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AFLP Analysis of Genetic Diversity Evaluation in Pear (Pyrus communis L.) in Kurdistan Region/Iraq

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ABSTRACT

The pear (*Pyrus communis* L.) is one of the most economically important pome fruits, belonging to the genus *Pyrus*. This study utilized 15 (Amplified Fragment Length Polymorphism) AFLP primer combinations to characterize 32 pea landraces collected across six different locations from the Kurdistan Region of Iraq. A high level of genetic diversity was observed among the tested pear landraces. A total of 8,016 amplified fragments were scored, with 7,399 identified as polymorphic. The polymorphism rate ranged from 86% to 100%, with an average of 92.49%. The Dice Coefficient of similarity and an AFLP-supported dendrogram were used to assess the genetic distances between the studied individuals. The dendrogram revealed two major clusters, with the highest similarity (0.880) between landraces C6 and C11, indicating the presence of both closely related and distantly connected pear landraces in the region. Additionally, a strong correlation was found between the dendrogram clusters and the similarity matrix. The findings from this study provide valuable insights into the origin and evolution of *Pyrus communis* L. Furthermore, the AFLP technique proves to be a novel and highly effective method for DNA fingerprinting, applicable to DNAs of any origin or complexity.

INTRODUCTION

The pear (*Pyrus communis*) is one of the Rosaceae family, and widely known for its ability to thrive in diverse environmental conditions around the world (Al Shoffe & Safadi, 2018). The pear (genus *Pyrus*), which comprises 20 to 45 species of trees and shrubs, includes the common pear (*Pyrus communis*), a widely cultivated fruit in temperate regions. Its origin and domestication are well documented (Silva, 2014). There are two main types of pears: European or Western pears, such as *P. communis* and Asian pears, like *P. pyrifolia* (James-Martin *et al.*, 2015). The taxonomy and morphological characteristics of pears show considerable genetic variation, which makes species classification challenging. Both types belong to the genus *Pyrus*, part of the Rosaceae family in the order Rosales. Pears are closely related to apples, sharing some traits, yet they possess unique characteristics that contribute to their distinct and delicate flavor (Silva *et al.*, 2014).

Morphological classification is an invaluable tool for plant breeders and gene bank managers in recognizing species relationships and providing deeper insights for future breeding programs aimed at specific goals, such as developing new commercial cultivars with improved fruit quality, or disease resistance (Hrotko *et al*., 2008; Magyar & Hrotko, 2008).

In the genus *Pyrus*, the basic chromosome number is x = 17, leading to a diploid chromosome count of 34 (2n = 2x = 34). However, variations with 51 or 68 chromosomes have occasionally been observed (Westwood, 1978). Traditional research methods involve morphological and agronomic assessments, conventional breeding techniques, and biochemical markers such as cytology and isoenzymes. However, these approaches are limited by environmental factors and provide a narrow range of markers, which restricts the scope of research on pear diversity. Morphological classifications often fail to establish genetic links between cultivars, resulting in phenotypic variations over time and across different geographic regions, largely influenced by the interaction between genotype and environment. According to a recent study by Kadkhodaei *et al.*, (2021) examined several morphological characteristics of *Pyrus* cultivars. Distinct traits such as fruit size, diameter, color, and ripening time were identified in these varieties (Mousavi *et al.*, 2015).

Regarding economic value and consumption rate, *Pyrus* species one of the most important tree and essential edible fruits (FAOSTAT, 2010). Pears are also, recognized as a medicinal crop or functional food due to their rich content of bioactive compounds, which are primarily concentrated in the peels (Hong *et al.*, 2021). Additionally, the toxic effects of certain secondary chemical compounds, such as phenolics extracted from the leaves of pear varieties have been identified. These compounds help confer resistance to pests in the plants (Al-Mallah, 2009) such as, the pear lace bug (*Stephanitis pyri F*.) is an insect that affects pear trees (Al-Mallah, 2010).

The genus *Pyrus* is believed to have first appeared in the mountains of southwestern and western China. However, evidence suggests that wild relatives of pears are native to Eastern Europe and the Caucasus Mountains (Silva *et al*., 2014).

Molecular markers are an effective tool for identifying and distinguishing pear landraces without impacting the environment. They are widely employed in various fields, including molecular taxonomy, evolutionary research, and genetic map construction. These markers have proven essential for investigating genetic diversity, as they reliably detect genetic variation within and across species and facilitate identification (Eleuch *et al.*, 2008; Al-Burki, 2020; Elias & Al-Jubouri, 2022).

In recent decades, molecular techniques have provided a means to predict phenotypes based on genotypes, reducing the need for extensive phenotypic characterization of thousands of individuals. Breeders use these techniques to develop cultivars with unique profiles of desirable traits, such as improved yield, tolerance to biotic and abiotic stress, growth behavior, taste, and nutritional quality (Ishitani *et al.*, 2004). Since the advent of DNA marker technology, several specialized DNA markers, including Restriction Fragment Length Polymorphism (RFLP), (Random Amplified Polymorphic DNA), Amplified Fragment Length Polymorphism (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Simple Sequence Repeats (SSR), have been developed. These markers are widely used for genetic characterization and fingerprinting of numerous organisms, including economically significant plants. AFLP is a DNA fingerprinting technique first developed by Vos *et al.* (1995). It is an

arbitrary PCR-based method that involves digesting genomic DNA, ligating adapters to the resulting fragments, and amplifying only a subset of them (Paun & Schönswe 2012). Comparative studies demonstrate that AFLP is highly effective for determining the genetic structure of natural populations and assessing genetic diversity in various plant species (Hu *et al*., 2012; Kumar *et al.*, 2015).AFLP has been successfully used to investigate genetic diversity in a range of fruit species, including apple (Goulao *et al.*, 2001), Asian pears (Bao *et al*., 2008), carrot (Solouki *et al*., 2012 and Altameme & Ibraheam, 2019), pomegranate (Sinjare & Jubrael, 2020), and fig (Hussein & Jubrael, 2021), In pome fruits, several DNA-based markers, such as RAPD, SSR and AFLP, are commonly used (Tartarini & Sansavini, 2002).

The main objective of this study is to identify pear cultivars by molecular marker. In this study, thirty-two pear landraces from various regions as in Duhok, Aakri, Erbil, Shaqlawa, Sulaymaniyah and Halabja were characterized using 15 AFLP markers to evaluate the genetic relationships among pear landraces of the genus *Pyrus* in the Kurdistan Region of Iraq.

MATERIALS AND METHODS

The Plant Material:

A total of 32 pear landraces were systematically collected from various geographic and mountain regions of Kurdistan, Iraq. The collection comprised four groups: the first group included 8 landraces and wild pears from the Duhok/Arza (Maten Mountain) and Aakre/Dinarta regions; the second group contained 8 landraces from different areas of Erbil; the third group featured 8 landraces from Sulaymaniyah in Khamze (Azamar Mountain); and the final group included 8 landraces collected from Halabj.

Genomic DNA Extraction and PCR-AFLP Analysis:

In Table 1 and Figure 1, the genomic DNA extraction from pear leaves was performed following the protocol described by Weigand *et al.* (1993), with some modifications. While most DNA extraction protocols recommend using smaller leaf sample sizes, we opted for 2 grams of fresh pear leaves to ensure a sufficient DNA yield, as the leaves from these specific landraces and wild varieties often exhibit lower DNA concentrations. This adjustment was necessary to obtain high-quality and high-quantity DNA for subsequent AFLP analysis, as noted by Sinjare & Jubrael (2020). Other studies have reported similar adjustments when working with challenging plant tissues (Sun *et al.*, 2013 and Kanoosh *et al*., 2019).

First, 2 grams of young, fresh pear leaves were collected from various pear trees across different regions of Kurdistan, as presented in Table 1 and Figure 1. The young leaves were ground into a fine powder using liquid nitrogen. This powder was then incubated at 60°C for 30 minutes in a shaking water bath containing a 2x CTAB extraction buffer (2x CTAB, 5M NaCl, 1M Tris-HCl, 0.5 M EDTA). The mixture was extracted with an equal volume of chloroform and isoamyl alcohol (24:1, v/v). After extraction, the mixture was centrifuged at 1400 xg for 30 minutes, transferred to a separate tube, and precipitated with 0.66 volumes of isopropanol. The nucleic acids were dissolved in TE buffer and stored at -20°C until further use.

Table 1; list of pear landraces name and origin (location).

P.L.	Name	G	Origin	Coordinate	P.L.	Name	G	Origin	Coordinate
C1	Krosk1	1	Duhok / banke Arza	37°10'44"N 43°08'15"E	C17	Qazam	3	Hawler/Ankawe	36°14'29"N 43°59'23"E
C2	Zarik	1	Duhok/ Bnankearza	37°10'44"N 43°08'15"E	C18	Spe gre	3	Hawler/ashqa	36°26'05"N 44°20'20"E
C3	Gelki	1	Duhok/Bnanke arza	37°10'44"N 43°08'15"E	C19	Bar-Awe	3	Hawler/Ankawe	36°14'29"N 43°59'23"E
C4	Hezel	1	Duhok/Bnank earza	37°10'44"N 43°08'15"E	C20	Naske	3	Hawler/ashqa	36°26'05"N 44°20'20"E
C5	Krosk2	1	Akre/Dinarta	36°46'35"N 43°56'09"E	C21	Kaska	3	Shaqlawe/	36°24'34"N 44°20'20"E
C6	Gelas	1	Akre/Dinarta	36°46'35"N 43°56'09"E	C22	Spe grie	3	Shaqlawe/Aqubani	36°25'14"N 44°20'23"E
C7	Sana Sive	1	Akre/Dinarta	36°46'35"N 43°56'09"E	C23	Sew harmi	3	Shaqlawe/Aqobane	36°25'14"N 44°20'23"E
C8	Krosk3	1	Akre/Dinarta	36°46'35"N 43°56'09"E	C24	Krosk	3	Shaqlawe/Aqobane	36°25'14"N 44°20'23"E
C9	Sork	2	Halabja /khurmal	35°16'27"N 45°59'38"E	C25	Qalate	4	Sulaymaniyah/khamza	35°40'25"N 45°26'53"E
C10	Kabrie	2	Halabja/khurmal	35°16'27"N 45°59'38"E	C26	Lasor	4	Sulaymaniyah/khamza	35°40'25"N 45°26'53"E
C11	Kroska	2	Halabja/khurmal	35°16'27"N 45°59'38"E	C27	Balegie	4	Sulaymaniyah/khamza	35°40'25"N 45°26'53"E
C12	Bebar	2	Halabja/khurmal	35°16'27"N 45°59'38"E	C28	Naske	4	Sulaymaniyah/khamza	35°40'25"N 45°26'53"E
C13	Bazingani	2	Halabja/serwan	35°13'59"N 45°59'04"E	C29	Do shawe	4	Sulaymaniyah/khamza	35°40'25"N 45°26'53"E
C14	Sebar	2	Halabja/serwan	35°13'59"N 45°59'04"E	C30	Gollawe	4	Sulaymaniyah/khamza	35°40'25"N 45°26'53"E
C15	Qazam	2	Halabja/serwan	35°13'59"N 45°59'04"E	C31	Sewharme	4	Sulaymaniyah/khamza	35°40'25"N 45°26'53"E
C16	Gwele	2	Halabja/Khurmal	35°16'27"N 45°59'38"E	C32	Shaxawan	4	Sulaymaniyah/khamza	35°40'25"N 45°26'53"E

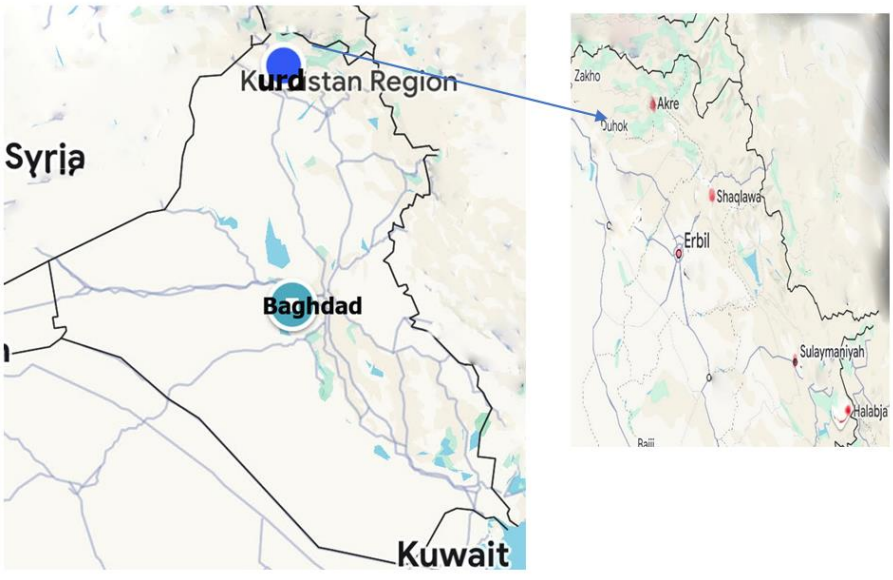

Fig.1: Geographic location of various pear landraces from the Kurdistan region of Iraq.

DNA Quantity and Quality:

The quality of the DNA was assessed using the Nanodrop instrument, which follows a specific formula: purity of DNA= O.D. 260 / O.D 280 = ≥ 1.8 with a range of readings between 1.8 and 2 (Sun *et al*., 2013 and Kanoosh *et al*., 2019), and DNA concentration was adjusted to a final concentration of 50 ng/ l

PCR-AFLP Analysis:

The AFLP analysis, including adaptor and primer sequences and PCR conditions for pre-selective and selective amplifications in (Table 2), was conducted based on the protocol described by Vos et al. (1995), with minor modifications. Specifically, 250 ng of DNA was double digested using 5 units each of the restriction enzymes Tru91 and PstI in a final reaction volume of 30 µl. This volume contained 1x One-Phor-All buffer (50 mM potassium acetate, 10 mM Tris-acetate at pH 7.5-, and 10-mM magnesium acetate, pH 7.5) (Pharmacia Biotech, Uppsala, Sweden). The reaction was incubated at 37°C for three hours. The amplification process was carried out using a specific program of 36 cycles. Each cycle included a 30-second phase of DNA denaturation at a temperature of 94°C, followed by a 30-second annealing step, and finally1-minute extension step at a temperature of 72°C. The annealing temperature in this program underwent variation during the first cycle, starting at 65°C. In each successive cycle for the next 12 cycles, it was systematically decreased by 0.7°C, following a touchdown PCR protocol. Subsequently, for the remaining 23 cycles, the temperature was maintained at 56°C. The amplified products were loaded on to denaturing polyacrylamide gels with an 8% concentration. The DNA fragments were then stained using silver staining kit from (Promega, Madison) the manufacturer's instructions. The silver-stained gels were digitally imaged after being dried in the air.

Table 2. the Primer sequences used in the first and second amplification for pear landraces

Pre-selective primers (5' ⟶ 3')		Selective amplification primers	
P00	GACTGCGTACATGCAG	P12	GAC TGC GTA CAT GCAGAC
MOO	GATGAGTCCTGAGTAA	P50	GACTGCGTACATGCAGCATG
		P81	GACTGCGTACATGCAGTAG
		P100	GACTGCGTACATGCAGAACC
		P107	GACTGCGTACATGCAGAATA
		P174	GACTGCGTACATGCAGCATG
		P71	GACTGCGTACATGCAGGGA
		P20	GACTGCGTACATGCAGGC
		M307	GATGAGTCCTGAGTAATCAG
		M95	GATGAGTCCTGAGTAAAAAA
		M88	GATGAGTCCTGAGTAATGC
		M237	GATGAGTCCTGAGTAAGATA
		M43	GATGAGTCCTGAGTAAATA
		M182	GATGAGTCCTGAGTAATAT
		M293	GATGAGTCCTGAGTAATACA
		M22	GATGAGTCCTGAGTAAGT
		M81	GATGAGTCCTGAGTAATAG
		M82	GATGAGTCCTGAGTAATAT

Data Analysis:

The bands were scored as either 1 (present) or 0 (absent) and recorded in a Microsoft Excel spreadsheet. This data was then analyzed using Notedit software, with statistical analysis performed using version 2.02 (Rohlf, 1998).

Three parameters were used to assess the discriminatory power of AFLP markers. First, the polymorphic information content (PIC) was calculated for each AFLP primer combination individually, using the formula: PIC = 1 - $\Sigma(^2pi)$, where *pi* represents the frequency of the allele. Second, Resolving Power (Rp), which measures each primer's ability to detect variation among individuals, was calculated according to the method by Prevost and Wilkinson (1999): Rp = ΣIb, where Ib (band informativeness) is determined by the formula: 1 - [2 × 0.5 - p], with *p* being the fraction of genotypes that include the band.

Finally, the Marker Index (MI) was calculated based on the method proposed by Powell et al. (1996) and

employed by Milbourne *et al.* (1997). The Marker Index incorporates both the polymorphic information content and the Effective Multiplex Ratio (EMR), where EMR = np(np/n), were *np* representing the number of polymorphic loci and *n* the total number of loci. The formula for MI is given as MI = PIC × EMR (Satish *et al*., 2015).

Additionally, the number of distinct fingerprints generated by each primer, as well as the number of elite lines with unique fingerprints for each primer, was record.

The AFLP technique, known for its high level of polymorphism, was selected for this study due to its ability to detect genetic diversity across pear landraces. The high polymorphism rate (92.49%) observed in this study was expected, as AFLP markers are sensitive to genetic variation, particularly in populations with high genetic divergence. This is consistent with previous studies where AFLP markers have been successfully applied to genetically diverse fruit species, allowing for a detailed analysis of genetic relationships.

RESULTS AND DISCUSSION

Twenty-four AFLP primer combinations tested, only 15 produced reproducible and clear polymorphic profiles across all 32 pear landraces, as shown in (Fig. 2).

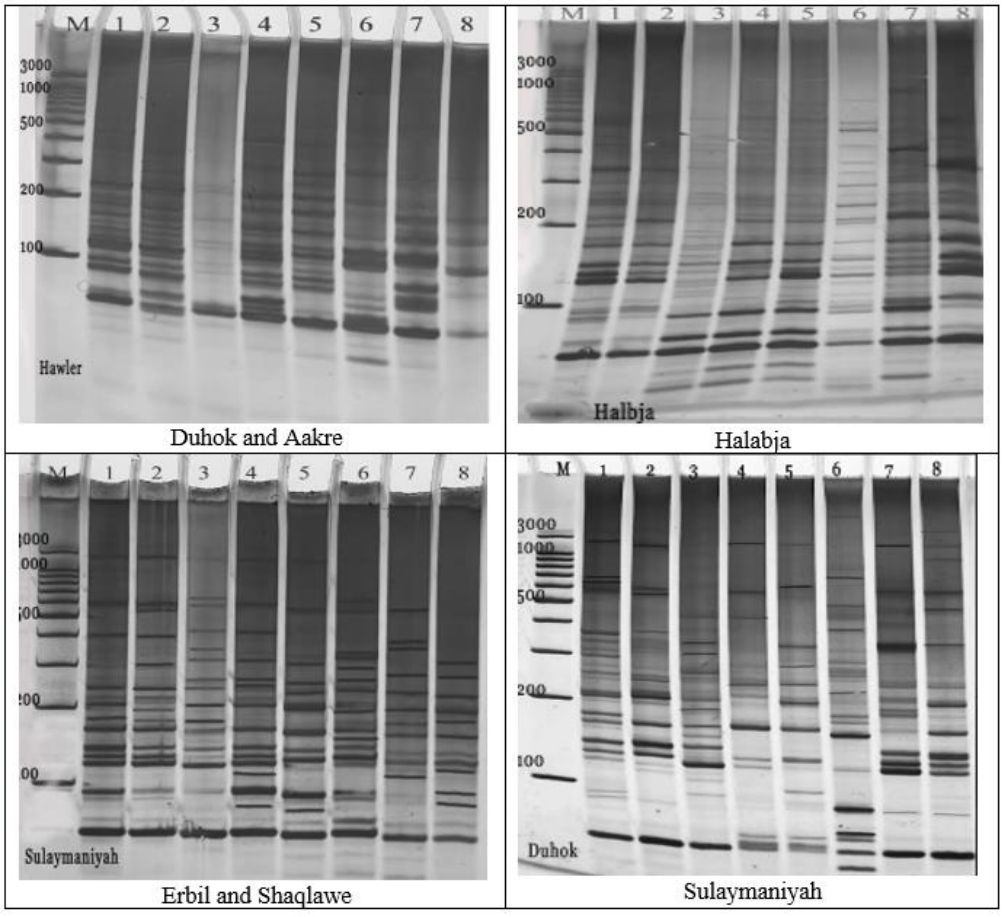

Fig.2: Represents a polyacrylamide gel Electrophoresis of the results of the 32 pear landraces using AFLP primer combinations (P100/M237). Lanes from1 to 32 represent (*pyrus communis)* pear landraces: (Duhok and Aakre region) 1. Krosk, 2. Zarik, 3. Gelki, 4. Hezel, 5. krosk2, 6. Gelas, 7. Sana sive, and 8. Krosk3 (Halabja rejon)1. Sork, 2. Kabrie ,3. Kroska, 4. Bebar,5. Bazingane ,6. Sebar ,7. Qazam and 8. Gwele. (Erbil and Shaqlawe region); 1. Qazam, 2. Spegre, 3. Bar-Awe,4. Naske, 5. kaska, 6. Spegrie, 7. Sew harmi and 8. Krosk, and (Sulaymaniyah region) 1. Qalate, 2. Lasor,3. Balegie,4. Naske,5. Doshawe 6. Gollawe, 7. Sewharmie and 8. Shaxawan. Lane M represents the molecular weight marker.

A total of 8,016 amplified fragments were generated, with the primer efficiency percentage of each primer calculated as: (total number of bands for each primer / total number of bands across all primers) × 100 (Amoon & Abdul-Hamed, 2020). Of these, 7,399 bands were polymorphic, with the number of fragments per primer pair averaging 493, ranging from 341 in the (P100/M237) combination to 588 in the (P100/M81) combination. The size of the polymorphic bands ranged from 40 to 1,200 bp, and the overall polymorphism rate was 92.45%. Primer efficiency varied between 4.25% and 8.28% as in (Table 3).

Table 3: Number of bands (NB), number of polymorphic bands (NPB), number of exclusive bands, primer efficiency(E), Percentage polymorphism (PPB %) polymorphic information content (PIC), resolving power (Rp) and marker index (MI) obtained per AFLP primer combination.

N	Primer combinations	TNB	NPB	E%	PPB %	PIC	RP	EMR	MI
1	P100/M237	341	341	4.25	100	0.32	21.3125	341	111.3
2	P107/M307	583	551	7.27	94.5	0.35	36.44	520.8	185.8
3	P12/M237	583	551	7.27	94.5	0.35	36.44	520.8	185.8
4	P50/M88	709	613	8.84	86.5	0.35	44.31	530	189.6
5	P81/M43	528	528	6.59	100	0.36	33	528	192.3
6	P107/M182	505	441	6.3	87	0.35	31.56	385	138.5
7	P20/M182	360	360	4.49	100	0.36	22.50	360	131
8	P107/M293	585	521	7.3	86	0.36	36.56	464	168.5
9	P81/M88	520	488	6.49	93.8	0.35	32.50	458	162.6
10	P20/M22	596	564	7.44	94.6	0.32	37.25	534	172.5
11	P174/M81	659	595	8.22	90	0.35	41.19	392	140.3
12	P71/M88	509	445	6.35	87.4	0.34	31.81	389	135.8
13	P100/M81	588	588	7.34	100	0.39	36.75	588	231.7
14	P20/M95	507	443	6.23	87.4	0.32	31.69	387	124.6
15	P100/82	443	379	5.53	85.55	0.44	27.69	324	142.8
Total		8016	7408						
Mean		534	494	6.6	92.5	0.35	33.4	448	161

The PIC value, which estimates the discriminating power of each marker and the capacity of a locus, takes into account not only the number of expressed alleles but also their relative frequencies. In this study, PIC values ranged from 0.32 to 0.44. Using the co-dominant marker calculation method developed by Botstein *et al.* (1980), the resolving power (Rp) of primers across different pear landraces varied from 21.31 (P100/M237) to 44.31 (P50/M88), with an average Rp of 33.4. The effective multiplex ratio (EMR) ranged between 324 (P100/82) and 588.9 (P100/M81), with an average of 566.1, indicating a robust multiplexing capability. Furthermore, the marker index (MI) values, which reflect the overall discriminating power of the primer pairs, varied significantly among the fifteen primer combinations. The average MI value across the 32 pear landraces was 160.87 (Table 3), highlighting the variability in the discriminatory efficiency of the different primer sets.

Based on these results, AFLP markers proved to be valuable tools for analyzing the genetic diversity of pears. Additionally, they have been found to be effective identifiers in various types of fruit plants (Jubrael *et al.*, 2005). The number of markers assessed in this study exceeded those reported for pears using RAPD markers in previous research: 57.32% in

Iran (Koushesh *et al*., 2017), 65.95% in Iran (Sisko *et al.*, 2009), 81.9% in China (Mingan *et al*., 2002), and 84% in Portugal (Monte-Coro *et al*., 2000). The AFLP marker technique is more efficient than RAPD for species identification, offering several advantages, such as the ability to analyze more loci and improved repeatability of banding patterns (Dolatowski *et al*., 2004).

Mahmodi *et al*. (2013) registered AFLP markers for 116 pear species (Pyrus spp.) in western Iran. In their study of 11 pear cultivars, AFLP markers revealed 98% polymorphic markers, a polymorphism rate similar to that observed in this study. By comparison, Luisa Monte-Coro *et al.* (2000) reported an 87% polymorphism rate among 25 *Pyrus communis* L. cultivars. This high level of polymorphism may result from long-term evolution, demonstrating how a single species can adapt and change in response to complex environmental factors over time.

The genetic relationships between the 32 pear landraces, as determined by Dice's similarity coefficient. These results offer valuable insights into the genetic diversity and evolutionary relationships within the pear landraces studied, are presented in Table 4 and Figure 3.

Table 4: Genetic similarity matrix for Pear genotypes based on AFLP data

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31
C1	0.00																														
C2	0.29	0.00																													
C3	0.39	0.33	0.00																												
C4	0.29	0.27	0.31	0.00																											
C5	0.31	0.30	0.35	0.24	0.00																										
C6	0.44	0.44	0.47	0.46	0.45	0.00																									
C7	0.33	0.31	0.41	0.37	0.32	0.40	0.00																								
C8	0.41	0.31	0.37	0.33	0.31	0.49	0.23	0.00																							
C9	0.66	0.59	0.63	0.54	0.64	0.77	0.72	0.57	0.00																						
C10	0.66	0.61	0.63	0.56	0.68	0.75	0.71	0.57	0.19	0.00																					
C11	0.82	0.73	0.68	0.75	0.84	0.88	0.81	0.71	0.37	0.36	0.00																				
C12	0.63	0.63	0.63	0.57	0.65	0.73	0.70	0.54	0.27	0.20	0.36	0.00																			
C13	0.64	0.55	0.66	0.61	0.65	0.69	0.69	0.62	0.31	0.30	0.45	0.25	0.00																		
C14	0.71	0.64	0.70	0.68	0.77	0.76	0.75	0.67	0.39	0.35	0.48	0.37	0.22	0.00																	
C15	0.71	0.60	0.67	0.65	0.67	0.73	0.74	0.64	0.37	0.34	0.47	0.30	0.35	0.31	0.00																
C16	0.65	0.63	0.62	0.59	0.61	0.74	0.67	0.56	0.26	0.23	0.41	0.25	0.35	0.37	0.25	0.00															
C17	0.65	0.58	0.63	0.59	0.62	0.75	0.67	0.59	0.51	0.47	0.57	0.51	0.52	0.56	0.58	0.47	0.00														
C18	0.71	0.62	0.64	0.65	0.61	0.70	0.70	0.57	0.62	0.52	0.67	0.52	0.53	0.62	0.66	0.57	0.26	0.00													
C19	0.76	0.67	0.66	0.65	0.66	0.75	0.77	0.65	0.66	0.69	0.77	0.66	0.62	0.73	0.81	0.63	0.46	0.36	0.00												
C20	0.70	0.59	0.63	0.59	0.63	0.69	0.70	0.56	0.51	0.48	0.68	0.47	0.49	0.59	0.68	0.52	0.29	0.29	0.30	0.00											
C21	0.69	0.63	0.63	0.63	0.61	0.67	0.72	0.56	0.51	0.49	0.62	0.51	0.50	0.58	0.67	0.52	0.36	0.30	0.37	0.19	0.00										
C22	0.68	0.60	0.65	0.57	0.62	0.71	0.71	0.60	0.52	0.54	0.62	0.53	0.48	0.55	0.64	0.54	0.35	0.36	0.37	0.24	0.23	0.00									
C23	0.67	0.63	0.60	0.59	0.62	0.67	0.67	0.63	0.60	0.61	0.60	0.63	0.54	0.57	0.69	0.53	0.34	0.39	0.37	0.26	0.28	0.21	0.00								
C24	0.69	0.62	0.68	0.58	0.63	0.67	0.71	0.59	0.54	0.55	0.68	0.54	0.54	0.64	0.72	0.53	0.38	0.36	0.33	0.25	0.23	0.25	0.26	0.00							
C25	0.58	0.55	0.63	0.63	0.61	0.70	0.59	0.52	0.64	0.60	0.68	0.65	0.56	0.64	0.66	0.60	0.55	0.60	0.60	0.53	0.55	0.60	0.59	0.60	0.00						
C26	0.64	0.60	0.65	0.68	0.67	0.75	0.71	0.56	0.57	0.54	0.66	0.59	0.58	0.59	0.63	0.56	0.48	0.56	0.67	0.53	0.52	0.56	0.58	0.57	0.23	0.00					
C27	0.69	0.59	0.69	0.70	0.75	0.75	0.76	0.57	0.54	0.51	0.75	0.55	0.58	0.64	0.63	0.54	0.50	0.56	0.58	0.44	0.52	0.57	0.60	0.55	0.30	0.20	0.00				
C28	0.64	0.60	0.67	0.66	0.69	0.76	0.74	0.60	0.56	0.52	0.71	0.56	0.57	0.64	0.61	0.55	0.54	0.59	0.64	0.54	0.55	0.58	0.58	0.56	0.29	0.21	0.19	0.00			
C29	0.71	0.61	0.72	0.64	0.72	0.78	0.73	0.56	0.53	0.49	0.65	0.54	0.56	0.62	0.63	0.52	0.46	0.52	0.59	0.46	0.50	0.54	0.52	0.51	0.28	0.26	0.23	0.21	0.00		
C30	0.63	0.54	0.59	0.62	0.63	0.67	0.56	0.55	0.53	0.68	0.62	0.53	0.60	0.60	0.52	0.47	0.59	0.61	0.49	0.50	0.56	0.51	0.54	0.28	0.27	0.25	0.21	0.19	0.23	0.00	
C31	0.70	0.66	0.66	0.70	0.68	0.77	0.72	0.61	0.60	0.60	0.76	0.65	0.64	0.69	0.68	0.60	0.56	0.65	0.64	0.57	0.55	0.65	0.63	0.60	0.38	0.29	0.28	0.30	0.29	0.20	0.00
C32	0.59	0.54	0.65	0.59	0.62	0.73	0.65	0.55	0.59	0.56	0.70	0.62	0.59	0.61	0.64	0.58	0.51	0.61	0.67	0.57	0.58	0.66	0.59	0.57	0.34	0.29	0.28	0.27	0.23	0.23	0.16

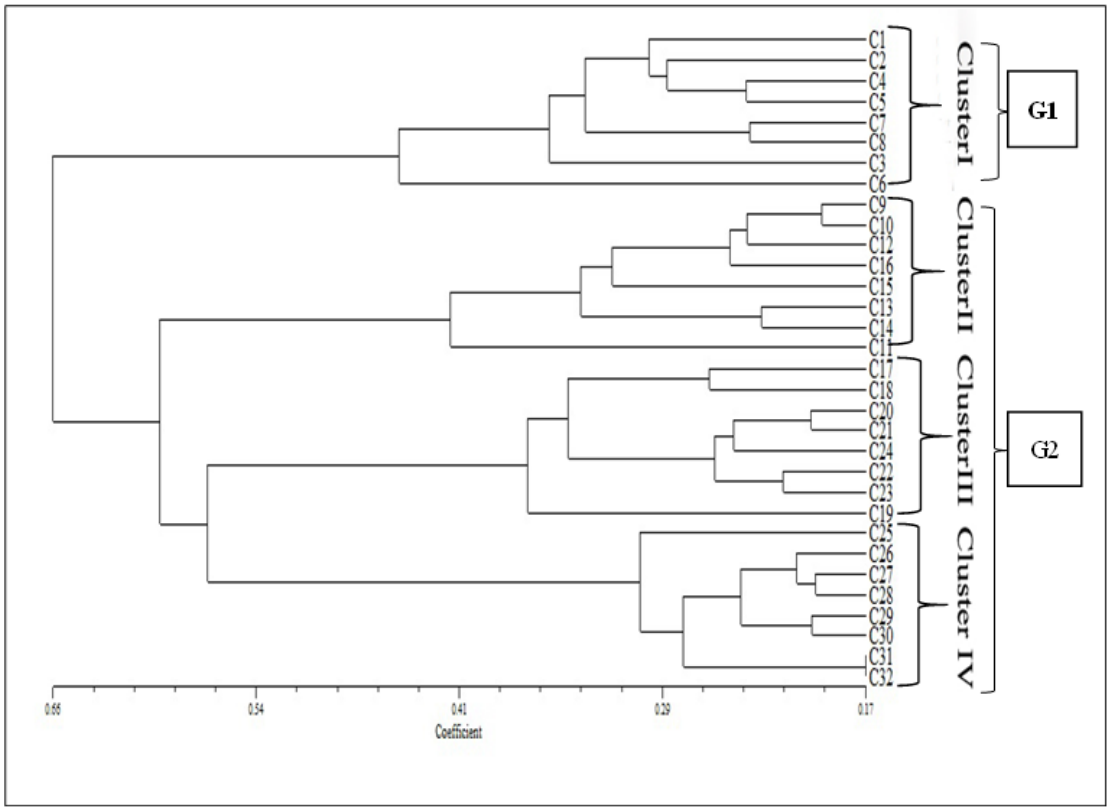

Fig. 3. Dendrogram of 32 pear landraces resulting from the unweighted pair-group method of arithmetic average cluster analysis based on the Dice similarity coefficient obtained from 8016 (TNB) of AFLP marker.

In the dendrogram the highest genetic distance within landraces of pear (*P. communis)* were 0.88 between Krosk from Halabja and Gelase from Akre, and the lowest genetic distance genetic was 0.16 between Sew Harmie and Shakhwan both from Sulaymaniyah, this shows that these two landraces are very different from the others. The genetic similarity between different pear can reveal key insights into their relatedness and evolutionary paths. In this case, the highest genetic distance, 0.88, between Krosk from Halabja and Gelase from Akre, indicates that these two genotypes are genetically very distinct from each other. This suggests that despite both being from the different region, these genotypes have undergone significant divergence, possibly due to environmental factors, human cultivation practices, or geographic isolation that have driven genetic differentiation.

On the other hand, the lowest genetic distance, 0.16, between Sewharmi and Shaxwan, both from Sulaymaniyah, implies that these two genotypes are highly similar. The close genetic relationship may be due to shared ancestry, similar cultivation conditions, or a more recent divergence. The similarity within the same region may suggest limited genetic variation among some local pear varieties, likely reflecting specific agricultural or environmental factors favoring certain traits.

Overall, this variation in genetic distance highlights both the diversity within pear genotypes and the potential influences of geography and human practices on the genetic structure of these populations.

The high level of genetic diversity observed among the pear in this study suggests the presence of considerable genetic variation even within a single species, such as *P. communis*. This finding raises taxonomic challenges, as traditional classification systems based on morphological traits may not capture the

true genetic relationships among varieties. As demonstrated in this study, AFLP markers provide a more precise tool for assessing genetic diversity and can help resolve uncertainties in species and genus classification. This research contributes to ongoing efforts to refine the taxonomy of the genus *Pyrus*. Additionally, a genetic similarity matrix using Dice's coefficient was determined, revealing variation between the pear landraces.

The dendrogram displayed two main groups. The First group was indicted as G1, according to Dice's similarity matrix and the UPGMA clustering method. G1, which includes the landraces of pear that share the maximum similarity distance, this groups consists of one cluster it divided in to sub-cluster; the lower includes only one (C6), and the upper included (C3), (C7 and C8 with similarity 0.236), C1 and (C4, C5 with similarity 0.239), and(C2).

Second group, indicated as G2, which included three cluster landraces of pear: first, the upper included (cluster 2), which included the taxa (C11) (C13, C14 with similarity 0.229), (C15), (C16), (C12) and (C9, C10 with similarly 0.192).

The second cluster 3 which included C19, (C17, C18 with similarity 0.26), C22, C23with similarity 0.216), (C20, C21 with similarity 0.199) and C24). The third cluster C4, which included (C25), (C31, C32 with similarity of 0.166), (C27, C28) with a similarity of 0.197), and (C29 and C30 with a similarity of 0.199).

In addition, the study showed that the location has a variety of pear genotypes that are both closely linked and distantly connected. It also demonstrated that there is a good match between the dendrogram clusters and the similarity matrix, suggesting that there is substantial genetic heterogeneity among the genotypes.

Previous study has shown that utilizing AFLP markers, by Mahmod *et al*. (2013) have also reported that the genetic similarity matrix, which was calculated using AFLP markers, has a strong correlation with the cophenetic matrix, which was made using the dendrogram. Furthermore, AFLP was a dependable and powerful technique for genotyping and distinguishing among pear cultivars. Moreover, it has been established that it is the most effective method for pears of the *P. communis* cultivar. (Monte-Corvo *et al*., 2002; Dolatotowski *et al*., 2004; Bao *et al*., 2008).

The Coordinate in (Table 1) indicates that genotypes located closer together geographically tend to be more genetically similar, likely due to factors such as gene flow, environmental pressures, and local adaptation. The observed clustering of genotypes based on geographic regions supports this idea, highlighting the influence of spatial proximity on genetic relatedness (Hoban *et al*.,2016)

Additionally, the clustering patterns may reflect differences between wild and cultivated varieties. Cultivated genotypes often undergo artificial selection for specific traits, leading to reduced genetic diversity compared to wild populations. Conversely, wild specimens may retain a broader genetic base, shaped by natural selection. The distinction between wild and cultivated genotypes could further contribute to the genetic divergence observed in different regions **(**Meyer *et al*., 2012)

Future studies should explore the role of morphological variation in these clustering patterns. Morphological traits, which can be influenced by both genetic and environmental factors, may offer additional insights into the relationship between genotype and phenotype. By examining the correlation between morphological and genetic diversity, researchers could better understand the evolutionary processes driving the differentiation of pear genotypes across geographic regions.

CONCLUSION

Although this investigation was conducted in various regions of Kurdistan, Iraq, these areas represent a wide range of

ecological and geographical conditions, which significantly contributed to the genetic diversity observed in the study. The diversity of environmental factors, such as altitude, climate, and soil composition, likely played a key role in shaping the genetic variation among the pear landraces.

The findings of this research underscore the effectiveness of AFLP-based fingerprinting as a highly reliable method for identifying pear landraces with precision. This technique has proven its value in genetic diversity analysis, offering a robust tool for distinguishing between pear varieties and understanding their evolutionary relationships. The ability to analyze such diversity is crucial for both conservation efforts and the sustainable use of genetic resources in breeding programs.

Moreover, this genetic information can serve practical applications beyond diversity analysis. It may be utilized for cultivar identification, ensuring the accurate classification of pear varieties in breeding programs. Additionally, it plays a crucial role in breeder rights protection, safeguarding the intellectual property and efforts of local breeders in Kurdistan. This, in turn, supports agricultural innovation and the development of new pear cultivars adapted to the region's unique conditions

Declarations:

Ethical Approval: Not applicable.

Competing interests: The authors declare no conflict of interest.

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