Genetic diversity of some grape varieties (*Vitis Vinifera* L.) grown in Salah al-Din Governorate using RAPD-PCR markers

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Abstract:

Grapes (*Vitis vinifera* L.) are an important economic food crop in Salah al-Din Governorate. With the use of five randomly primers for randomly amplified polymorphic DNA (RAPD), the presented work intends to investigate the molecular characterization and genetic diversity of six grape cultivars. A total of 75 bands were formed in the amplification results regarding the random primers, and the primers OP-V19 as well as OP-P04 had the highest polymorphism rate, reaching 100%. Ten unique bands in all, split between the primers OP-P04 and OP-T20, were found. The genetic distances across the grape types under investigation ranged between 0.44444 and 0.64583, whereas the size of the amplified fragments varied between 150 and 1250bp. With regard to the groups, the cluster analysis indicated two primary groupings. According to the research, RAPD marker is fast, easy, and strong marker to identify genetic diversity as well as perform genetic analysis on grapes.

Key words : Vitis vinifera , Genetic diversity, RAPD-PCR, polymorphisms.

Introduction:

One of the most economically significant horticultural crops worldwide, grapes (*Vitis vinifera* L.) take up 6.93 million hectares of cultivated land and yield 73.5 million tons annually (FAO, 2023). Because of its environmental appropriateness, historical records and evidence suggest that it was first cultivated in the Near East 6,000–8,000 years ago, before its wild ancestor expanded throughout West and Central Asia and Southern Europe (Kahraman and Atak, 2014; Riaz et al., 2018). There are reportedly more than 10,000 grape varieties in nature, and they exhibit great genetic diversity (Teixera et al., 2013). The grape is native to western Asia and is divided into two main geographical groups, called the American and Eurasian groups, which differ significantly in their agronomic characteristics (Smith, 2010; Byng and Christenhusz, 2016). The grape species belongs to the genus Vitis, which is part of 14 genera within the Vitaceae family (Rahemi et al., 2022). Rich in ritol, ascorbic acid, and caffeic acid—a potent antioxidant that is employed as an

anticancer agent because of the presence of resveratrol—and low in sodium, fat, and cholesterol, it is a significant food crop (Glevitzky et al., 2019; Syed et al., 2021). Grape varieties can be analyzed and compared based on the morphological characteristics of leaves, clusters, and branches of mature plants (Mohammed et al., 2021). However, cultivar identification takes a long time. Since cultivars are often similar or homogeneous (This et al., 2006), phenotypic characteristics are not sufficient to establish the origin of cultivars, especially closely related cultivars. Therefore, using molecular markers has become an important process as they reveal a wealth of information at the DNA level, providing accurate results in research (Roychowdhury et al., 2014; Guan et al., 2019; Santos et al., 2020; Margaryan et al., 2021). Molecular markers have greater properties compared to morphological and biochemical markers (Al-Anbari et al., 2017). It is used to elucidate the genetic structure, classify varieties and germplasm groups, clarify the evolutionary relationships between species and closely related species, and understand the evolution of plant families by determining the nucleotide sequences in DNA (Eldessoky et al., 2017; Zidan, 2023). Various DNA marker techniques are used to characterize various grape varieties. Randomly amplified polymorphic DNA (RAPD-PCR) is one of the polymerase chain reaction (PCR)-based approaches that are utilized in grape genetic research (Maan, 2017). RAPD is widely used for the molecular characterization regarding animals as well as plants due to its efficiency, simplicity, speed, and absence of prior knowledge about genetic sequences and DNA. Furthermore, it creates unique fingerprints using polymorphic fragments (USAMA et al., 2020). The research's goal was to examine the genetic diversity as well as evolutionary relationships between grape varieties using RAPD technology for DNA sequencing.

Materials and Methods:

Samples:

Fresh leaves of six grape varieties, namely Red Glabe, Ajami, Dis Anz, Halwani, Kamali and Yaqoot, were collected during the fifth month of 2024 from Salah Al-Din Governorate.

DNA extraction:

50 to 100 mg of plant leaves were placed in a 1.5 ml tube and liquid nitrogen was added to it. The plant sample was then crushed in a ceramic mortar, after which the

genetic material was isolated using ZR Plant / Seed DNA MiniPrepTM- D6020 - Zymo/USA.

Polymerase chain reaction test:

Five different primers consisting of 10 nitrogenous bases were selected from Bioneer (Table 1). Several experiments were conducted to reach the optimal degree of degradation and binding of DAN. The range of DNA template was 1.50–2µl. The polymerase chain reaction's optimal conditions have been determined (Table 2). Through measuring the absorbance at 260nm and 280nm, the purity regarding the DNA has been determined (Table 3).

Table (1): Shows the random primers, their sequence, temperature, and percentage of GC bases.

No	Primers	Sequence	Temperature	GC
1	OP-V19	GGGTGTGCAG	37.7	70
2	OP-R12	ACAGGTGCGT	38.2	60
3	OP-M14	AGGGTCGTTC	33.8	60
4	OP-P04	GTGTCTCAGG	30.6	60
5	OP-T20	GACCAATGCC	33.1	60

Table (2): Optimal conditions for polymerase chain reaction

No	Condition	Tm	Time	No. of cycle
1	Initial Denaturation	94^{o}C	5min.	1 cycle
2	Denaturation -2	94^{o}C	1min.	40 cycles
3	Denaturation -2	36^{o}C	1min.	
4	Extension-1	72^{o}C	1min.	
5	Extension -2	72^{o}C	10min.	1 cycle

Table (3): Shows the degree of purity and concentration of DNA in plant samples.

NO	Types	DNA concentration (ng /ml)	Purity
1	Red glabe	12.7	1.171
2	Al-Ajami	6.6	1.008
3	Dis Anz	11.3	1.092

4	Halwani	7.7	0.813
5	Kamali	6.8	1.078
6	Yaqoot,	6.3	1.167

Estimation of molecular sizes of pieces:

PCR bands' molecular size has been determined using Photocapt software, and the results were compared to DNA ladder's size (Cerasela et al., 2011).

Analysis of results:

Following converting the descriptive results into digital data, the results that emerged in the gel have been examined. The data was organized in a table that contained results of every primer for the samples under study, with 1 denoting the presence of the band and 0 denoting its absence on the agarose gel. With the use of Jaccard coefficient and the next equation, the genetic distance coefficient between the samples has been determined:

Genetic Distance= $1-(\frac{2*N_{xy}}{N_y+N_x})$

In which: G.D. denotes the genetic dimension, Nxy denotes the number of bands shared between the two models x and y that denote two samples, Nx denotes total number of bands in sample x and Ny denotes the total number of bands in sample y, and draw a cluster analysis diagram according to the UPGMA method (Sokal and Sneath, 1973), with the use of ready-made program NTSYS-pc (Numerical Taxonomy System) for obtaining the kinship tree or genetic dimension.

Results and discussion:

The amplification of five random primers for six grape varietals produced a total of 75 bands, with sizes ranging between 150 and 1250 bp, according to Figure (1) and Table (4)'s data . The primer OP-R12 had 11 bands overall, while primer 0P-P04 had 21 bands. In contrast to the primer OP-T20, which yielded an 86.66% polymorphism percentage, the primers OP-V19 and OP-P04 yielded the greatest proportion, 100%. In contrast, the primers OP-P04 and OP-T20 had nine and one distinct band, respectively. The multiplicity of bands depends on the primer sequence and the variation between genotypes, as Eldessoky et al. (2017) stated that the total number of bands depends on the primer type and the nucleotide sequence of the primer. The

starter efficiency ranged from 14.66 to 28% for starters OP-R12 and OP-P04, respectively (Maan, 2017).

Table (4) shows the type of primer, nucleotide sequence, number of total and different bands, band size, polymorphism of the primer, efficiency of the primer, and discrimination ability of the primer.

Primer	T N B	NDB	NUB	SRB (bp)	PP %	PE %	PDA %
OP-V19	15	15	-	300-1100	100	20	21.126
OP-R12	11	10	-	150-1200	90.909	14.66	14.084
OP-M14	13	12	-	300-1250	92.307	17.33	16.901
OP-P04	21	21	9	225-1150	100	28	29.577
OP-T20	15	13	1	200-1150	86.666	20	18.309
	75	71	10				

TNB = Total number of Bands, NDB= Number of different bundles, NUB= Number of unique Bands, SRB= Size range of bands, PP= polymorphism of the primer, PE= primer efficiency, PDA= Primer discriminating ability

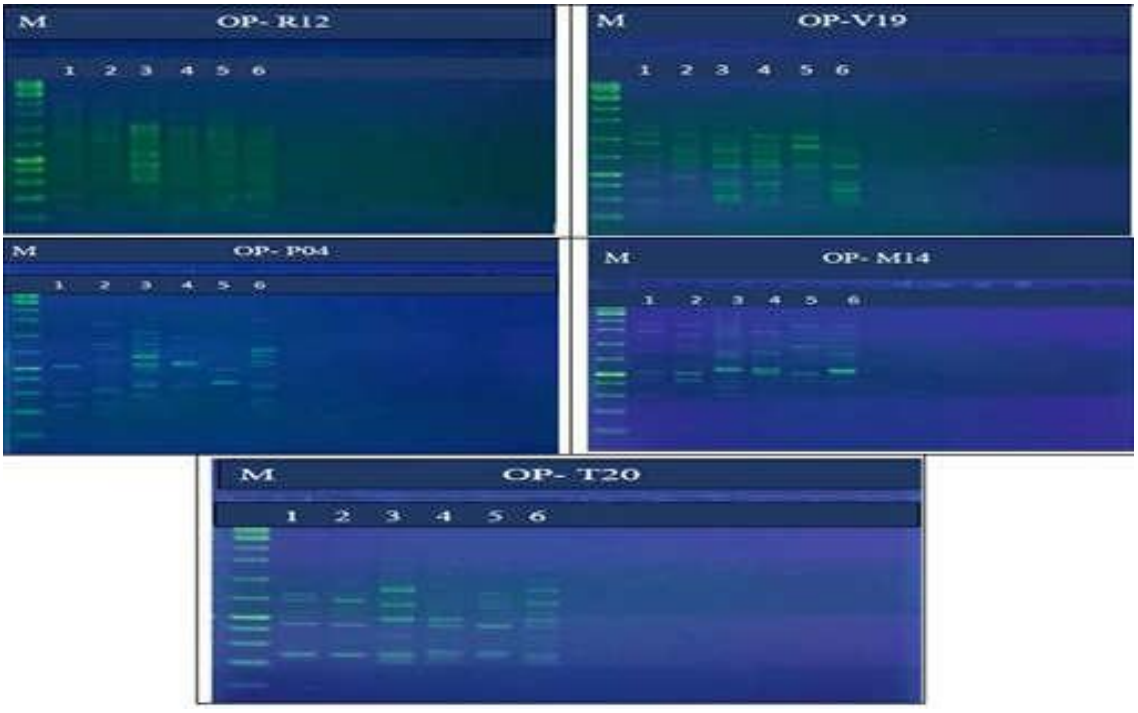

Figure (1) The result of amplifying five random primers on agarose gel using the size marker DNA (100-1500 bp) for grape varieties 1- Red Glabe, 2- Al-Ajami, 3- Dis Anz, 4- Al-Halwani, 5- Al-Kamali, 6- Al-Yaqout.

The phylogenetic tree diagram (Fig. 2) and the similarity percentage (Table 5) based on the degree of similarity in the RAPD plots reveal the diversity in grapevines. It is noted that there are two main groups with a similarity coefficient of 0.48485. The first main group included two subgroups with a similarity coefficient of 0.50746. The first subgroup contained the Kamali variety and the second subgroup included two subgroups. The first subgroup contained the Halwani variety and the second subgroup contained the Yaqout and Dis Anz varieties with a similarity coefficient of 0.64583. The second main group included the Red Glabe and Ajami varieties with a similarity coefficient of 0.55738. Table 5 shows that the highest similarity rate was between the cultivars Des Anz and Yaqoot with a similarity rate of 0.64583, and the lowest similarity rate was between the cultivars Ajami and Halwani with a similarity rate of 0.44444, which represents the genetic distance values. Regardless of these two groups of genotypes, we note that there is a great similarity between the cultivars of the first main group in terms of fruit shape, and this similarity may indicate that the origin of the genotypes is from parents with less genetic divergence, and that genetic diversity may be a result of selection, crossing, or environmental conditions (Fanizza et al., 2000).

Table (5) The percentage of similarity between grape varieties detected by RAPD indicators

	Red glabe	Al-Ajami	Dis Anz	Halwani	Kamali	Yaqoot
Red glabe	1					
Al-Ajami	0.55738	1				
Dis Anz	0.45	0.51852	1			
Halwani	0.51613	0.44444	0.53659	1		
Kamali	0.55385	0.48485	0.51765	0.50746	1	
Yaqoot	0.52632	0.46753	0.64583	0.61538	0.54321	1

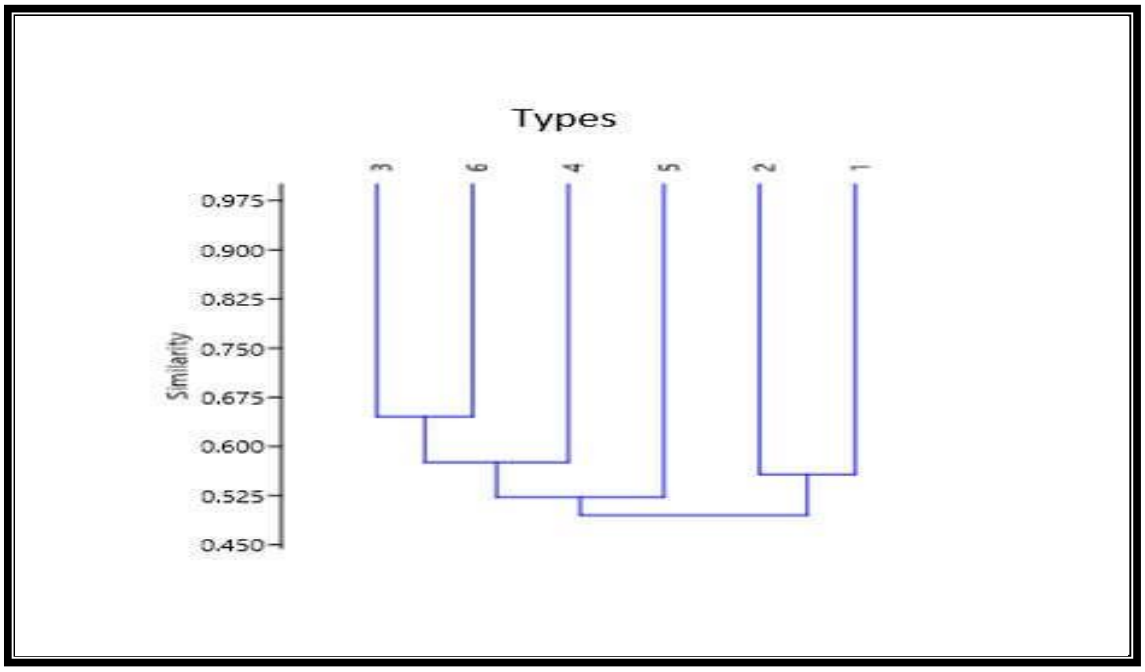

Figure (2) Evolutionary tree diagram of grape varieties 1-Red Glabe 2-Ajami 3-Dis Anz 4-Al-Halwani 5-Al-Kamali 6-Al-Yaqut

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