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Investigation of the genetic diversity of jewel orchid in Vietnam using RAPD and ISSR markers

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Abstract. *Tran TKP, Pham MH, Trinh TH, Widiarsih S, Ho VT. 2022. Investigation of the genetic diversity of jewel orchid in Vietnam using RAPD and ISSR markers. Biodiversitas 23: 4816-4825.* Jewel orchid is a general name for several genera belonging to the Orchidaceae family, namely *Anoectochilus, Doddinia, Goodyera, Ludisia,* and *Macodes*. These plants are generally used as medicinal herbal for common diseases in Vietnam. Due to over-exploitation, the natural resources of jewel orchids are gradually depleted. Therefore, genetic resource research for proper development of this medicinal plant is necessary. This study aimed to investigate the genetic diversity of jewel orchids in Vietnam using RAPD and ISSR markers to provide basic data conservation of genetic resources and the development of this medicinal plant. A total of 20 jewel orchid samples were genetically characterized by using 10 RAPD and 10 ISSR primers. The amplified bands were binary coded and used to construct phylogenetic trees with NTSYSpc 2.1 software. Each RAPD primer produced 5-11 bands with an average of 8.0, and similarity coefficients ranged from 0.52 to 0.81. Each ISSR primer produced 5-10 bands with an average of 7.6, and the similarity coefficients ranged from 0.54 to 0.87. Combined RAPD and ISSR markers produced similarity coefficients ranging from 0.53 to 0.83. Based on the PIC values, the primers used by both markers are highly informative. However, the topology of cluster analysis of 20 jewel orchid accessions does not correspond to the taxa studied. The findings could be potential to employ in future classification, conservation, and development of this plant.

Keywords: Genetic diversity, ISSR, jewel orchid, molecular markers, RAPD

INTRODUCTION

Jewel orchid is a common name for several genera in the Orchidaceae family, namely *Anoectochilus*, *Dossinia*, *Goodyera*, *Ludisia*, and *Macodes* (De and Pathak 2018). This plant is used for several health care purposes, such as anti-fatigue, anti-oxidant, anti-hyperliposis, anti-tumor, and immune modulation agents (Winarto and Samijan 2018). Due to over-exploitation, this plant group was included in the Vietnam Red Book of endangered species and banned from exploitation for commercial purposes (Vietnam Academy of Science Technology 2007). *In-situ* conservation measures in Vietnam are not effective because it is often illegally exploited by local people, and conservation status in some special-use forests is better than outside special-use forests (Pham et al. 2010).

The most popular method to distinguish jewel orchid species for conservation and cultivar development purposes is based solely on morphological features, such as leaf shape, color, and vein structure. Although this method is time-saving and cost-effective, morphological identification has several disadvantages since the plant's growing habitat could influence plant morphology. In addition, most distinctive morphological features appear only during a certain period in the development stage or after reaching maturity (Ko et al. 2013). Thus, the inaccuracy of jewel orchid classification based on visual appearance is due to the high similarity among different species (Lin 1988). Bhattacharjee and Chowdhery (2013) reported that the previous morphology-bases taxonomy has resulted in misidentification between two jewel orchid species in India, namely *Zeuxine goodyeroides* Lindl. and *Zeuxine nervosa* (Wall. ex Lindl.) Benth. ex Trimen.

Recently, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) molecular markers have been used for the plant classification, such as *Olea europaea* L. (Martins-Lopes et al. 2007), *Cassia tora* L. (Kumar and Roy 2018), and *Ilex aquifolium* L. (Tsaktsira et al. 2021). Those markers have an unlimited number of obtained polymorphisms, are free from environmental influence, and require only a small amount of DNA for analysis. RAPD was successfully utilized to characterize the genetic structure of different species in Papua's endemic orchid (Abbas et al. 2017), *Dendrobium* (Choopeng et al. 2019), *Coelogyne pandurata* Lindl., *C. rumphii* Lindl. and their hybrids (Hartati and Mulawati 2020), and micropropagated *Dendrobium fimbriatum* Hook. (Tikendra et al. 2021). ISSR marker is also widely used for its simplicity, rapidity, ease of implementation, and cost-effectiveness compared to other methods. For example, the marker was used to screen the somaclonal variation of *Ludisia discolor* A.Rich. for long-term conservation (Rajan et al. 2022) or to investigate the homogeneity of *Anoectochilus elatus* Lindl. regenerated

from somatic embryogenesis (Sherif et al. 2018). More recently, a study from Malaysia reported the application of this marker in confirming the genetic stability of the in vitro regenerated plants to the mother plant, *Macodes limii* J.J. Wood & A.L. Lamb., a jewel orchid endemic to Sabah, Malaysia (David et al. 2022).

Some studies reported that different species of jewel orchids contain various chemical compositions (Refish et al. 2015) and different medicinal values (Ye et al. 2017). Thus, analyzing genetic diversity could be useful for the conservation and authentication of this medicinal plant. Therefore, this study aimed to investigate the genetic diversity of 20 jewel orchids in Vietnam using RAPD and ISSR markers. The achieved results would be a valuable contribution to assisting the conservation efforts of precious jewel orchid germplasm and future breeding programs of jewel orchids in Vietnam.

MATERIALS AND METHODS

Plant materials

The leaves of 20 jewel orchid accessions included in three genera were collected from different places in Vietnam in wild and cultivated areas (Table 1), then dried in silica gel, and kept in a cool place until use. Although all samples are named jewel orchids, the leaf morphology presents a large variation in the vein structure, size, and color (Figure 1).

Procedures

DNA extraction

DNA was extracted with the CTAB method (Cetyl Trimethyl Ammonium Bromide) described by Madhou et al. (2013). DNA quality was checked on 1.5% agarose gel by staining with 0.5 µg/mL GelredTM and observed under ultraviolet light using Quantum gel reader - ST4 3000 (Montreal- Biotech, Canada). DNA concentration was then determined with a spectrophotometer (Optima SP3000 nano UV-VIS, Japan).

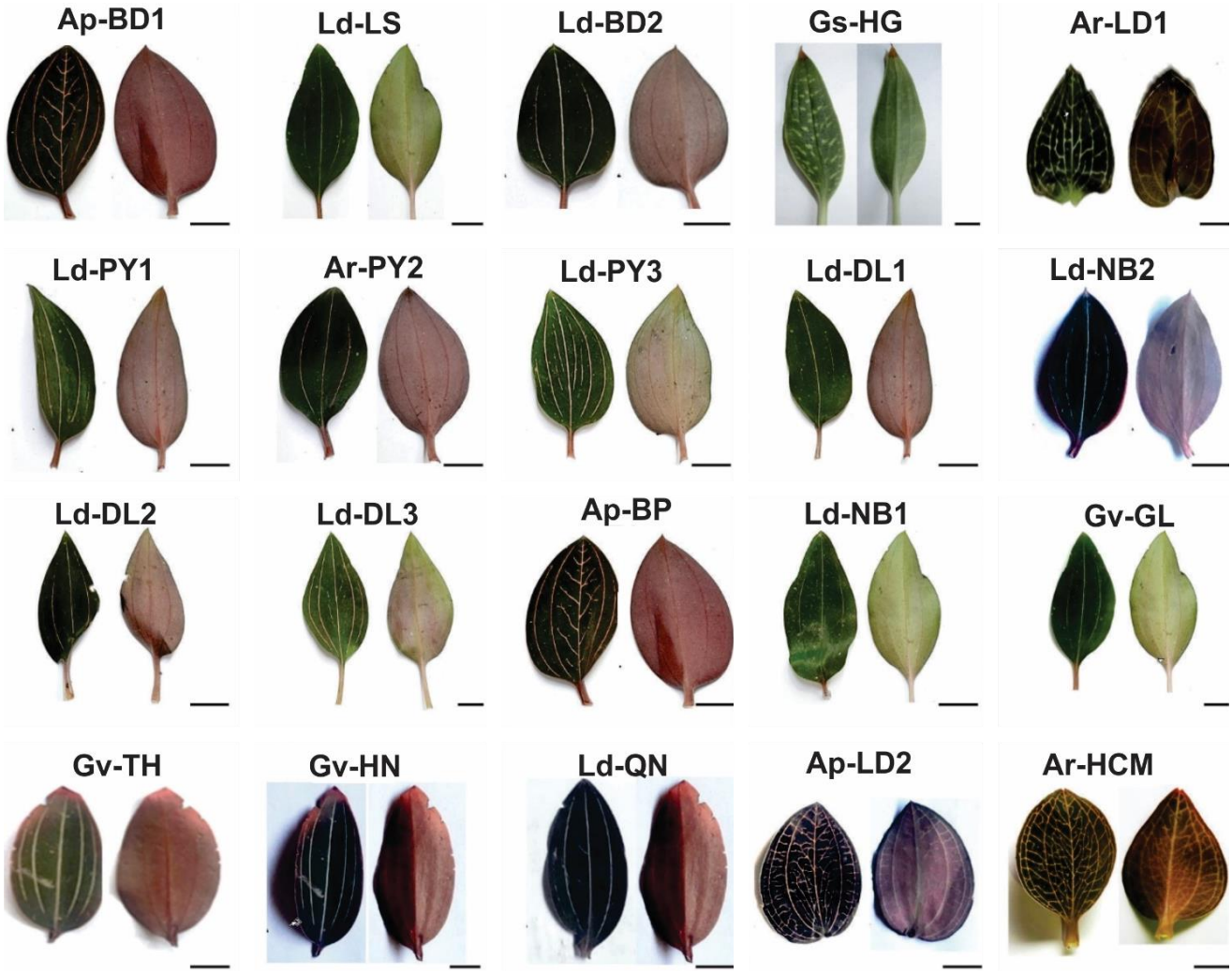

Figure 1. Leaf morphology of the jewel orchid samples used in the study (bar = 1cm). The scientific name of each abbreviated accessions studied are presented in Table 1.

RAPD and ISSR amplification

The compositions of PCR reactions of both RAPD and ISSR markers were followed by Ho and Tu (2019). Each reaction was performed in a volume of 25 µL containing 12.5 µL 2X Mytaq Red Mix (Bioline, UK), 1 µL of 20 ng DNA, 0.55 µL of 10 µM primer, and sterilized distilled water. The RAPD conditions were executed as follows: 94°C in 2 min for pre-denaturation; then 35 cycles of 94ºC in 30 sec for denaturation, 35ºC for 30 in for primer annealing, and 72ºC in 50 sec for primer extension. Finally, 72ºC in 5 min was added to complete the reactions. While the ISSR conditions were similar to those of RAPD reactions except that temperature annealing for primers was set at 55ºC. PCR reactions were performed using SureCycler 8800 Thermal Cycler (Agilent, USA). PCR amplifications were separated using 1.2% agarose gel and the product sizes were estimated based on 1 kb DNA ladder (Bioline, UK). Sequences of ten RAPD and ten ISSR primers used in this study are presented in Table 2.

Data analysis

Only the clear amplification bands from gel electrophoresis were encoded "1" for bands present and "0" for bands absence in specific positions. Information on primer quality is determined by the PIC (Polymorphism Information Content) coefficient according to the formula established by Chesnokov and Artemyeva (2015). The dendrogram was created by UPGMA (Unweighted Pair-group Method with Arithmetical Averages) method in the SAHN (Sequential, Hierarchical, Agglomerative, and Nested Clustering) module of NTSYSpc 2.1 software. The same program was also applied to determine the principal coordinate analysis (PCA) for constructing three-dimensional charts with DCENTER module (Rohlf 2000). The cut-off values of dendrograms were determined based on the calculation method described by Jamshidi and Jamshidi (2011). In this analysis, one data was performed from single or combined markers. The correlation between RAPD and ISSR similar matrices was determined using the ade4 package of the R-4.1.1 program.

Table 1. Jewel orchids in Vietnam that were used in the study

Accession code	Scientific name*	Collection site (ward, district, province)	Coordinate
Gs-HG	*Goodyera schlechtendaliana* R.Br.	Thuong Son, Vi Xuyen, Ha Giang	22°42'35.2"N 104°49'01.6"E
Ld-LS	*Ludisia discolor* A.Rich.	Dong Kinh, Lang Son City, Lang Son	21°50'44.4"N 106°45'45.6"E
Gv-HN	*Goodyera velutina* R.Br.	Vinh Phu, Ba Dinh, Ha Noi	21°02'51.0"N 105°48'24.1"E
Ld-NB1	*Ludisia discolor* A.Rich.	My Yen, Yen Mo, Ninh Binh	20°07'55.9"N 106°00'05.6"E
Ld-NB2	*Ludisia discolor* A.Rich.	Nam Son, Tap Diep, Ninh Binh	20°08'33.2"N 105°53'08.8"E
Gv-TH	*Goodyera velutina* R.Br.	Dong Yen, Dong Son, Thanh Hoa	19°47'39.5"N 105°42'57.5"E
Ld-QN	*Ludisia discolor* A.Rich.	Binh Trung, Binh Son, Quang Ngai	15°18'07.4"N 108°43'05.2"E
Ap-BD1	*Anoectochilus pingbianensis* Blume	Hoai Chau, Hoai Nhon, Binh Dinh	14°34'00.8"N 108°59'43.7"E
Ld-BD2	*Ludisia discolor* A.Rich.	Tay Phu, Tay Son, Binh Dinh	13°50'16.1"N 108°51'14.9"E
Gv-GL	*Goodyera velutina* R.Br.	Ia Bang, Chuprong, Gia Lai	13°48'35.9"N 107°59'30.4"E
Ld-PY1	*Ludisia discolor* A.Rich.	Eacha Rang, Son Hoa, Phu Yen	13°05'20.7"N 108°53'13.3"E
Ar-PY2	*Anoectochilus roxburghii* Blume	Phuoc Tan, Son Hoa, Phu Yen	13°15'27.6"N 108°53'30.7"E
Ld-PY3	*Ludisia discolor* A.Rich.	Son Long, Son Hoa, Phu Yen	13°12'28.1"N 109°06'22.2"E
Ld-DL1	*Ludisia discolor* A.Rich.	Cu Prong, Ea Kar, Dak Lak	12°44'16.7"N 108°37'37.4"E
Ld-DL2	*Ludisia discolor* A.Rich.	Ea O, Ea Kar, Dak Lak	12°42'35.7"N 108°29'23.7"E
Ld-DL3	*Ludisia discolor* A.Rich.	Ea Pan, Ea Kar, Dak Lak	12°42'23.4"N 108°34'53.4"E
Ap-BP	*Anoectochilus pingbianensis* Blume	Phuoc Binh, Phuoc Long, Binh Phuoc	11°49'07.6"N 106°56'04.6"E
Ar-LD1	*Anoectochilus roxburghii* Blume	Dạ Sar, Da Lat, Lam Dong	11°59'58.4"N 108°30'52.3"E
Ap-LD2	*Anoectochilus pingbianensis* Blume	Hoa Nam, Di Linh, Lam Dong	11°27'58.1"N 107°53'37.1"E
Ar-HCM	*Anoectochilus roxburghii* Blume	Pham Van Coi, Cu Chi, Ho Chi Minh	11°01'10.5"N 106°31'45.2"E

Note: Scientific names of accessions studied are from Ho et al. (2021)

Table 2. Sequences of RAPD and ISSR markers that were used in this study

RAPD	Sequence (5'-3')	References	ISSR	Sequence (5'-3')	References
OPB-04	GGACTGGAGT	Crochemore et al. (2003)	UBC880	GGAGAGGAGAGGAGA	Levi et al. (2004)
OPB-07	GGTGACGCAG	Crochemore et al. (2003)	UBC825	ACACACACACACACACT	Levi et al. (2004)
OPM-18	CACCATCCGT	Crochemore et al. (2003)	UBC841	GAGAGAGAGAGAGAGACTC	Levi et al. (2004)
OPA 05	AGGGGTCTTG	Crochemore et al. (2003)	UBC810	GAGAGAGAGAGAGAGAT	Levi et al. (2004)
OPF-06	GGGAATTCGG	Crochemore et al. (2003)	UBC855	ACACACACACACACACCTT	Shukla et al. (2017)
RAPD-09	GACCGCTTGT	Crochemore et al. (2003)	UBC809	AGAGAGAGAGAGAGAGG	Shukla et al. (2017)
OPB-18	GGGAATTCGG	Crochemore et al. (2003)	UBC834	AGAGAGAGAGAGAGACYT	Shukla et al. (2017)
OPN 18	GGTGAGGTCA	Crochemore et al. (2003)	UBC814	CTCTCTCTCTCTCTCTA	Shukla et al. (2017)
RAPD-01	TCCTACGCAC	Crochemore et al. (2003)	UBC 868	GAAGAAGAAGAAGAAGAA	Kumar et al. (2015)
OPM 06	CTGGGCAACT	Crochemore et al. (2003)	UBC 890	ACGACTACGGTGTGTGTTTGTGT	Shukla et al. (2017)

RESULTS AND DISCUSSION

RAPD analysis

The RAPD analysis results showed that all 10 markers are potential for genetic study of jewel orchids since all markers produced clear bands that appeared on the agarose gel after electrophoresis (Figure 2). The band numbers varied from 5 to 11, all polymorphic. Meanwhile, the information index (PIC) of these 10 primers varied from 0.76 to 0.92 (Table 3). Polymorphic DNA bands accounted for 100%, averaging 8.0 bands per primer. The calculated genetic similarity coefficients among samples showed a large variation in the similarity coefficients between samples from 0.37-0.81. The lowest similarity coefficient was found in the Gv-HN and Ar-PY2 samples (0.37), and the highest was from Gv-HN and Gs-HG (0.81) (Table 4). A dendrogram has divided 20 accessions into four clusters (Figure 4A). On the other hand, 20 examined jewel orchid accessions were grouped into three main clusters by PCA analysis (Figure 5A).

The PIC value is an important parameter showing the effectiveness of specific primers in genomic characterization. In this study, the PIC value of 10 RAPD primers varied from 0.76 to 0.92 (Table 3). It means that all these primers are deemed suitable for genetic diversity study according to the classification of Botstein et al. (1980) that PIC $\geq$ 0.5 is considered very high information, 0.5> PIC $\geq$ 0.25 is medium information, and PIC <0.25 is little information. In addition, polymorphic DNA bands, which are up to 100%, held important information for the molecular study, which shows that they could provide more detailed information about the genetic makeup of the individuals being analyzed.

In line with this study, numerous studies using RAPD for analyzing the genetic relationship of jewel orchid species, such as the Chinese group using 28 RAPD markers to reveal genetic variation in germplasms of *Anoectochilus roxburghii* Blume (Wang et al. 2015). The genetic diversity among 20 accessions of *Anoectochilus calcareus* Aver. collected in Ha Giang-Vietnam, a northernmost province of Vietnam near the China border, has found a narrow genetic diversity (Nguyen et al. 2014). Furthermore, RAPD was previously used to authenticate the herbal plant species *Patrinia* in Korea (Moon et al. 2016). Therefore, RAPD is generally considered a preferred method for genetic investigation in plants with no or little genetic information. In 2020, a study on *Dendrobium* orchids reported that RAPD markers are better than SSR markers in the phylogenetic analysis since former results are more similar to descriptive morphological characters (Basavaraj et al. 2020). Nevertheless, this marker contains a serious drawback based on its working principle, such as low reproducibility and being highly influenced by experimental conditions leading to a lack of resolution (Konzen et al. 2017).

ISSR analysis

The sequences of ISSR primers are longer and bind to conserved regions between SSR regions, meaning that this method has superior characteristics such as simplicity and high reproducibility. This fact has made ISSR more effective for distinguishing genotypes with high genetic similarity due to the high mutation rate commonly seen in ISSR loci (Verma et al. 2017). In this study, all ISSR reactions produced clear amplified bands, and are displayed in Figure 3. A total of 76 bands were generated from 10 primers ranging from 200 to 2,000 bp, all polymorphic. In particular, UBC880 and UBC825 primers produced the highest band number (10 bands), while UBC810 and UBC890 primers generated the lowest band number (5 bands). The PIC coefficient of the examined primers ranged from 0.79 (UBC810) to 0.91 (UBC825) (Table 3), with a total average of 0.88. The similarity coefficient varied from 0.33 (between Gv-HN and Ar-PY2) to 0.87 (between Gv-HN and Ld-BD2), the full data is presented in Table 4. The PIC coefficient of the examined primers is higher than that of previous studies on orchid species such as subtribe Laeliinae (Fajardo et al. 2014) and *Dendrobium* genus (Dharmarathna et al. 2018). Thus all primers are considered informative for the genetic study of jewel orchids.

The result from the phylogenetic analysis of ISSR was not corresponding to that of RAPD (Figure 4B), in which 20 jewel orchid accessions were classified into five clusters. Nevertheless, only two main groups were established after analysis by PCA (Figure 5B). ISSR markers were not only used for evaluating the genetic variation of jewel orchids. This method was also previously utilized for investigating the somaclonal variation during tissue culture of jewel orchids (Zhang et al. 2010). Furthermore, the classifying capacity of ISSR in jewel orchids was also formerly reported. For example, Lin et al. (2007) successfully used the ISSR marker to distinguish four lines with different biochemical values belonging to *Anoectochilus formosanus* Hayata, one member of the jewel orchid group.

Combined RAPD and ISSR data

Both data from RAPD and ISSR markers were integrated for analysis by using the UPGMA algorithm, the Jaccard's coefficient varies from 0.35 (between Ar-PY2 and Gv-HN) to 0.83 (between Gv-HN and Gs-HG) (Table 5). The value from Mantel's test with 1000 replicates 0.83 with a p-value < 0.05, indicating the significant relatedness between these two markers. The dendrogram built based on combined data is shown in Figure 4C. At the cut-off level of 0.60, the dendrogram is divided into four clusters. The analysis of the three main components shows that 20 accessions formed three clusters (Figure 5C) which are relatively similar to RAPD analysis (Figure 5A).

Plotting diagrams from PCA are not corresponding to dendrogram developed directly from RAPD or ISSR markers, proposing that the algorithm in the PCA method reduces minor effectors and only keeps the three most significant factors relevant to genetic relatedness of jewel orchid accessions from data to build the plots. Thus, PCA analysis could be used as an alternative method for sample classification. Furthermore, the low factors used for calculation in PCA could be advantageous for jewel orchid classification. However, the results show considerable

differences in detecting polymorphism capacity between RAPD and ISSR markers. This could be due to the distinct working mechanisms of these two methods, where RAPD and ISSR markers amplify the whole genome and between two satellite regions, respectively (Khalik et al. 2014). This phenomenon was reported in different plant species such as sweet potato landraces (Moulin et al. 2012), bamboos (Goyal and Sen 2015), *Buxus hyrcana* Pojark. (Shanjani et al. 2018).

The topology of cluster analysis of 20 jewel orchid accessions between PCR-based methods (RAPD and ISSR) is not corresponding to taxa identification based on DNA barcoding from Ho et al. (2021). It could be due to the difference in working principles of RAPD and ISSR markers compared to molecular taxonomy method based on DNA barcode region or the large variation in the genetic composition of jewel orchid. This phenomenon was also reported previously in some plant genera such as *Ocimum* (Chen et al. 2013), *Triticum* (Kyrienko et al. 2018), and *Salvia* (Sunar et al. 2020). Although plant identification based on DNA barcode is recommended, the accuracy of this method is variable and depends on plant species and barcode loci (Schori and Showalter 2011). So that RAPD and ISSR are still preferred markers supplementing DNA barcodes in plant identification (Techen et al. 2014; Ramasetty et al. 2016) since the capacity of polymorphism of RAPD and ISSR is higher than that of DNA barcodes (Sevindik et al. 2020).

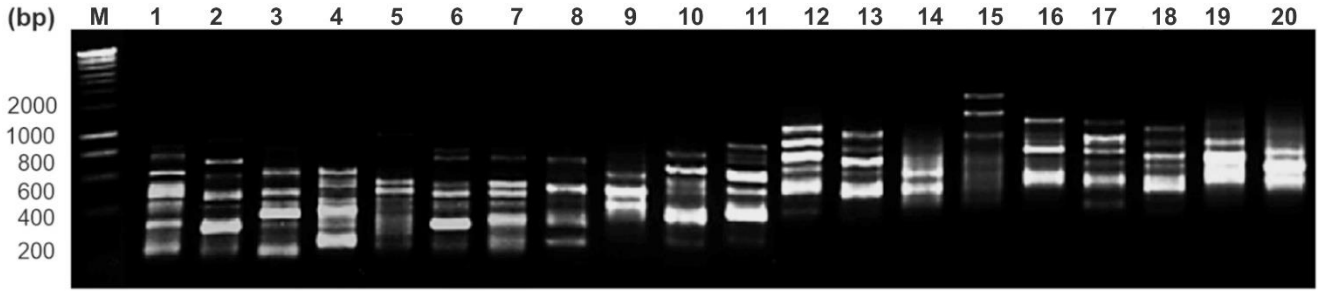

Figure 2. Result of RAPD marker with OPN18 primer. The number is corresponding to the sample number in Table 1; M: 1 kb DNA marker (Bioline, UK)

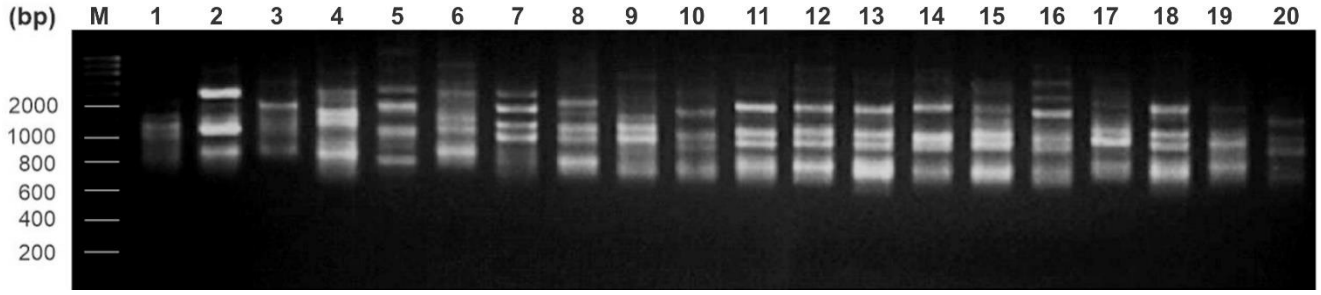

Figure 3. Result of ISSR marker with UBC811 primer. The number corresponds to the sample number in Table 1; M: 1kb DNA marker (Bioline, UK)

Table 3. Characteristics of DNA profiles generated by RAPD and ISSR markers

RAPD marker	SB	NPB	PPB (%)	PIC	ISSR marker	SB	NPB	PPB (%)	PIC
OPB04	10	10	100	0.87	UBC880	10	10	100	0.87
OPB07	10	10	100	0.92	UBC825	10	10	100	0.91
OPM18	9	9	100	0.89	UBC841	9	9	100	0.88
OPA05	5	5	100	0.79	UBC810	5	5	100	0.79
OPF06	11	11	100	0.86	UBC855	7	7	100	0.90
RAPD09	5	5	100	0.76	UBC809	8	8	100	0.92
OPB18	7	7	100	0.90	UBC834	9	9	100	0.89
OPN18	7	7	100	0.80	UBC814	7	7	100	0.87
RAPD01	9	9	100	0.88	UBC868	6	6	100	0.85
OPB04	7	7	100	0.88	UBC890	5	5	100	0.89
Sum	80	80	-	-		76	76	-	-
Average	8.0	8.0	100	0.86		7.6	7.6	100	0.88

Note: SB: Total scored bands for each marker; NPB: number of polymorphic bands for each marker; PPB: percentage of polymorphic bands based on the ratio of NPB to SB; PIC: polymorphism information content

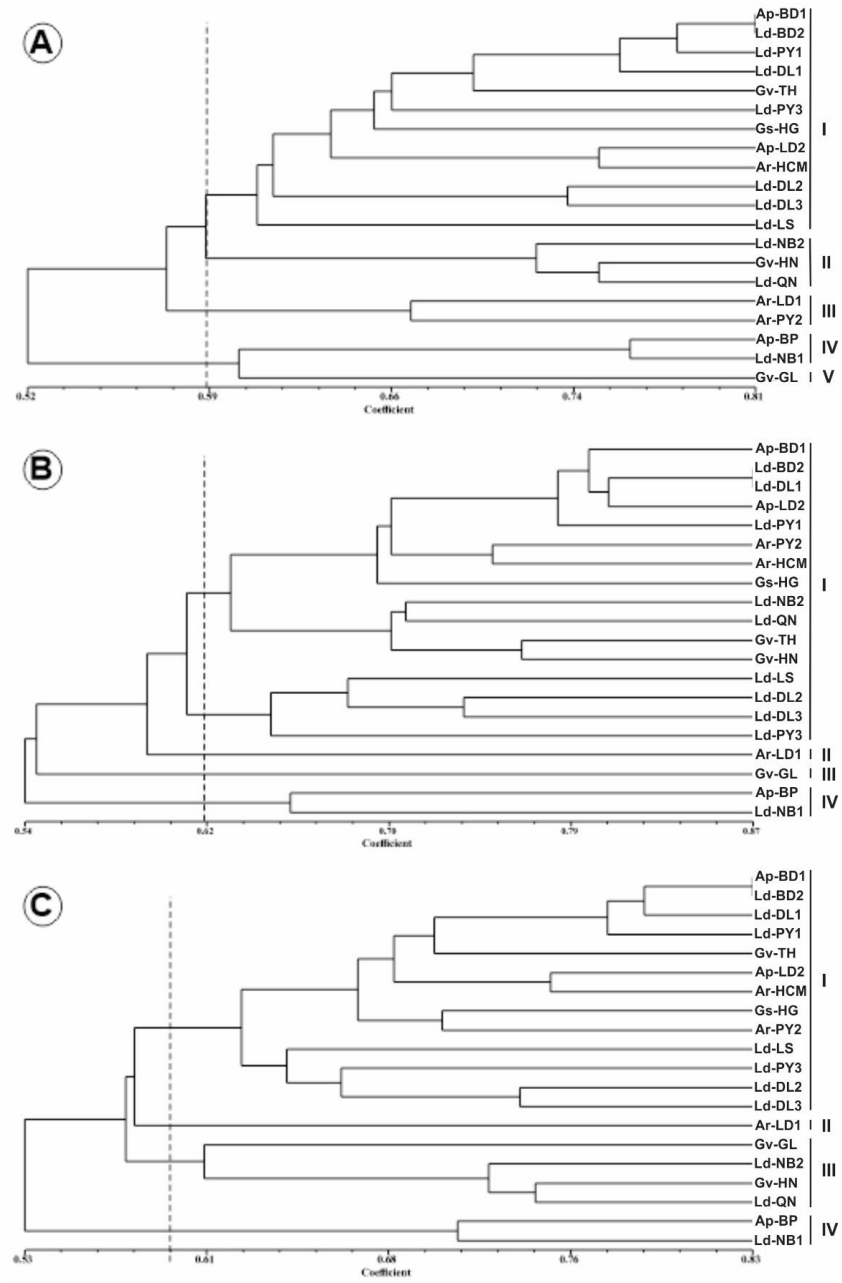

Figure 4. The dendrograms are produced by UPGMA method based on Jaccard's coefficient with: (A) 10 RAPD primers, (B) 10 ISSR primers, and (C) RAPD + ISSR combination. The vertical lines indicate the cut-off values of each dendrogram and the scale shown at the bottom is the measure of genetic similarity.

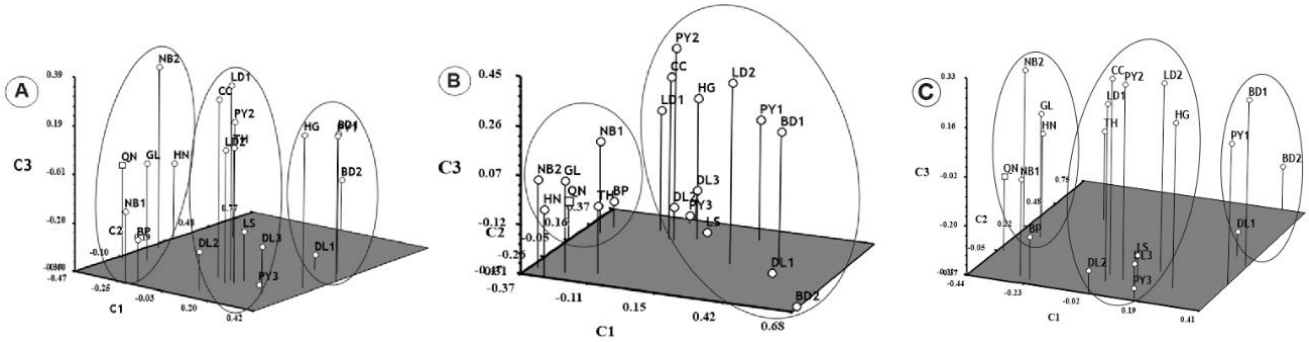

Figure 5. Three-dimensional plot of the principal coordinate analysis (PCA) of genetic distance among 20 jewel orchids by using: (A) 10 RAPD primers, (B) 10 ISSR primers, and (C) combiner RAPD and ISSR primers

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

	Gs-HG	**Ld-LS**	**Gv-HN**	**Ld-NB1**	**Ld-NB2**	**Gv-TH**	**Ld-QN**	**Ap-BD1**	**Ld-BD2**	**Gv-GL**	**Ld-PY1**	**Ar-PY2**	**Ld-PY3**	**Ld-DL1**	**Ld-DL2**	**Ld-DL3**	**Ap-BP**	**Ar-LD1**	**Ap-LD2**	**Ar-HCM**
Gs-HG	1.00	0.64	0.86	0.67	0.63	0.76	0.75	0.64	0.75	0.59	0.57	0.37	0.58	0.53	0.59	0.62	0.59	0.59	0.78	0.66
Ld-LS	0.59	1.00	0.68	0.55	0.57	0.62	0.58	0.61	0.63	0.71	0.66	0.49	0.51	0.46	0.50	0.58	0.53	0.63	0.63	0.67
Gv-HN	0.81	0.66	1.00	0.71	0.57	0.80	0.74	0.63	0.87	0.61	0.58	0.33	0.49	0.59	0.63	0.76	0.68	0.61	0.84	0.67
Ld-NB1	0.70	0.59	0.66	1.00	0.64	0.72	0.74	0.61	0.68	0.66	0.61	0.51	0.57	0.54	0.58	0.66	0.58	0.55	0.68	0.67
Ld-NB2	0.59	0.52	0.53	0.62	1.00	0.61	0.72	0.62	0.57	0.62	0.49	0.53	0.63	0.47	0.54	0.57	0.51	0.64	0.59	0.61
Gv-TH	0.77	0.67	0.78	0.72	0.59	1.00	0.78	0.67	0.78	0.67	0.70	0.39	0.61	0.53	0.54	0.70	0.62	0.64	0.78	0.66
Ld-QN	0.67	0.59	0.58	0.67	0.67	0.62	1.00	0.66	0.68	0.66	0.66	0.46	0.67	0.59	0.66	0.71	0.66	0.66	0.74	0.75
Ap-BD1	0.68	0.63	0.67	0.63	0.53	0.66	0.63	1.00	0.66	0.71	0.63	0.57	0.59	0.43	0.50	0.58	0.58	0.66	0.61	0.54
Ld-BD2	0.76	0.58	0.77	0.63	0.51	0.73	0.58	0.72	1.00	0.66	0.63	0.43	0.51	0.54	0.58	0.74	0.63	0.55	0.76	0.62
Gv-GL	0.53	0.61	0.54	0.63	0.53	0.56	0.63	0.65	0.62	1.00	0.74	0.59	0.62	0.54	0.53	0.61	0.63	0.66	0.66	0.62
Ld-PY1	0.70	0.62	0.61	0.65	0.49	0.67	0.59	0.66	0.68	0.73	1.00	0.62	0.62	0.41	0.55	0.58	0.47	0.61	0.61	0.64
Ar-PY2	0.43	0.48	0.37	0.46	0.46	0.41	0.43	0.54	0.52	0.59	0.56	1.00	0.66	0.45	0.54	0.43	0.46	0.59	0.41	0.47
Ld-PY3	0.54	0.57	0.46	0.49	0.52	0.52	0.59	0.58	0.48	0.61	0.54	0.76	1.00	0.61	0.59	0.62	0.59	0.70	0.54	0.66
Ld-DL1	0.47	0.42	0.56	0.44	0.49	0.47	0.49	0.48	0.51	0.53	0.44	0.56	0.65	1.00	0.67	0.62	0.67	0.54	0.57	0.53
Ld-DL2	0.61	0.46	0.65	0.53	0.61	0.56	0.61	0.49	0.57	0.52	0.51	0.49	0.53	0.61	1.00	0.66	0.74	0.71	0.63	0.59
Ld-DL3	0.65	0.54	0.78	0.59	0.47	0.65	0.57	0.58	0.71	0.53	0.54	0.43	0.49	0.57	0.68	1.00	0.76	0.68	0.68	0.67
Ap-BP	0.65	0.59	0.73	0.52	0.42	0.62	0.54	0.58	0.66	0.53	0.54	0.46	0.52	0.59	0.71	0.72	1.00	0.74	0.68	0.59
Ar-LD1	0.59	0.59	0.63	0.47	0.52	0.59	0.59	0.61	0.53	0.61	0.54	0.61	0.70	0.54	0.73	0.62	0.75	1.00	0.58	0.62
Ap-LD2	0.68	0.63	0.70	0.61	0.56	0.73	0.63	0.62	0.70	0.62	0.66	0.54	0.61	0.58	0.59	0.61	0.61	0.63	1.00	0.75
Ar-HCM	0.66	0.56	0.62	0.63	0.56	0.63	0.58	0.52	0.62	0.57	0.66	0.52	0.56	0.51	0.65	0.61	0.58	0.58	0.75	1.00

Table 5. Simple matching coefficients of similarity among 20 jewel orchid accessions with combined data from RAPD and ISSR primers

	Gs-HG	**Ld-LS**	**Gv-HN**	**Ld-NB1**	**Ld-NB2**	**Gv-TH**	**Ld-QN**	**Ap-BD1**	**Ld-BD2**	**Gv-GL**	**Ld-PY1**	**Ar-PY2**	**Ld-PY3**	**Ld-DL1**	**Ld-DL2**	**Ld-DL3**	**Ap-BP**	**Ar-LD1**	**Ap-LD2**	**Ar-HCM**
Gs-HG	1.00																			
Ld-LS	0.62	1.00																		
Gv-HN	0.83	0.67	1.00																	
Ld-NB1	0.68	0.57	0.68	1.00																
Ld-NB2	0.61	0.54	0.55	0.63	1.00															
Gv-TH	0.77	0.65	0.79	0.72	0.71	1.00														
Ld-QN	0.71	0.59	0.66	0.70	0.70	0.70	1.00													
Ap-BD1	0.66	0.62	0.65	0.62	0.57	0.66	0.65	1.00												
Ld-BD2	0.75	0.61	0.82	0.66	0.54	0.75	0.63	0.69	1.00											
Gv-GL	0.56	0.66	0.57	0.65	0.57	0.61	0.65	0.68	0.64	1.00										
Ld-PY1	0.63	0.64	0.59	0.63	0.49	0.68	0.63	0.65	0.66	0.74	1.00									
Ar-PY2	0.40	0.48	0.35	0.48	0.49	0.40	0.45	0.55	0.48	0.59	0.59	1.00								
Ld-PY3	0.56	0.54	0.47	0.53	0.57	0.56	0.63	0.59	0.50	0.61	0.58	0.71	1.00							
Ld-DL1	0.50	0.44	0.57	0.49	0.48	0.50	0.54	0.46	0.52	0.54	0.43	0.50	0.63	1.00						
Ld-DL2	0.60	0.48	0.64	0.55	0.57	0.55	0.63	0.50	0.57	0.52	0.53	0.52	0.56	0.64	1.00					
Ld-DL3	0.63	0.56	0.77	0.63	0.52	0.67	0.64	0.58	0.72	0.57	0.56	0.43	0.55	0.59	0.67	1.00				
Ap-BP	0.62	0.56	0.71	0.55	0.46	0.62	0.60	0.58	0.65	0.58	0.51	0.46	0.55	0.63	0.72	0.74	1.00			
Ar-LD1	0.59	0.61	0.62	0.51	0.58	0.62	0.63	0.63	0.54	0.63	0.57	0.60	0.70	0.54	0.72	0.65	0.74	1.00		
Ap-LD2	0.73	0.63	0.77	0.65	0.57	0.75	0.68	0.61	0.73	0.64	0.63	0.48	0.57	0.57	0.61	0.65	0.65	0.61	1.00	
Ar-HCM	0.66	0.61	0.65	0.65	0.58	0.65	0.66	0.53	0.62	0.59	0.65	0.50	0.61	0.52	0.62	0.64	0.59	0.60	0.75	1.00

In conclusion, after studying 20 jewel orchid accessions in Vietnam we found that RAPD and ISSR markers are not significant relatedness. However, the RAPD marker was superior to the ISSR marker in generating more informative bands. Consequently, this marker shows higher clustering analysis power. Nevertheless, the combination of RAPD and ISSR markers seems to be more effective for the clustering analysis of jewel orchid populations. Therefore, in the future, developing Sequence Characteristics Amplification Area (SCAR) markers from polymorphic RAPD and ISSR bands should be considered to provide specific primers that can improve the accuracy of jewel orchid authentication.

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