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MOLECULAR CLASSIFICATION OF THE GENUS *ROSA* L. (*ROSA*CEAE) GROWN IN NORTHERN IRAQ BY USING RAPD MARKERS

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SUMMARY

The presented research, comprising characterization of nine species of the wild rose (*Rosa* L.), came from various regions of Northern Iraq. The study proceeded in 2021–2022 at the College of Education of Pure Science, University of Mosul, Mosul, Iraq. Employing the random amplification polymorphism DNA (RAPD) helped determine the genetic variation relationships among the species using the statistical program Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.02). The use of 10 random primers attained amplifications observed with agarose gel electrophoresis. The RAPD primers generated 523 random bands, making it possible to separate *Rosa* species from each other. Among the studied species, the genetic distance ranged from 0.067 to 1.027. The least genetic dimension (0.067) came from the species *Rosa canina* var. deseglisei and *Rosa canina* var. canina, with the highest genetic breadth reaching 1.027 between *Rosa x centifolia* and *Rosa foetida*. The dendrogram revealed three main clusters based on the genetic distance values, with the third one getting split into three distinct groups. RAPD proved as an effective method for studying the relatedness among the species.

Key words: molecular study*, Rosa, Rosa*ceae*,* RAPD markers, genetic distance, multivariate analysis, species association

Key findings: Molecular diagnosis using RAPD-PCR indicators helped identify and distinguish the nine species of the genus *Rosa* (*Rosa*ceae) growing in Northern Iraq. Based on the similarities and genetic differences among the species, the RAPD primers generated 523 random bands for the possible separation of *Rosa* species from each other. Among the species, the genetic distance ranged from 0.067 to 1.027.

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INTRODUCTION

The genus *Rosa* belongs to the family Rosaceae, which also consists of four subgenera distributed in the Northern Hemisphere's temperate and subtropical zones (Jagodzinski *et al*., 2016). Christenhusz *et al*. (2017) reported that the family Rosaceae consists of 90 genera and 2,950 species. Bisby *et al*. (2020) also stated that there are 5,819 species in 109 genera, with 310 belonging to the genus *Rosa*.

The importance of roses is due to the abundance of shapes, sizes, and colors of their flowers, which are almost available throughout the year. Perfume extracts also come from the flowers of some varieties for perfume and rose water manufacture (Zhu *et al*., 2015). The rose, considered one of the most valued ornamental woody plants, has antioxidant and anti-inflammatory properties, widely used as a horticultural and medicinal plant, hence, having a vast economic value worldwide (Scalliet *et al*., 2008; Zhu *et al*., 2015; Xiang *et al*., 2017). The *Rosa* L. is one of the largest genera, with alternate, stipulate, palmate, or pinnate leaves and flowers distinguished by their colorful appearance and flowering nature (Xiang *et al*., 2017; Al-Mathidy *et al*., 2023; Al-Musawi and Al-Tamimi, 2023).

Globally, much research has progressed on varieties and species of roses in their diagnostic field, especially after the emergence of biotechnologies. The study of biodiversity has become more relevant and accurate, using the RAPD technology in several studies to characterize plant genetic resources. Emadpour *et al*. (2009) explained that conducting RAPD analyses studied the genetic relationship among the 15 commercial cultivars of roses using 10 primers, resulting in 126 bands, with 73 showing polymorphic, and the degree of genomic similarity ranged from 0.12 to 0.53.

In another study, the RAPD technique assessed the genetic relationship among five genotypes of wild roses using 27 primers (Riaz *et al*., 2011). This technology has also helped in evaluating the genetic variations in seven species of roses through the use of 10 primers, as the number of polymorphic bands reached 111 bands, with the degree of genetic similarity of the studied species ranging from 0.42 to 0.83 (Mirzaei and Rahmani, 2011).

Elhawary *et al*. (2021) indicated that genetic primers successfully analyzed the diversity among three varieties of the genus *Rosa* L. cultivated in Egypt. The genotypes expressed the highest degree of similarity among the *Rosa banksiae* var. 'Banksiae' and *R. polyantha* Thunb. The RAPD technology also assessed the genetic variation in the species and cultivars of various crop plants, i.e., genus *Morus* L., *Pyrus malus* L., *P. communis* L., and *Prunus persica* L. Batsch (Al-Jumeily, 2015; Al-Badrany, 2020; Shehab, 2020; Al-Jumeily and Al-Maathidy, 2021). Therefore, the existing research aims to separate the nine species of the genus *Rosa* in North Iraq using the RAPD markers technique.

MATERIALS AND METHODS

Plant material

Fresh leaf samples collection for nine species (*Rosa dumalis* subsp. *Biossieri, Rosa canina* var. canina*, Rosa canina* var. *deseglisei, Rosa canina* var. *dumetorm, Rosa gallica, Rosa hemisphaerica, Rosa foetida, Rosa* × *damascene, Rosa* × *centifolia*) of the genus *Rosa* growing in different regions of Northern Iraq ensued.

DNA extraction

The DNA extracted from young leaves of the *Rosa* species used a Plant Genomic DNA Extraction Min kit by Favorgen, Korea, transpired in the RNA Lab. After transferring 5 µL of isolated DNA into the agarose gel at a concentration of 1%, measuring the optical density followed at the wavelength of 260–280 nm, yielding a purity of 1.6–1.8.

DNA analysis

Ten primers ran for random amplification, prepared by the Korean Company Macrogen, and their sequences (nitrogen bases) appear in

Table 1. Primers for amplification of RAPD genes.

Primers	Sequences 5' 3'	Resources
OPA-01	5'-CAGGCCCTTC-3'	
OPA-02	5'-TGCCGAGCTG-3'	
OPD-02	5'-GGACCCAACC-3'	
OPB-10	5'-CTGCTGGGAC-3'	
OPC-04	5'-CCGCATCTAC-3'	Mirzaei and Rahmani (2011)
OPE-08	5'-TCACCACGGT-3'	Elhawary *et al*. (2021)
OPI-08	5'-TTTGCCCGGT-3'	
OPK-10	5'-GTGCAACGTG-3'	
OPM-17	5'-TCAGTCCGGG-3'	
OPO-15	5'-TGGCGTCCTT-3'	

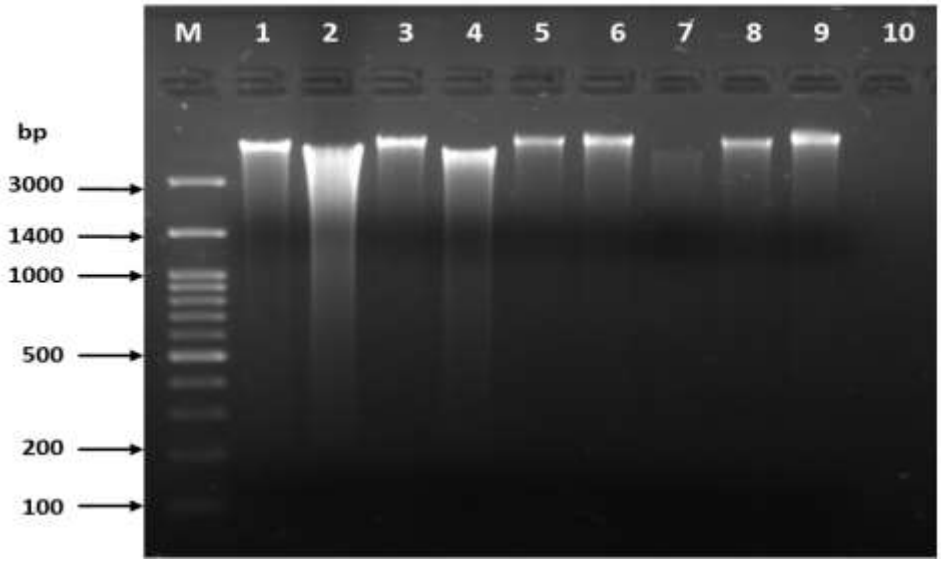

Figure 1. Genomic DNA isolated from the studied *Rosa* taxa. 1. *R. dumalis* subsp. *Biossieri,* 2. *R. canina* var. *canina,* 3. *R. canina* var. *deseglisei,* 4. *R. canina* var. *dumetorm,* 5. *R. gallica,* 6. *R. hemisphaerica,* 7. *R. foetida,* 8. *Rosa* × *damascena,* 9. *Rosa* × *centifolia.*

Table 1. The amplification mixture's volume and component concentrations calculation followed the processing company's guidelines. According to Mirzaei and Rahmani (2011), the random amplification reactions began on DNA samples isolated from the studied species and varieties of roses, with the gel electrophoresis performed on an agarose gel and stained with ethidium bromide for 20–30 minutes. Later, exposing the gel to a UV-transilluminator provided a final estimation of the diameters of the amplified DNA bands by comparing their positions within the gel to those of a standard volumetric guide (DNA Ladder), as shown in Figure 1.

Statistical analysis

In determining the genetic similarity among samples, engaging the Jaccard's coefficient translated the data from the agarose gel that resulted from the interaction of the primers with the RAPD tests, where '1' indicates the presence and '0' the absence of the band, using the following formula:

$$(NAB / [NAB + NA + NB]) = Sij$$

Where:
NAB is the total number of bands present in each sample

NA is the number of amplified subsets present in each sample
B using NTSYSpc 2.02.

The cluster analysis helped draw a dendrogram using the UPGMA (Unweighted pair group method with arithmetic average) (Yao *et al*., 2007).

RESULTS AND DISCUSSION

The RAPD interactions studied the genetic relationship among the nine species of roses. The 10 primers used in the study, along with the sequences of nitrogen bases in them, are in Table 1. The sum and proportion of polymorphic and total bands and variations resulting from DNA amplification, according to the sequence of nitrogen bases of the random primers used for the species and varieties of roses under study, appear in Figure 2. The results of random amplification of DNA showed different patterns of bands, and the total number of sites recognized by the primers on the sample genome was 121 sites, with an average of 12.1 bands for each primer, 11 of which were monomorphic, having an average of 1.1 for each primer. Likewise, 110 were polymorphic sites, with an average of 11 packages for each primer.

The primer OPB-10, characterized by the highest productive sites, amounted to 17. The primers OPD-02 and OPO-15 were the less fruitful sites, amounting only to nine and having total bands of 523, wherein 117 are monomorphic and 406 bands (73%) polymorphic. The primers OPI-08 and OPM-17 showed the highest contrast ratio, which amounted to 100%. Some varieties and species were distinguishable by distinct bands, with their total number resulting from the primers being 30 different bands, 24 unique bands, and six absent bands. These bands were among the most crucial diagnostic characteristics accepted in distinguishing species and varieties.

The primer OPA-01 showed unique bands for the species, i.e., *R*. *hemisphaerica, R. foetida,* and *Rosa* × *damascena* at molecular sizes of 2,000, 1,380, 580, 330, and 136 bp. The primer showed an absent band in the species *Rosa* × *damascena* and had a molecular size of 1,500 bp. However, the contrast ratio for this primer was 85.42. As for the discriminatory potential, its determination based on the number of bands showed, concerning the total number of polymorphic bands expressed by other primers, was 13.05%. Meanwhile, its relative efficiency amounted to 11.85%, based on the number of entire bands.

The primer OPA-02 showed 44 polymorphic bands, obtaining two binding sites of different molecular sizes, as these bands occurred at the same level in all varieties and species and were respectively 640 and 500 bp. It indicates the presence of this sequence in a similar location on the DNA for the studied variations and species. The primer also distinguished the two species, *R. foetida* and *Rosa* × *damascena,* with unique bands and molecular sizes of 1,075 and 390 bp, making this a diagnostic characteristic for these two species from the rest of the varieties and species. For the contrast ratio, it was 85.71, and the relative efficiency of this primer was 11.85%, with a discriminatory potential of 10.84% (Table 2).

The primer OPD-02 was illustrative of 31 polymorphic bands and four binding sites of different molecular sizes, i.e., 820, 750, 440, and 358 bp. The primer distinguished the species *R. hemisphaerica* with a unique band of molecular size (166 bp). Further, it differentiated the species *R. foetida* with the absence bands in molecular sizes of 1,000 and 200 bp, respectively. The lowest contrast ratio appeared in this primer at 55.55%, and this is a diagnostic advantage for this type over the rest of the varieties and species, as its efficiency was 12.81% and discriminatory potential at 7.64%.

The results of the primer OPB-10 were similar to the primer OPA-01 in terms of the number of monomorphic and polymorphic bands, the efficiency of the primer, and its discriminatory potential, with the contrast ratio at 94.11%. Additionally, the primer OPC-04 found 51 polymorphic and main bands with a molecular size of 742 bp. The primers' results showed the varieties and species, *R. canina*

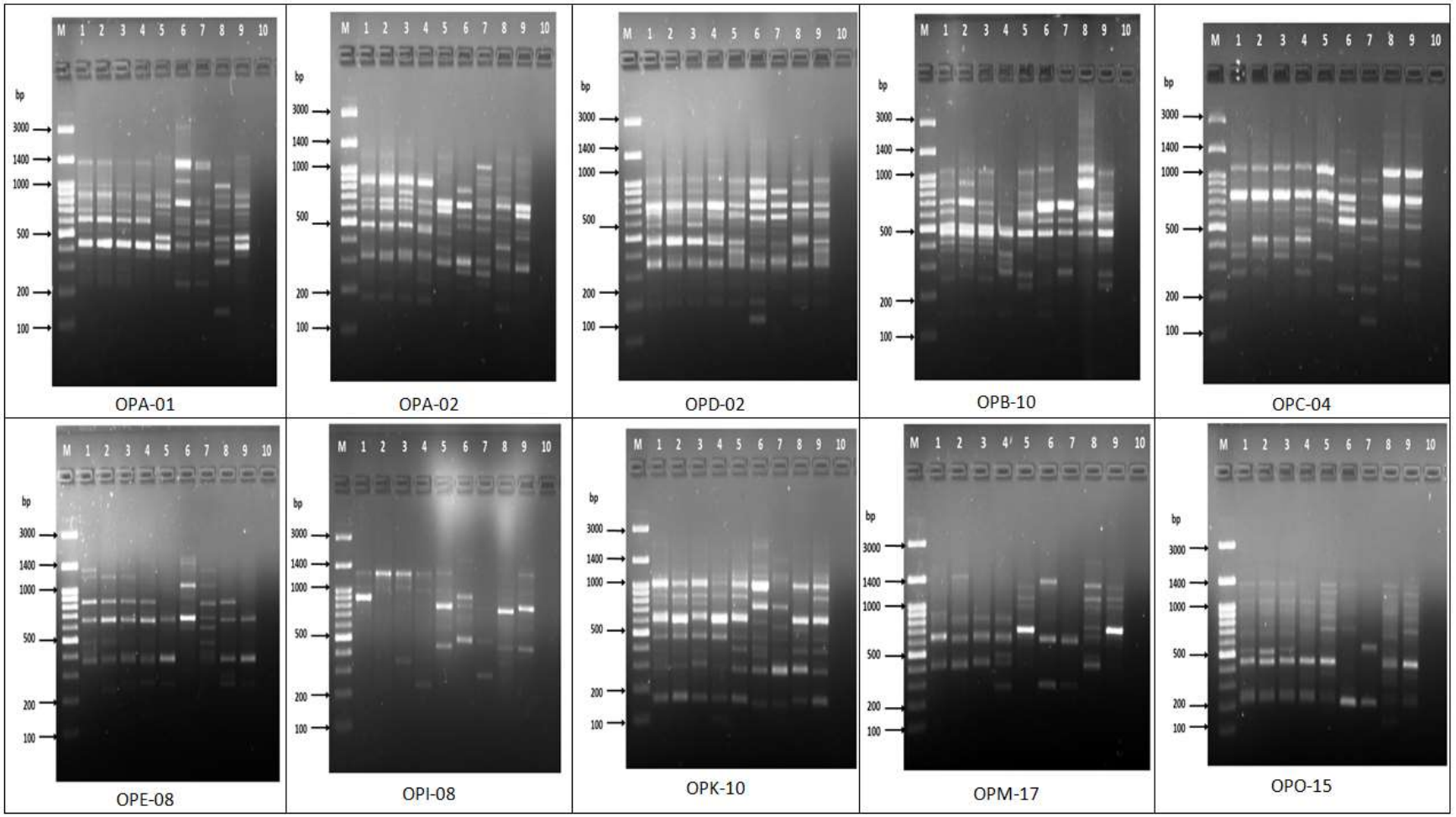

Figure 2. Profiles of nine species of genus *Rosa* with primers.
1. R. dumalis subsp. *Biossieri,* 2. *R. canina* var. *canina,* 3. *R. canina* var. *deseglisei,* 4. *R. canina* var. *dumetorm,* 5. *R. gallica,* 6. *R. hemisphaerica,* 7. *R. foetida,* 8.*Rosa* × *damascena,* 9.*Rosa* × *centifolia.*

Table 2. Results of primers used in RAPD reactions.

No.	Primers	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism ratio (%)	Premier proficiency (%)	Discriminatory ability (%)	Band size
1	OPA-01	62	9	53	85.42	11.85	13.05	2000 136
2	OPA-02	62	18	44	85.71	11.85	10.84	1300 176
3	OPD-02	67	36	31	55.55	12.81	7.64	1000 166
4	OPB-10	62	9	53	94.11	11.85	13.05	1022 212
5	OPC-04	60	9	51	93.75	11.47	12.56	1700 139
6	OPE-08	36	9	27	90.00	6.88	6.65	1586 378
7	OPI-08	27	0	27	100.0	5.16	6.65	1500 234
8	OPK-10	67	18	49	83.33	12.81	12.07	1638 182
9	OPM-17	33	0	33	100.0	6.31	8.13	1716 300
10	OPO-15	47	9	38	88.88	8.99	9.36	1375 244
	Total	523	117	406	73.29	100%	100%	

var. *canina*, *R. hemisphaerica*, *R. foetida*, and *R. damascena,* had unique bands at molecular sizes of 1,409, 484, 264, and 139 bp, respectively. The contrast ratio of this primer was 93.75%, its efficiency was 11.47%, and its discriminatory potential was 12.56%.

Both primers OPE-08 and OPI-08 showed similar results for the number of polymorphic bands (27), and the discriminatory potential amounted to 6.65% (Table 2). The primer OPK-10 was distinct, with 49 polymorphic bands and two binding sites of different molecular sizes (295 and 182 bp, respectively). The *R. hemisphaerica* primer distinguished unique bands with molecular sizes of 1,638, 1,348, and 348 bp, with the species *R. foetida* characterized by the absence of bands at the molecular range of 1,063 bp. The contrast ratio was 83.33%, with a relative efficiency of 12.81% and discriminatory potential of 12.07%.

The primer OPM-17's outcomes are similar to primer OPI-08 in that it does not bind at any site of the DNA of the varieties and species, and the primer OPM-17 distinguished the variations and species *R. dumalis* subsp. *Biossieri, R. canina* var. *canina,* and *R. damascena,* with unique bands at molecular sizes of 1,716, 840, and 500 bp, respectively. The relative efficiency of this primer was 6.31%, while its discriminatory potential was 8.13%. As for the primer OPO-15, it showed 38 polymorphic and monomorphic bands for all varieties and species whose molecular size was 244 bp and distinguishing the primer of the variety *Rosa canina* var. *dumetorm,* with an absent band at a molecular mass of 1,375 bp and a unique band in *Rosa foetida* at a molecular size of 581 bp. The contrast ratio was 88.88%, the relative efficiency was 8.99%, and its discriminatory potential was 9.36% (Table 2).

Amplified DNA fragments varied in size from 136 to 2,000 bp, and the fraction of polymorphic bands ranged from 55% to 100% (Table 2). These fragments benefitted calculation and statistical analysis by including the outputs of the digital results, represented by '1' for the presence of the band and '0' for the absence, within the UPGMA statistical program. Table 3 indicates the genetic dimension values among the studied species and varieties of roses using the NTSYS statistical analysis coefficient, with ranges from

Table 3. The genetic distance between species of the genus *Rosa* L.

Species	P1	P2	P3	P4	P5	P6	P7	P8	P9
P1	0								
P2	0.074	0							
P3	0.077	0.067	0						
P4	0.257	0.214	0.270	0					
P5	0.389	0.345	0.372	0.432	0				
P6	0.736	0.751	0.798	0.803	0.780	0			
P7	0.800	0.809	0.816	0.713	0.996	0.454	0		
P8	0.539	0.519	0.514	0.578	0.405	0.772	0.936	0	
P9	0.431	0.438	0.397	0.467	0.078	0.768	1.027	0.364	0

1. *R. dumalis* subsp. *Biossieri,* 2. *R. canina* var. *canina,* 3. *R. canina* var. *deseglisei,* 4. *R. canina* var. *dumetorm,* 5. *R. gallica,* 6. *R. hemisphaerica,* 7. *R. foetida,* 8.*Rosa* × *damascena,* 9. *Rosa* × *centifolia.*

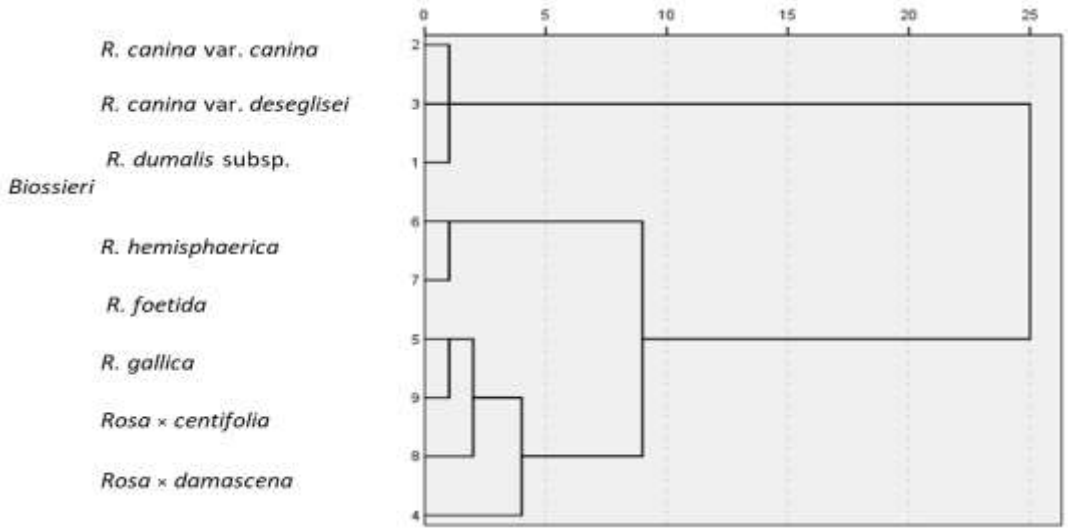

Figure 3. UPGM dendrogram showing the relationship among species of the genus *Rosa*
1. *R. dumalis* subsp. *Biossieri,* 2. *R. canina* var. *canina,* 3. *R. canina* var. *deseglisei,* 4. *R. canina* var. *dumetorm,* 5. *R. gallica,* 6. *R. hemisphaerica,* 7. *R. foetida,* 8.*Rosa* × *damascene,* 9.*Rosa* × *centifolia.*

0.067 to 1.027. Elhawary *et al*. (2021) also confirmed the RAPD primers' use determines the genetic variation among three varieties of the genus *Rosa* cultivated in Egypt.

The least genetic dimension (0.067) occurred between the species *R. canina* var. *deseglisei* and *R. canina* var. *canina*, indicating the highest similarity between the two species included in the study. The highest genetic dimension reached 1.027 between the species *Rosa* × *centifolia* and *R. foetida*, observed as the minimum genetic similarity (Table 3). Moderate values emerged for the rest of the rose species and their varieties, and these results were consistent with the findings of Mirzaei and Rahmani (2011), who reported studying some species of the genus *Rosa* at the molecular level. Yonemoto *et al*. (2007) also observed equal findings and mentioned that the RAPD primers are essential in knowing and determining the genetic similarity in crop plants. Emadpour *et al*. (2009) also revealed similar outcomes by studying the genetic variation of 15 cultivars of roses using 10 primers.

Through cluster multivariate analysis to determine the nature of the genetic relationship (Figure 3), the tree of genetic

variation showed that the studied species and their varieties divide into two central groups. The first group includes varieties *R. canina* var. *canina* and *R. canina* var. *deseglisei* under the species *R. dumalis* subsp*. Biossieri;* a second group consisting of three secondary groups, the first consisting of the species *R. hemisphaerica* and *R. foetida;* the second consisting of the species *R. gallica, Rosa* × *centifolia,* and the *Rosa* × *damascena, and* the third secondary including the cultivar variety *Rosa canina* var*. dumetorm* (Figure 3).

It indicates a higher degree of similarity within the genetic material than other varieties, which in turn depends on the number of common bands resulting from the primers used in the interactions of the RAPD technology. The study results agree with a similar study by Mohapatra and Rout (2005) using 10 RAPD primers that could classify 34 rose cultivars into nine clusters despite any apparent detectable morphological differences within rose (*R. damascene* Mill.) genotypes grown in Turkey. Baydar *et al*. (2004) observed no genetic variations, employing AFLP (amplified fragment length polymorphism) and microsatellite marker, which also agree with those of Pirseyedi *et al*. (2005), Mohapatra and Rout (2005), and Babaei *et al*. (2007).

CONCLUSIONS

Based on these findings, the presence of shared bands among the species and their varieties suggested their genetic material was highly similar in that region of the genome. It could signify shared phenotypic traits related to productivity, reproduction, disease resistance, and genetic adaptation to environmental demands for growth and production.

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