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Genetic Relationship and Gene Stability Among Some Pomegranates (*Punica granatum* L.) Cultivars Cultured in Duhok Province, Iraq

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ABSTRACT

The study was conducted at the University of Duhok, College of Science, Department of Biology, from October 2021 to February 2023, focused on investigating the micropropagation and genetic diversity of seven pomegranates (*Punica granatum* L.) Masafik, Melisse, Radisho, Armishte, Shahraban, Halapja, and Dwarf. The experiments were conducted in the Plant Tissue Culture and Plant Molecular Laboratories at the Scientific Research Center (SRC), College of Science, University of Duhok,. The main aim of this study was to assess the genetic diversity, genetic relationships, and gene stability of the pomegranate cultivars being investigated, using Sequence Related Amplified Polymorphism (SRAP) DNA markers.

The results showed that only four tested SRAP primer combinations produced consistent and distinct polymorphic patterns in all 21 pomegranate genotypes, including the seven cultivars, the produced callus, and the regenerated plantlets formed from the callus. In the seven cultivars of *Punica granatum*, the combination of EM29-ME5 primers produced the highest number of banding patterns at 14, while primer EM29-ME17 produced only seven bands, totaling 42 bands. There were 3 monomorphic bands and 39 polymorphic bands, averaging 91.88%. The genetic relationship between species was determined using the calculated genetic distance, which ranged from the lowest value of 0.11989 between Armishte and Melisse to the highest value of 0.73162 between Melisse and Masafik. The dendrogram recognized three clusters for similarity between the cultivars.

***Keywords*:** SRAP, Pomegranate, genetic markers, micropropagation, PCR.

INTRODUCTION

In recent years, molecular markers have systematically reconstructed evolutionary relationships among plants. This knowledge is essential for understanding the evolution and breeding of pomegranates. Various molecular markers, such as RAPD, AFLP, ISSR, and SSR, have been utilized to identify genetic diversity among pomegranate cultivars [1,2,3,4,5,6 and 7]. The Sequence-Related Amplified Polymorphism (SRAP) method has proven to be a relatively simple and highly reproducible DNA marker technology for gene tagging and plant mapping. It offers more formativeness in detecting genetic diversity than other PCR-based approaches [8, 9,10].

Although additional molecular markers have been used to study the genetic diversity of Iraqi pomegranates, the use of SRAP markers has not been attempted. Thus, the present study was conducted to establish a reproducible protocol for large-scale *in vitro* production of propagules of a pomegranate without any seasonal barrier using different concentrations and combinations of plant growth regulators. In addition, use SRAP markers to evaluate the genetic linkages and population genetic structure of seven different pomegranate genotypes. Therefore, the major aims of this study include the evaluation of the genetic diversity, genetic relationship, and gene stability among some local pomegranate cultivars by using an SRAP DNA marker. In addition, evaluate the genetic stability of natural pomegranate cultivars compared to those obtained through the micropropagation technique.

Materials And Methods

Fresh young leaf samples from seven Pomegranate (*Punica granatum* L.) cultivars grown in Duhok province, Kurdistan region of Iraq, were previously obtained from various locations in Iraq for molecular analysis. DNA samples from the callus and regenerated shoots were also collected from plants resulting from tissue culture experiments. Genomic DNA was extracted and CTAB method, as described by [11]. was followed, and the DNA sample was analyzed using a nanodrop spectrophotometer at wavelengths of 260/230 and 260/280 nm. An optical density (OD) was calculated using 1 µl of the DNA sample, A260/A230 ratio of 2.0 - 2.2. Then agarose gel electrophoresis was run, and DNA bands were separated using a 1% agarose gel electrophoresis. finally, after the migration was complete, DNA bands were visualized by ultraviolet illumination.

In this study, four SRAP primer pairs were used. The sequences and the names of the loci are listed in Table 1.

Table (1): Name and sequence of SRAP primers [8].

Reverse	5'	3'	Forward	5'	3'
EM29	CGT AGC GCG TCA ATT ATG		ME17	TGA GTC CAA ACC GGG TA	
EM29	CGT AGC GCG TCA ATT ATG		ME10	TGA GTC CTT TCC GGT CC	
EM29	CGT AGC GCG TCA ATT ATG		ME22	TGA GTC CAA ACC GGC TA	
EM29	CGT AGC GCG TCA ATT ATG		ME5	TGA GTC CAA ACC GGA AG	

The PCR reaction mixture consists of a 50 ng/µl DNA template, 1.5 µl each of reverse and forward primers, and 10 µl of Taq master at a 2X concentration. The Taq master solution is prepared using the following components: 20 mM Tris-HCl (pH 8), 10 mM KCl, 0.2% Triton X-100, 4 mM $MgCl_2$, protein stabilizer, sediment loading dye, Taq DNA polymerase, and 0.5 mM each of dATP, dCTP, dGTP, and dTTP. The volume is then adjusted with sterilized deionized distilled water to 20 µl. The PCR reaction was conducted by first mixing the DNA samples in an ice bath using a pipette and then vortexing to collect the liquid at the bottom of the PCR Eppendorf tube. After that, 18 µl of the mixture was added into 0.25 ml of new PCR Eppendorf tubes along with 2 µl of each DNA sample individually, and gently mixed. The tubes were then placed in the thermocycler to carry out the following amplification program: Initial denaturation step of 5 min at 94˚C; Five cycles of denaturation for 1 min at 94˚C; Annealing for 1 min at 35˚C; Elongation for 1 min at 75˚C; Followed by 35 cycles for 1 min at 94˚C; Annealing for 1 min at 50˚C; Elongation for 1 min at 72˚C; Final extension for 5 min at 72˚C and the final temperature holds at 4˚C.

The NTSYS pc2.1 program, created by the Numerical Taxonomy System, applied biostatistics in Setauket, New York, and was used to analyze the data. PCR products were obtained from SRAP, and the presence of each fragment was determined as either present (1) or absent (0). The genetic separation between the accessions was calculated using this information, and the findings were presented using a dendrogram. The original DICE similarity index was used as the basis for calculating the similarity coefficient using the NTSYS-PC version 2.20 software.

Results And Discussion

DNA extraction

The quantity of DNA in the solution ranged from 166.307 to 10399.69 ng/µl, and its purity ranged between 1.24 and 2.23 (Table 2). DNA bands were separated using 1% agarose gel electrophoresis. Uncut lambda (λ) CI85Sam at a concentration of 25 ng/µl was used as a reference to determine the molecular weight of DNA samples. The concentration of the DNA samples was generally around 50 ng/µl.

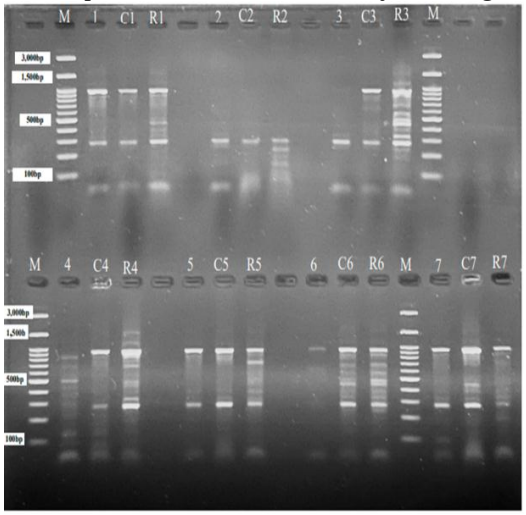
Several methods were reported for extracting DNA from plant matter and other living organisms. Generally, a good technique should consider several points, such as maximizing yields, maintaining purity, and reducing deterioration, in addition to the price, duration, labor, and compatibility for repeated sample extraction [12,13,14,15]

The current method for DNA isolation involves many processes and chemicals, including the use of liquid nitrogen. Liquid nitrogen readily crystallizes the water content of cells, aids in breaking down their walls, and inactivates any enzymes [16]. Additionally, DNA was successfully isolated using CTAB and other components of the extraction buffer. CTAB, acting as an ionic detergent at 65˚C, removes proteins, polysaccharides, and other elements from DNA, forming a complex known as the CTAB-nucleic acid complex, which keeps the DNA in an aqueous phase. Furthermore, EDTA functions as a chelating agent in the extraction buffer, binding to divalent ions such as Mg^2, which play a significant role in DNase activity. After centrifuging, chloroform, an organic solvent, retains the DNA in an aqueous phase, and isopropanol assists in DNA precipitation, causing high molecular weight DNA to appear as a white mass, which can be gathered with a glass hook or by centrifuging[11].

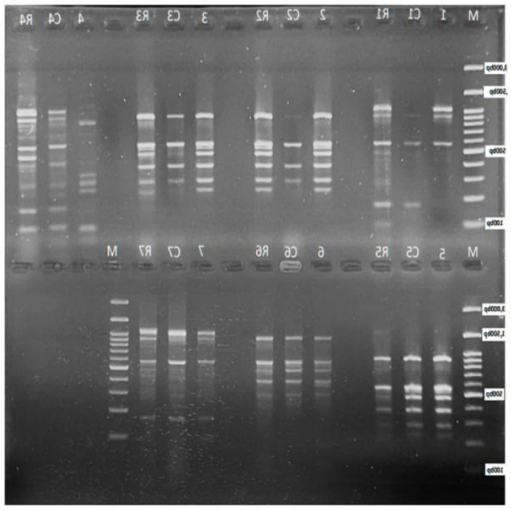
Table (2): DNA concentrations in samples were determined using a Nanodrop Spectrometer.

No.	Sample	DNA conc. (ng/µl)	260/230 nm	260/280 nm
1	Masafik	927.427	0.55	1.32
2	Melissa	871.338	0.50	1.24
3	Radish	3995.547	0.47	1.91
4	Armishte	3550.738	0.44	1.60
5	Shahraban	166.307	0.10	1.54
6	Halapja	3407.331	0.47	1.51
7	Dwarf	624.095	1.49	2.23
8	Masafik (callus)	1036.214	0.87	1.85
9	Melissa (callus)	2472.177	0.90	1.81
10	Radish (callus)	10399.694	1.43	1.72
11	Armishte(callus)	904.027	0.68	1.46
12	Shahraban (callus)	1385.645	1.50	1.95
13	Halapja (callus)	1382.941	0.97	1.76
14	Dwarf (callus)	3146.983	1.34	2.02
15	Masafik (regenerated)	321.524	1.03	1.45
16	Melissa (regenerated)	418.755	0.92	1.69
17	Radish (regenerated)	1177.651	1.76	2.13
18	Armishte(regenerated)	842.172	1.45	1.98
19	Shahraban (regenerated)	613.288	1.05	1.83
20	Halapja (regenerate)	941.421	1.08	1.69
21	Dwarf (regenerated)	309.475	1.38	2.19

The amplification results were analyzed using 2% Agarose gel electrophoresis, as depicted in Figure 1.



A



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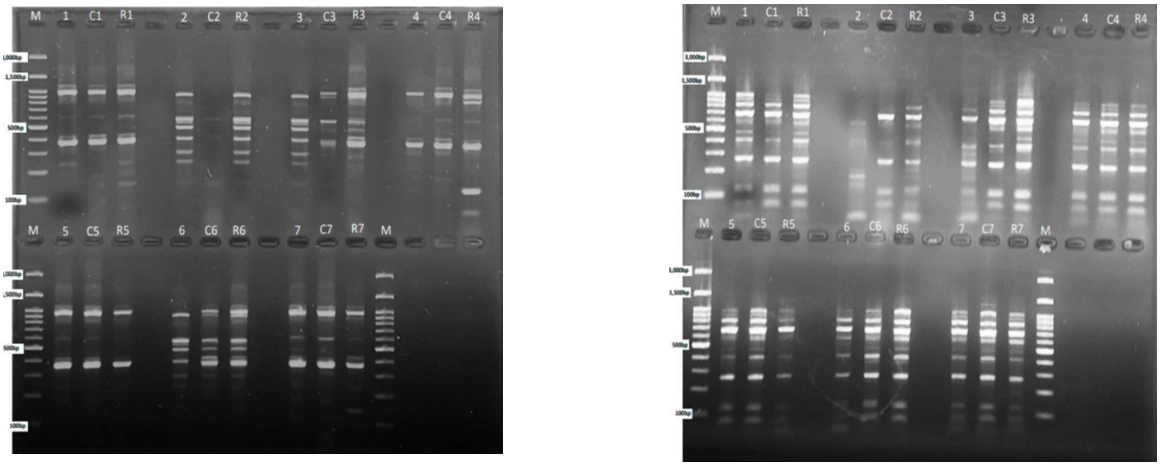

Figure (1): The amplified PCR products of Pomegranate cultivars, using the combination of forwarding and reverse primer (ME17), (M) Molecular weight marker (100 – 3000 bp), the lanes 1. Masafik, C1. Masafik callus, R1. Masafik regenerates; 2. Melisse, C2. Melisse's callus, R2. Melisse regenerates; 3. Radisho, C3. Radisho callus, R3. Radisho regenerates; 4. Armishte, C4. Armishte callus, R4. Armishte regenerates; 5. Shahraban, C5. Shahraban callus, R5. Shahraban regenerates; 6. Halapja, C6. Halapja callus, R6. Halapja regenerates; 7. Dwarf, C7. Dwarf callus, R7. Dwarf regenerates. A: Forwarding primer (EM29) and reverse primer (ME17); B: Forwarding primer (EM29) and reverse primer (ME10) ; C: Forwarding primer (EM29) and reverse primer (ME22); D: Forwarding primer (EM29) and reverse primer (ME5).

SRAP marker data analysis

In early data, only four selected SRAP primer combinations were found to produce distinct polymorphic patterns in all 21 pomegranate genotypes consistently.

For the Masafik cultivar, the callus and regenerated plantlets showed that the combination of EM29-ME5 primers produced 11 banding patterns, while primer EM29-ME17 (Figure 1) generated five bands. In total, there were 29 bands across all primers, with 18 being monomorphic and 21 being polymorphic. The average polymorphism rate was 40.73%, as indicated in Table 3.

Table (3): The number of bands and degree of polymorphism revealed by each SRAP primer combination of the Masafik cultivar with its callus and regenerated plant.

Primer combination	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)
EM29-ME17	5	3	2	40
EM29-ME10	7	2	5	71.42
EM29-ME22	6	4	2	33.33
EM29-ME5	11	9	12	18.18
Total	29	18	21	
Average	7.25	4.5	5.25	40.73

The combination of EM29-ME5 primers in Melisse cultivars produced a higher number of banding patterns at 10, while the combination of EM29-ME17 primers (Figure 1) resulted in a lower number of bands at five. In total, there were 31 bands across all combinations, with seven being monomorphic bands and 24 being polymorphic bands. This resulted in an average polymorphism rate of 75.16%, as indicated in Table 4.

Table (4): The number of bands and degree of polymorphism revealed by each SRAP primer combination of Melisse cultivar with its callus and regenerated plant.

Primer combination	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)

EM29-ME17	5	1	4	80
EM29-ME10	7	4	3	42.86
EM29-ME22	9	2	7	77.78
EM29-ME5	10	0	10	100
Total	31	7	24	
Average	7.75	1.75	6	75.16

In Radisho cultivars, the rootstock and its *In vitro* callus with the regenerated plantlets were analyzed using primers EM29-ME5 and EM29-ME22, which resulted in a higher number of banding patterns (12 bands) as shown in Figure 1. Using primer EM29-ME10 (Figure 1) yielded a lower number of bands (7 bands). The total number of bands observed was 42, with 15 being monomorphic and 27 being polymorphic. This represented an average 62.42% polymorphism rate, as detailed in Table 5.

Table (5): The number of bands and degree of polymorphism revealed by each SRAP primer combination of the Radisho cultivar with its callus and regenerated plant.

Primer combination	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)
EM29-ME17	11	2	9	81.81
EM29-ME10	7	4	3	42.86
EM29-ME22	12	4	8	66.67
EM29-ME5	12	5	7	58.33
Total	42	15	27	
Average	10.5	3.75	6.75	62.42

In Armishte cultivars, using the EM29-ME10 primers resulted in 14 banding patterns, while the EM29-ME17 primers produced 8 bands. In total, there were 40 bands, with 14 being monomorphic and 26 being polymorphic, resulting in an average polymorphism rate of 64.17%.

Table (6): The number of bands and degree of polymorphism revealed by each SRAP primer combination of Armishte cultivar with its callus and regenerated plant.

Primer combination	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)
EM29-ME17	8	0	8	100
EM29-ME10	14	3	11	78.57
EM29-ME22	9	4	5	55.55
EM29-ME5	9	7	2	22.22
Total	40	14	26	
Average	10	3.5	6.5	64.17

EM29-ME5 primers produced nine banding patterns in the Shahraban cultivars' callus and regenerated plantlets. On the other hand, primer EM29-ME17 (Figure 1) only yielded four bands. In total, 26 bands were observed, with 18 being monomorphic and 8 being polymorphic, resulting in an average polymorphism rate of 32.17%, as shown in Table 7.

Table (7): The number of bands and degree of polymorphism revealed by each SRAP primer combination of the Shahraban cultivar with its callus and regenerated plant.

Primer combination	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)
EM29-ME17	4	2	2	50
EM29-ME10	8	6	2	25
EM29-ME22	5	4	1	20
Em29-ME5	9	6	3	33.33

Total	26	18	8	
Average	6.5	4.5	2	32.17

In Halapja cultivars, its callus, and the regenerated plantlets, the combination of EM29-ME22 primers resulted in a higher number of 10 banding patterns. On the other hand, primer EM29-ME17 (Figure 1) showed a lower number of bands, specifically six bands. In total, there were 31 bands, with 18 being monomorphic and 13 being polymorphic. This resulted in an average polymorphism rate of 43.78%, as indicated in Table 8.

Table (8): The number of bands and degree of polymorphism revealed by each SRAP primer combination of the Halapja cultivar with its callus and regenerated plant.

Primer combination	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)
EM29-ME17	6	1	5	83.33
EM29-ME10	7	6	1	14.29
EM29-ME22	10	6	4	40
EM29-ME5	8	5	3	37.5
Total	31	18	13	
Average	7.75	4.5	3.25	43.78

Finally, the combination of EM29-ME5 primers resulted in a higher number of banding patterns (11). The EM29-ME17 primer (Figure 1) exhibited a lower number of bands, with only five bands in Dwarf cultivars with its callus and regenerated plantlets. In total, 31 bands were attained, out of which 19 were monomorphic and 12 were polymorphic. The average polymorphism rate was 41.91%, as shown in Table 9.

Table (9): The number of bands and degree of polymorphism revealed by each SRAP primer combination of the Dwarf cultivar with its callus and regenerated plant.

Primer combination	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)
EM29-ME17	5	2	3	60
EM29-ME10	7	4	3	42.86
EM29-ME22	8	5	3	37.5
EM29-ME5	11	8	3	27.27
Total	31	19	12	
Average	7.75	4.75	3	41.91

Based on the previous results, it is evident that the combination of EM29-ME5 primers produced a higher number of banding patterns, reaching 14, in the seven cultivars of *Punica granatum* mother plants. On the other hand, Primer EM29-ME17 resulted in a lower number of bands, specifically seven. The total number of bands observed was 42, with 3 being monomorphic bands and 39 being polymorphic bands, with an average of 91.88%. These results are summarized in Table 10.

Table (10): The number of bands and degree of polymorphism revealed by each SRAP primer combination of seven cultivars of *Punica granatum* L.

Primer combination	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)
EM29-ME17	7	1	6	85.71
EM29-ME10	10	0	10	100
EM29-ME22	11	2	9	81.81
EM29-ME5	14	0	14	100
Total	42	3	39	
Average	10.5	0.75	9.75	91.88

As a fundamental molecular marker of genetic variation among pomegranate genotype cultivars, Sequence-Related Amplified Polymorphism (SRAP) is being utilized for the first time in Iraq and the Kurdistan region. The results of the current investigation indicate that the average polymorphism in the Masafik cultivar, along with its callus and regenerated plantlets derived from in vitro callus, is approximately 40.75%. In comparison, the polymorphism in the Melisse cultivar reached 75.16%, in Radisho 62.42%, in Armishte 64.17%, in Shahraban 32.17%, in Halapja 43.78%, and in the dwarf variety it reached 41.91%.

The visual variation observed in this study could be attributed to genetic variability in the somaclonal variation in addition to the germplasm of the seeds used as a source of the explants. [17] assessed the genetic integrity of in vitro raised pomegranate plants using Random Amplified Polymorphic DNA (RAPD) by utilizing cotyledons as an explant for callus induction and regenerated shoots from the callus. Their study found 74% similarity in the genetic integrity of plantlets and concluded that the variation was due to somaclonal variation.

Returning to the current results, our approach revealed a significant amount of polymorphism (91.88%) in the seven *Punica granatum* genotypes collected from the field, which is higher than the rates of 57.3%, 57.0%, 56%, and 22.0%, respectively, reported by utilizing RAPD markers for pomegranate cultivars in Iran [1, 18], Jordan [19], and Turkey [20]. Additionally, it was observed that 86.5% polymorphism among 24 Iraqi pomegranate cultivars collected from different geographical areas around the Sulaimani region using 12 random combination primers.

According to [2], 34 pomegranate cultivars were collected from 14 locations in Tunisia and studied using AFLP markers. They found 94.7% polymorphism, indicating a similar range of polymorphism to the current study. [21] used eight AFLP primer combinations and reported an average of 73.26% polymorphism across 85 Chinese pomegranate cultivars. [22]. Also observed 73% polymorphism among 19 genotypes using four primer combinations. However, [23] found an average of 86.6% polymorphism in 15 Iraqi pomegranate cultivars using 10 AFLP primers. On the other hand, [24] reported 66% polymorphism in ten local Iraqi pomegranate genotypes from different geographical regions using the ISSR marker.

Although the samples used in this study came from similar regions, the results from the above analyses can be compared with the polymorphism rate. The variations in the number of samples examined and the number of primer combinations in this research may explain the differences in polymorphism percentages. This genetic diversity among pomegranate cultivars is essential for germplasm conservation and can be combined with consumer-oriented fruit traits to improve existing pomegranate cultivars through breeding.

Genetic distance

The genetic relationship between species was evaluated using calculated genetic distance (refer to Table 11). The genetic distance varied, with the lowest value of 0.11989 recorded between [Armishte & Melisse, and the highest value of 0.73162 documented between Melisse and Masafik. The NTSYS-PC program, which is based on Neis's (1972) standard genetic distance, was used to estimate the genetic relationships among *Punica* genotypes.

Table (11): Genetic distance between Pomegranate cultivars using SRAP markers.

	Masafik	*Melisse*	*Radisho*	*Armishte*	*Shahraban*	*Halapja*	*Dwarf*
Masafik	0.00000						
Melisse	0.73162	0.00000					
Radisho	0.49549	0.28265	0.00000				
Armishte	0.64964	0.11989	0.69314	0.00000			
Shahraban	0.20273	0.56971	0.38237	0.67005	0.00000		
Halapja	0.27363	0.30164	0.17883	0.60962	0.24275	0.00000	
Dwarf	0.14590	0.69520	0.48538	0.55438	0.61620	0.33252	0.00000

Dendrograms were created using the SRAP data in this study to measure genetic distance and classify cultivars. The genetic similarity matrices were built based on the number of bands produced by the SRAP marker. This approach allows for directly calculating the most accurate genetic distance between cultivars. The bands in the SRAP marker were considered regions of shared or non-shared genomic material among the cultivars.

The study's findings demonstrate a range of genetic similarities between cultivars, with values ranging from close to 0 to close to 1. A value of 0 is theoretically supposed to represent the complete similarity between two cultivars, although, in practice, complete similarity does not exist. On the other hand, a value of 1 means total dissimilarity between two cultivars, which does occur. The clustering of cultivars resulting from the genetic distance table is shown in Figure (2):

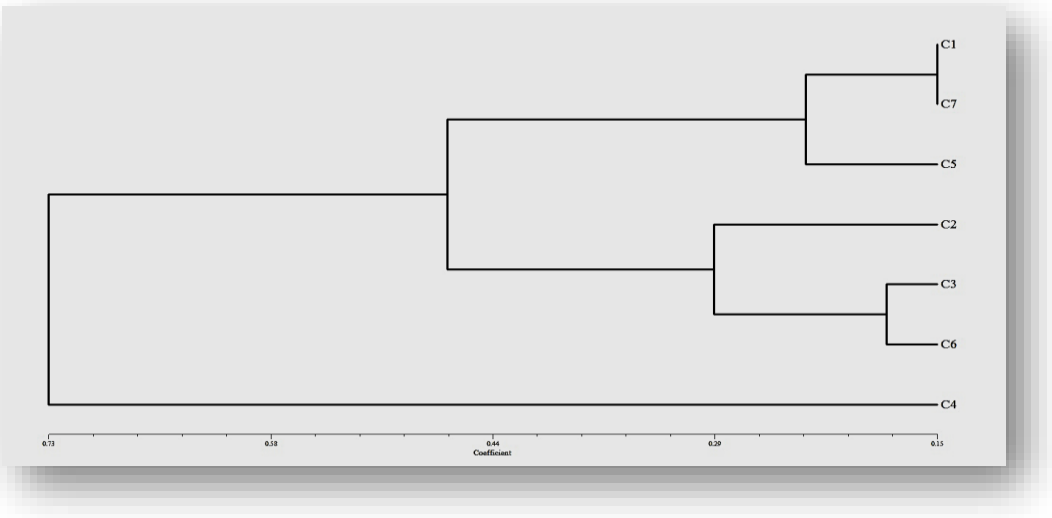

Figure (2): Genetic dendrogram clusters of pomegranate cultivars in this study: C1: Masafik cultivar 2. C2: Melisse cultivar 3. C3: Radisho cultivar 4. C4: Armishte cultivar 5. C5: Shahraban cultivar 6. C6: Halapja cultivar 7. C7: Dwarf cultivar.

Based on the figure (2), three clusters are identified: the first cluster comprises the cultivars Masafik (C1), Dwarf (C7), and Shahraban (C5). The similarity coefficient of 0.15 between cultivars C1 and C7 indicates a high degree of genetic similarity. The second cluster consists of Melisse (C2), Radisho (C3), and Halapja (C6), while the third cluster only includes the Armishte (C4) cultivar. In a study on pomegranate plants using RAPD markers, Sarkhosh *et al*. (2006) demonstrated a similarity coefficient ranging from 0.29 to 0.89, signifying significant genetic variance among genotypes. The dendrogram clusters and similarity matrix showed a strong relationship.

These results align with those of other researchers (Yuan *et al*., 2007; Jbir *et al*., 2008; Narzary *et al*., 2009; Bargish and Rahmani 2016; Amar & El-Zayat 2017; Wang *et al*., 2019; Hajari *et al*., 2020), indicating that the clustering of cultivars is not influenced by geography.

The notable disparities in phenotypic traits such as fruit color, flavor, weight, and seed hardiness across pomegranate genotypes may be attributed to environmental factors, significantly impacting quantitative traits. The study's clustering tree analysis revealed that geographical distribution and morphological characterization clustered independently. Despite different names and visual characteristics, cultivar C7 (Dwarf), classified as *Punica granatum* var. nana, and cultivar C1 from Duhok exhibited the highest similarities. The genetic makeup of C5, despite having different names from the same region, also showed remarkable similarity to other cultivars. This challenges the notion that commonly used names by farmers are accurate. Clusters 2 and 3 demonstrated similar scenarios.

These findings are consistent with research by other scholars examining pomegranates using morphological traits and DNA markers. For instance, Jbir *et al*. (2008) research in Tunisia revealed that cultivars were primarily grouped independently of their geographical and denominational origins. They further suggested that various marker systems, such as SSRs and AFLPs, could display genetic variation between closely associated pomegranate genotypes (Almiahy & Juma, 2017; Patil *et al*., 2020; Patil *et al*., 2022).

Conclusion

The study successfully demonstrated the effectiveness of PCR-SRAP markers in assessing the genetic diversity among various pomegranate cultivars. The analysis identified four reliable SRAP primer combinations that generated distinct polymorphic patterns across 21 genotypes, including multiple cultivars, callus tissue, and regenerated plantlets. The dendrogram analysis revealed three distinct genetic clusters, highlighting a close genetic relationship between the Masafik and Dwarf cultivars. In contrast, Melisse, Radisho, and Halapja were grouped, while the

Armishte cultivar was distinctly separate. These findings provide valuable insights into the genetic diversity and relationships among pomegranate cultivars, which can inform breeding and conservation strategies.

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العلاقة الوراثية والثبات الجيني بين بعض أصناف الرمان (*Punica granatum* L.) المزروعة في محافظة دهوك ,العراق

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الخلاصة
أُجريت هذه الدراسة في جامعة دهوك، كلية العلوم، قسم قسم علوم الحياة خلال الفترة الممتدة من أكتوبر 2021 إلى فبراير 2023، وتركّزت على إكثار الأنسجة النباتية والتنوع الوراثي لسبعة أصناف من الرمان***(Punica granatum L.)*** ، وهي: مصافك، ميليسي، راديشو، أرميشتي، شهرابان، حلبجة، ولصنق القزمي. نُفّذت التجارب في مختبر زراعة الأنسجة النباتية ومختبر البيولوجيا الجزيئية النباتية في مركز البحوث العلمية ***(SRC)*** ، كلية العلوم، جامعة دهوك**.**
هدفت الدراسة إلى تقييم التنوع الوراثي، والعلاقات الوراثية، والاستقرار الجيني للأصناف المدروسة، وذلك باستخدام واسمات الحمض النووي من نوع ***SRAP*** .
أظهرت النتائج أن أربعة فقط من بين التوليفات المختبرة من بادئات ***SRAP***أنتجت أنماط تعددية الأشكال متّسقة ومتميزة في جميع العينات الـ 21 من الرمان، والتي شملت الأصناف السبعة، والكالس المنتج منها ، والشتلات المتجددة من الكالس. في الأصناف السبعة من ***Punica granatum***، أنتجت توليفة البادئات ***EM29-ME5*** أعلى عدد من أنماط الحزم، حيث بلغت 14، في حين أن البادئ ***EM29-ME17*** أنتج سبع حزم فقط، ليصل إجمالي عدد الحزم إلى 42. وُجدت 3 حزم أحادية الشكل و39 حزمة متعددة الأشكال، بمتوسط تعددية شكل بلغ 91.88٪**.**
تم تحديد العلاقة الوراثية بين الأصناف باستخدام المسافة الوراثية المحسوبة، والتي تراوحت بين أدنى قيمة (0.11989) بين أرميشتي وميليسي، وأعلى قيمة (0.73162) بين ميليسي ومصافك. كما أظهر التحليل التفرعي ***(Dendrogram)*** وجود ثلاث مجموعات تصنيفية تعكس درجة التشابه بين الأصناف المدروسة**.**

الكلمات المفتاحية: الاكثار الدقيق، التنوع الوراثي، تمايز الكالس، الواسمات الوراثية ,SRAP *Punica granatu*