PHYLOGENETIC RELATIONSHIP ANALYSIS OF THREE SPECIEOF *ROSA* L.,1735 CULTIVATED IN IRAQ BASED ON ITS, matK and trnT-trnL PRIMERS

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ABSTRACT

This study was investigate to demonstrate the phylogenetic relationship among three species of *Rosa* that are *R. canina* L.,1735, *R. damascene* Mill. and *R. centifolia* L., cultivated in Iraq by using three primers of ITS, matK and trnL. With regard to the nuclear ITS sequencing reaction results showed that the identified identities of the investigated samples were *R. canina*, *R. damascena*, and *R. centifolia* the presence of seven nucleic acid variants (g.392G>T, g.439G>A, g.442T>G, g.458C>T, g.477C>T, g.495T>C, and g.496C>T) of *R. canina* sample, one nucleic acid variant (g.15A>C) in *R. centifolia* sample compared with the referring sequences respectively (GenBank acc. no. MT796505.1 and MZ230175.1). Sequencing reaction results for the matK amplicons revealed that the samples under investigation were *R. canina*, *R. damascena*, and *R. centifolia*. This finding suggested the presence of two nucleic acid variations (g.165C>T and g.687G>T) of the *R. centifolia* sample compared with the referring sequences (GenBank acc. no. MZ261886.1). These amplicons be could quickly and accurately identify the biological diversity of a larger genotype of *Rosa* sequences.

Keywords: Molecular identification, rosaceae, plastid primers.

تحليل علاقة النشوء والتطور لثلاثة أنواع من الروز *Rosa* L.,1735 المزروعة في العراق بالأعتماد على البادئات ITS و matK و trnT-trnL

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المستخلص

هذه الدراسة تهدف الى كشف علاقة النشؤ والتطور ما بين ثلاث من أنواع الروز المستزرعة في العراق وهي *R. canina* و *R. damascena* و *R. centifolia* بالأعتماد على البادئات ITS و matK و trnT-trnL. أوضحت نتائج تفاعل التسلسل لل ITS النووي للانواع المدروسة الى وجود سبع متغايرات من الأحماض النووية للنوع *R. canina* وهي: (g.392G>T, g.439G>A, g.442T>G, g.458C>T, g.477C>T, g.495T>C, g.496C>T)، ووجود متغاير واحد للحمض النووي للنوع *R. centifolia* (g.15A>C)وذلك بعد مقارنتها مع التسلسلات المرجعية الموجودة في بنك الجينات وهي no. MT796505.1 و MZ230175.1، وبينت نتائج تفاعل التسلسل لل matK امبليكون لنفس الأنواع قيد الدراسة الى وجود متغايرين أثنين من الأحماض النووية وهي g.165C>T و g.687G>T للنوع *R. centifolia* بالمقارنة مع للتسلسلات المرجعية لبنك الجينات no. MZ261886.1. ونجد أن هذه الأمبليكونات يمكن أن تستعمل في الكشف عن التنوع الحيوي لأنواع أخرى من الروز.

الكلمات المفتاحية: التشخيص الجزيئي، العائلة الوردية، بادئات البلاستيدات.

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INTRODUCTION

Rosaceae is a large family between plants about 2825 – 3500 species in 95 – 125 genera global, many rosaceous crops have been improved by breeders via sexual hybridization and asexual propagation, members of Rosaceae are trees, shrubs or herbs, flowers mainly actinomorphic, bisexual or rarely unisexual (21, 1) .The genus *Rosa* L. that include 200 species is widely spread from subtropical to cold-temperate regions. This genus could be placed in three classical groups 1. Wiled roses. 2. Old garden roses 3. Modern garden roses According to Shahbaz (20) and Al-Rawi and Chakravrty (4). Garden roses are a broad category since they are grown and cherished not just for their flowers but additionally as potted, plants, hedging, landscaping, hip production, and even as a source of ingredients for cosmetics and food products (27, 28). *R. canina* (dog rose) regarded as important rootstock due to its high resistance environmental stresses and pathogens, and classified as a native wild type in Iraq (2, 25) .The *R. damascena* with significant value not only in ornamental field but also in perfume and cosmetic industry (for rose oil and rose water), pharmaceutical uses as antimicrobial, analgesic, anti-inflammatory and anticancer effects of essential oil (9, 14), while *R. centifolia* (cabbage roses) planted for large number of petals with scented white or pink flowers (26). Molecular identification of the genus *Rosa* based on the nucleotide sequence of multiple markers of nuclear ribosomal DNA (rDNA) like the internal transcribed spacer (ITS), for the creation and application of universal primers in plant research, this area has been used (8, 13). The universality of the ITS region attributed to high number of copies per cell that produce large sized amplicons and characterized by high level of variability that provide high interspecific resolution with lo interspecific variability (18). The ITS gene is applied to diagnose and confirm bacterial and fungal diseases by molecular characterization (3, 22) .Since the gene order and genome sequence of land plants are conserved thanks to the slower pace of evolution, the chloroplast genome is a valuable source for consensus primers. One of the most promising options for a plant barcode is the matK. The matK gene is nearly 1570 bp in length and codes for a maturase protein hence it's utility to identify at family, genus and even species levels although it is sometimes difficult to amplify and sequence this region (12, 32). An appropriate target area for phylogenetic relationships may be the chloroplast trnL (UAA) intron because its primers are substantially conserved and its amplification technique is robust but low-resolution (24, 31) and could be exhibit a series limitation at intraspecific level with low evolutionary ratio of this molecule (19).Phylogenetic analysis is a valuable tool to study plant genome sequences and to detect genetic variation that considered as the basic for natural selection and hybredisation with prefer for the desired triates (11, 16) .The target of this study is to demonstrate the phylogenetic association between three of *Rosa* species (*R. canina, R. damascene* and *R. centifolia*) cultivated in Iraq via three primers ITS, matK and trnT-trnL.

MATERIALS AND METHODS

This study was based on young leaves of three *Rosa* species that include *R. canina* , *R. damascene* and *R. centifolia* respectively for molecular analysis and the samples were collected in April and May (2020-2021). DNA extraction was performed by the cetyltrimethylammonium bromide (CTAB) protocol with some modifications (15) using Genomic DNA Mini Kit (plant) of Geneaid. The quality and quantity of extracted DNA was confirmed using Nanodrop under 260/280 nm wavelength..The PCR amplification was performed for ITS, matK and trnT-trnL by AccuPower PCR PreMix (Bioneer) . Amplifications were conducted using MyGenie96/384 Thermal Block (Bioneer) . The thermal cycling program for amplification of the ITS region was as follows: 95 ◦C for 2 min, 34 cycles of 95 ◦C for 45 s, 58 ◦C for 45 s, and 72 ◦C for 90 s, and a final extension at 72 ◦C for 5 min; that for the matK region was as follows: 95 ◦C for 3 min, 40 cycles of 94 ◦C for 30 s, 49 ◦C for 1 min, and 72 ◦C for 1 min, and a final extension at 72 ◦C for 10 min ; that for the trnT-trnL region was as follows: 95 ◦C for 5 min, 15 cycles of 95 ◦C for 45 s and 60 ◦C for 1 min, with an extension at 72 ◦C for 2 min, followed by 20 cycles of 95 ◦C for 45 s

and 54 ◦C for 1 min, and a final extension at 72 ◦C for 2 min (8). The three primers used in this study elucidated in Table (1).

Table 1. DNA primer sequences (13) used in molecular analysis. F, forward; R, reverse.

Primer	Primer sequence (5 -3)
ITS F	GCACGCGCGCTACACTGA
R	TAGAATTCCCCGGTTCGCT CGCCGTTAC
matK F	ACCCAGTCCATCTGGAAACT TGGTTC
R	CGTACAGTACTTTTGTG TTTA CGAG
trnT-trnL F	TCTACCGATTTCGCCA TATC
R	CATTACAAATGCGATG CTCT

The resolved PCR amplicons were sequenced using commercial equipment from both termini (forward and reverse orientations), adhering to the manufacturer's instructions (Macrogen Inc. Geumchen, Seoul, South Korea). Only chromatographs from Applied Biosystems (ABI) that is clear. Utilizing BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA), the sequencing findings of the PCR products of the targeted specimens were edited, aligned, and assessed along with the corresponding sequences in the reference database. All the investigated and analyzed sequences were submitted to the NCBI Bankit portal and all the instructions depicted by the portal were followed as described by the server (5). To obtain specific GenBank accession numbers for the sequences under investigation, each submitted sequence was supplied as nucleic acid sequences and translated to its matching reading structure in the NCBI. In the present study, a particular comprehensive tree was built using the neighbor-joining methodology as described by Davidson R. and Martin (7). Using the NCBI-BLASTn server, the detected variations were compared to their nearby homologous reference sequences (33). Every arrangement of species that were included in the comprehensive tree was given the suitable color. The *R. canina*, *R. damascene* and *R. centifolia* assigned as A1, A2 and A3 respectively in figures and diagrams for ITS results and assigned as B1, B2,and B3 respectively for matK results.

RESULTS AND DISCUSSION

Nucleic acids sequencing of the ITS amplicons (the ribosomal PCR amplicons): Three samples were used in the current analysis at this location. To amplify the rRNA sequences of the *Rosa* sp., these samples were screened. *Rosa* could be able to adapt to different genetic diversity, hence the variation of the rRNA sequences can be utilized to characterize *Rosa* sp. . Following NCBI BLASTn for these PCR amplicons, the sequencing procedures confirmed the exact similarity. The NCBI BLASTn engine revealed 98% to 99% sequence similarity between the sequenced samples and the desired reference target sequences for the examined ribosomal amplicons. The precise locations and other information of the retrieved PCR fragments were determined by comparing the obtained nucleic acid sequences of these examined samples with the retrieved nucleic acid sequences (GenBank acc. MT796505.1, MG584526.1, and MZ230175.1) (Fig. 1). Remarkably, the ribosomal sample alignment outcomes demonstrated that, in contrast to the most similar referring reference nucleic acid sequences, the studied A1 sample had seven nucleic acid variations exhibited by 7 nucleic acid substitutions. These variations were g.392G>T, g.439G>A, g.442T>G, g.458C>T, g.477C>T, g.495T>C, and g.496C>T) compared with the referring sequences of Rosa canina (GenBank acc. no. MT796505.1). In addition, one nucleic acid mutation (g.15A>C) was found in the A3 sample when compared to the Rosa centifolia referencing sequences (GenBank acc. no. MZ230175.1).

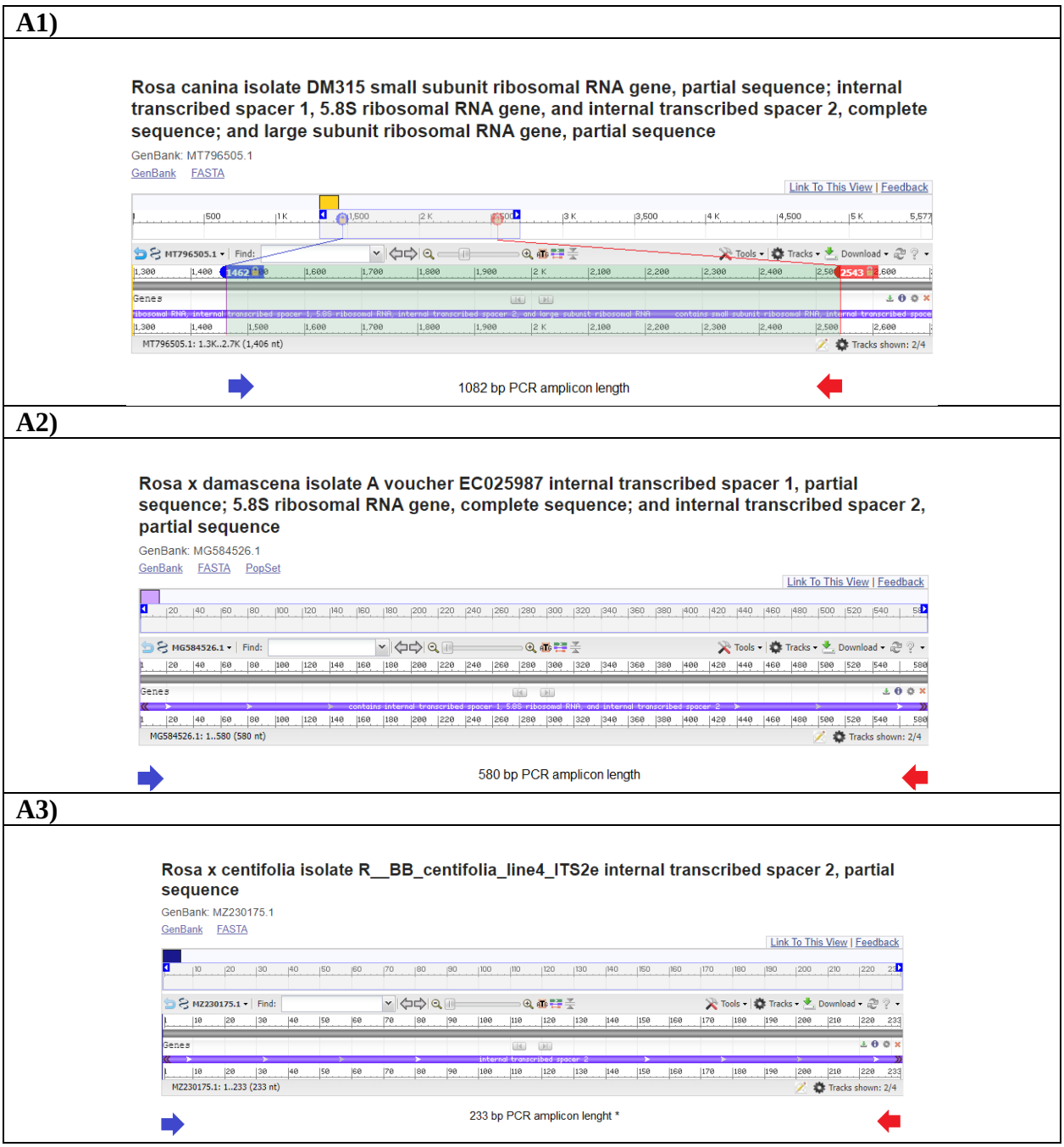

Fig. 1.The exact position of the retrieved ribosomal amplicons within *R. canina, R. damascena,* and *R. centifolia* genomic sequences (GenBank acc. no. MT796505.1, MG584526.1, and MZ230175.1). The blue arrow denotes the amplicon's beginning, and the red arrow designates its termination. * Due to the limited data of *R. centifolia*, only 233 bp was found in the NCBI.

Results indicated the presence of a total of eight nucleic acid variants, seven in the A1 sample and one in the A3 sample. All the investigated one rRNA sequences were deposited in the NCBI web server, and unique accession numbers were obtained for the analyzed A1 to A3 sequences. The deposited sequences received the GenBank accession number op491881, op491882 and op491883 to represent the A1, A2, and A3 samples respectively. Based on nucleic acid changes seen in the amplified ITS amplicons, a thorough phylogenetic tree was created in this current research. This phylogenetic tree contained the A1, A2, and A3 samples alongside other relative nucleic acid sequences of *Rosa canina* , *Rosa x damascena* and *Rosa centifolia* sequences respectively. Three primary clades of incorporated sequences within the cladogram were formed by incorporating our researched samples within this tree together with additional relative sequences. The data for this tree indicated the presence of relatively high homology between

the three incorporated clades within the same tree. Aside from this result, the data showed that rRNA-based amplicons could detect the *Rosa* sequences present in the A1, A2, and A3 samples without confounding them with sequences from other species belonging to the same genus. In this complete tree, twenty-three aligned nucleic acid sequences were present. As indicated above, our investigated A1, A2, and A3 samples were clustered into three phylogenetic clades of close phylogenetic distances within three closely related clades of *Rosa canina* , *Rosa damascene* and *Rosa centifolia* sequences. Two types of cladograms were generated to explain three different representations of the incorporated *Rosa* sequences, a rectangular cladogram (Fig. 2), Within the *Rosa* sequences, the examined samples were grouped into three major clades.

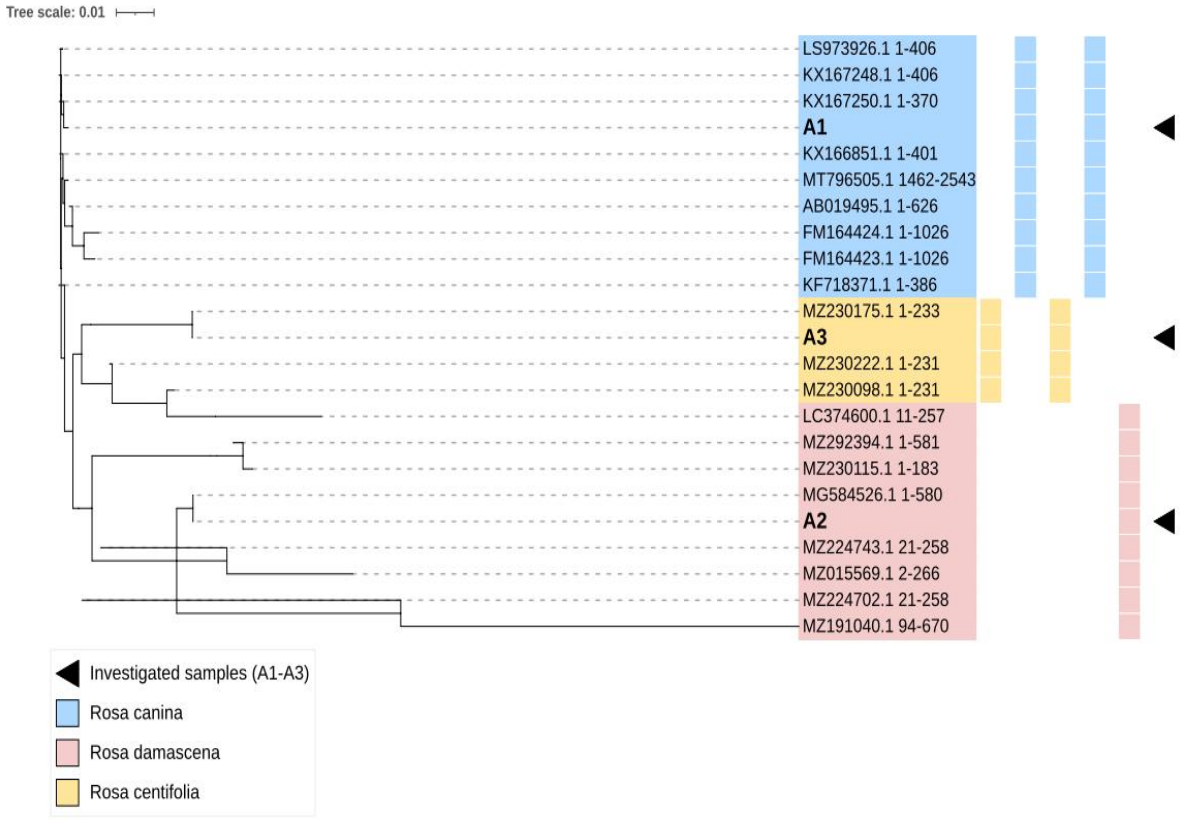

Fig.2. A thorough rectangular cladogram phylogenetic tree constructed from the genetic variations in one sample's rRNA sequences *from R. canina, R. damascena, and R. centifolia*. The triangle in black corresponds to the examined versions. All the numbers represented the GenBank accession numbers for the corresponding species. The scale range among the comprehensive tree-categorized organisms is shown by the number "0.01" at the top of the tree. The investigated sample's code is indicated by the letter "A#".

Within the major clade of *Rosa canina*, ten sequences of the same species were incorporated. The A1 sample was suited to various strains of the *Rosa canina* sequences that have been deposited from Chinese (GenBank acc. no. KF718371.1), Japanese (GenBank acc. no. AB019495.1), Denmark (GenBank acc. no. MT796505.1) and UK (GenBank acc. no. KX167250.1 and KX166851.1) origins. In the present study the investigated sequences of the A1 sample exerted a noticeable tilt of phylogenetic distributions within this major clade. This was due to the presence of seven nucleic acid substitutions (g.392G>T, g.439G>A, g.442T>G, g.458C>T, g.477C>T, g.495T>C, and g.496C>T) when these samples were compared to the *Rosa canina* reference sequences. The A2 sample was suited beside one of the *Rosa damascena* sequences that has been deposited from India (GenBank acc. no. MG584526.1). The A3 sample was suited to various strains of the *Rosa centifolia* sequences that have been deposited from Chinese (GenBank acc. no. MZ230175.1, MZ230222.1, and MZ230098.1) origins. However, no tilt was observed between the A3 sample and the Chinese strain of the *Rosa*

centifolia (GenBank acc. no. MZ230175.1). Compared to the referencing sequences of Rosa centifolia sequences, there was only one nucleic acid variant present (g.15A>C). Interestingly, the utilized ITS (rRNA) sequence-based amplicons provide high accuracy in the detection and discrimination of this type of organism in sufficient detail (10, 17 , 29) . All three major clades showed distinct positions without overlapping with each other. This observation demonstrated that the ribosomal sequences in use could distinguish between the sequences currently under investigation without causing any phylogenetic confusion between them. This phylogenetic tree has confirmed sequencing reactions because it explains the actual neighbor-joining-based positioning in such observed nucleic acid variations. It was impossible to ignore the widespread European and Asian dispersion of the present studied samples. This in turn provides more evidence that the rRNA sequence-specific primers now in use are able to explain the Rosa sequences under investigation and their phylogenetic locations (6, 34).

Nucleic acids sequencing of the *matK* PCR amplicons: Three samples were used in the current analysis at this location. To amplify the *matK* Rosa sequences, these samples were tested. Due to *Rosa* potential ability to respond to diverse genetic variety as was found in different species within *Rosa* sequences, the variance of the *matK* sequences caould be employed for *Rosa* characterization. After executing NCBI blastn for these PCR amplicons, the sequencing reactions confirmed the precise identification. The NCBI BLASTn engine revealed that the sequences of the sequenced samples and the intended reference target sequences for the 886 bp amplicons were 99% to 100% identical. The precise locations and other information of the retrieved PCR fragments were discovered by comparing the observed nucleic acid sequences of these examined samples with the retrieved nucleic acid sequences (GenBank acc. NC_047295.1, MG947055.1, and MZ261886.1) (Fig. 3). Remarkably, the 886 bp samples alignment findings demonstrated that when analyzed in comparison to the most similar referring reference nucleic acid sequences, the studied B3 sample included two nucleic acid variations represented by 2 nucleic acid substitutions. The obtained results showed that the tested B3 sample included two nucleic acid variations, while both B1 and B2 did not show any variations compared with the referring DNA sequences. The observed variants in the investigated B3 samples were 165C>T and 687G>T. All the investigated *matK* sequences were deposited in the NCBI web server, and unique accession numbers were obtained for the analyzed B1, B2, and B3 sequences. The deposited sequences received the GenBank accession number op503880, op53881, op503882 to represent the B1, B2, and B3 samples respectively. According to nucleic acid differences found in the amplified 886 bp of the *matK* amplicons, a thorough phylogenetic tree was created in the current work. The B1, B2, and B3 samples were included in this phylogenetic tree along with three closely related *Rosa* species. The sample under the present investigation was included in this tree along with additional related sequences to form three major clades of incorporated sequences in the cladogram that was produced. These three clades were represented by *R. canina*, *R. damascena* and *R. centifolia*. This observation obtained from this tree indicated the presence of extremely high homology among the three incorporated clades within the same tree.

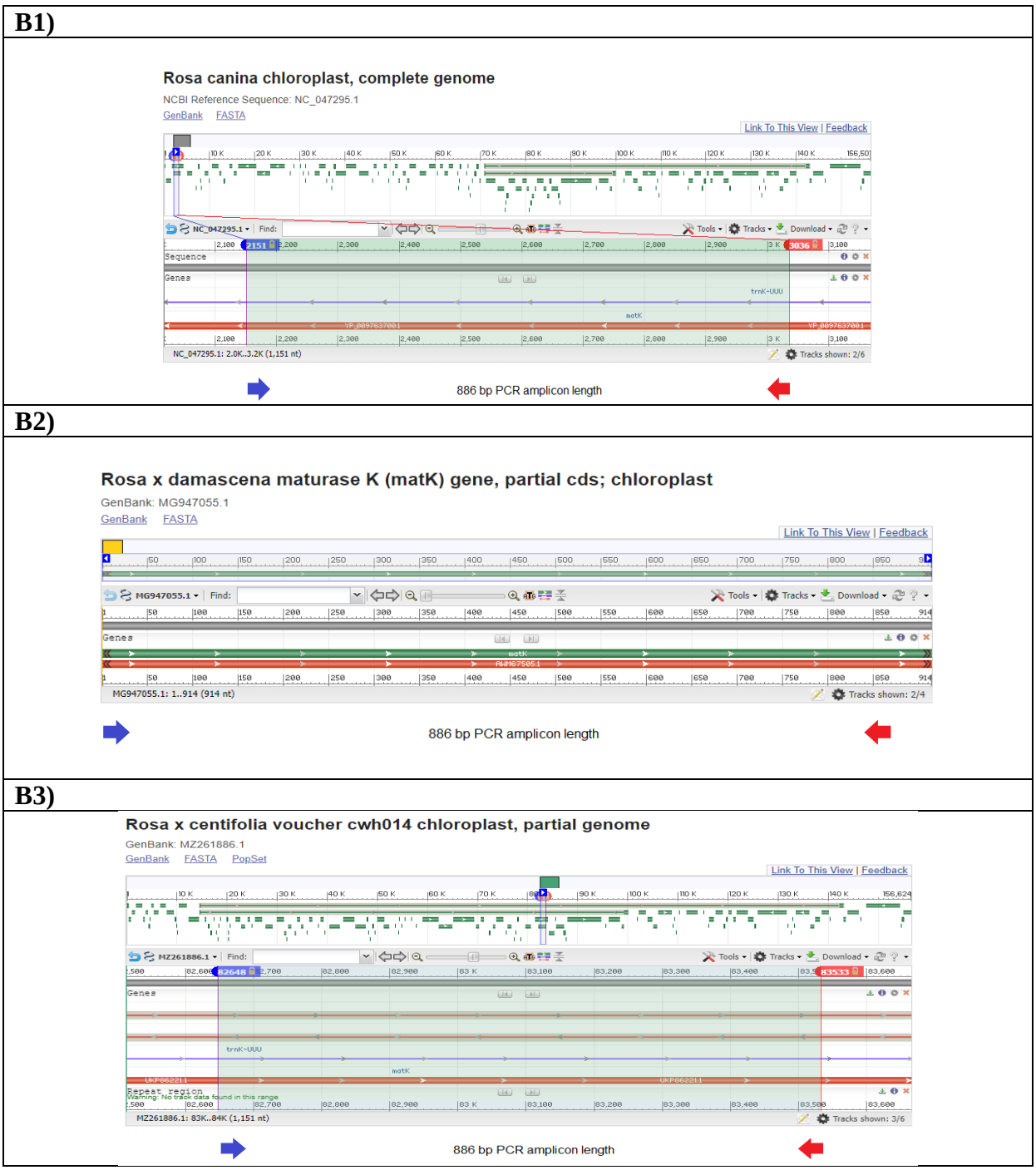

Fig. 3.The exact position of the retrieved *matK* amplicons within *R. canina, R. damascena,* and *R. centifolia* genomic sequences (GenBank acc. no. NC_047295.1, MG947055.1, and MZ261886.1). The blue arrow denotes the amplicon's beginning, while the red arrow designates its termination.

Aside from this result, the data showed that matK sequence-based amplicons were capable of detecting the sequences of the B1, B2, and B3 samples under investigation without confounding them with sequences from other species of the Rosa genus. This comprehensive tree contained 25 aligned nucleic acid sequences in total (Fig. 4).

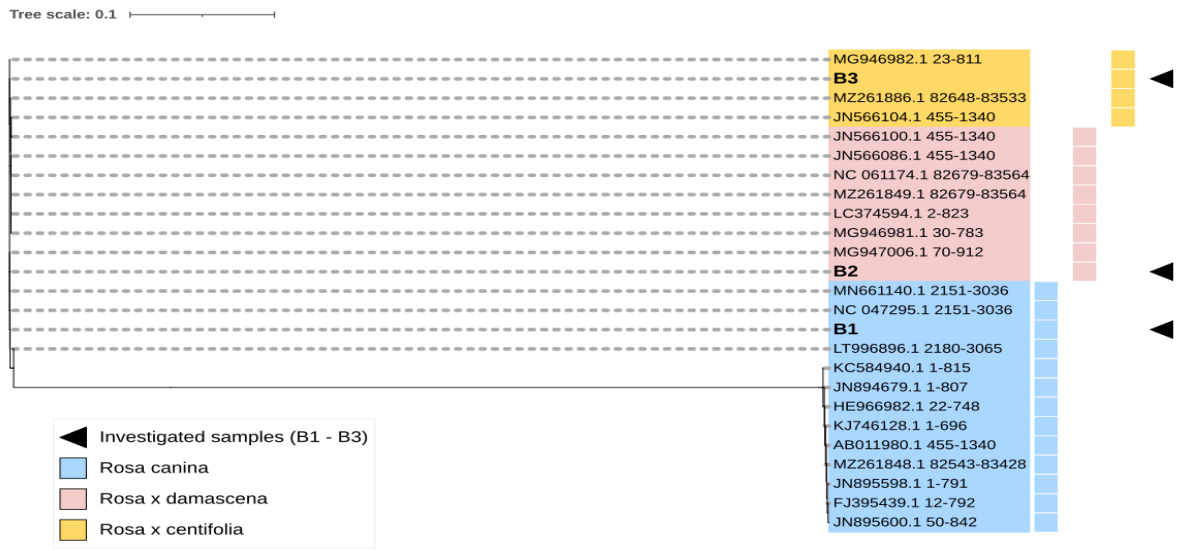

Fig. 4. A thorough rectangular cladogram showing the matK gene's genetic variations in three Rosa sequence-containing species. The analyzed versions are shown by the triangle in black. All of the numbers were the corresponding GenBank entry numbers for the referred species. The complete tree categorized creatures' degree of scale range is shown by the number "0.001" at the top of the tree. The code for the sample under investigation is indicated by the letter "B#".

Within the major clade of *R. canina*, thirteen sequences of the same species were incorporated. In this study the investigated sequences exerted a specific pattern of phylogenetic distributions within the same major clade. The B1 sample was suited to three strains of the *R. canina* sequences that have been deposited from China (GenBank acc. no. MN661140.1), USA (GenBank acc. no. NC_047295.1), and South Africa (GenBank acc. no. LT996896.1). However, no tilt was observed between the B1 sample and these strains due to the absence of any detectable genetic variations. B2 sample was suited within the clade *of Rosa damascena* beside various strains deposited from the USA (GenBank acc. no. MG947006.1, MG946981.1, and NC_061174.1), Saudi Arabia (GenBank acc. no. LC374594.1), and China (GenBank acc. no. MZ261849.1, JN566086.1, and JN566100.1). Regarding the incorporated B3, it was found that this sample did not show any tilt in its positioning within the clade of *R. centifolia* despite the presence of two nucleic acid variations (g.165C>T and g.687G>T). The reason behind the absence of any deviation within this clade was attributed to the possible absence of any detected phylogenetic role for the observed variants of g.165C>T and g.687G>T in the investigated B3 sample. Despite the close phylogenetic distances observed between the three clades, the *matK* gene-based tree was able to generate non-confused positioning among them (17, 23, 30). This observation indicated the high resolution of the currently utilized PCR products of the *matK* sequences in the efficient detection of *Rosa* sequences and its discrimination into three distinct species of *R. canina* , *R. damascena*, and *R. centifolia*. Because it verified the real neighbor-joining-based positioning in these discovered nucleic acid changes, the current observation of this phylogenetic tree has corroborated sequencing reactions. Notably, it was unable to disregard the various distributions of the samples it was studied. Interestingly, using *matK* sequences in the present research has provided more evidence that the organisms being now under investigation can be accurately identified. The divergence of these species from distinct geographical sources can also be supported by these observations. This in turn provides more evidence that the *matK* sequence-specific primers now in use can describe the sequences of the Rosa species under investigation and their phylogenetic locations.

CONCLUSION

The results of this phylogenetic study confirmed the ability of the ITS and matK molecular markers to identify the *Rosa* sp. even closely related ones while the trnT-trnL failed to give us un applicable result. *Rosa* sp. can be detected and distinguished with high accuracy using ITS (rRNA) sequence-based amplicons, and sequencing reactions have

been confirmed because the phylogenetic tree explained the actual neighbor-joining-based positioning in such observed nucleic acid variations. These results indicated the high resolution of the currently utilized PCR products of the *matK* sequences in the efficient detection of *Rosa* sequences and its discrimination into three distinct species of *R. canina* , *R. damascena* and *R. centifolia*.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

REFRENCES

1. Ali, Z. A. 2020. A Comparative Taxonomic Study of Seeds of Some Plants of Rosaceae Family in Iraq. Plant Archives, 20: 589- 595.

2. Al-Mayah, A. A., 2013. Medicinal Plants and Herbal Therapy. College of Science, University of Basrah. Pp. 172-174.

3. Al-qaysi, Safaa Al-deen Ahmed Shanter and Adel Hamdan Alwan., 2016. Molecular identification of rhizosphere *Trichoderma* spp. and their antagonistic impact against some plant pathogenic fungi. Baghdad Science Journal, 13(1): 53-65. http://dx.doi.org/10.21123/bsj.2016.13.1.0053

4. Al-Rawi , Ali and H. L. Chakravrty., 2014. Medicinal Plants of Iraq. Ministry of Agriculture. Irqi National Herbarium. 3th. Edit., Minitery of Agrigulture, Iraq National Herbarium: "pp. 82-83".

5. Benson, D. A.; M. Cavanaugh; K. Clark and et al. 2016. GenBank. Nucleic acids research. 28;45(D1):D37-42.

6. Bruneau, A.; J. R. Starr, and S. Joly., 2007. Phylogenetic relationships in the genus Rosa: New evidence from chloroplast DNA sequences and an appraisal of current knowledge. Syst. Bot., 32(2):366–378. https://doi.org/10.1600/036364407781179653

7. Davidson R and A. Martín del Campo., 2020. Combinatorial and computational investigations of neighbor-joining bias. Front. Genet. 11:584785. https://doi.org/10.3389/fgene.2020.584785

8. EL-Banhawy, A.; C. Acedo ; S. Qari and A. Elkordy., 2020. Molecular identification and phylogenetic placement of *Rosa arabica* Crép. (Rosaceae), a Critically Endangered Plant Species. Life, 10: 335. https://doi.org/10.3390/life10120335

9. Elhawary, E.A.; N. M. Mostafa; R. M. Labib and A. N. Singab., 2021. Metabolomic profiles of essential oils from selected *Rosa* varieties and their antimicrobial activities. Plants, 10, 1721. https://doi.org/10.3390/plants10081721

10. Fougere- Danezan, M., S.; Joly, A. Bruneau, X. F.; Gao, and L. B. Zhang, 2015. Phylogeny and biogeography of wild roses with specific attention to polyploids. Annals of Botany, 115: 275–291. https://doi.org/10.1093/aob/mcu245

11. Guan, B.; J. Wen; H. Guo and Y. Liu., 2024. Comparative and phylogenetic analyses based on the complete chloroplast genome of *Cornus subg. Syncarpea* (Cornaceae) species. Front. Plant Sci. 15:1306196. https://doi.org/10.3389/fpls.2024.1306196

12. Haider, N., 2011. Chloroplast-specific universal primers and their uses in plant studies. Biologia Plantarum, 55 (2): 225-236. https://doi.org/10.1007/s10535-011-0033-7

13. Jian, H. Y.; L. Zhao; H. Zhang and et al. 2022. Phylogeography and population Ggenetics of *Rosa chinensis* var. spontanea and *R. lucidissima* complex, the important ancestor of modern Roses. Front. Plant Sci. 13:851396. https://doi.org/10.3389/fpls.2022.851396

14. Kovacheva, N.; K. Rusanov, and I. Atanassov, 2010. Industrial cultivation of oil bearing rose and rose oil production in Bulgaria during 21ST Century, directions and challenges, Biotechnology & Biotechnological Equipment, 24 (2): 1793-1798. https://doi.org/10.2478/V10133-010-0032-4

15. Li, J.; S. Wang; J. Yu and et al. 2013. A modifed CTAB protocol for Plant DNA extraction. Chin Bull Bot 48:72–78. https://doi. org/10.3724/SP.J.1259.2013.00072

16. Pham, T.; Q. T. Nguyen ; D. M. Tran and et al. 2022. Phylogenetic Analysis Based on DNA Barcoding and Genetic Diversity Assessment of *Morinda officinalis* How in Vietnam Inferred by Microsatellites. Genes 2022, 13, 1938. https://doi.org/10.3390/genes13111938

17. Qiu, X. Q.; H. Zhang; Q. G. Wang and et al. 2012. Phylogenetic relationships of wild roses in China based on nrDNA and matK

data. Sci. Hort., 140:45–51. https://doi.org/10.1016/j.scienta.2012.03.014

18. Scibetta, S.; L. Schena,; A. Abdelfattah and et al. 2018. Selection and experimental evolution of universal primers to study the fungal microbiom of higher plants. Phytobiomes Journal, 2(4): 225-236. https://doi.org/10.1094/PBIOMES-02-18-0009-R

19. Sevindik, E. and K. Okan, 2020. Genetic diversity and phylogenetic analyzes of *Laurus nobilis* L. (Lauraceae) populations revealed chloroplast (cpDNA) trnL Intron and trnL-F Region. International Journal of Fruit Science, 20(S2): S82-S93. https://doi.org/10.1080/15538362.2019.1707745

20. Shahbaz, S. Esmael., 2010. Trees and Shrubs. Univesity of Duhok Publication. "pp. 131-149''.

21. Shulaev, V.; S. S. Korban ; B. Sosinski and et al. 2008 . Multiple Models for Rosaceae Genomics. Plant Physiology, 147: 985–1003. https://doi.org/10.1104/pp.107.115618

22. Sinjare, D. Y. and J. M. Jubrael., 2023. PCR-RFLP analysis of the 16S rRNA and ITS regions in bacterial blight (*Xanthomonas axonopodis* pv. punicae) across pomegranate farms in Kurdistan regions-Iraq. Iraqi Journal of Agricultural Sciences , 54(2):339- 347. https://doi.org/10.36103/ijas.v54i2.1707

23. Suprun, I. I.; S. A. Plugatar; I. V. Stepanov, and T.S. Naumenko., 2020. Analysis of genetic relationships of genotypes of the genus *Rosa* L. from the collection of Nikita Botanical Gardens using ISSR and IRAP DNA markers. Вавиловский журнал генетики и селекции, 24(5):474-480. doi. 10.18699/VJ20.639

24. Taberlet, P.; E. Coissac,; F. Pompanon and et al. 2007 . Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Research, 35(3): e14. https://doi.org/10.1093/nar/gkl938

25. Townsend, C. C. and E. Guest., 1966. Flora of Iraq (vol. 2). Ministry of Agriculture Republic of Iraq. pp. 141-151.

26. Verhoeven, H. A.; J. Blaas and W. A. Brandenburg., 2003. Fragrance and pigments| fragrance profiles of wild and cultivated Roses. Encyclopedia of Rose Science. "pp. 240-248".

27. Vukosavljeva, M.; J. Zhanga; G. D. Esselinka; W. P. C. Westendea and et al. 2013 . Genetic diversity and differentiation in roses: A garden rose perspective. Scientia Horticulturae 162) 320–332. http://dx.doi.org/10.1016/j.scienta.2013.08.015

28. Wang, H.; Y. Yang; M. Li ; J. Liu and W. Jin., 2017. Residents' preferences for roses, features of rose plantings and the relations between them in built-up areas of Beijing, China. Urban Forestry & Urban Greening, 27 : 1–8. http://dx.doi.org/10.1016/j.ufug.2017.06.011

29. Wissemann, V. and C. M. Ritz., 2005. The genus *Rosa* (Rosoideae, Rosaceae) revisited: molecular analysis of nrITS-1 and atpB-rbcL intergenic spacer (IGS) versus conventional taxonomy. *Botanical Journal of the Linnean Society*, 147:275–290. https://doi.org/10.1111/j.1095-8339.2005.00368.x

30. Yin, X. ; B. Liao,; S. Guo and et al. 2020. The chloroplasts genomic analyses of Rosa laevigata, R. rugosa and R. canina. Chin Med, 15:18. https://doi.org/10.1186/s13020-020-0298-x

31. Yu, J.; X. Wu,; C. Liu; Newmaster, S. and et al. 2021. Progress in the use of DNA barcodes in the identification and classification of medicinal plants. Ecotoxicology and Environmental Safety, 208 : 111691. https://doi.org/10.1016/j.ecoenv.2020.111691

32. Yu, J.; J. Xue and S. Zhou., 2011. New universal matK primers for DNA barcoding angiosperms Journal of Systematics and Evolution 49 (3): 176–181 . 10.1111/j.1759-6831.2011.00134.x

33. Zhang, Z.; S. Schwartz; L. Wagner and W. Miller., 2000. A greedy algorithm for aligning DNA sequences. J Comput Biol. 7(1-2):203-14. https://doi.org/10.1089/10665270050081478

34. Zhu, Z. M.; X. F. Gao and M. Foug_ere-Danezan., 2015. phylogeny of *Rosa* sections chinenses and stynstylae (Rosaceae) based on chloroplast and nuclear markers. Mol. Phylogenet. Evol. , 87:50–64. 1624. https://doi.org/10.1016/j.ympev.2015.03.014