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Genetic diversity analysis of black pepper (*Piper* spp.) with RAPD markers

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Abstract. Black pepper is a well-known export commodity in Vietnam, but pepper production has been decreasing in recent years. The lack of knowledge about the origin and genetic characteristics of pepper varieties may create variety degradation and loss of product quality. Therefore, it is necessary to study the genetic diversity of existing local and imported pepper varieties and effectively propagate and create new varieties with high yields and quality. In this study, RAPD markers were used with 100 RAPD UBC primers to study genetic diversity. Twelve RAPD primers were selected to amplify 39 pepper cultivars, and 40 polymorphic DNA bands were created with sizes ranging from 200 to 1400 bp. Five of the 12 primers amplified all 39 cultivars. The genetic diversity of lines/cultivars in the pepper population is relatively high. The phylogenetic tree of the 39 cultivars has two branches showing similarity ranging from 41.8 to 51%. The first branch includes five pepper individuals, and the second consists of 34 individuals. There is a high diversity among the pepper cultivars in the same population.

Keywords: piper spp., black pepper, RAPD, genetic diversity

1 Introduction

Black pepper (*Piper* spp.) is known as the most valuable and important spice crop worldwide. The production, transportation and consumption of black pepper have influenced both economy and culture of nations and their people [1]. Geographically, black pepper originated from humid, tropical evergreen forests of the Western Ghats in South India. At present, pepper is cultivated in most tropical and subtropical areas, with a large production, primarily in Vietnam, Indonesia, Brazil, India, Sri Lanka, China, Malaysia, and Cambodia. Besides, it is widely used in medicine, the food industry, the military, etc. [2].

The chemical constituents of pepper can be classified into three major groups: compounds that make spiciness (spicy taste), compounds that give a characteristic aroma, and those that constitute the predominant ingredient (starch). Especially, piperine, a piperidine alkaloid, is responsible for the pungent taste and constitutes about 98% of the total alkaloids in black pepper [3]. It exhibits numerous physiological and pharmacological properties, including antioxidant, anti-inflammatory, antimutagenic, antitumor, antiapoptotic, antigenotoxic, antiarthritic, antifungal, antidepressant, anti-HVB (hepatitis B), and gastro-protective activities [4].

Vietnam is one of the dominant pepper suppliers in the world market, but its production is decreasing. According to estimated data from

International Pepper Community (IPC), Vietnam's pepper production in 2021 was 220,000 tons, reducing 8% compared with 2020 (20,000 tons). In 2020, Vietnam's pepper turnover reached 240,00 tons, decreasing by 15% compared with 2019 [5]. The cause of this decline is low seedling quality. Currently, most of the pepper varieties are old and need to be selected in terms of yield and disease resistance. Besides, the technique of selection and propagation is still spontaneous and monotonous. Most pepper-growing households are not aware of the origin and basic characteristics of pepper varieties and only call the variety with local names; consequently, the breed names are easily confused. Sometimes, the same breed has different names. Therefore, it is necessary to investigate the genetic diversity of existing local and imported pepper varieties and, thus, effectively propagate and create new varieties with high yields and quality. In addition, it is crucial to develop methods for testing, managing, and protecting the genetic resources of domestic and imported pepper varieties.

To study genetic diversity, we used morphological, isozyme, or molecular marker methods. Nevertheless, the limitations associated with pedigree data and morphological, physiological and cytological markers for assessing genetic diversity in cultivated and wild plant species have primarily been circumvented by the development of molecular markers such as restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNAs (RAPD), amplified fragment length polymorphisms (AFLP), and simple sequence repeats (SSR) [6]. Among these types of molecular markers, RAPD is a dominant molecular marker commonly used in genetic diversity research with numerous advantages, such as rapidness, simplicity, effectiveness, and a small amount of template DNA required. Furthermore, it is unnecessary to know the genome sequence and the primer sequence. In addition, the application of the RAPD technique has been carried out on different animals, plants, and microorganisms [7].

2 Materials and methods

2.1 Material

A total of 39 lines/cultivars of pepper were used in this study, and their information is presented in Table 1. In addition, 100 UBC RAPD primers (University of British Columbia) synthesized by Bioneer company (Korea) were used for the genetic diversity survey of the pepper cultivars (Table 2).

Table 1. Lines/cultivars of pepper used in this study

No.	Code	Scientific name [8]	Local name	Original collected place	Collected location
1	HUIB_PN10 (PN10)	*Piper nigrum*	Vinh Linh	Do An, Quang Tri	Gia Lai, Vietnam
2	HUIB_PN20 (PN20)	*Piper nigrum*	Vinh Linh	Cam My, Dong Nai	Gia Lai, Vietnam
3	HUIB_PN21 (PN21)	*Piper nigrum*	Tien Phuoc	Tien Phuoc, Quang Nam	Quang Nam, Vietnam
4	HUIB_PN27 (PN27)	*Piper nigrum*	Vinh Linh	Vinh Linh, Quang Tri	Quang Tri, Vietnam
5	HUIB_PN29 (PN29)	*Piper nigrum*	Cua	Cua, Quang Tri	Quang Tri, Vietnam
6	HUIB_PH30 (PH30)	*Piper hancei*	Wild pepper with round leaves	Huong Hoa, Quang Tri	Quang Tri, Vietnam
7	HUIB_PN34 (PN34)	*Piper nigrum*	Sri Lanka	Pepper Research and Development Center, Gia Lai	Dak Lak, Vietnam

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No.	Code	Scientific name [8]	Local name	Original collected place	Collected location
8	HUIB_PD36 (PD36)	*Piper divaricatum*	South American wild pepper	Pepper Research and Development Center, Gia Lai	Gia Lai, Vietnam
9	HUIB_PN45 (PN45)	*Piper nigrum*	Loc Ninh	Pepper Research and Development Center, Gia Lai	Gia Lai, Vietnam
10	HUIB_PH46 (PH46)	*Piper hancei*	Wild pepper with long leaves	Huong Hoa, Quang Tri	Quang Tri, Vietnam
11	HUIB_PN47 (PN47)	*Piper nigrum*	Tan Lam	Tien Phuoc, Quang Nam	Quang Nam, Vietnam
12	HUIB_ PN52 (PN52)	*Piper nigrum*	Binh Phuoc	Hon Quan, Binh Phuoc	Binh Phuoc, Vietnam
13	HUIB_PN55 (PN55)	*Piper nigrum*	Ha Tien	Phu Quoc	Phu Quoc, Vietnam
14	HUIB_PN56 (PN56)	*Piper nigrum*	Ba le	Ba to, Quang Ngai	Quang Ngai, Vietnam
15	HUIB_PN69 (PN69)	*Piper nigrum*	India	Chu Prong, Gia Lai	Gia Lai, Vietnam
16	HUIB_PN70 (PN70)	*Piper nigrum*	India	Xuyen Moc, Ba Ria – Vung Tau	Gia Lai, Vietnam
17	HUIB_PN84 (PN84)	*Piper nigrum*	Local	Cam My, Dong Nai	Gia Lai, Vietnam
18	HUIB_PN87 (PN87)	*Piper nigrum*	Local	Cam My, Dong Nai	Gia Lai, Vietnam
19	HUIB_PN89 (PN89)	*Piper nigrum*	Local	Buon Ho, Dak Lak	Gia Lai, Vietnam
20	HUIB_PN91 (PN91)	*Piper nigrum*	Se dia phuong	CuKuin, DakLak	Gia Lai, Vietnam
21	HUIB_PN93 (PN93)	*Piper nigrum*	Bau May	Xuyen Moc, Ba Ria – Vung Tau	Gia Lai, Vietnam
22	HUIB_PN95 (PN95)	*Piper nigrum*	Ma Lai	Xuan Loc, Dong Nai	Gia Lai, Vietnam
23	HUIB_PN96 (PN96)	*Piper nigrum*	Ma Lai	Cambodia	Gia Lai, Vietnam
24	HUIB_PN97 (PN97)	*Piper nigrum*	Sri Lanka	Loc Ninh, Binh Phuoc	Gia Lai, Vietnam
25	HUIB_PN101 (PN101)	*Piper nigrum*	Phu Quoc	Chu Prong, Gia Lai	Gia Lai, Vietnam
26	HUIB_PN102 (PN102)	*Piper nigrum*	Phu Quoc	Duc co, Gia Lai	Gia Lai, Vietnam
27	HUIB_PN105 (PN105)	*Piper nigrum*	No name	Dak Nong	Gia Lai, Vietnam
28	HUIB_PN113 (PN113)	*Piper nigrum*	Sri Lanka	Sri Lanka	Gia Lai, Vietnam
29	HUIB_PN114 (PN114)	*Piper nigrum*	Indo 2	(PRDC) Indonesia	Gia Lai, Vietnam
30	HUIB_PN115 (PN115)	*Piper nigrum*	Nata 1	(PRDC) Indonesia	Gia Lai, Vietnam
31	HUIB_PN116 (PN116)	*Piper nigrum*	Tieu chum	Ba Ria – Vung Tau	Gia Lai, Vietnam
32	HUIB_PN38 (PN38)	*Piper nigrum*	Vĩnh Linh	Ban Me Thuot, Dak Lak	Quang Tri, Vietnam
33	HUIB_PN42 (PN42)	*Piper nigrum*	Loc Ninh	Pepper Research and Development Center, Gia Lai	Gia Lai, Vietnam
34	HUIB_PN43 (PN43)	*Piper nigrum*	India	Pepper Research and Development Center, Gia Lai	Gia Lai, Vietnam
35	HUIB_PN35 (PN35)	*Piper nigrum*	Local	Ban Me Thuot, Dak Lak	Gia Lai, Vietnam

No.	Code	Scientific name [8]	Local name	Original collected place	Collected location
36	HUIB_PN54 (PN54)	*Piper nigrum*	Vĩnh Linh	Xuan Loc, Dong Nai	Dong Nai, Vietnam
37	HUIB_PR48 (PR48)	*Piper retrofractum*	Bau may	Huong Hoa, Quang Tri	Quang Tri, Vietnam
38	HUIB_PR41 (PR41)	*Piper retrofractum*	Tieu lot	Huong Hoa, Quang Tri	Quang Tri, Vietnam
39	HUIB_PN50 (PN50)	*Piper nigrum*	Indo 1	Hon Quan, Binh Phuoc	Gia Lai, Vietnam

Table 2. UBC RAPD used primers

No.	Primer	Sequence (5′-3′)	No.	Primer	Sequence (5′-3′)	No.	Primer	Sequence (5′-3′)
1	UBC#301	ACGGCAGTGG	35	UBC#335	TTGCTTGGCG	69	UBC#369	GCGCATAGCA
2	UBC#302	ACTTCCTCCA	36	UBC#336	CACGGCTGCG	70	UBC#370	TCAGCCAGCG
3	UBC#303	GGTCTCCTAG	37	UBC#337	GGAGCCCCCT	71	UBC#371	TCTCGATTGC
4	UBC#304	CCTCACCTGT	38	UBC#338	TGACGCGCTC	72	UBC#372	CCCACTGACG
5	UBC#305	CTAGGGGCTG	39	UBC#339	ACGGCAGTGG	73	UBC#373	CTGAGGAGTG
6	UBC#306	CGGAGAGCGA	40	UBC#340	ACTTCCTCCA	74	UBC#374	GGTCAACCCT
7	UBC#307	GTGGCCGCGC	41	UBC#341	GGTCTCCTAG	75	UBC#375	CCGGACACGA
8	UBC#308	CCGGCATAGA	42	UBC#342	CCTCACCTGT	76	UBC#376	CAGGACATCG
9	UBC#309	ATCTAGGGAC	43	UBC#343	CTAGGGGCTG	77	UBC#377	GACGGAAGAG
10	UBC#310	GCCGCTACTA	44	UBC#344	CGGAGAGCGA	78	UBC#378	GACAACAGGA
11	UBC#311	GACATCTCGC	45	UBC#345	GTGGCCGCGC	79	UBC#379	GGGCTAGGGT
12	UBC#312	ACAGGGAACG	46	UBC#346	CCGGCATAGA	80	UBC#380	AGGAGTGAGA
13	UBC#313	TCTAAGCTCG	47	UBC#347	ATCTAGGGAC	81	UBC#381	ATGAGTCCTG
14	UBC#314	CGGATCTCTA	48	UBC#348	GCCGCTACTA	82	UBC#382	ATACACCAGC
15	UBC#315	ATACGGCGTC	49	UBC#349	GACATCTCGC	83	UBC#383	GAGGCGCTGC
16	UBC#316	ATGGCCTTAC	50	UBC#350	ACAGGGAACG	84	UBC#384	TGCGCCGCTA
17	UBC#317	GCGAACCTCC	51	UBC#351	CTCCCGGTGG	85	UBC#385	ACCGGGAACG
18	UBC#318	GGTGGTTTCC	52	UBC#352	CACAACGGGT	86	UBC#386	TGTAAGCTCG
19	UBC#319	GCCTAGTCAC	53	UBC#353	TGGGCTCGCT	87	UBC#387	CGCTGTCGCC
20	UBC#320	AACGCGTAGA	54	UBC#354	CTAGAGGCCG	88	UBC#388	CGGTCGCGTC
21	UBC#321	GAATGCGACG	55	UBC#355	GTATGGGGCT	89	UBC#389	CGCCCGCAGT
22	UBC#322	ATGGCAAAGC	56	UBC#356	GCGGCCCTCT	90	UBC#390	TCACTCAGAG
23	UBC#323	TGGACCACCC	57	UBC#357	AGGCCAAATG	91	UBC#391	GCGAACCTCG
24	UBC#324	GCCACGGAGA	58	UBC#358	GGTCAGGCCC	92	UBC#392	CCTGGTGGTT
25	UBC#325	TCCCGAACCG	59	UBC#359	AGGCAGACCT	93	UBC#393	TTCCATGCCT
26	UBC#326	CTGTGGCGGT	60	UBC#360	CTCTCCAGGC	94	UBC#394	TCACGCAGTT
27	UBC#327	CTCACTTGGG	61	UBC#361	GCGAGGTGCT	95	UBC#395	TCACTTGAGG
28	UBC#328	GAGAGGCACC	62	UBC#362	CCGCCTTACA	96	UBC#396	GAATGCGAGG
29	UBC#329	CTGGGGCCGT	63	UBC#363	ATGACGTTGA	97	UBC#397	GGGCTGTGCC

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No.	Primer	Sequence (5′-3′)	No.	Primer	Sequence (5′-3′)	No.	Primer	Sequence (5′-3′)
30	UBC#330	GAGATCCCTC	64	UBC#364	GGCTCTCGCG	98	UBC#398	CAGTGCTCTT
31	UBC#331	TGTTAGGCTC	65	UBC#365	TAGACAGAGG	99	UBC#399	TTGCTGGGCG
32	UBC#332	TGTTAGGCAC	66	UBC#366	CCTGATTGCC	100	UBC#400	GCCCTGATAT
33	UBC#333	GCGTGACCCG	67	UBC#367	ACCTTTGGCT			
34	UBC#334	TAGGCGAACG	68	UBC#368	ACTTGTGCGG			

2.2 Methods

Genomic DNA isolation

Genomic DNAs were extracted from fresh leaves following the cetyl-trimethylammonium bromide procedure published by Raz and Ecker [9]. For each cultivar, a genomic DNA was extracted from two different plants and used in the PCR (Polymerase Chain Reaction). Then, the extracted DNA was incubated with an SYBR Green I nucleic acid gel stain (Invitrogen, USA) for 10–20 min and separated on 1% agarose gels for 30 minutes at 120 V and photographed under UV light (HyperLadder™ 100 bp (Meridian Bioscience) was used as a molecular weight marker). The DNA containing impurities was purified with a QIAquick gel extraction kit (Qiagen, Germany), and its concentration was determined on a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA). A large quantity of DNA extracts was used for RAPD amplification.

RAPD amplification

First, 3 out of 39 cultivars were randomly selected to screen for 100 RAPD primers to select the ones with the highest polymorphism rate. The selected polymorphic primers were then used to amplify 39 pepper lines/cultivars to assess genetic diversity.

The PCRs were conducted according to the protocol by Truong et al. [10] in a volume of 15 μL containing 25 mM $MgCl_2$ (Bioline-Meridian, UK), 200 μM deoxyribonucleotide triphosphate mix (Bioline-Meridian, UK), 5X PCR buffer, 1U of Taq DNA polymerase (Bioline-Meridian, UK), 10 pmol of RAPD primer, 5–10 ng of total DNA, and the addition of just 15 μL sterile distilled water. The used PCR program consisted of an initial strand separation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 37 °C for 1 min, and elongation at 72 °C for 2 min, and a final extension at 72 °C for 7 min. The amplified products were incubated with a 1:10,000 dilution of the SYBR Green I nucleic acid gel stain (Invitrogen, USA) for 20 min and resolved by using electrophoresis on a 1% agarose gel, run in 0.5×TBE buffer for 4 h at 120 V, and photographed under UV light. A 100 bp ladder was used as a molecular weight marker.

Data analysis

The electrophoretograms show two types of bands. The clear and undistorted bands were assigned "1", and the unclear or faint bands were assigned "0". The size of each PCR product band was estimated with a standard marker. This logical matrix data were used for all analyses by using POPGENE software (version 1.32 [11]) to calculate genetic diversity indices and NTSYS software (version 2.1 [12]) to construct a phylogenetic tree.

POPGENE provides the following parameters: Nei's gene diversity (*h*), Shannon's information index (*Ho*), the observed number of alleles (*na*), and the effective number of alleles (*ne*) [13]. The data in the form of a logical matrix was put into NTSYS to build a phylogenetic tree by using the UPGMA algorithm, with a distance matrix established based on the *SM* similarity coefficient (Simple Matching, $SM \in [0;1]$) [14]:

$$SM = \frac{a+b}{a+b+c+d}$$

where a is the number of DNA segments present in both the ith and the jth cultivars; b is the number of DNA segments absent in both the ith and the jth cultivars; c is the number of bands appearing in the ith cultivar but not in the jth cultivar; d is the number of bands appearing in the jth cultivar but not in the ith cultivar.

3 Results and discussion

3.1 DNA extraction and purification

The electrophoretogram of the extracted product shows strong, clear DNA with a little smear (Fig. 1). Besides, the 260/280 ratio of extracted DNA is ~1.8. Therefore, the extracted DNA exhibits excellent quality and is suitable for further experiments [15].

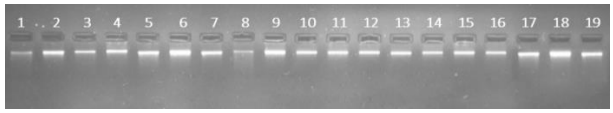

Fig. 1. Electrophoretogram of extracted genomic DNA; 1: HUIB_PN10, 2: HUIB_PN20, 3: HUIB_PN69, 4: HUIB_PN70, 5: HUIB_PN89, 6: HUIB_PN91, 7: HUIB_PN93, 8: HUIB_PN95, 9: HUIB_PN96, 10: HUIB_PN97, 11: HUIB_PN101, 12: HUIB_PN102, 13: HUIB_PN105, 14: HUIB_PN113, 15: HUIB_PN114, 16: HUIB_PN115, 17: HUIB_PN116, 18: HUIB_PN55, 19: HUIB_PN56

3.2 RAPD analysis

Primer screening

Only 12 out of 100 primers surveyed with three random pepper varieties were selected (Table 3). These primers can produce the most pronounced, bold and polymorphic bands. They were then used for genetic diversity assessment with RAPD for 39 lines/cultivars of pepper.

Table 3. Selected polymorphic primers

No.	Primer	Sequence (5′-3′)
1	UBC#303	GGTCTCCTAG
2	UBC#317	GCGAACCTCC
3	UBC#322	ATGGCAAAGC
4	UBC#329	CTGGGGCCGT
5	UBC#333	GCGTGACCCG
6	UBC#347	ATCTAGGGAC
7	UBC#352	CACAACGGGT
8	UBC#359	AGGCAGACCT
9	UBC#363	ATGACGTTGA
10	UBC#377	GACGGAAGAG
11	UBC#382	ATACACCAGC
12	UBC#392	CCTGGTGGTT

Electrophoresis of PCR products amplified with selected polymorphic RAPD primers

Among the 39 target individuals of the study, HUIB_PN29 had the most amplification bands (22, 3.259% of the total number of formed bands); HUIB_PN21 and HUIB_PN47 both had 21 bands (3.111%). HUIB_PN27, HUIB_PN34, and HUIB_PN45 had 20 DNA bands (2.963%); HUIB_PN96, HUIB_PH46, HUIB_PN84, and HUIB_PN87 had 19 DNA bands (2.815%); HUIB_PN10, HUIB_PN70, and HUIB_PN93 had 14 bands (Table 4).

Table 4. Number of amplified DNA bands of each pepper cultivar with each primer

Material symbol	UBC #303	UBC #317	UBC #329	UBC #322	UBC #333	UBC #352	UBC #359	UBC #363	UBC #377	UBC #347	UBC #382	UBC #392	Total
HUIB_PN10	3	1	0	1	0	1	2	0	0	2	2	2	**14**
HUIB_PN20	4	1	0	1	0	1	3	0	1	1	2	2	**16**
HUIB_PN69	3	1	0	1	0	3	3	0	1	2	2	1	**17**
HUIB_PN70	3	1	0	1	0	3	2	0	1	2	0	1	**14**

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Material symbol	UBC #303	UBC #317	UBC #329	UBC #322	UBC #333	UBC #352	UBC #359	UBC #363	UBC #377	UBC #347	UBC #382	UBC #392	Total
HUIB_PN89	3	0	1	1	0	2	3	2	1	2	1	2	**18**
HUIB_PN91	3	0	0	1	0	1	3	0	2	2	1	2	**15**
HUIB_PN93	3	0	0	1	0	1	3	0	2	2	1	1	**14**
HUIB_PN95	3	1	0	1	0	1	3	2	1	2	1	2	**17**
HUIB_PN96	2	1	0	1	0	2	3	1	2	2	2	3	**19**
HUIB_PN97	3	0	0	1	0	3	2	0	3	2	1	1	**16**
HUIB_PN101	3	1	1	1	0	1	3	0	2	2	2	1	**17**
HUIB_PN102	4	1	0	1	0	2	2	0	2	2	0	1	**15**
HUIB_PN105	3	1	0	1	0	1	3	1	2	1	1	2	**16**
HUIB_PN113	3	1	0	1	0	2	3	0	2	2	1	3	**18**
HUIB_PN114	3	1	.0	1	0	1	3	1	2	1	1	2	**16**
HUIB_PN115	2	1	0	1	0	2	3	0	1	2	1	2	**15**
HUIB_PN116	2	1	1	1	0	2	2	0	2	2	2	1	**16**
HUIB_PN55	3	1	1	1	0	1	3	0	2	2	2	1	**17**
HUIB_PN56	3	0	1	1	0	1	3	0	2	2	2	1	**16**
HUIB_PH30	3	2	1	0	2	1	2	1	1	1	1	2	**17**
HUIB_PD36	3	0	0	0	3	1	4	1	1	1	1	2	**17**
HUIB_PH46	3	2	3	1	2	1	2	1	1	1	1	1	**19**
HUIB_PN84	1	1	1	1	2	2	4	1	1	1	1	3	**19**
HUIB_PN87	2	1	2	1	1	2	3	1	2	1	2	1	**19**
HUIB_ PN52	1	0	1	1	2	1	3	1	3	1	2	2	**18**
HUIB_PN21	1	1	2	1	2	1	3	1	3	1	2	3	**21**
HUIB_PN27	3	1	1	1	2	1	3	1	2	1	2	2	**20**
HUIB_PN29	2	1	1	1	4	1	3	2	2	1	2	2	**22**
HUIB_PN34	2	0	1	1	2	1	3	1	2	2	2	3	**20**
HUIB_PN45	2	1	1	1	2	1	3	1	2	2	2	2	**20**
HUIB_PN47	3	1	1	1	2	1	3	1	2	2	2	2	**21**
HUIB_PN38	3	1	0	1	0	1	2	0	0	2	2	2	**14**
HUIB_PN42	2	1	1	1	2	1	3	1	2	2	2	2	**20**
HUIB_PN43	3	1	0	1	0	3	3	0	1	2	2	1	**17**
HUIB_PN35	1	1	1	1	2	2	4	1	1	1	1	3	**19**
HUIB_PN54	4	1	0	1	0	1	3	0	1	1	2	2	**16**
HUIB_PR48	3	2	1	0	2	1	2	1	1	1	1	2	**17**
HUIB_PR41	3	0	0	0	3	1	4	1	1	1	1	2	**17**
HUIB_PN50	3	1	0	1	0	1	3	1	2	1	1	2	**16**
Total	**104**	**33**	**23**	**35**	**35**	**56**	**112**	**25**	**62**	**61**	**57**	**72**	**675**

The results in Table 5 show that 40 DNA bands were amplified from 12 random primers. They are all polymorphic bands (the average number of polymorphic bands per primer is 3.333). The band size ranges from 200 to 1400 bp. The rate of amplified cultivars is relatively high (83.547%). Primers UBC#303, UBC#352, UBC#359, UBC#347, and UBC#392 amplified all the cultivars with 5, 3, 5, 2, and 4 DNA bands, respectively, followed by UBC#377 (96.774%) with five polymorphic bands. Primer UBC#333 had the lowest number of amplified individuals (38,710%) (Table 5, Fig. 2).

According to Nei et al., the greater the number of amplified bands, the greater the ability to separate different cultivars in the phylogenetic tree [16]. With the 12 used primers, we obtained 40 polymorphic DNA bands from 39 different pepper lines/cultivars for genetic diversity research and phylogenetic tree building.

Table 5. Number of cultivars and number of amplified bands in each primer

No.	Primer	Number of amplified cultivars	The rate of amplified cultivars (%)	Total of amplified bands	Number of polymorphic bands	Size (bp)	Per cent polymorphism (%)
1	UBC#303	39	100.00	5	5	220–1040	100
2	UBC#317	30	76.92	2	2	370–800	100
3	UBC#329	19	48.72	3	3	320–450	100
4	UBC#322	35	89.74	2	2	400–720	100
5	UBC#333	16	41.03	5	5	200–1400	100
6	UBC#352	39	100.00	3	3	300–1200	100
7	UBC#359	39	100.00	5	5	230–1050	100
8	UBC#363	22	56.41	2	2	400–460	100
9	UBC#377	37	94.87	5	5	420–1100	100
10	UBC#347	39	100.00	2	2	500–590	100
11	UBC#382	37	94.87	2	2	300–420	100
12	UBC#392	39	100.00	4	4	330–900	100
Mean			**83.547**	**40**	**40**	**200–1400**	**100**

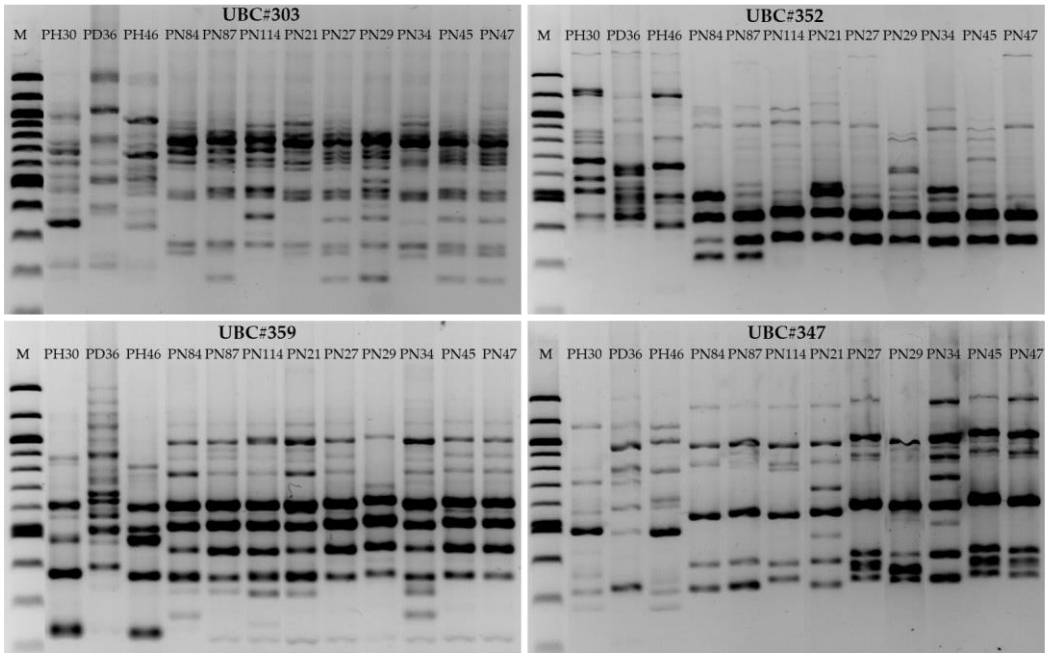

Fig. 2. PCR products of UBC#303, UBC#352, UBC#359, UBC#347 with their polymorphisms: HUIB_PH30, HUIB_PD36, HUIB_PH46, HUIB_PN84, HUIB_PN87, HUIB_PN114, HUIB_PN21, HUIB_PN27, HUIB_PN29, HUIB_PN34, HUIB_PN45, HUIB_PN47; M: 100 bp Ladder

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Genetic diversity indices

The diversity indices are presented in Table 6. The results of the analysis with POPGENE show that the genetic diversity of individuals in the population was relatively high (mean of *Ho* = 0.41) (Table 6). Among the 12 used RAPD primers, UBC#329 shows the highest diversity (*Ho* = 0.5332), followed by UBC#317 (*Ho* = 0.4676). The lowest diversity is in primer UBC#322 (*Ho* = 0.3311). The diversity in the population of a certain species has also been studied by numerous authors. The results of our study are similar to those of Verma et al. in Eremostachys superba, in which the diversity in the populations was also high (*Ho* is 0.31–0.42) and accounted for 83.01% of the species diversity [17]. This relatively high diversity can be found in other studies [18, 19]. These authors reported a correlation between geographical distance and genetic diversity. The populations in geographically distant regions had significant genetic diversity.

Phylogenetic tree

Jaccard's pair-wise similarity coefficient values for 39 common pepper accessions were calculated, and they were between a minimum of 0.418 and a maximum of 1. The phylogenetic tree generated by using UPGMA cluster analysis divided the 39 cultivars into two major branches showing similarity in the range of 41.8–51% among themselves (Fig. 3). Each branch has subgroups with different levels of similarity.

Table 6. Diversity indices in each primer

Primer	*na**	*ne**	*h**	*Ho**
UBC#303	2	1.5058	0.2926	0.4476
UBC#317	2	1.5249	0.3073	0.4676
UBC#329	2	1.6443	0.3614	0.5332
UBC#322	2	1.3268	0.2035	0.3311
UBC#333	2	1.3180	0.2114	0.3468
UBC#352	2	1.4115	0.2636	0.4195
UBC#359	2	1.5444	0.3029	0.4531
UBC#363	2	1.3851	0.2341	0.3697
UBC#377	2	1.4649	0.2754	0.4209
UBC#347	2	1.4246	0.2594	0.4072
UBC#382	2	1.5435	0.3075	0.4625
UBC#392	2	1.4751	0.2850	0.43976
Mean	**2**	**1.44**	**0.266**	**0.41**
SE	**0.172**	**0.343**	**0.171**	**0.226**

Note: Nei's gene diversity (**h*), Shannon's information index (**Ho*), observed number of alleles (**na*), effective number of alleles (**ne*)

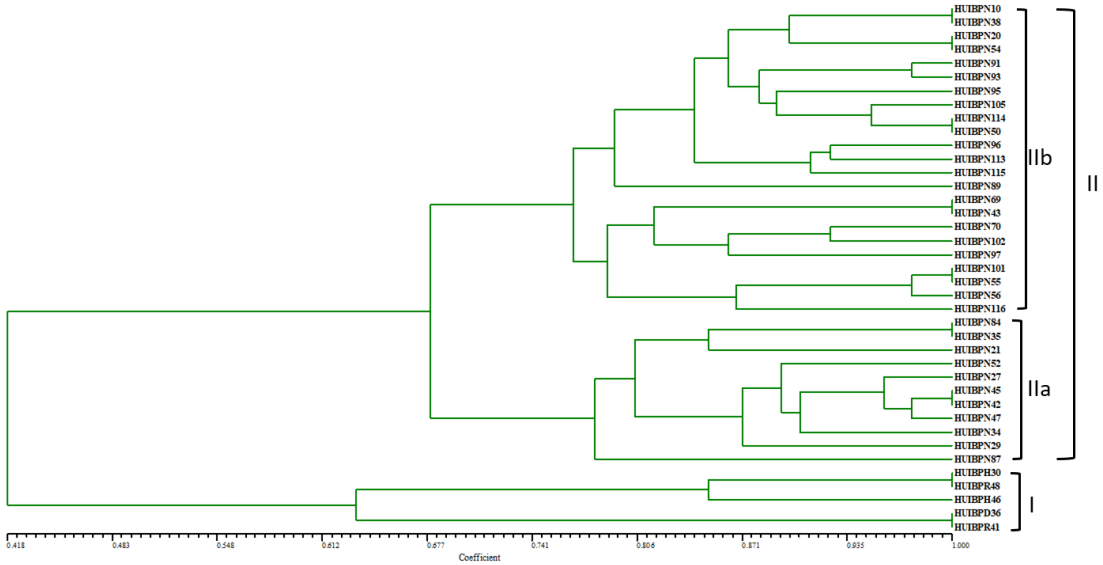

Fig. 3. UPGMA tree showing relationship among 39 cultivars of pepper

Branch I includes five individuals of pepper, namely HUIB_PH30, HUIB_PH46, HUIB_PD36, HUIB_PR41, and HUIB_PR48. However, these five pepper individuals are divided into two subgroups with the genetic similarity coefficient of about 0.635. The first subgroup includes HUIB_PH30, HUIB_PH46 and HUIB_PR48, and the second subgroup has two individuals (HUIB_PD36 and HUIB_PR41), all having the same genetic similarity coefficient of 1.

Branch II includes the remaining 34 individuals. This branch has a genetic similarity coefficient of 0.677. The pepper individuals were segregated into two subgroups: IIa (11 individuals) and IIb (23 individuals). In which, cultivars with the highest similarity coefficient are HUIB_PN10, HUIB_PN20, HUIB_PH30, HUIB_PN54, HUIB_PN114, HUIB_PN50, HUIB_PN43, HUIB_PN69, HUIB_PN84, HUIB_PN101, HUIB_PN35, HUIB_PN45 and HUIB_PN45, and the lowest cultivars is HUIB_PN87 (0.772).

Thus, the results of DNA pedigree analysis show a high diversity among individuals in the same population. Genetic variation may be due to differences in reproductive conditions and seedling origin.

4 Conclusions

In this study, twelve RAPD primers were selected to amplify 39 pepper cultivars. All 40 created DNA bands ranged from 200 to 1400 bp. Five primers (UBC#303, UBC#352, UBC#359, UBC#347, and UBC#392) amplified all cultivars.

The genetic diversity of the individuals in the population is relatively high (*Ho* is between 0.3311 and 0.5332).

The phylogenetic tree has two major branches with 41.8–51% similarities. Branch I includes five individuals, and the remaining 34 individuals belong to branch II, which has two large subgroups with 11 and 23 individuals.

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