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SEQUENCING ANALYSIS OF SIX INTERGENIC SPACER SEQUENCES OF IRAQI *TAMARIX* L. GENUS (TAMARICACEAE)

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SUMMARY

Tamarix L. (Tamaricaceae) is a taxonomically challenging genus. Six Iraqi species of *Tamarix* include *T. amplexicaulis* L., *T. aphylla* L. Karst., *T. arceuthoides* Bunge, *T. gracilis* L., *T. mascatensis* Bunge, and *T. ramosissima* Ledeb, which incurred evaluation for genomic diversity and taxonomic identities. The following study presents an analysis of the taxa from all previously described *Tamarix* species using nuclear and plastid DNA markers on fresh samples of *Tamarix* species. DNA genomic isolation proceeded by the ABIO pure extraction protocol. This study amplified one specific PCR fragment partially covering transposable pBS ISS to assess the pattern of genetic polymorphism in *Tamarix* species. Building a specific tree assessed the accurate genotyping of the observed variants and their phylogenetic distribution. Researchers focused on GenBank-searched sequences as DNA barcodes for a meta-analysis. Rectangular and circular cladograms explored displayed the different representations of integrated Tamarix sequences. The presented investigations revealed many interesting facts. This study pursued sequencing to reconstruct the phylogeny by integrating the sequencing results with robust evidence for the species classification and developing molecular markers based on ISS analysis of Tamarix conducted in Iraq.

Keywords: DNA barcodes, DNA sequences, phylogenetic analysis, ISS analysis, Tamarix, species, taxonomy

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Key findings: According to ISS, the Iraqi *Tamarix* species' isolation occurred by matching with GenBank-searched sequences as DNA barcodes for a meta-analysis. The highlighted details of their sequences had the total length of the amplified amplicons determined. These results were greatly analogous to earlier observations based on the phylogenetic analyses that supported the accurate placement of these isolates within their respective clades and correcting past phylogenetic findings on the relationships among Iraqi *Tamarix* species.

INTRODUCTION

Tamarix, commonly known as salt cedar and tamarisk, is considerably the largest genus belonging to the Tamaricaceae family that contains about 54 species (Gaskin and Shafroth, 2005). These plants are native to Eurasia and Africa but have been introduced to various parts of the world, including North America and Australia (Villar *et al.,* 2014). *Tamarix* species often seemed invasive. The name came from the word Tambro and from a river in Spain on the border of the Pyrenees, where *Tamarix* species are abundant (Little, 1949). Meanwhile, the species *Tamarix aphylla,* as written by Carl Linnaeus, is a derivative of the ancient Greek word for leaf, Phyllon (Liddel and Scott, 1980).

Tamarix species prevail in North Africa, Central Asia, and Southeastern Europe and are native to salt deserts, river banks, dunes, salt marshes, canals, and coastal plains (Orwa *et al.,* 2009). Classified as a halophyte plant, they grow in soils rich in sodium chloride salts. The dissolved salts in the soil reached absorption in high concentrations into the plant tissues, causing the death of the plant. Hence, to protect themselves from these conditions, the leaves have adapted to produce a system of salt glands, which secrete excess salt outside the plant tissues (Townsend and Guest, 1980). These glands, which complete their secretion, will age and die, with new glands replacing them that will secrete salts (Bosabalidis, 1992).

The genus *Tamarix* contains various chemicals, including polyphenols, flavonoids, and tannins. On the other hand, the bioactive compounds make the genus beneficial in traditional medicine in Yemen, where an enema of fresh leaves of *T. nilotica* has been helpful to treat diarrhea (Ghazanfar, 1994; Sultanova *et al.,* 2002). Many studies have shown some *Tamarix* species have antioxidant and antimicrobial activities, such as *T. aphylla* (Mohammedi and Atik, 2011; Zain *et al.,* 2012) and *T. gallica* (Ksouri *et al.,* 2009). However, a highly oxygenated bioactive flavonoid, gardenin, as isolated from the aerial parts of *T. dioica,* showed antiviral and anti-invasive activity against solid tumors (Parmar *et al.,* 1994). In general, most species of the genus *Tamarix* are applicable in traditional medicine as an appetite stimulant, diaphoretic, and diuretic. Moreover, they are very effective in treating vitiligo (leucoderma), spleen problems, and eye diseases (Lefahal *et al.,* 2010). In Egypt, cooking the leaves and young branches of Tamarix helped treat splenic edema, and mixing with ginger treated uterine infections. After mixing it with vinegar, a decoction of the bark can be a natural remedy to get rid of head lice (Bakr *et al.,* 2013). The people living in the coastal Mediterranean region of Egypt use the bark of *T. aphylla* as an astringent and tonic, which is also effective in treating inflammation of the head from eczema. Furthermore, the use of *T. aphylla* in the Mediterranean region helps treat fever and eye inflammation, with the powdered extract of *T. aphylla* leaves also used to treat toothache (Kamal *et al.,* 2009). *T. nilotica* application in traditional Egyptian medicine serves as an antiseptic agent and an antifungal, anti-inflammatory, and wound-healing stimulant (Yusufoglu and Al-Qasoumi, 2011). *T. ramosissima* showed antioxidant and antimicrobial activities, while an extract of *T. aphylla* served as an antioxidant (Sultanova *et al.,* 2002).

Despite the numerous morphological and anatomical studies of the *Tamarix*, genetic biodiversity studies have long been underrepresented because the taxonomy of the genus is characteristically complex, with the family Tamaricaceae now included in the

Caryophyllales (Arianmanesh *et al.,* 2015). The taxonomy and phylogenetic relationship of related taxa are considerable for realizing the diversity of a species.

Genetic diversity with locative distribution may offer an important genetic resource to investigating evolutionary and genetic relationships among Tamaricaceae taxa and insights into the history of range shifts and morphological and anatomical variations (Lee *et al.,* 2019). However, the studies focusing on the ISS genomes of *Tamarix* remain limited. Hence, to fill this knowledge gap, the presented study examined the genetic variations in six Iraqi patterns of *Tamarix,* seeking to the following: a) establish *Tamarix*'s phylogeny to evaluate the correctness and advantage of the current classification; b) identify the weakest points in the taxonomy of said genus as an indicator tool for possible future lines; and c) assess the importance of morphological characters used in Tamarix systematics.

MATERIALS AND METHODS

Plants' collection

The collection of fresh leaf samples of *Tamarix* species transpired in the middle of Iraq at latitudes 31 °C–38 °C and longitudes 40 °C–45 °C, with the help of some information obtained from the Iraqi flora (Gest, 1966). The mature samples (Z1–Z8) obtained tagging and proper documentation using a field notebook containing the dates (June to October 2022) place of collection, the local name, the economic importance, and anthropogenic activity.

Genomic DNA extraction and quantification

Genomic DNA isolation from fresh leaves followed the protocol of the ABIO pure extraction. The use of a Quantus Fluorometer helped detect the concentration of extracted DNA to know the quality of samples for downstream applications. The procedure was as follows: mix 1 µl of DNA and 200 µl of diluted Quantifluor Dye, then, incubate for 5 min at room temperature, and detect the DNA concentration values.

PCR amplification

One specific PCR fragment partially covering the transposable pBS intergenic spacer sequences reached amplification in this study. The amplified PCR fragments bore direct exposure to Sanger sequencing experiments to assess the pattern of genetic polymorphism in these *Tamarix* species. Building a specific comprehensive tree guided in assessing the accurate genotyping of the observed variants and their phylogenetic distribution.

Sequencing method

Nucleic acids sequencing of PCR amplicons

The resolved PCR amplicons incurred commercial sequencing from both directions, forward and reverse directions, following the instruction manual of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (Applied Biosystem) sequence files attained further analysis, ensuring the annotation and variations. By comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences, identifying the virtual positions and other details of the retrieved PCR fragments was successful.

Interpretation of sequencing data

The sequencing results of the PCR products of the targeted samples depended on the protocol (Al-Shuhaib and Hashim, 2023) as follows: edited, aligned, and analyzed according to the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). In each sequenced sample, the observed variations sustained numbering in PCR amplicons as well as in their corresponding position within the referring genome. The observed nucleic acids' numbering ensued in

PCR amplicons, including their corresponding positions within the referring genome. The identified sequences within the *Tamarix* sequences underwent annotations by the Snap Gene Viewer ver. 4.0.4 (https://www.snapgene.com).

Deposition of sequences to GenBank

Submitting all the investigated and analyzed sequences continued to the NCBI Bankit portal, with all the instructions described by the portal followed, as described by the server (Benson *et al.,* 2016). The submitted sequence, provided as nucleic acid sequences in the NCBI, received a unique GenBank accession number for the investigated sequences.

Comprehensive phylogenetic tree construction

This study constructed a specific comprehensive tree according to the neighbor-joining protocol (Sarhan *et al*., 2019). The observed variants' comparison with their neighbor homologous reference sequences used the NCBI-BLAST server (Zhang *et al.,* 2000). Then, a full inclusive tree, including the observed variants, built by the neighbor-joining method, proceeded visualization as rectangular and circular cladograms using the iTOL suit (Letunic and Bork, 2019). In the comprehensive tree, the sequences of each classified phylogenetic group bore appropriate color labels.

RESULTS AND DISCUSSION

The results indicated the exact identity of the six amplified samples. Sequencing reactions stated the identity of the investigated samples has the highest similarities with the reference sequences of *Tamarix aphylla* (for Z1 sample), *Tamarix arceuthoides* (for Z2 sample), *Tamarix amplexicaulis* (for Z3, Z5, and Z7 samples), *Tamarix gracilis* (for Z4 sample), *Tamarix mascatensis* (for Z6 sample), and *Tamarix ramosissima* (for Z8 sample).

Phylogenetic analyses also confirmed their positions within their corresponding clades accordingly. The multinational origins from which the investigated samples attained isolation were clear, and the sequencing reactions indicated the exact identity after performing the NCBI BLASTn search for these PCR amplicons (Ye *et al.,* 2012).

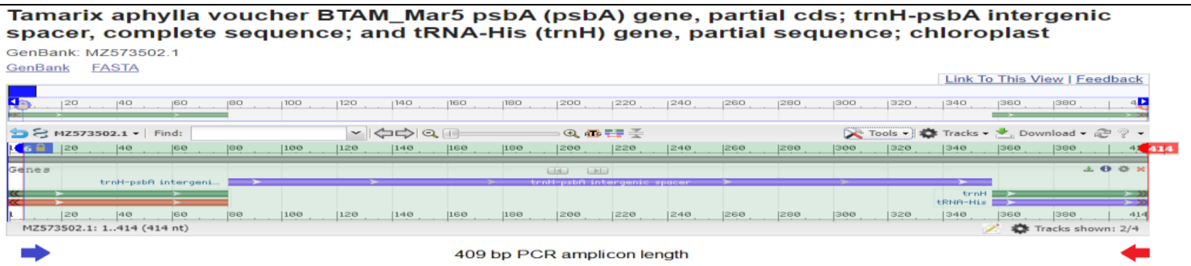
The NCBI BLASTn engine showed the highest sequence similarities between the Z1 sample and the reference Intergenic spacer sequences of *Tamarix aphylla* (GenBank acc. MZ573502.1) (Figure 1.1). The NCBI BLASTn detailed the ultimate sequence similarities between the Z2 sample and the reference intergenic spacer sequences of *Tamarix arceuthoides* (GenBank acc. MZ573503.1) (Figure 1.2). The utmost sequence similarities between the *Tamarix* samples Z3 and Z5 and the reference intergenic spacer sequences of *Tamarix amplexicaulis* (GenBank acc. MZ573498.1), as indicated by the NCBI BLASTn (Figure 1.3).

The topmost sequence similarities, as shown by the NCBI BLAST, were between the Z4 samples and the reference intergenic spacer sequences of *Tamarix gracilis* (GenBank acc. NC_084253.1) (Figure 1.4). The results were also consistent with past findings based on the molecular phylogenetic investigation of the U.S. invasive *Tamarix* (Gaskin and Schaal, 2003). The NCBI BLASTn exerted the highest sequence similarities between the Z6 samples and the reference intergenic spacer sequences of *Tamarix mascatensis* (GenBank acc. MT136597.1) (Figure 1.5).

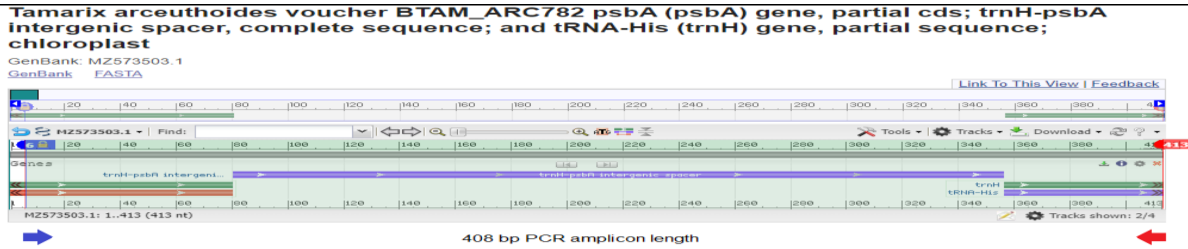
The NCBI BLASTn exhibited the maximum sequence similarities between the Z6 samples and the reference intergenic spacer sequences of *Tamarix ramosissima* (GenBank acc. NC_067942.1) (Figure 1.6). The accurate positions and other details of the retrieved PCR fragments were also evident. The total length of the targeted locus' localization in the NCBI server signified confirming the positions of the start and end of the targeted locus within the most homologous target.

After positioning the Intergenic spacer amplicons' sequences within the genomic

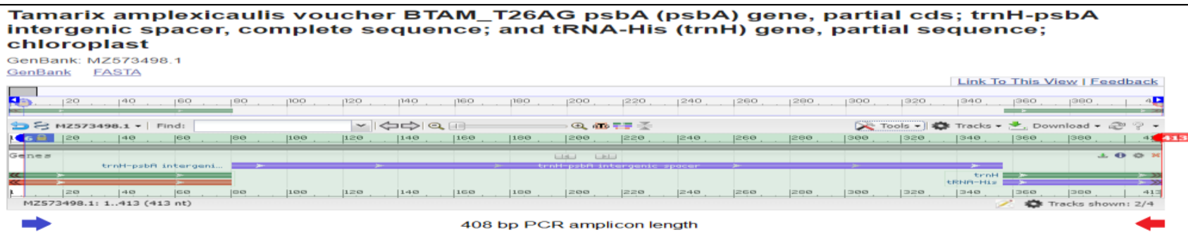
1) *Tamarix aphylla* (for Z1 sample)



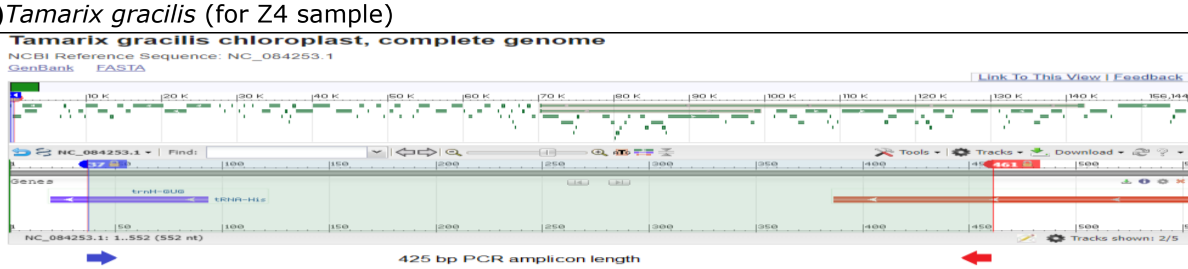
2) *Tamarix arceuthoides* (for Z2 sample)



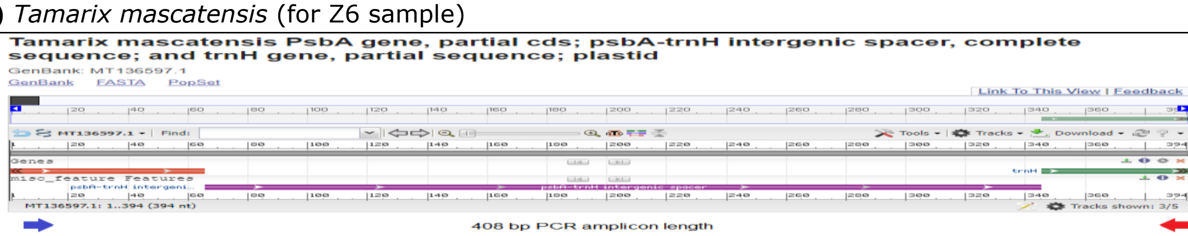
3) *Tamarix amplexicaulis* (for Z3, Z5, and Z7 samples)



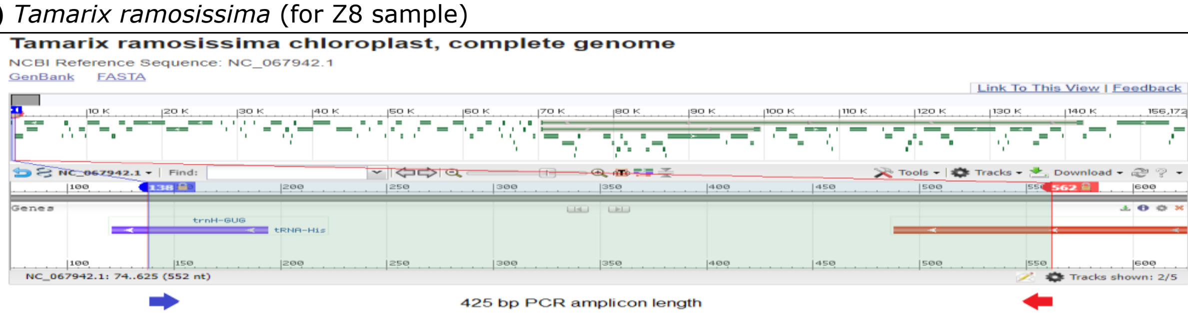
4)*Tamarix gracilis* (for Z4 sample)



5) *Tamarix mascatensis* (for Z6 sample)



6) *Tamarix ramosissima* (for Z8 sample)



sequences of the amplified *Tamarix* sequences, an emphasis on the details of their sequences appeared, with the total length of the amplified amplicons also determined (Table 1). These results were analogous to the observations based on the molecular investigation of invasive *Tamarix* in the USA made by Gaskin and Schaal (2003). However, the presented results were contrary to past findings on studying the phylogenetic relationships among the *Tamarix* species in China (Sun *et al.*, 2016).

Table 1. The position and length of the PCR amplicons used to partially amplify the intergenic spacer sequences within the amplified *Tamarix* genomic sequences.

Amplicon	Reference locus sequences (5′ - 3′)	Length
1) *Tamarix aphylla* sequences (for Z1 sample)	ATGACCGCGCATGGTGGATTCACAATCCACTGCCTTGATCCACTTGGCTACATCCGCCCCTCT AAGATTTACTTTTTTAGATATTAAATATTAATAAAAAGAATTGTTCAGAGCCTAGATATTATAAT TAAAATTAAAAAACGTAAAAAAAAATACATAATCATAAATTCATAAGATTTTTTTATGAAATTGA AGTCAAAAAAAAACTTATGCTTATGTAAGCAAAATTACTATTCAATTAGTATTAAAGGAGCAATA ACCAATCTCTTTTTTTATCAAGAGGATTGATATTGCTCCTTTCTTTTTAAAGACTCGTATACACT AAGAAAAAAATCTTATCCATTTATAGATGGAGCTTCAATAGCAGCTAAGTCTAGAGGGAAGTT GTGAGCATTACGTTCATGCATAAC	409 bp
2) *Tamarix arceuthoides* sequences (for Z2 sample)	ATGACCGCGCATGGTGGATTCACAATCCACTGCCTTGATCCACTTGGCTACATCCGCCCCTCT AAGATTTACTTTTTTAGATATTAAATATTAATAAAAATAATTGTTCAGAGCCTAGATATTCTAAT TAAAATTCAAAAACGTAAAAAACAATACATAATCATAAATTAATCAGATTTTTTTATGAAATTGA AGTAAAAAAAAACTTATGCTTATGTAAGCAAAATTACTATTAAATTAGTATTAAAGGAGCAATAA CCAATCTCTTTTTTTATCAAGAGGATTGATATTGCTCCTTTCTTTTTAAAAACTCGTATACACTA AGAAAAAAATCTTATCCATTTATAGATGGAGCTTCAATAGCAGCTAGGTCTAGAGGGAAGTTG TGAGCATTACGTTCATGCATAAC	408 bp
3) *Tamarix amplexicaulis* sequences (for Z3, Z5, and Z7 samples)	ATGACCGCGCATGGTGGATTCACAATCCACTGCCTTGATCCACTTGGCTACATCCGCCCCTCT AAGATTGACTTTTTTAGATATTAAATATTAATAAAAATAATTGTTCAGAGCCTAGATATTCTAAT TAAAATTCAAAAACGTAAAAAACAATACATAATCATAAATTAATAAGATTTTTTTATGAAATTGA AGTAAAAAAAAACTTATGCTTATGTAAGCAAAATTACTATTAAATTAGTATTAAAGGAGCAATAA CCAATCTCTTTTTTTATCAAGAGGATTGATATTGCTCCTTTCTTTTTAAAAACTCGTATACACTA AGAAAAAAATCTTATCCATTTATAGATGGAGCTTCAATAGCAGCTAGGTCTAGAGGGAAGTTG TGAGCATTACGTTCATGCATAAC	408 bp
4) *Tamarix gracilis* sequences (for Z4 sample)	GAACCCGCGCATGGTGGATTCACAATCCACTGCCTTGATCCACTTGGCTACATCCGCCCCTCT AAGATTTACTTTTTTAGATATTAAATTTTTTTAGATATTAAATATTAATAAAAATAATTGTTCAGA GCCTAGATATTCTAATTAAAATTCAAAAACGTAAAAAACAATACATAATCATAAATTAATCAGAT TTTTTTATGAAATTGAAGTAAAAAAAAACTTATGCTTATGTAAGCAAAATTACTATTAAATTAGTA TTAAAGGAGCAATAACCAATCTCTTTTTTTATCAAGAGGATTGATATTGCTCCTTTCTTTTTAAA AACTCGTATACACTAAGAAAAAAATCTTATCCATTTATAGATGGAGCTTCAATAGCAGCTAGGT CTAGAGGGAAGTTGTGAGCATTACGTTCATGCATAAC	425 bp
5) *Tamarix mascatensis* sequences (for Z6 sample)	ATGATCGCGCATGGTGGATTCACAATCCACTGCCTTGATCCACTTGGCTACATCCGCCCCTCT AAGATTTACTTTTTTAGATATTAAATATTAATAAAAATAATTGTTCAGAGCCTAGATATTCTAAT TAAAATTCAAAAACGTAAAAAACAATACATAATCATAAATTAATCAGATTTTTTTATGAAATTGA AGTAAAAAAAAACTTATGCTTATGTAAGCAAAATTACTATTAAATTAGTATTAAAGGAGCAATAA CCAATCTCTTTTTTTATCAAGAGGATTGATATTGCTCCTTTCTTTTTAAAAACTCGTATACACTA AGAAAAAAATCTTATCCATTTATAGATGGAGCTTCAATAGCAGCTAGGTCTAGAGGGAAGTTG TGAGCATTACGTTCATGCATAAC	408 bp
6) *Tamarix ramosissima* sequences (for Z8 sample)	GAACCCGCGCATGGTGGATTCACAATCCACTGCCTTGATCCACTTGGCTACATCCGCCCCTCT AAGATTTACTTTTTTAGATATTAAATTTTTTTAGATATTAAATATTAATAAAAATAATTGTTCAGA GCCTAGATATTCTAATTAAAATTCAAAAACGTAAAAAACAATACATAATCATAAATTAATAAGAT TTTTTTATGAAATTGAAGTAAAAAAAAACTTATGCTTATGTAAGCAAAATTACTATTAAATTAGTA TTAAAGGAGCAATAACCAATCTCTTTTTTTATCAAGAGGATTGATATTGCTCCTTTCTTTTTAAA AACTCGTATACACTAAGAAAAAAATCTTATCCATTTATAGATGGAGCTTCAATAGCAGCTAGGT CTAGAGGGAAGTTGTGAGCATTACGTTCATGCATAAC	425 bp

* Sequences of the forward primer, as placed in the forward direction, ** Sequences of the reverse primer, as placed in the reverse direction.

The alignment results of the intergenic spacer sequences samples revealed the presence of two nucleic acid variations in the investigated samples compared with the most similar referring reference nucleic acid sequences of *T. arceuthoides* and *T. ramosissima* (GenBank acc. no. NC_024447.1). However, the other investigated *Tamarix* samples showed 100% similarity with their corresponding reference sequences within the genus *Tamarix*. These research results further explored one nucleic acid variation observed in the investigated *Tamarix* samples Z3, Z5, and Z7, as compared with the most homologous reference sequences of *T. amplexicaulis*. The variation appears as substituting guanine with adenine at the 192nd position in the PCR amplicon (Figure 2.3). The results were compatible with past findings, which confirmed the differences among the taxa belonging to the existence of hybridization (Gaskin and Schaal, 2003; Gaskin and Kazmer, 2009; Mayonde *et al.,* 2015).

The presence of one nucleic acid variation was evident in the investigated *Tamarix* sample Z6, as compared with the most homologous reference sequences of *T. ramosissima*. This variation also occurs as substituting adenine with cytosine at the 190th position in the PCR amplicon (Figure 2.6). For confirmation of these variations, the sequencing chromatogram of the investigated samples as well as their detailed annotations reached verification and documentation. Pirie (2015) also did not connect the datasets among the morphological, anatomical, and molecular data generating these differences. The previously proposed sections and series (based on morphology) emerged to be polyphyletic (Baum, 1978).

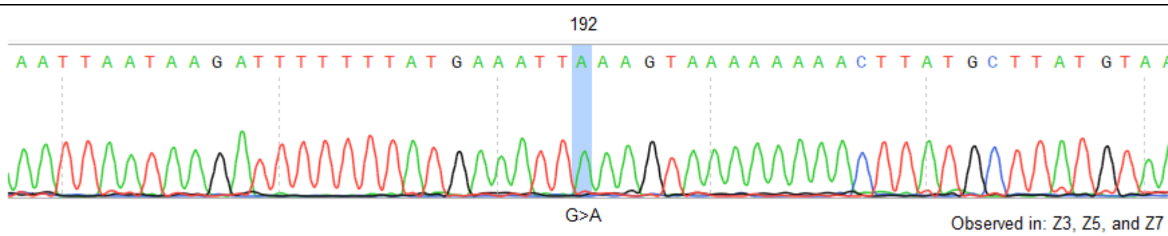
1)*Tamarix aphylla* (for Z1 sample)

--NO VARIATION DETECTED--

2)*Tamarix arceuthoides* (for Z2 sample)

--NO VARIATION DETECTED--

3) *Tamarix amplexicaulis* (for Z3, Z5, and Z7 samples)



5) *Tamarix gracilis* (for Z4 sample)

--NO VARIATION DETECTED--

5) *Tamarix mascatensis* (for Z6 sample)

--NO VARIATION DETECTED--

6) *Tamarix ramosissima* (for Z8 sample)

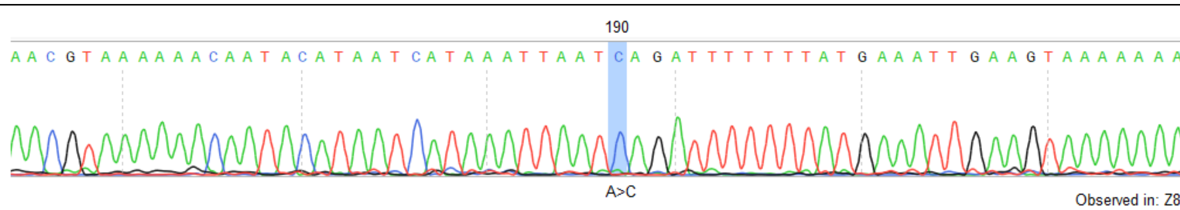


Figure 2. The chromatogram of the investigated *Tamarix* sequences in the amplified PCR products. The symbol ">" refers to substitution mutation.

Indeed, the key identifications based on the morphological features, such as floral part, size, and shape, insertion of the staminal filaments on the nectariferous disc, raceme inflorescence, bract size, and the leaf shape, appear scattered across the phylogenetic trees. Despite incongruence to the presented findings, Villar *et al.* (2019) reported that *Tamarix* is robustly monophyletic in all the trees. This study generated a phylogenetic tree based on the nucleic acid sequences identified in the amplified intergenic spacer amplicons to enhance one's understanding about phylogenetic relationships among examined samples and the most pertinent reference strains among amplified *Tamarix* samples.

The phylogenetic tree included the amplified samples and other relevant nucleic acid sequences from their respective sequences. Two distinct types of cladograms, namely a rectangular cladogram and a circular one, underwent development to illustrate the two different representations of the integrated *Tamarix* sequences. In both instances, the constructed cladograms organized the incorporated sequences into six distinct phylogenetic clades (Figures 3a, b). The total number of aligned nucleic acid sequences in the generated tree was 22. The most interesting fact observed in these investigations was that *Tamarix* isolates appeared to be correlated with the positioning of the samples into a distinct phylogenetic clade within the tree. This sort of diversity emerged from the observed phylogenetic effects of the viewed nucleic acid sequences, due to their ability to cause such distribution in their positioning in the generated clades. Thus, these sequences have constituted an important contrast between these *Tamarix* sequences and other related sequences. The presented results also coincide with the past finding of the six taxa having the highest number of positive amino acid sites (Wanga *et al.,* 2023; Feng *et al.,* 2024).

Within the major *Tamarix aphylla* clade, it was visible that the investigated *Tamarix* sample Z1 had positioned in the immediate vicinity of one strain that has been isolated from China (GenBank NC_084252.1). In the relative vicinity of the *Tamarix aphylla* clade, the other related *Tamarix* clades' incorporation occurred. One of them was the *T. amplexicaulis* clade, where incorporating the samples Z3, Z5, and Z6 ensued. All three samples were within the vicinity of one strain isolated from Sweden (GenBank EU531725.1). In the *Tamarix arceuthoides* clade, the sample Z2's integration was within the vicinity of one strain isolated from Iran (GenBank MZ573504.1). In the *T. mascatensis* clade, assimilating the sample Z6 took place beside one strain isolated from Spain (GenBank MT136597.1). The *T. gracilis* clade had the sample Z6 incorporated beside one strain isolated from China (GenBank NC_084253.1).

Accordingly, the identification of multinational sources of the latest investigated *Tamarix* samples succeeded. Using these intergenic sequences, several clades were evident to be positioned away from each other, suggesting the high specificity about the identification of these *Tamarix* species, showing no confusion among the identified species**.** Phylogenetic analyses also confirmed their positions within their corresponding clades, consistently. The multinational origins from which the investigated samples bore isolation were notable. These results were also consistent with previous findings based on the complete chloroplast genome sequence of *T. taklamakanensis* (Pang *et al.*, 2021).

The presented results demonstrated that plastome studies can provide useful data for future phylogenetic, taxonomic, and evolutionary studies on the family Tamaricaceae. However, the complete Cp genomes proved not distinct with *Tamarix* taxa, indicating that the molecular identification of the genus *Tamarix* could require selecting highly variable regions in Cp genomes to include additional nrDNA sequences (Feng *et al.,* 2024). The outcome of the study signifies the species' different sections according to morphological characters found together genetically. Thus, the definition of taxa according to morphological data must need reevaluation because of invalidity. Probably, this goes to change the intermediate states for several morphological characters that exist from season to season; however, the morphological information does not always

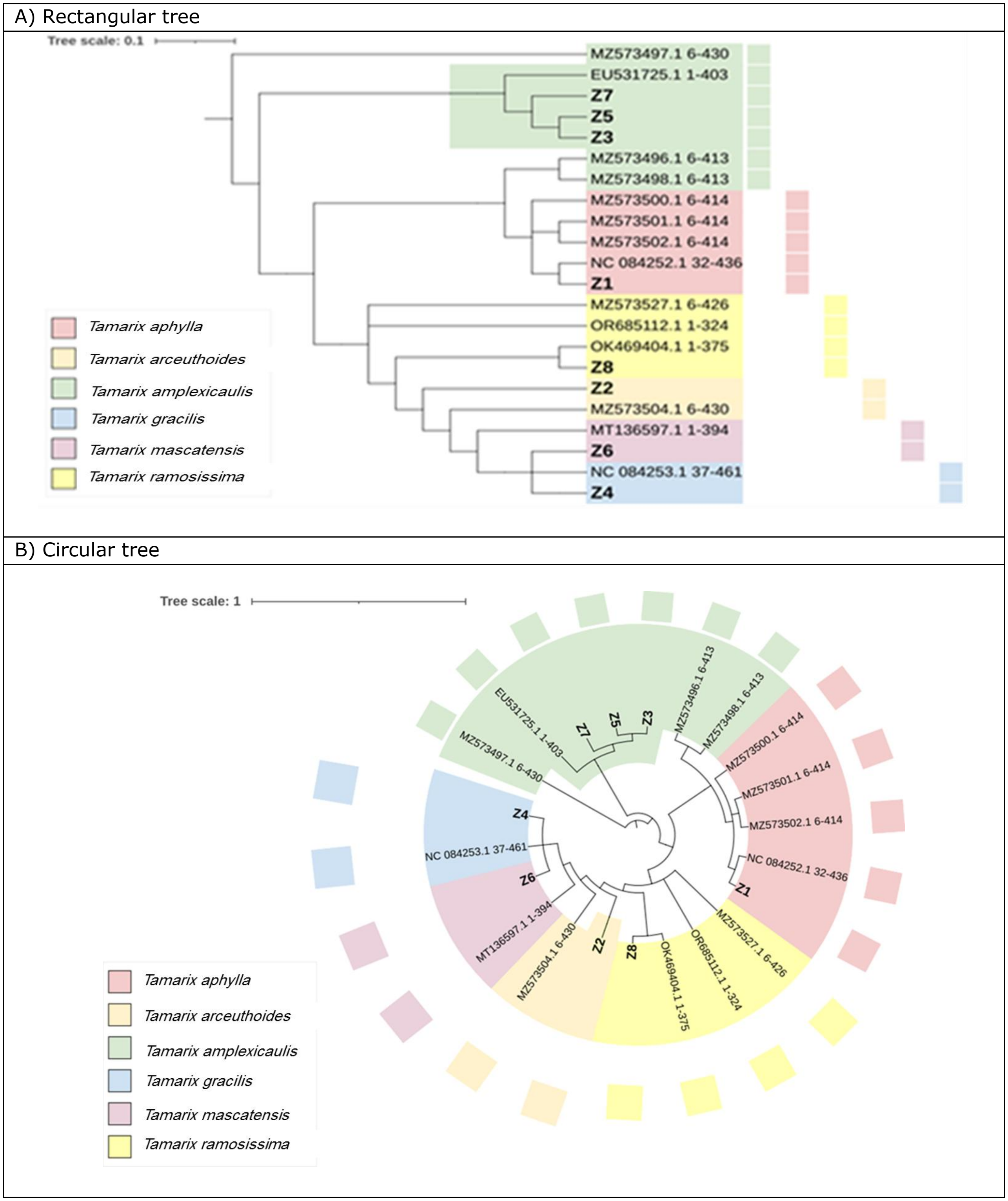


Figure 3. The comprehensive rectangular (in branch A) and circular (in branch B) cladogram phylogenetic tree of intergenic spacer sequences of *Tamarix*. All the mentioned numbers referred to GenBank accession number of each referring species. The number at the top portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. The letter "Z#" refers to the code of the investigated samples.

correlate with the DNA sequence data. *Tamarix* remains an exceedingly complex genus, as this analysis reveals the morphology within *Tamarix.* Although leaf morphology and the number of floral parts have been considered useful for the taxonomy of *Tamarix* species, and future subgeneric classification of *Tamarix* must include molecular data (Arianmanesh *et al.*, 2015).

CONCLUSIONS

Through the use of specific PCR amplification and Sanger sequencing, we accurately identified the genetic characterization within these *Tamarix* isolates. The results revealed distinct identities for the isolates, identified as *Tamarix aphylla*, *Tamarix arceuthoides*, *Tamarix amplexicaulis*, *Tamarix gracilis*, *Tamarix mascatensis*, and *Tamarix ramosissima*. Phylogenetic analyses further supported the accurate placement of these isolates within their respective clades, suggesting their origination from multiple geographical sources worldwide. This study enhances our understanding of the biological diversity of the *Tamarix* isolates in the region and provides valuable insights into their genetic variations.

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