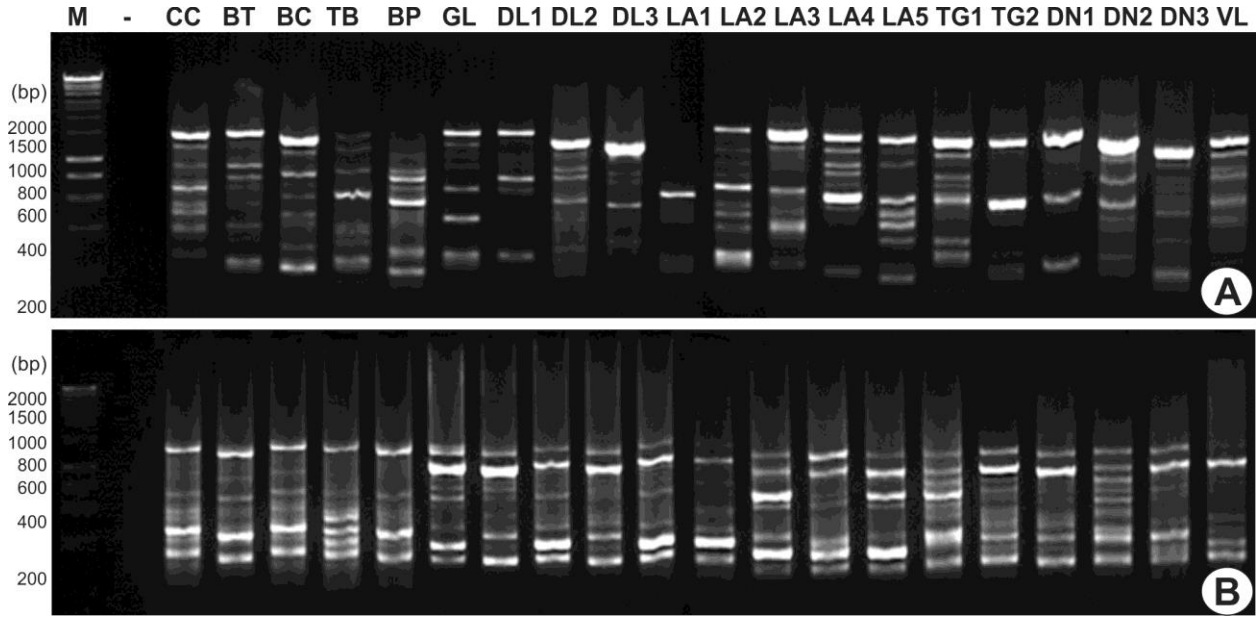

Figure 5. Representative ISSR result with UBC 880 primer (A) and UBC 811 primer (B). The number is corresponding to sample number in Table 1; M: DNA marker; "-": Negative control without DNA.

Table 4. Simple matching coefficients of similarity among 20 passion fruit accessions with 10 RAPD primers (above diagonal) and ISSR (below diagonal).

Accessions	CC	BT	BC	TB	BP	GL	DN1	DK1	DK2	DK3	LA1	LA2	LA3	LA4	LA5	TG1	TG2	DN2	DN3	VL
CC		0.66	0.72	0.71	0.69	0.61	0.68	0.64	0.66	0.68	0.70	0.57	0.57	0.54	0.55	0.63	0.61	0.54	0.52	0.55
BT	0.78		0.66	0.67	0.78	0.71	0.79	0.64	0.72	0.72	0.68	0.57	0.64	0.59	0.47	0.61	0.61	0.54	0.56	0.62
BC	0.75	0.74		0.69	0.69	0.63	0.68	0.62	0.68	0.62	0.62	0.51	0.57	0.52	0.51	0.59	0.63	0.54	0.48	0.53
TB	0.65	0.67	0.76		0.74	0.68	0.69	0.67	0.73	0.69	0.65	0.54	0.63	0.55	0.52	0.64	0.62	0.53	0.53	0.54
BP	0.61	0.68	0.69	0.74		0.62	0.78	0.61	0.71	0.69	0.67	0.50	0.56	0.53	0.54	0.64	0.62	0.57	0.53	0.59
GL	0.64	0.61	0.69	0.74	0.63		0.73	0.69	0.71	0.73	0.67	0.54	0.65	0.55	0.48	0.62	0.60	0.55	0.57	0.56
DN1	0.68	0.61	0.65	0.69	0.75	0.61		0.70	0.83	0.79	0.74	0.60	0.64	0.59	0.49	0.65	0.69	0.61	0.54	0.60
DK1	0.60	0.53	0.54	0.61	0.67	0.67	0.64		0.70	0.79	0.72	0.64	0.70	0.61	0.53	0.71	0.67	0.69	0.63	0.68
DK2	0.68	0.64	0.65	0.67	0.66	0.71	0.75	0.74		0.81	0.79	0.66	0.70	0.65	0.57	0.67	0.67	0.63	0.56	0.64
DK3	0.69	0.66	0.62	0.65	0.56	0.64	0.63	0.62	0.76		0.79	0.66	0.70	0.67	0.57	0.73	0.73	0.67	0.61	0.64
LA1	0.60	0.62	0.64	0.63	0.60	0.67	0.60	0.59	0.74	0.77		0.68	0.68	0.65	0.62	0.69	0.67	0.65	0.61	0.60
LA2	0.67	0.69	0.66	0.64	0.64	0.74	0.62	0.59	0.69	0.72	0.76		0.74	0.73	0.62	0.71	0.63	0.65	0.59	0.64
LA3	0.63	0.69	0.62	0.58	0.59	0.64	0.56	0.53	0.63	0.66	0.74	0.84		0.78	0.64	0.73	0.71	0.76	0.71	0.77
LA4	0.63	0.64	0.58	0.55	0.59	0.64	0.58	0.58	0.64	0.66	0.72	0.84	0.80		0.63	0.66	0.66	0.62	0.57	0.63
LA5	0.59	0.56	0.55	0.55	0.53	0.56	0.61	0.57	0.68	0.69	0.74	0.70	0.69	0.78		0.67	0.65	0.71	0.65	0.57
TG1	0.64	0.57	0.58	0.58	0.57	0.60	0.62	0.63	0.65	0.62	0.64	0.68	0.69	0.65	0.67		0.74	0.70	0.66	0.69
TG2	0.60	0.62	0.61	0.66	0.67	0.60	0.67	0.59	0.69	0.70	0.69	0.73	0.67	0.65	0.70	0.69		0.77	0.66	0.69
DN2	0.56	0.53	0.55	0.57	0.58	0.54	0.58	0.58	0.58	0.58	0.69	0.65	0.66	0.64	0.68	0.69	0.74		0.77	0.76
DN3	0.62	0.62	0.58	0.68	0.62	0.58	0.57	0.61	0.62	0.67	0.69	0.71	0.67	0.64	0.69	0.75	0.76	0.74		0.76
VL	0.54	0.56	0.62	0.60	0.56	0.59	0.61	0.62	0.68	0.61	0.67	0.67	0.63	0.63	0.68	0.65	0.69	0.61	0.77	

(Full names/information of accession abbreviations are shown in Table 1)

Table 5. Simple matching coefficients of similarity among 20 passion fruit accessions with combined data from RAPD and ISSR primers (below diagonal) and geographical distances in km (above diagonal).

Accessions	CC	BT	BC	TB	BP	GL	DN1	DK1	DK2	DK3	LA1	LA2	LA3	LA4	LA5	TG1	TG2	DN2	DN3	VL
CC		128	59	50	124	420	178	372	372	360	70	77	68	73	70	105	89	235	239	155
BT	0.73		82	91	236	532	290	483	484	471	68	67	64	65	63	64	70	330	335	30
BC	0.74	0.70		10	132	451	210	403	404	391	14	16	15	14	14	46	41	249	247	105
TB	0.68	0.67	0.73		147	442	201	394	395	382	22	25	24	22	22	56	51	240	244	114
BP	0.65	0.72	0.69	0.74		349	125	323	306	294	144	149	151	147	148	179	173	164	167	236
GL	0.63	0.66	0.66	0.71	0.62		265	188	188	173	463	466	461	467	469	498	492	251	255	555
DN1	0.68	0.69	0.67	0.69	0.76	0.67		214	215	202	222	231	228	225	218	256	251	51	59	313
DK1	0.62	0.58	0.58	0.64	0.64	0.68	0.67		10	18	415	422	417	419	4221	449	444	164	167	506
DK2	0.67	0.68	0.67	0.70	0.68	0.71	0.78	0.72		19	417	418	421	423	416	450	441	166	173	507
DK3	0.69	0.69	0.62	0.67	0.62	0.68	0.70	0.69	0.78		403	412	409	414	405	437	432	159	164	495
LA1	0.65	0.65	0.63	0.64	0.63	0.67	0.67	0.65	0.76	0.78		4	7	8	12	44	47	261	268	91
LA2	0.63	0.64	0.59	0.60	0.58	0.65	0.61	0.61	0.67	0.69	0.73		6	3	5	42	46	271	274	98
LA3	0.60	0.67	0.60	0.60	0.58	0.65	0.59	0.61	0.66	0.68	0.71	0.80		2	8	51	51	262	270	92
LA4	0.59	0.62	0.56	0.55	0.57	0.60	0.58	0.59	0.65	0.67	0.69	0.79	0.79		10	48	49	268	272	87
LA5	0.58	0.52	0.53	0.54	0.53	0.52	0.56	0.55	0.63	0.64	0.68	0.67	0.67	0.71		46	48	264	268	90
TG1	0.63	0.58	0.58	0.60	0.60	0.61	0.63	0.67	0.66	0.67	0.67	0.69	0.71	0.66	0.67		9	295	303	95
TG2	0.60	0.61	0.62	0.64	0.65	0.60	0.68	0.63	0.68	0.72	0.68	0.68	0.69	0.66	0.68	0.72		290	294	100
DN2	0.55	0.53	0.55	0.55	0.58	0.55	0.59	0.63	0.60	0.62	0.67	0.65	0.70	0.63	0.69	0.69	0.75		8	353
DN3	0.58	0.59	0.53	0.61	0.58	0.58	0.56	0.62	0.59	0.64	0.66	0.66	0.69	0.61	0.67	0.71	0.72	0.75		361
VL	0.55	0.58	0.58	0.58	0.57	0.58	0.60	0.65	0.66	0.62	0.64	0.66	0.69	0.63	0.63	0.67	0.69	0.67	0.76	

(Full names/information of accession abbreviations are shown in Table 1)

clusters. The first cluster comprised of 11 accessions, namely CC, BC, BT, BP, TB, GL, DK1, DK3, LA1, LA2, and DK2. The remaining nine accessions belonged to second cluster. This grouping is further supported by PCA in Figure 4A.

ISSR analysis

Genetic composition of 20 passion fruit accessions was further analyzed by 10 ISSR markers. The PCR reactions showed clear and reproducible bands (Figures 5A and 5B). All of 118 generated bands were polymorphic. The amplification sizes ranged from 200 to 2000 bp. The number of bands varied from eight to fifteen, PIC values varied from 0.78 to 0.96 and RP values varied from 4.9 to 8.3 with average of 6.3 (Table 3).

According to Jaccard's coefficient matrix (below diagonal of Table 4), both DK1 and BT accessions show the lowest coefficient (0.53) whereas LA3 had the highest coefficient with LA2 (0.84). Based on UPGMA analysis with ISSR data, the dendrogram was constructed. At a cut-off value of 0.65, these 20 accessions were then divided into three main groups: the first group consisted of seven accessions (CC, BT, BC, TB, GL, BP, and DK1); most of accessions belonged to the second groups with 12 accessions (DK3, LA1, LA2, LA3, LA4, LA5, TG1, TG2, DN1, DN2; DN3, and VL), only DK2 was classified in the third group (Figure 3B). PCA analysis also showed a similar clustering pattern (Figure 4B).

Combined data

In order to gain better understanding of the effectiveness of combined RAPD and ISSR markers, all amplified bands were combined and later subjected for UPGMA analysis. The resulted Jaccard's coefficient is shown in Table 5 (below diagonal) and the dendrogram based on the pooled data is shown in Figure 3C. At cut-off value of 0.64, the 20 genotypes were grouped into two groups. The cluster pattern and PCA of the pooled data were divided into two groups which were relatively similar to RAPD analysis (Figures 3C and 4C). Mantel's test analysis showed a high correlation between two similarity matrices of RAPD and ISSR markers ($P < 0.0001$). There was no association between geographical location of collected samples and genetic data from either RAPD ($P = 0.1$); ISSR ($P = 0.25$) or combination of RAPD and ISSR markers ($P = 0.1$).

DISCUSSION

Although RAPD and ISSR markers have been widely used for genetic characterization of passion fruit in several different countries (Fajardo *et al*., 1998; Cerqueira-Silva *et al*., 2010; dos Santos *et al*., 2011; Costa *et al*., 2012; Wulandari *et al*., 2017; Vianna *et al*., 2019), no similar study has been done in Vietnam. In this study, the potential use of these markers was compared to identify suitable markers for characterizing genetic composition of passion fruit accessions collected from southern Vietnam. The results showed that RAPD markers generated more bands and polymorphic bands than those of ISSR. However, the percentage of polymorphic bands of ISSR was higher than that of RAPD. The complex marker patterns of ISSR would be advantageous for distinguishing closely related species (Rayar *et al*., 2015). Previous studies also reported that ISSR primers were more informative than RAPD in different plants such as wheat (Nagaoka and Ogihara, 1997); coconut (Manimekalai *et al*., 2006); *Dalbergia oliveri* (Phong *et al*., 2011).

Both RAPD and ISSR primers showed high PIC values from 0.85 to 0.88. It means that all these primers are suitable for studying genetic diversity of passion fruit. Botstein *et al* (1980) suggested that ISSR marker is slightly more informative than RAPD. Since RAPD markers use several arbitrary short primers (8–12 nucleotides) which enable random binding of primers in plant genome, generating a high number of DNA bands. On the other hand, ISSR is

also considered more reproducible than RAPD markers (Goulão and Oliveira, 2001) as ISSR primers are longer and require higher annealing temperature, resulting in higher consistency. Furthermore, ISSR primers are specifically designed to complement the corresponding microsatellite sequence, thus leading to less random annealing during PCR reaction. Nevertheless, in this study, RAPD markers showed higher resolving power value (7.78) than ISSR markers (6.31). This implied that RAPD markers could distinguish more genotypes and produced higher polymorphism (Debnath *et al*., 2008). This finding further suggested that different marker types used in the study could amplify genetically distinct regions in genome of passion fruit thus resulting in distinct amplification profiles.

The results from clustering analysis of RAPD and ISSR data were not identical. Their differences could result due to the different genomic targets within the passion fruit genome of each marker system. This data suggested that the variation was due to the characteristics of a specific genotype (Debnath *et al*., 2008). Another explanation for this result could be the difference between the obtained data generated by two marker types where RAPD and ISSR amplify non-repetitive and repetitive regions of plant genome, respectively (Ghislain *et al*., 2006).

In general, several accessions were not grouped based on geographical location (Figures 3 and 4). These results are further supported by Mantel test between genetic data and geographical locations of collected samples. This could be due to the complex exchanging of seedlings or breeding materials from distinct location, leading to non-correlation between the genetic structure and the geographical location where the samples were collected. Our result is consistent with previous findings in different crops such as sweet potato (Gichuki *et al*., 2003) and potato (Moulin *et al*., 2012). The obtained PCA plotting presents similar grouping patterns to the cluster analysis for RAPD, ISSR, and RAPD + ISSR markers (Figures 3 and 4). Thus, the PCA analysis could reveal another level of separation of sample and it could be a benefit to use more than one type of analysis method.

The genetic diversity of passion fruit accessions in southern Vietnam implies the diverse gene pool of this crop in the region. This genetic richness will be useful for breeding programs as the breeders would have more materials for expanding the genetic base of new passion fruit cultivars. Furthermore, distinguishable DNA banding patterns of each genotype could be used in marker assisted selection in breeding. This strategy could significantly reduce the number of required generations for traits or gene introgression and facilitate the rapid identification of genetically different parents to generate heterosis (Levi *et al*., 2004).

In conclusion, both RAPD and ISSR markers in this study showed their suitability for generating a high number of polymorphic markers. These markers were used for evaluating genetic variation of passion fruits. The combination of both markers could increase the effectiveness of genetic analysis in passion fruits. In the future, the development of sequence characterized amplified region (SCAR) should be considered to provide markers with higher level of authenticity for passion fruit identification by using specific primers designed from RAPD and ISSR. It is also feasible to apply these markers to choose parents for developing mapping populations and use in marker-assisted selection in future passion fruit breeding programs in Vietnam.

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