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Research Article
High Genetic Diversity of 16 Indian lettuce (*Lactuca indica* L.) Accessions from Vietnam

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Abstract

Background and Objective: Plant genetic resources provide the raw material for crop improvement and plant breeding program largely depends on it. Therefore, the evaluation of plant genetic resources plays a critical role in crop improvement and also in conserving valuable genetic resources for the future. In this study, the genetic diversity of 16 *Lactuca indica* L. accessions collected in Vietnam was investigated by using ISSR and RAPD markers. **Materials and Methods:** Genetic diversity of 16 *Lactuca sativa* L. genotypes collected in Vietnam were evaluated using Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) molecular markers. **Results:** In this study, 42 RAPD and ISSR primers were initially used, of which 12 and 9 primers, respectively were finally selected as they produced scorable patterns. RAPD markers produced a total of 113 loci, out of which 52 loci (45.96%) were polymorphic. The average percentage of the polymorphic band for RAPD primer is 45.96% and the genetic similarity based on simple matching coefficient ranged from 69.0-94.7%. ISSR analysis detected a total of 60 loci, out of which 22 loci (36.32%) were polymorphic and the genetic similarity ranged from 56.7-95.0%. In general, ISSR markers amplified fewer loci and showed lower variation in the percentage of polymorphism compares to the RAPD assay. **Conclusion:** These results indicate that the 16 collected Indian lettuce genotypes are genetically diverse. Because of these genetic diversities, the collected genotypes could be used for preserving or crossing programs to improve this precious medicinal plant in Vietnam.

Key words: DNA markers, genetic diversity, Indian lettuce (*Lactuca indica* L.), crossing programs, RAPD assay, ISSR markers

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lactuca indica L. is wild lettuce of genus *Lactuca*, which was originated in Africa. The genus *Lactuca* L. consists of approximately 100 species that are widespread across the world, especially in temperate and warm areas in Asia, Africa, Europe, North America and Australia[1,2]. In Asia, there are total of 51 species of the genus *Lactuca* L.[3].

Plant genetic resources are playing an important role in crop improvement. Therefore, the conservation of biodiversity of plant genetic resources, including wild relatives, is considered as the most important milestone for sustainable agricultural production[4]. There is increasing interest for conservation, evaluation and utilization of germplasm collections in *Lactuca sativa* and wild *Lactuca* spp. For example, the wild lettuces *L. serriola*, *L. saligna* and *L. virosa* are an important genetic resource as donors of resistance genes for breeding the cultivated lettuce (*L. sativa*)[3].

In Vietnam, Indian lettuce (*Lactuca indica* L.) is an undomesticated wild plant. This plant is cultivated as a leaf vegetable and more recently can also grow as a medicinal plant. Because Indian lettuce has multiple biological effects including antioxidant, antibacterial and anti-inflammatory, digestive, diuretic, necrotic properties, it has been used as components in traditional treatments of various diseases in Vietnam and other countries[5-7]. In addition, the plant also has minerals, reducing sugar, free amino acids, organic acids, vitamin C, phenolic and flavonoids[8,9].

Since the role of genetic diversity in plant breeding is well recognized, investigation and exploitation of the genetic diversity is the key to assessing the potential value of germplasm[3]. Many techniques can characterize and evaluate the differences between individuals based on morphological traits, biochemical traits and DNA-based markers, among those, DNA markers are the prominent techniques for characterization and evaluation[2].

Many molecular markers have been applied to analyze the diversity of *Lactuca* germ plasm, including: Sequence Characterized Amplified Region (SCAR), Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphism (AFLP), Internal Transcribed Spacers (ITS-1) and Single Nucleotide Polymorphisms (SNPs)[2,10-13]

In this study, genetic diversity and relationships among 16 *Lactuca indica* L. accessions collected in Vietnam were analyzed using 12 random amplified polymorphic DNA and 9 Inter Simple Sequence Repeat (ISSR) molecular markers. In addition, the present study also compared the potential of using RAPD and ISSR markers and their relative efficiency in exploring the genetic diversity of *Lactuca indica* L.

MATERIALS AND METHODS

Study area: This work was conducted at the laboratory and greenhouse at the Faculty of Biotechnology, Vietnam National University of Agriculture and Fruit and Vegetable Research Institute, from December, 2019-July, 2021.

Plant materials: Genetic diversity among 16 accessions, collected in 4 provinces of Vietnam, was assessed using RAPD and ISSR primers.

Research procedure

DNA extraction and PCR amplification: DNA was extracted from frozen leaves, following the method described by Masoomi-Aladizgeh *et al.*[14] with minor modifications. Briefly, about 50-100 mg of leaf tissue was ground in pestle and mortar with extraction buffer. Extraction buffer was prepared by dissolving 0.5 g CTAB, 1 g EDTA, 2.5 g Tris base and 5 g NaCl in 100 mL autoclaved water. Freshly add 10 µL of 2-mercaptoethanol and 15 mg PVPP to 1 mL of the extraction buffer before adding to the sample. Ground samples were incubated at 65°C for 10 min with occasional gentle swirling. After incubation, samples were centrifuged at 13700 g at 4°C for 10 min. The supernatant was taken to a new tube and the same volume of phenol: Chloroform: Isoamyl alcohol (25:24:1) was added to each tube, homogenized by vortexing and then centrifuged at 13700 g at 4°C for 10 min. The supernatant was transferred to a new tube and the same volume of chloroform: Isoamyl alcohol (24:1) was added to each tube, vortexed and centrifuged at 13700 g at 4°C for 10 min. The supernatant was collected and added double volume of ethanol to each tube, inverted tubes 8-10 times to mix the solution and then centrifuged at 13700 g at 4°C for 10 min. Discard the supernatant and wash the precipitated pellet twice with 70% EtOH. The dried pellet was dissolved in 100 µL Tris-EDTA buffer and stored at -20°C for further use.

RAPD decamer oligonucleotides (designed by Operon Technologies Inc. Alameda CA, USA), ISSR UBC primers (designed by University of British Columbia, Canada) and 2 ISSR primers (ISSR1 and ISSR 14)[15] were used. Amplification was done in an Eppendorf mastercycler personal machine. Amplification parameters were 94°C for 5 min followed by

40 cycles at 95°C for 60 sec, annealing temperatures (Ta) for 60 sec as expressed in Table 1 and 2 and 72°C for 2 min. A 72°C incubation for 10 min was included as a final step. Twenty-five µL amplification reactions contained 2X PCR master mix including tracking dye that contains components required for PCR amplification (2X PCR master mix solution (i-Taq)-Intron Biotechnology), 10 ng of genomic DNA for each reaction and 0.5 µM primer. Amplification products were analyzed by gel electrophoresis in 1.2% agarose gels containing RedSafe™ nucleic acid staining solution at 90V and photographed by UVP BioDoc-It Imaging Systems.

Data analysis: The amplified bands were scored manually as 1 (present) and 0 (absent) of RAPD or ISSR marker bands. A binary matrix was obtained for RAPD and ISSR. Data analyses including simple matching coefficient matrix, pair-wise similarity matrices and cluster analysis via unweight pair-group method using arithmetic average (UPGMA) were performed using NTSYS-pc version 2.1 program package. Estimation of genetic distance matrix correlation between markers was done using a mantel test[16] and Principal Component Analysis (PCA) was done by using XLSTAT Addinsoft[17].

The resolving power of the markers was calculated as described by Prevost and Wilkinson, the polymorphism information content (PIC value) for each marker is calculated as:

$$PIC\ (i) = 1-\sum(Pij)^2$$

Where, Pij is the frequency of the i_{th} pattern revealed by the j_{th} primer summed across all patterns revealed by the primers[18].

RESULTS AND DISCUSSION

Levels of polymorphism and genetic relationships among 16 Indian lettuce: While medicinal plants have globally been playing the important role of primary healthcare, only a very small number of plant species are cultivated for this purpose, the main resource is still wildly exploited. To estimate genetic relationships among 16 Indian lettuce accessions, originating from the North area of Vietnam, RAPD and ISSR analysis was performed. 20 RAPD and 22 ISSR primers were initially used, of which 12 and 9 primers, respectively were finally selected as they produced scorable patterns. RAPD and ISSR profiles are represented in Fig. 1a and b, respectively.

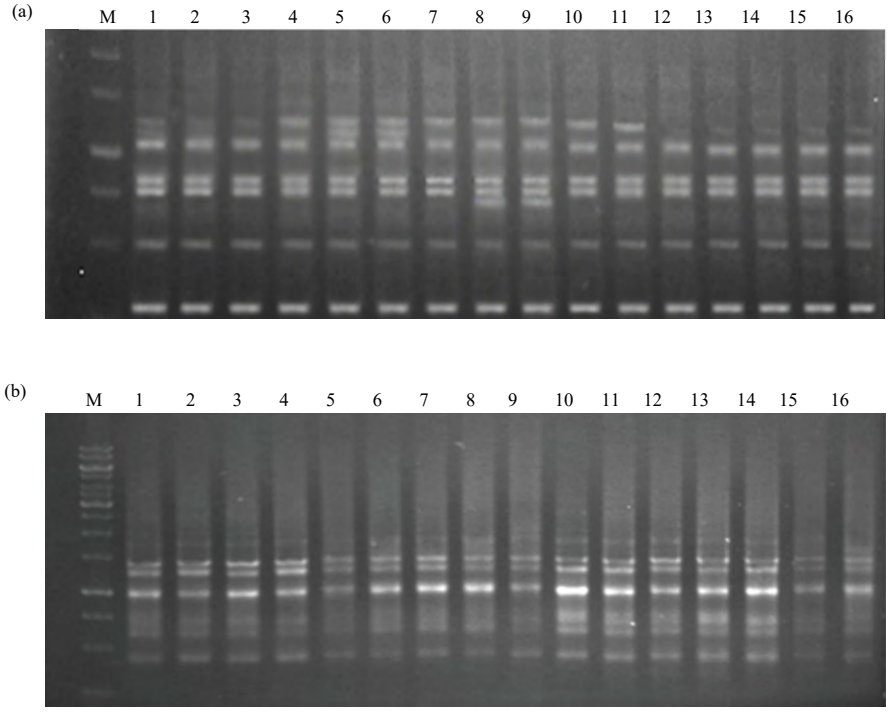

Fig. 1(a-b): RAPD and ISSR patterns of the sixteen Indian lettuce accessions, (a) RAPD profile obtained with primer OPB06 and (b) ISSR profile obtained with primer UBC866

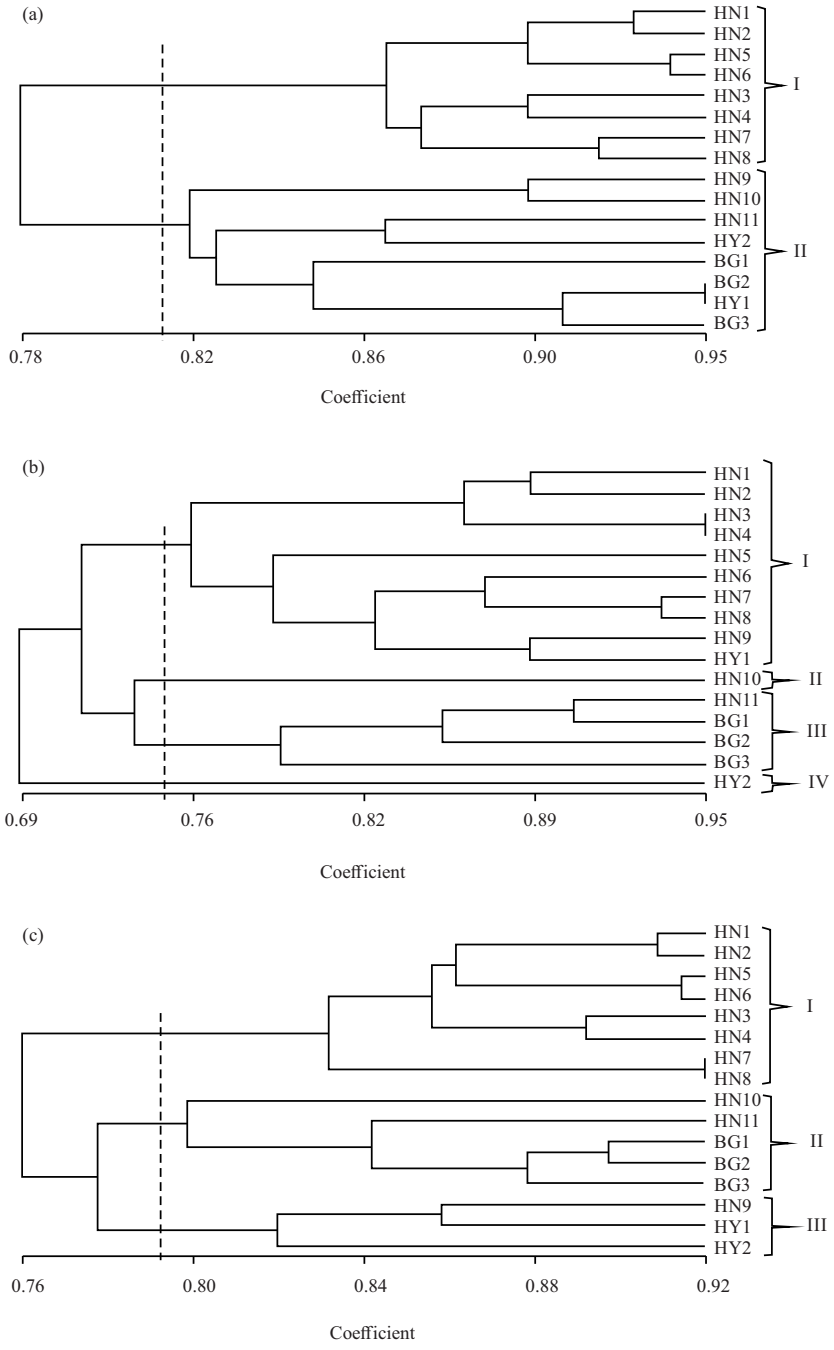

Fig. 2(a-c): Dendrogram constructed with UPGMA clustering method of sixteen accessions of Indian lettuce, (a) RAPD, (b) ISSR and (c) Combined RAPD and ISSR primers

The results of RAPD and ISSR assays and combined RAPD and ISSR data in fingerprinting of the 16 Indian lettuce accessions are presented in Table 1, 2 and Fig. 2.

RAPD analysis obtained a total of 113 loci, out of which 52 loci (45.96%) were polymorphic. The number of bands for each primer, ranged from 5 (OPC02) to 14 (OPB02), with an average of 9.42 bands per primer. The percentage of polymorphism ranged from 12.50-75.00% in OPD03 and OPB06, respectively and the average percentage of the polymorphic band for RAPD primer is 45.96%. The lowest and the highest PIC values were found for primer OPB06 (0.07) and OPB05 (0.23), respectively in Table 1. The percentage of the polymorphic band for RAPD primer in our study was following Padmalatha and Prasad[19], when they studied the genetic

Table 1: Performance of RAPD primers in the genetic diversity analysis of Indian lettuce

Primer names	Sequence (5′-3′)	Annealing temperature (°C)	Number of amplified loci	Number of polymorphic loci	Polymorphic band (%)	Total amplified bands/primer	Bands/ accession	PIC value	Resolving power
OPB02	TGATCCCTGG	30	14	6	42.9	125	7.81	0.17	15.625
OPB04	GGACTGGAGT	30	10	5	50.0	131	8.19	0.17	16.375
OPB05	TGCGCCCTTC	32	10	4	40.0	119	7.44	0.23	14.875
OPB06	TGCTCTGCCC	32	8	6	75.0	102	6.38	0.07	12.750
OPC01	TTCGAGCCAG	30	11	6	54.5	119	7.44	0.16	14.875
OPC02	GTGAGGCGTC	32	5	3	60.0	64	4.00	0.20	8.000
OPC03	GGGGGTCTTT	30	7	3	42.9	89	5.56	0.13	11.125
OPC04	CCGCATCTAC	30	11	6	54.5	123	7.69	0.16	15.375
OPC05	GATGACCGCC	32	11	7	63.6	144	9.00	0.16	18.000
OPD01	ACCGCGAAGG	32	9	2	22.2	83	5.19	0.23	10.375
OPD02	GGACCCAACC	32	9	3	33.3	96	6.00	0.18	12.000
OPD03	GTCGCCGTCA	32	8	1	12.5	93	5.81	0.19	11.625
Total			113	52		1288			

Table 2: Performance of ISSR primers in the genetic diversity analysis of Indian lettuce

Primer names	Sequence (5′-3′)	Annealing temperature (°C)	Number of amplified loci	Number of polymorphic loci	Polymorphic band (%)	Total amplified bands/primer	Bands/ accession	PIC value	Resolving power
UBC 811	GAGAGAGAGAGAGAGAC	50	8	2	25.0	74	4.63	0.35	9.25
UBC 813	CTCTCTCTCTCTCTCTT	49	6	2	33.3	59	3.69	0.23	7.38
UBC 827	ACACACACACACACACG	56	7	2	28.6	77	4.81	0.27	9.63
UBC 866	CTCCTCCTCCTCCTCCTC	56	8	4	50.0	94	5.88	0.16	11.75
UBC 872	GATAGATAGATAGATA	35	6	1	16.7	58	3.63	0.38	7.25
UBC 873	GACAGACAGACAGACA	45	6	2	33.3	73	4.56	0.19	9.13
ISSR1	CACACACACACACACAAG	56	6	3	50.0	64	4.00	0.20	8.00
UBC 808	AGAGAGAGAGAGAGAGC	50	8	4	50.0	95	5.94	0.17	11.88
ISSR 14	CGTCACACACACACACACA	44	5	2	40.0	42	2.63	0.18	5.25
Total			60	22		636			

Table 3: Matrix of genetic similarity among the sixteen Indian lettuce accessions revealed by ISSR markers (above diagonal) and RAPD markers (below diagonal) calculated by similarity coefficient of Sokal-Michener

	HN1	HN2	HN3	HN4	HN5	HN6	HN7	HN8	HN9	HN10	HN11	BG1	BG2	BG3	HY1	HY2
HN1		0.883	0.833	0.850	0.750	0.833	0.783	0.850	0.783	0.683	0.733	0.700	0.683	0.717	0.767	0.683
HN2	0.929		0.883	0.867	0.733	0.817	0.733	0.800	0.733	0.700	0.750	0.717	0.700	0.700	0.717	0.600
HN3	0.867	0.885		0.950	0.817	0.767	0.683	0.717	0.650	0.717	0.767	0.767	0.750	0.750	0.667	0.617
HN4	0.858	0.858	0.903		0.833	0.817	0.700	0.767	0.700	0.667	0.783	0.717	0.700	0.767	0.717	0.633
HN5	0.885	0.903	0.912	0.867		0.817	0.733	0.767	0.833	0.700	0.683	0.650	0.600	0.733	0.783	0.733
HN6	0.912	0.912	0.867	0.876	0.938		0.850	0.883	0.850	0.717	0.767	0.733	0.683	0.750	0.800	0.683
HN7	0.867	0.850	0.876	0.885	0.841	0.903		0.933	0.833	0.733	0.717	0.717	0.733	0.800	0.783	0.733
HN8	0.858	0.841	0.867	0.876	0.850	0.876	0.920		0.900	0.767	0.717	0.650	0.667	0.767	0.783	0.667
HN9	0.832	0.796	0.841	0.761	0.841	0.832	0.805	0.850		0.700	0.683	0.617	0.600	0.700	0.883	0.733
HN10	0.788	0.770	0.850	0.805	0.814	0.805	0.814	0.788	0.903		0.717	0.717	0.733	0.767	0.650	0.567
HN11	0.761	0.743	0.770	0.690	0.752	0.726	0.699	0.726	0.823	0.796		0.900	0.817	0.783	0.733	0.717
BG1	0.699	0.699	0.796	0.735	0.743	0.717	0.708	0.699	0.779	0.823	0.761		0.883	0.783	0.733	0.750
BG2	0.796	0.761	0.805	0.743	0.788	0.779	0.752	0.743	0.823	0.850	0.876	0.867		0.800	0.717	0.733
BG3	0.779	0.814	0.805	0.779	0.805	0.796	0.752	0.726	0.788	0.814	0.805	0.832	0.912		0.817	0.700
HY1	0.832	0.814	0.823	0.779	0.805	0.814	0.788	0.779	0.823	0.850	0.841	0.850	0.947	0.912		0.817
HY2	0.805	0.805	0.779	0.717	0.779	0.805	0.761	0.752	0.850	0.805	0.867	0.752	0.867	0.814	0.885	

polymorphism of *Rauvolfia tetraphylla* L.f, using RAPD markers and reported the percentage of the polymorphic band for RAPD primer was 51.6%.

ISSR analysis generated a total of 60 loci, out of which 22 loci (36.32%) were polymorphic. The number of bands for each primer, ranged from 5 (ISSR 14) to 8 (UBC 811, UBC 866, UBC 808), with an average of 6.67 bands per primer. The percentage of polymorphism ranged from 16.67-50.00% in UBC 872 and UBC 866, ISSR 1, UBC 808, respectively. The lowest and the highest PIC values were found for primer UBC 866 (0.16) and UBC 872 (0.38), respectively. The lowest and the highest resolving power values were found for primer ISSR 14 (5.25) and UBC 808 (11.88), respectively in Table 2.

The genetic similarity matrix, based on a simple matching coefficient for RAPD markers ranged from 69.0 (HN4 vs. HN11) to 94.7% (BG2 vs. HY1) Table 3.

Table 4: Comparison of the discriminating capacity of RAPD and ISSR of sixteen Indian lettuce (*Lactuca indica* L.) accessions

	Marker system	
Index types	RAPD	ISSR
Total number of primers	12	9
Total number of amplified loci	113	60
The average number of amplified loci/primer	9.42	6.67
Total number of amplified bands	1288	636
Average amplified bands/primer	107.33	70.67
Average amplified bands/accession/primer	6.71	4.42
Total number of polymorphic bands	52	22
The average number of polymorphic bands/primer	4.33	2.44
The average percentage of polymorphic band/primer (%)	45.96	36.32
Total number of monomorphic bands	61	38
Average resolving power/primer	13.42	8.83
Average PIC value/primer	0.17	0.24

Dendrogram analysis shows that, at the average of 81,6% similarity level, the 16 Indian lettuce accessions were clustered into 2 main clusters. The 1st cluster had 8 accessions and all of them were collected in Hanoi. The 2nd cluster contained 8 accessions, among them 3 accessions from Hanoi, 3 from Bac Giang and 2 from Hung Yen province. The 2nd cluster has more genetic diversity than the 1st one in Fig. 2a. Sharma *et al.*[10], analyzed the genetic diversity of 25 *Lactuca sativa* L. genotypes by using 22 RAPD primers and found the genetic similarity coefficients varied between 13.7-84.10% which indicates a broad genetic diversity. The result gained in current study revealed the degree of polymorphism ranged from 69.0 (HN4 vs. HN11) to 94.7% (BG2 vs. HY1) which is lower in genetic diversity compared to the work obtained by Sharma *et al.*[10].

In general, the selected ISSR markers amplified fewer loci and showed lower variation in the percentage of polymorphism compares to the RAPD assay. Interestingly, the PIC values conducted by ISSR were higher when compared to RAPD. The genetic similarity matrix, based on a simple matching coefficient ranged from 56.7 (HN10 vs. HY2) to 95.0% (HN3 vs. HN4) in Table 3. Dendrogram analysis shows that, at the average of 74.8% similarity level, the 16 Indian lettuce (*Lactuca indica* L.) accessions were grouped into 4 clusters. The 1st cluster had 10 accessions and nine of them were sampled in Hanoi and only HY1 was from Hung Yen province. While clusters 2 and 4 have only 1 accession, the 3rd cluster contained 4 accessions, among them 1 accession collected in Hanoi and 3 from Bac Giang in Fig. 2b.

Combined data from RAPD and ISSR together showed a genetic similarity matrix varied from 67.1 (HN4 vs. HY2) to 92.5% (HN7 vs. HN8). Dendrogram analysis shows that, at the average of 79.2% similarity level, the 16 Indian lettuce (*Lactuca indica* L.) accessions were divided into 3 major clusters. The 1st cluster had 8 accessions and all of them were sampled in Hanoi. The 2nd cluster contained 5 accessions, among them 2 accessions collected in Hanoi and 3 from Bac Giang and the 3rd cluster contained 2 samples from Hung Yen province and 1 from Hanoi in Fig. 2c.

Comparative study of RAPD and ISSR-based results: Many molecular markers are used for genetic diversity analysis and their application in crop breeding in general[20,21] and *Lactuca* spp.[22] or even specifically developed for Indian lettuce (*Lactuca indica* L.)[12].

Here random amplified polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) approaches used to analyze the genetic diversity of 16 Indian lettuce (*Lactuca indica* L.) accessions. While the RAPD marker grouped 8 accessions (HN1 to HN8) into 1 major cluster, ISSR markers grouped 10 accessions of which eight of them were the same with RAPD and plussed HN9 and HY accessions in Table 4. This result indicates that the dendrogram based on RAPD markers was different from ISSR. Interestingly, combined RAPD and ISSR also grouped 8 accessions (HN1-HN8) into 1 major group which is similar to RAPD.

The arithmetic means of all the marker indexes are presented in Table 4. Several indexes generated by RAPD showed better than ISSR such as average amplified bands/accession/primer index (6.71 vs. 4.42), the average number of polymorphic bands/primer (4.33 vs. 2.44) and the average percentage of polymorphic band/primer (%) (45.96 vs. 36.32), respectively. These results suggest that ISSR markers were less efficient than the RAPD assay in polymorphism detection (Table 4). Current results agreed with results obtained by Gajera *et al.*[23], who analyzed the genetic diversity of different endangered *Mangifera indica* genotypes of the Indian Gir forest region.

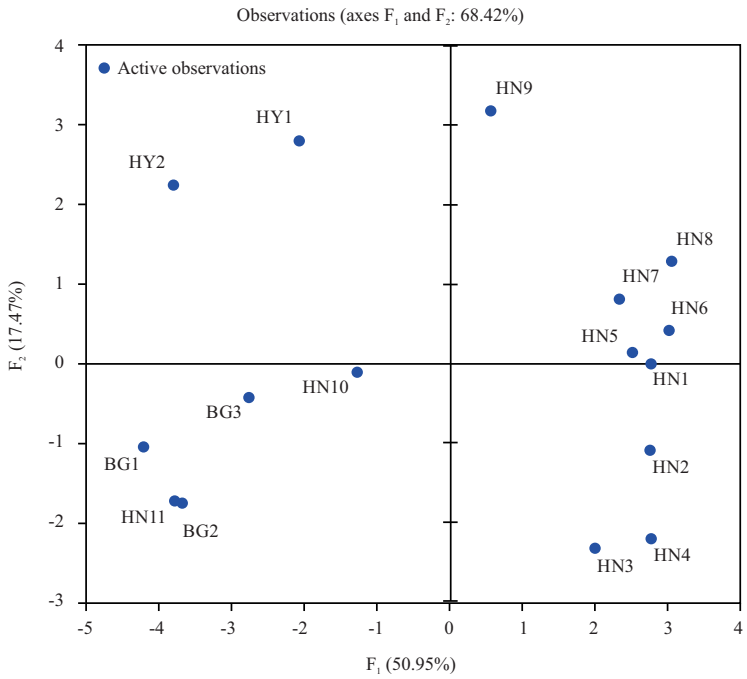

Fig. 3: Relationship among sixteen accessions determined by the principal component analysis

Current study shows the average number of bands/primer reached 9.42 for RAPD and 6.67 for ISSR marker while another study, worked on 20 black gram (*Vigna mungo* L. Hepper) varieties and reported the average number of bands/primer was 8.5 and 9.8 for RAPD and ISSR marker, respectively[24]. However, current results differed from those of Sharma *et al.*[10], who worked on 25 *Lactuca sativa* L. genotypes and showed the highest number of amplified fragments per RAPD primer was 2.6 which is much lower compared to current data. In contrast, while our data showed only 45.96% of amplified loci were polymorphic, another study reported 100% polymorphic of all amplified loci[10].

The Polymorphism Information Content (PIC) value is commonly used in genetics as a measure of polymorphism. While most of the indexes of the RAPD marker were higher compared to ISSR, the average PIC value/primer of the RAPD marker was lower than ISSR (0.17 vs. 0.24).

Genetic similarity and relationships: To estimate the correlation between the genetic distances determined by the RAPD and ISSR, the matrix of Nei's distance data was used for the Mantel test and a coefficient of correlation (r) of 0.387 ($p<0.0001$), which suggested a low correlation between the 2 markers in Table 5. Genetic matrices of RAPD and combined RAPD-ISSR resulted moderate correlation ($r = 0.696$, $p<0.0001$). However, the ISSR matrix exhibited correlation with pooled RAPD-ISSR matrix ($r = 0.801$, $p<0.0001$) indicating higher effectiveness of ISSR over RAPD in evaluating the genetic diversity. Lalhruaitluanga and Prasad[25] used RAPD and ISSR to survey 12 accessions of *Melocanna baccifera* and reported a poor fit between RAPD and ISSR markers. This suggested that the 2 groups of markers investigate genetic variation differently. It is possible that RAPD and ISSR target different areas of genome. The low correlation between RAPD and ISSR markers was also described in black gram (*Vigna mungo* (L.) Hepper)[26], *Dysosma tonkinense*[18] and in *Triticum aestivum*, respectively[27]. In addition, the effectiveness of ISSR was better than RAPD markers have been documented[28].

To add another layer of data analysis, this study determined the relationship among 16 accessions by the Principal Component Analysis (PCA) in Excel using the XLSTAT software[24]. In PCA scatter plots based on pooled RAPD and ISSR markers, the 1st 2 principal components accounted for 50.95 and 17.47% of the total genetic variation of the total variation, respectively. Following the dendrogram result (Fig. 2c), 8 accessions (HN1, HN2, HN3, HN4, HN5, HN6, HN7 and HN8) analyzed with by PCA were also grouped into 1 major group in Fig. 3. Moreover, the PCA also shows the far distance between accessions from the others. These results again reveal that the 16 collected Indian lettuce genotypes are genetically diverse. Because of these genetic diversities, the collected genotypes could be used for preserving or crossing programs to improve this precious medicinal plant in Vietnam.

Table 5: Matrix comparisons of Mantel test/Two-tailed test between markers

Treatments	Matrix correlation (r)	p-value (two-tailed)	Alpha
RAPD vs. ISSR	0.387	<0.0001	0.05
RAPD vs. pooled RAPD and ISSR	0.696	<0.0001	0.05
ISSR vs. pooled RAPD and ISSR	0.801	<0.0001	0.05

CONCLUSION

Indian lettuce (*Lactuca indica* L.) is an undomesticated medicinal plant in Vietnam. In this study, RAPD and ISSR were used to unravel the genetic variability and relationships across 16 Indian lettuce accessions, originating from the North area of Vietnam. There was a low correlation between RAPD and ISSR markers, however, ISSR markers exhibited higher effectiveness over RAPD in determining the genetic diversity. 16 collected Indian lettuce genotypes are genetically diverse. Because of these genetic diversities, the collected genotypes could be used for preserving or crossing programs to improve this precious medicinal plant in Vietnam.

SIGNIFICANCE STATEMENT

This study showed the genetic diversity of 16 *Lactuca sativa* L. genotypes were evaluated using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) molecular markers. Based on these results, the 16 Indian lettuce genotypes collected in Vietnam are genetically diverse. This study helps to improve preserving or crossing programs of this precious medicinal plant in Vietnam.

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