Research Article

Morphological and Molecular Barcoding Characterization of Four Invasive Species of Solanaceae in the Southern Region of Saudi Arabia

Manal A. Alshaqhaa[1]*, Mona S. Al Wahaibi[2], Muhammad Afzal[3] and Mohammed O. Alshaharni[1]

[1]*Biology Department, College of Science, King Khalid University, Saudi Arabia*

[2]*Botany and Microbiology Department, College of Science, King Saud University, Saudi Arabia*

[3]*College of Food and Agricultural Sciences, King Saud University, Saudi Arabia*

*Corresponding Author

Manal A. Alshaqhaa, Biology Department, College of Science, King Khalid University, Saudi Arabia.

Abstract

Invasive plants are the second greatest threat to plant diversity, after habitat loss due to human activities. Plant diversity hotspots and islands, as well as farmlands, woodlands, forests, grasslands, and populated areas, are all threatened by invasive species. Over the centuries, and especially in the last few decades, trade and travel have increased dramatically, hastening the introduction of alien species. Although comprehensive sampling is necessary for DNA barcoding, it is rarely done due to a lack of materials. In practice, only a few species from each genus must be identified, rather than all of them. In many of Saudi Arabia's most problematic habitats in the southwestern region, where the presence of invasive species was significant. As a result, in the current application of DNA barcodes, identifying a given species using a limited sample is important. In the case of incomplete sampling, DNA barcodes can provide reliable specific-species discrimination. For that purpose, the current study was designed to identify the four invasive species in the southern part of Saudi Arabia using morphological and 4 molecular barcoding (ITS, PsbA, rbcla, matk,) markers were used for the identification and characterization of invasive species. The results suggested identifying the 4 invasive Solanaceae plants species in the southwestern region. A phylogenetic tree was also constructed from the identified four species along with reference sequences of the blast results from the NCBI confirmed the four Solanaceae species i.e., Datura innoxia, Datura stramonium, Nicotiana glauca, and Withania somnifera, recorded invasive in the southern part of Saudi Arabia. Except for the matK tree, all the trees clustered individuals of the same species together and separately from the other species, with strong bootstrap support indicating that they were more closely related species.

1. Introduction

Invasive plant species are defined as those that spread into local plant systems, establish self-sustaining populations, and become dominant within those systems [1]. Around the world, invasive plants are widely recognized as major threats to biodiversity conservation [1]. Biodiversity loss is mainly caused by invasive alien species [2]. Invasive plants are second only to habitat loss and degradation in endangering native plants [3]. They also exhibit rapid growth rates, powerful production capabilities, and a high tolerance for environmental stress [4]. It is estimated that up to 50% of invasive species, in general, are environmentally endangered, based on their actual effects [5]. Globally, invasive alien plants threaten agricultural and natural systems. Many invasive species are not dominant competitors in their natural systems, but they competitively exclude their new neighbors in their new environments [6,7].

Due to lack of research and information, several unexpected and invasive species were present in the Kingdom Saudi Arabia, but not previously collected and reported under field conditions. However, some species are not yet known to Saudi Arabia and are considered invasive plants [8]. The southern region of Saudi Arabia is unique in its nature, land shape, water availability, and climate and is considered significant for the presence of high diversity among plant species [9]. This diversity results might be due to geomorphological characteristics, including islands, dunes, sand plains, low rocky hills, and high mountains [10]. The region maintains a diverse topography and as a result, the climate fluctuates dramatically from hot dry in the lowlands to cold to highlands in the region and according to Hosni & Hegazi a total of 218 species belonging to 48 species present in southern Saudi Arabia [11]. Among them, Solanaceae is the most widespread plant including 5424 species and 40 genera i.e., trees, shrubs, and grasses spread in the tropics and temperate regions of the world

[12]. The distribution found throughout tropical and temperate regions, but with a more concentration of diversity in Australia and Latin America [13]. It includes *Solanum, Atropa, Capsicum, Datura, Withania, Hyoscyamus, Nicotiana*, and *Miscellaneous* and has medicinal characteristics worldwide as well as a source of food [14]. Forty-eight alien species have been recorded from Saudi Arabia, some of which have been introduced and settled for decades. Due to the harsh environmental, climate change effects, the ecosystem is harmful to the biodiversity of the species including Solanaceae species *Nicotiana glauca, Physalis acuminate, Datura forex, Datura stramonium, Datura metal,* and *Datura innoxia* [8]. A taxonomic analysis at the molecular level, using molecular biology tools and techniques (polymerase chain reaction (PCR), Q-PCR, DNA barcoding, and high throughput DNA sequencing tools), provides a more profound understanding than chemotaxonomy [15]. As a result, molecular taxonomy is considered the most appropriate method for identifying and evaluating the genetic potential of species among the various DNA analysis methods from a taxonomic point of view. Therefore, the current study was designed to collect the specimen of four invasive Solanaceae species in Southern Saudi Arabia and evaluate their morphological and molecular identification using barcoding genes. To the best of my knowledge, these species are recorded for the first time in the southern part of Saudi Arabia.

2. Materials and Methods
2.1 Climate Study Area
The study area is in the southwestern part of the Kingdom of Saudi Arabia between longitudes 41 25' and 44 37' east and latitudes 17' 25' and 20 57' north. It extends for 380 km from the northeast at the point 20'55 6" and 43' 45" 44 to the southeast of the point 17' 23" 00 N and 43' 30' 41" E. The western mountainous heights are considered one of the most important features of the terrain in the Kingdom of Saudi Arabia. This region is characterized by low temperatures, with an annual average of 17 m, a rainfall rate of 250 mm, and average relative humidity of 65% [16]. As for the western plains, they are in the west of the Kingdom of Saudi Arabia east of the Red Sea, along the Tehama plain, as it extends between Sarawat Mountains and the Red Sea beach [17,18]. In an arid climate and rain sparse country such as Saudi Arabia, rainfall plays a crucial role in several important socio-economic aspects, including water resources management and agriculture [19]. From (Figure 1a) the annual rainfall in the southwestern region of Saudi Arabia varies greatly between the years 2010-2020. It reached 243.9 mm/year in 2016 as the highest rainfall rate, while 2015 was the lowest, reaching 25.9 mm/year. Through temperature data in the period between 2010-2020 in the southwestern region of the Kingdom, the lowest average temperature was recorded in 2010, where it was between 8.4-21 °C, while in 2017 the highest average temperature was recorded between 18.5-31.2 °C (Figure 1b).
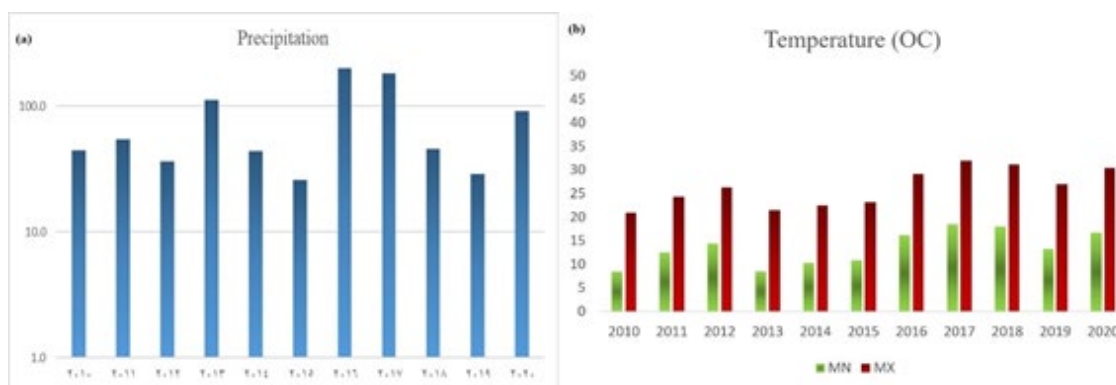

Figure 1: (a, b) Average annual rainfall and average minimum and maximum temperature (oC) in the southwestern region of the Kingdom between the years 2010-2020

3. Preparation of Plant Samples
Plant samples were collected from their natural locations in the southwestern region of the Kingdom of Saudi Arabia (KSA). The detailed information about the collection locations is presented in (Table 1).

Taxa	Collected location	Coordinates
Datura innoxia	Tehama (Bellow the obstacle logo)	18.2615426,42.3876575
Datura stramonium	Tabbab Village	18.4129811,42.3452565
Nicotiana glauca	Extension of Soda Park Road	18.2615426,42.3876575
Withania somnifera	Abha Valley	18.2631911,42.5601736

Table 1: Plant species and their places of collection

4. Morphological Characteristics

The plant samples of each species used for the current study were collected from their natural habitats(Figure 2), then the appearance of the shoot system of each of the stem and leaves was examined in terms of the shape, edge, base, and tip of the blade, then the inflorescence and flower density on the axis were examined, the calyx, corolla, stamens, and pistils were examined, and all results were recorded using standard procedure.

Figure 2: Study plants in their natural locations
(a) D. innoxia (b) D. stramonium (c) N. glauca (d) W. somnifera

5. DNA Barcoding Analysis

The DNA was extracted from each species using the DNA was extracted using the Gene JET plant genomic DNA purification mini kit (Thermo Scientific) following the protocol instructions suggested by the manufacturers. For species identification, five DNA barcoding markers (ITS, psbK-psbl, matK, trnH-psbA, and rbcl) were used. The amplicon size was estimated using the protocol suggested by [20]. The polymerase chain reaction (PCR) was used with the following instructions 50 ng genomic DNA, five pmol of each marker (forward & reversed), 1X PCR Master Mix (Promega, Fitchburg, WI, USA), and a volume of 25 µL were used in the PCR reaction.

Marker	Primer sequence 3'	Amplicon size	MT	References
psbK-psbI	F: TTAGCATTTGTTTGGCAAG R: AAAGTTTGAGAGTAAGCAT	544 bp	51oC	(Wang et al., 2010)
trnH-psbA	F: GTTATGCACGAACGTAATGCTC R: CGCGCGTGGTGGATTCACAATCC	300 bp	55 oC	(Wang et al., 2010)
matK	F: CGTACTGTACTTTTATGTTTACGAG R: ATCCGGTCCATCTAGAAATATTGGTTC	862 bp	55 oC	(Suzuki et al., 2014)
atpF-atpH	F: ACTCGCACACACTCCCTTTCC R: GCTTTTATGGAAGCTTTAACAAT	675 bp	53 oC	(Wang et al., 2010)
rbcL	F: GTAAAATCAAGTCCACCACG R: ATGTCACCACAAACAGAGACTAAAGC	580 bp	56 oC	(Suzuki et al., 2014)
ITS	F: ATG CGA TAC TTG GTG TGA AT R: GAC GCT TCT CCA GAC TAC AAT	300 bp	55 oC	(Wang et al., 2010)

Table 2: List of DNA barcode primers used for the identification of invasive species

The reaction conditions include initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 1 minute, with the annealing temperature set for each marker (Table 2), and extension at 72°C for 1 minute for 35 cycles. The final extension temperature was set at 72°C for 5 minutes. The PCR product purification and sequencing were done by the company Macrogen in South Korea (https://www.macrogen.com/en/main/index.php) (accessed on 6 May 2021), ABI3730 automated sequencer was used for the sequencing of the same marker used as in the PCR reactions. The strands were sequenced, and ambiguous nucleotides were removed after cleaning. ClustalW alignment software was used to generate multiple DNA sequence alignments by removing 30 bp from the ends of each read, measuring the length of amplicon products [21,22]. The phylogenetic tree was constructed using the neighbor-joining (NJ) with 1000 bootstrap values with Mega X using Matrix Representation with Parsimony method [23,24]. The cleaned sequences are then submitted to NCBI for accession numbers.

6. Evolutionary Analysis by Maximum Likelihood Method
The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [25]. The tree with the highest log likelihood (-17226.03) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with a superior log-likelihood value. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 32 nucleotide sequences. There were a total of 2328 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [24].

7. Results
Datura innoxia is an annual to perennial herb 1-2 m high, with a spreading crown up to 2 m in diameter. Stem with dense, spreading glandular hairs. Mature leaves broadly ovate, the lamina up to 20 cm long, almost entire, slightly sinuate, or irregularly lobed towards the base. Solitary terminal or axillary flowers, pentamerous hermaphrodite, peduncles height is up to 10 mm. Cylindrical Calyx, 8-9cm long, Corolla white with green veins, infundibuliform, 17-19 cm long. Stamens not exerted, anthers 8-10 mm long. Style 10-14 cm long, stigma well below anthers.
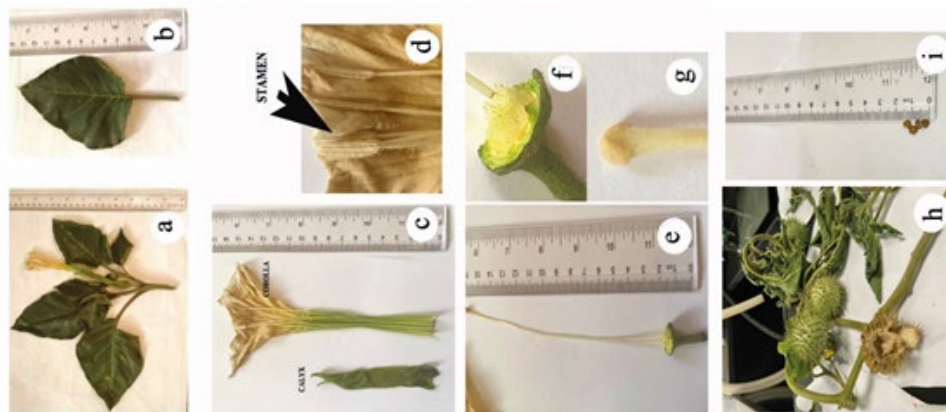

Figure 3: *D. innoxia* (a) Branch of a plant (b) leaf (c) Calyx and Corolla (d) stamens (e) gymnasium (f) ovary (g) stigma (h) Fruits (i) seeds

The fruit shape is a globose or ovoid spiny capsule with numerous slender spines, about 3-5 cm in diameter, deflexed, spiny; spines numerous, slender, sharp, all about equal in length, to 10 mm long; the persistent base of the calyx to 20 mm long, very prominent. Capsule breaking irregularly when ripe, releasing brown seeds. Seeds D-shaped Table (3; Figure 3).

Datura stramonium is an annual to perennial herb 1-1.5 m high, with a spreading crown up to 1.5 m in diameter. Stems smooth. Mature leaves ovate, the lamina is up to 20 cm long, almost dentate, slightly lobed, or irregularly lobed towards the base. Solitary terminal or axillary flowers, pentamerous hermaphrodite, peduncles height is up to 9-10 mm, solitary terminal or axillary flowers, pentamerous hermaphrodite, peduncles height is up to 9-10 mm. The calyx is narrowly cylindrical. Corolla is white, infundibuliform, with a tube 14-15 cm long. Five epipetalous stamens, anthers 7-9 mm long. Style 10-14 cm long, stigma well below anthers. The fruit is a globose or ovoid spiny capsule with numerous slender spines, about 3-5 cm in diameter, deflexed, spiny; spines numerous, slender, sharp, all about equal in length, to 10-15 mm long; the persistent base of the calyx to 20 mm long, very prominent. Capsule erect breaking irregularly when ripe, releasing black seeds (Table (3; Figure 4).
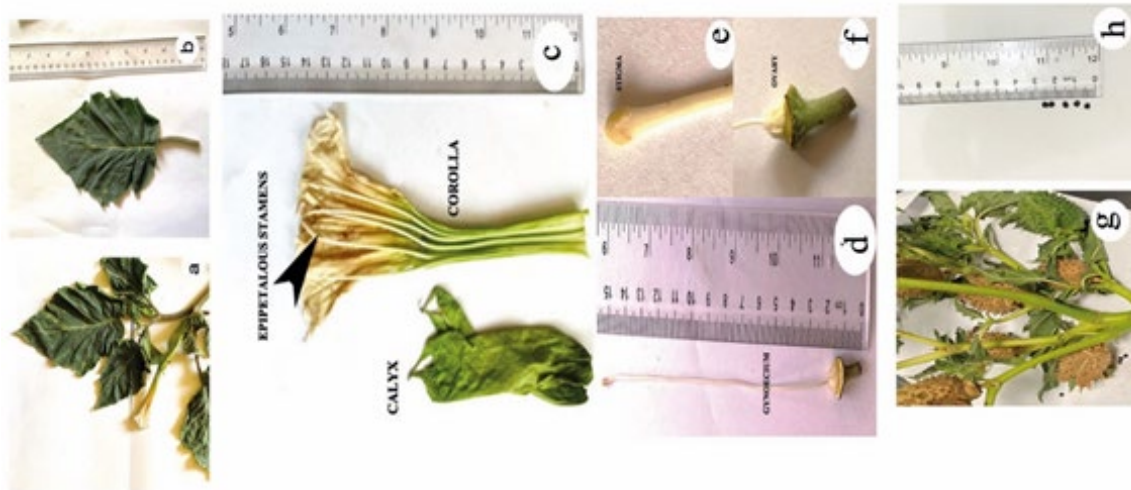
Figure 4: *D. stramonium* (a) Branch of a plant (b) leaf (c) Calyx, Corolla, and stamens (d) gymnasium (e) stigma (f) ovary (g) Fruits (h) seeds

Nicotiana glauca is a shrub or small tree, up to 6 m tall, with stems that are laxly branched, smooth green hairless branches, glaucous leaves. The leaves are alternate, elliptical to lanceolate or oval, pointed, bluish, or greyish green. The tubular flowers are greenish-yellow 30–40 mm long. Many are borne in a lax panicle. The corolla is tubular with a short-lobed limb. The fruit is an egg-shaped, two-valved capsule, 7–10 mm long and slightly longer than the persistent papery calyx. It produces many small seeds, which can be dispersed by wind and water (Table 3; Figure 5).
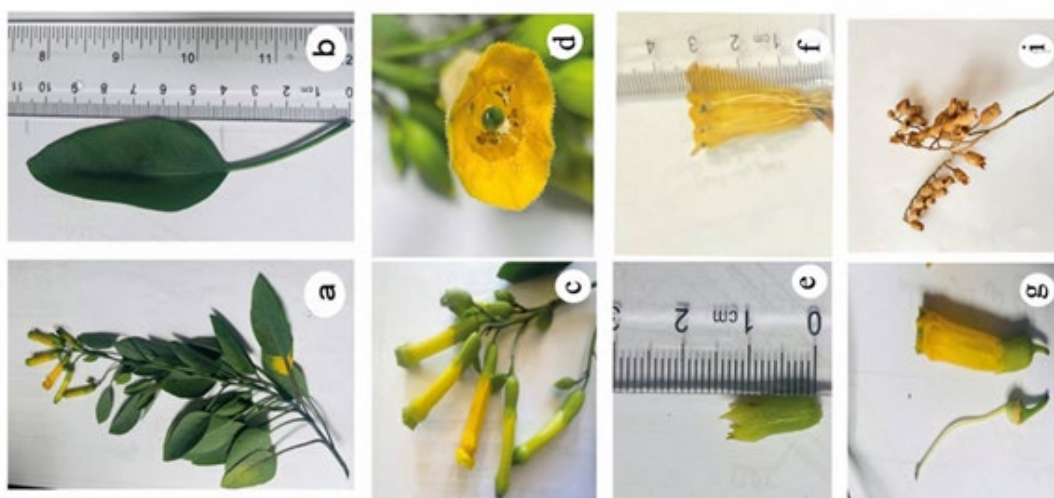

Figure 5: *N. glauca* (a) Branch of a plant (b) leaf (c) inflorescence (d) flower (e) calyx (f) corolla and stamens (g) gynoecium (h) Fruits

Withania somnifera recorded an erect, branched, grayish, stellate-tomentose under-shrub, 90- 150 cm high. Leaves are simple, petiolate with the leaf blade varying in shape from elliptic-ovate to broadly ovate, entire along margins, acute to obtuse at apex, cuneate or oblique at base, clothed with a persistent grayish tomentum on sides, 4-10 cm long and 2-7 cm broad. Leaves on vegetative shoots are alternate and large and those floral branches are opposite, arranged somewhat laterally in pairs of one large and one small leaf, bearing in their axile a cymose cluster of 5-25 inconspicuous pale green bisexual flowers. W. somnifera has exhibited stigma-another proximity caused by elongation of filaments to cover the bilobed stigmatic surface with dehiscing anthers. High pollen load on the stigma and stiff pollen competition within a flower strongly favor self-pollination (Table 3; Figure 6).
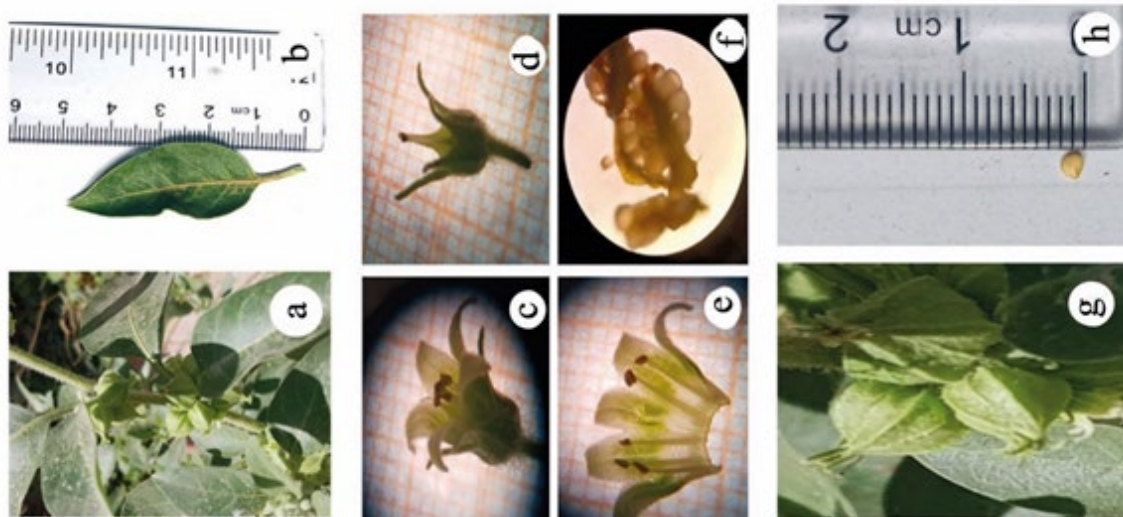
Figure 6: W. somnifera (a) Branch of a plant (b) leaf (c) Corolla (d) Calyx (e) stamens (f) ovary (g) Fruits (h) seeds

Taxa	Life form		Height Of the Plant M	The Plant Color	Stem					leaf shape	Inflorescence	pedicel Height (mm)	Calyx Height (mm)	Corolla Height (mm)	Fruit		
	Herbaceous	Shrub			An erect	glabrous	Pubescent	branched	unbranched						spiny surface	brownColor	CapsuleType
D. innoxia	+	-	1.2	Gray Green	+	-	+	+	-	ovate	-	20-25	80-90	17-19	spiny	brown	Capsule
D. stramonium	+	-	1.5	Yellowish Green	+	+	-	+	-	ovate	-	10-15	70-80	14-15	spiny	brown	Capsule
N. glauca	-	+	4.3	Bluish to Gray Green	+	+	-	+		ovate-elliptic	raceme	10-15	13-15	13-15	smooth	brown	Berry/Capsule
W. somnifera	+	-	0.6	gray green	+	-	+	+	-	ovate- broadly ovate	axillary	2-4	11-15	5-15	smooth	red	Berry/Capsule

Table 3: Morphological characteristics of the invasive plant species recorded during the study

8. DNA Barcoding Identification

The DNA barcoding results for the identification and their accession numbers of 4 invasive species were presented in Table 4. All the primers identified the four invasive species as i.e., D. inoxia, D. stramonium, N. glauca, and W. somnifera respectively. The sequences generated from each primer and species. The cleaned sequenced were then aligned and a species-level phylogenetic tree was constructed using a divergence score matrix representing each separate species in one group and represents as D. inoxia, D. stramonium, N. glauca, and W. somnifera respectively.

Collection Site	ITS	matK	be	rbcl
18.2615426,42.3876575	D.inoxia	D.inoxia	D.inoxia	D.inoxia
	OM674667	OM616890	OM616891	OM616892
18.4129811,42.3452565	D.stramonium	D.stramonium	D.stramonium	D.stramonium
	OM843134	OM616887	OM616888	OM616889
18.2615426,42.3876575	N. glauca	N. glauca	N. glauca	N. glauca
	OM281764	OM646550	OM646552	OM646551
18.2631911,42.5601736	W. somnifera	W. somnifera	W. somnifera	W. somnifera
	OM017149	OM047180	OM305000	OM305001

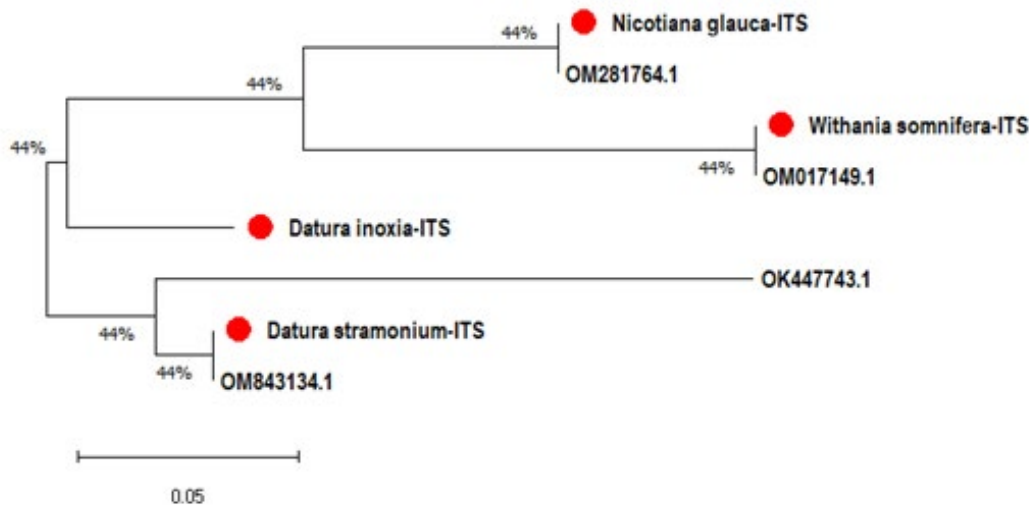**Table 4: DNA barcoding results and their accession Numbers**

Figure 7: Phylogenetic tree constructed based on concatenated ITS sequences (Nicotiana glauca, Withania somnifera, Datura Inoxia, and Datura stramonium) along with reference sequence from NCBI using maximum likelihood and neighbor-joining with 1000 bootstrap value with Maga X.

The phylogenetic tree for ITS sequences represents, all the four species belong to different genera and remains separate in the group but showed maximum similarity with NCBI corresponding reference sequences (Figure 7). Furthermore, the combined phylogenetic tree was also constructed using all the sequences generated from the barcode primers as well as ITS spacer sequences along with the corresponding NCBI reference sequence. There were four clear groups formed based on (ITS, matK, psbA, and rbcL) sequences. Each group contains the four identified by each primer but represents as separate species. However, Datura Inoxia-matK remains separate and does not make any group with any one of the species except the HQ602920.1 and KC146630.1 (Figure 8).
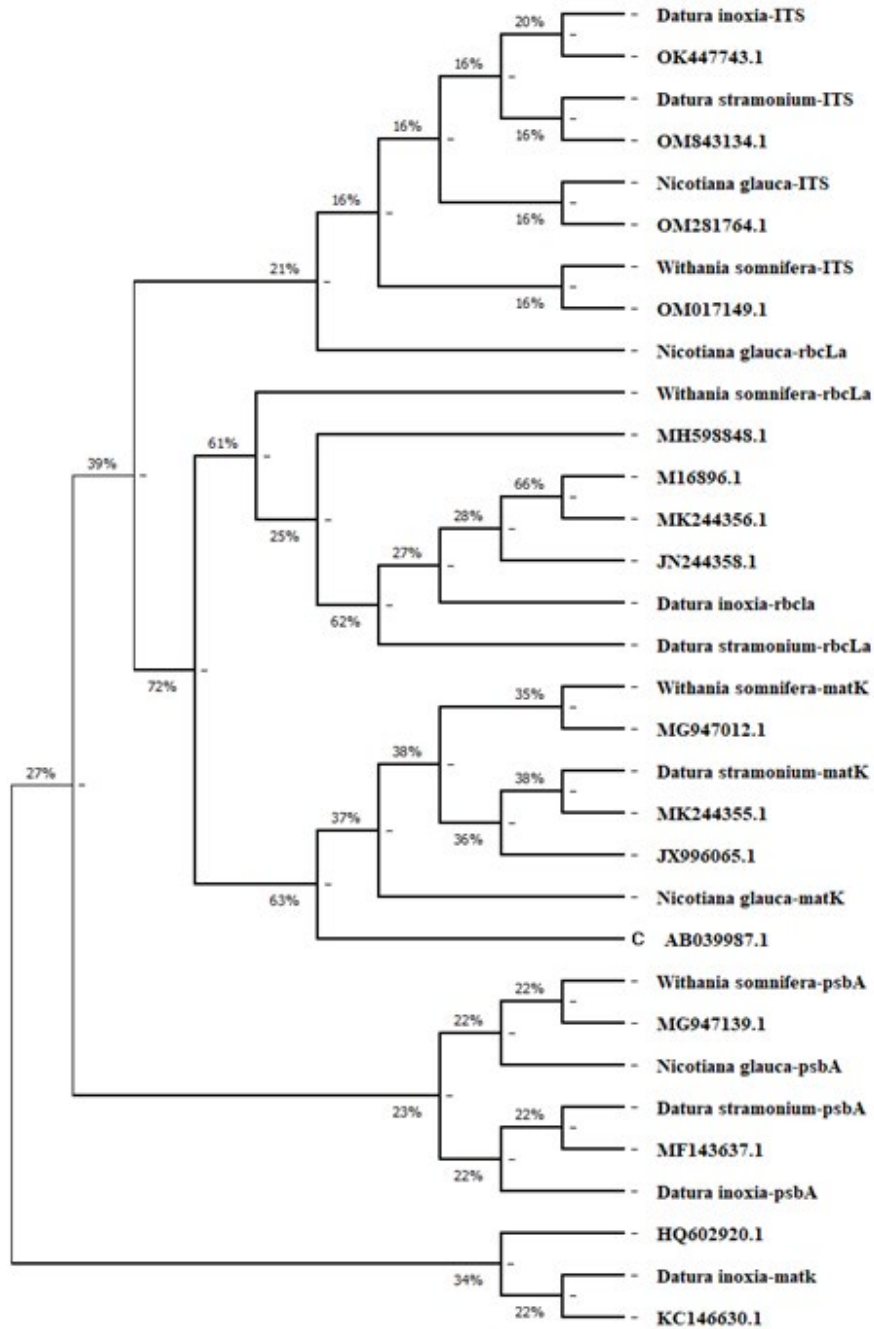
Figure 8: Combine phylogenetic tree constructed using Maga X with 1000 bootstrap value of concatenated ITS, matK, psbA, and rbcl generated sequences from four Solanum species and their represented sequences from NCBI. The percentage of bootstrap value from 1000 replicates is represented by the number of nodes. The horizontal bar represents the genetic distance.

9. Discussion

The invasive species of the different studied genera of Solanaceae spread in their environments in the form of close individuals, and are always seen in monodominant stands, and form dominant groups that compete or disappear with the local species, and this is consistent with the nature of invasive plants where the rates of fruiting and seed vitality are high leads to an increase in the rate of seed growth [26]. Similarly, the low plains such as in the Tehama plains, are located between the valleys, dunes, and coastal areas, have the Datura innoxia, while in the highlands, which are between 800-2700, other species such as Datura stramonium and Withania somnifera are widespread species [26]. As for Nicotiana glauca, it grows between 0.000 to 3700 meters above sea level [27-29]. On average, all species were found in most habitats, but they differ in terms of abundance and density, as they were common in neglected lands, valleys, and on the sides of high roads. It was also noted that the extent of plant growth, size, and the speed of its detection was related to the amount of rain in the respective environment [30]. Invasive plant species increased their distribution range in both lowlands and highlands and are therefore considered the most troublesome in Saudi Arabia [8]. The studied invasive species have adapted to drought and salinity, some of them are sparsely hairy plants in species D. innoxia and W. somnifera, to reduce

water loss [27,28]. Whereas in other species, D. stramonium, N. glauca, the appearance of the plant was smooth, glabrous, and consistent with what was mentioned in the study, the leaves of N. glauca are covered with a waxy substance to preserve the water inside [26,31].

Based on the morphological and molecular identification results suggested that these species are different from each other. Similar results were suggested by for identifying species based on morphological traits, however, a detailed key is available. However, the use of this key is significant for the identification of species around the world is limited due to a lack of experience and expertise in morphological taxonomy [32]. Due to a lack of morphological markers, we used five different plastid markers to identify Solanaceae species in Saudi Arabia.

Similarly, Johnson and Soltis, (1994) suggested that the barcoding markers such as matK, rbcL, psbA-trnH showed a higher rate of nucleotide substitutions, making it one of the fastest evolving protein-coding regions in chloroplast DNA (cpDNA). However, intergenic spacers evolve more quickly than coding regions, with psbA-trnH being one of the cpDNA's fastest evolving intergenic spacers [33,34]. Except for the matK tree, all the trees clustered individuals of the same species together and separately from the other species, with strong bootstrap support indicating that they were more closely related. Previous phylogenetic studies based on the combined analysis recorded similar results i.e., ITS, waxy, and trnS-trnG sequences and ITS, waxy, ndhF-rpL32, trnStrnG, and trnT-trnF sequences studies [35,36]. Similar results were reported by suggested that the ability of matK and psbA-trnH to produce species-specific groups singly could be explained by sufficient phylogenetic grouping, but this was not the case for rbcL [37]. matK had higher bootstrap support for the S. melongena group than psbA-trnH. In contrast to matK, psbA-trnH trees were able to resolve the close intraspecific relationships for S. violaceum. These results might be due to the markers originating from the chloroplast genome and having the same evolutionary history, and this was confirmed with concatenated without a congruence sequence test [38].

10. Conclusion
It is difficult for observers to be sure about the status of invasion in the field, especially as these are often side observations made while performing other work in restoration, animal, or plant surveys or just field expeditions. The lack of expertise on biological invasions in Saudi Arabia may also lead to confusion between invasive, established knowledge available on invasive alien floras and plant species is highly heterogeneous in the country. The Solanaceae species collected from the southern parts of Saudi Arabia were morphologically and molecularly characterized in this study. Also, historical differences among regions and differences in land use might explain some differences in the number of invasive species. When a country is as large as Saudi Arabia, has challenges, assessing its biota due to its size and diversity of ecosystems. This research assessment of plant invasions in Saudi Arabia is far from complete or fully accurate. Furthermore, the application of barcode genes identification helps the identification and phylogenetic relationship among the closely related genera. The study is of official invasive species lists is important to provide evidence and support towards researchers and institutions communities to build-up prevention and early detection techniques and regional strategies to mitigate the threats posed by biological invasions [39-47].

Data Accessibility and Benefit -Sharing
Sequence reads and date are deposited to (NCBI
National Center for Biotechnology Information (nih.gov).
All data have been shared with the broader public via appropriate biological databases.
Benefits from this research accrue from the sharing of our data and results on public databases as described above.

Reference
1. Reichard, S. H., & White, P. (2001). Horticulture as a pathway of invasive plant introductions in the United States: most invasive plants have been introduced for horticultural use by nurseries, botanical gardens, and individuals. BioScience, 51(2), 103-113.
2. Boy, G., & Witt, A. (2013). Invasive alien plants and their management in Africa. CABI Africa.
3. Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., & Losos, E. (1998). Quantifying threats to imperiled species in the United States. BioScience, 48(8), 607-615.
4. Geesing, D., Felker, P., & Bingham, R. L. (2000). Influence of mesquite (Prosopis glandulosa) on soil nitrogen and carbon development: Implications for global carbon sequestration. Journal of arid environments, 46(2), 157-180.
5. Richardson, D. M., Pyšek, P., Rejmanek, M., Barbour, M. G., Panetta, F. D., & West, C. J. (2000). Naturalization and invasion of alien plants: concepts and definitions. Diversity and distributions, 6(2), 93-107.
6. Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. Trends in ecology & evolution, 17(4), 164-170.
7. Solorzano, J., Hernandez, M., Go, V., & Roberts, E. (2018). Distribution of Nicotiana Glauca at Eaton Canyon.
8. Thomas, J., El-Sheikh, M. A., Alfarhan, A. H., Alatar, A. A., Sivadasan, M., Basahi, M., ... & Rajakrishnan, R. (2016). Impact of alien invasive species on habitats and species richness in Saudi Arabia. Journal of Arid Environments, 127, 53-65.
9. Abulfatih, H. A. (1981). WildplantsofAbhaanditssurround-ings.
10. Masrahi, Y. S. (2015). A new species of Leptadenia (Apocynaceae) and two other new records from southwestern Saudi Arabia. Saudi Journal of Biological Sciences, 22(5), 631-636.
11. Hosni, H. A., & Hegazy, A. K. (1996). Contribution to the flora of Asir, Saudi Arabia. Candollea, 51, 169-202.
12. Siqueira-Silva, A. I., Pereira, E. G., Modolo, L. V., Lemos-Filho, J. P., & Paiva, E. A. S. (2016). Impact of cement dust pollution on Cedrela fissilis Vell.(Meliaceae): A potential bioindicator species. Chemosphere, 158, 56-65.
13. Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome

fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20(2), 176-183.

14. Shree, P., Mishra, P., Selvaraj, C., Singh, S. K., Chaube, R., Garg, N., & Tripathi, Y. B. (2022). Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants–Withania somnifera (Ashwagandha), Tinospora cordifolia (Giloy) and Ocimum sanctum (Tulsi)–a molecular docking study. Journal of Biomolecular Structure and Dynamics, 40(1), 190-203.

15. Bog, M., Appenroth, K. J., & Sree, K. S. (2019). Duckweed (Lemnaceae): its molecular taxonomy. Frontiers in Sustainable Food Systems, 3, 117.

16. Chowdhury, S., & Al-Zahrani, M. (2015). Characterizing water resources and trends of sector wise water consumptions in Saudi Arabia. Journal of King Saud University-Engineering Sciences, 27(1), 68-82.

17. Alwhibi, M. S., Al Otaibi, B., Al Harbi, S., Aljulefi, A., & Aldosari, A. (2017). Plant Diversity in the King Saud University Female Campus. International Journal of Science and Research (IJSR), 7(10), 1066-1072.

18. Almazroui, M., Nazrul Islam, M., Athar, H., Jones, P. D., & Rahman, M. A. (2012). Recent climate change in the Arabian Peninsula: annual rainfall and temperature analysis of Saudi Arabia for 1978–2009. International Journal of Climatology, 32(6), 953-966.

19. Athar, H. (2015). Teleconnections and variability in observed rainfall over Saudi Arabia during 1978–2010. Atmospheric Science Letters, 16(3), 373-379.

20. Wang, W., Wu, Y., Yan, Y., Ermakova, M., Kerstetter, R., & Messing, J. (2010). DNA barcoding of the Lemnaceae, a family of aquatic monocots. BMC Plant Biology, 10, 1-11.

21. Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research, 22(22), 4673-4680.

22. Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2003). Multiple sequence alignment using ClustalW and ClustalX. Current protocols in bioinformatics, (1), 2-3.

23. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, 35(6), 1547.

24. Ragan, M. A. (1992). Phylogenetic inference based on matrix representation of trees. Molecular phylogenetics and evolution, 1(1), 53-58.

25. Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular biology and evolution, 10(3), 512-526.

26. Alsenidi, M. D., Moustafa, M. F., & Alrumman, S. A. (2018). Assessing genetic diversity of invasive plant Nicotiana glauca in Abha region, Saudi Arabia by RAPD, ISSR and mixed biomarkers. International Journal of Agriculture and Biology, 20(7), 1471-1478.

27. Alharthi, A. S., Abd-ElGawad, A. M., & Assaeed, A. M. (2021). Influence of the invasive shrub Nicotiana glauca Graham on the plant seed bank in various locations in Taif

28. Ibrahim, H. M., Abdo, N. A., Al Masaudi, E. S., & Al-Gifri, A. N. A. (2016). Morphological, epidermal and anatomical properties of Datura Linn. leaf in Sana'a City-Yemen and its taxonomical significance. Asian Journal of Plant Science and Research, 6(4), 69-80.

29. Maslo, S., & Šarić, Š. (2019). Datura innoxia Mill. (Solanaceae), a new alien species in the flora of Bosnia and Herzegovina. Thaiszia, 29, 225-230.

30. Olmstead, R. G., Bohs, L., Migid, H. A., Santiago-Valentin, E., Garcia, V. F., & Collier, S. M. (2008). A molecular phylogeny of the Solanaceae. Taxon, 57(4), 1159-1181.

31. Chaudhary, S. A. (2001). Flora of the Kingdom of Saudi Arabia. Ministry of Agriculture and water. National Herbarium, National Agriculture and Water Research Center, Riyadh, KSA.

32. Landolt, E. (1980). Key to the determination of taxa within the family of Lemnaceae. Veröffentlichungen des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel, Zürich, 70, 13-21.

33. Johnson, L. A., & Soltis, D. E. (1994). matK DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. Systematic botany, 143-156.

34. Sun, X. Q., Bai, M. M., Yao, H., Guo, J. L., Li, M. M., & Hang, Y. Y. (2013). DNA barcoding of populations of Fallopia multiflora, an indigenous herb in China. Genet Mol Res, 12(3), 4078-4089.

35. Levin, R. A., Myers, N. R., & Bohs, L. (2006). Phylogenetic relationships among the "spiny solanums"(Solanum subgenus Leptostemonum, Solanaceae). American journal of botany, 93(1), 157-169.

36. Aubriot, X., Singh, P., & Knapp, S. (2016). Tropical Asian species show that the Old World clade of 'spiny solanums'(Solanum subgenus Leptostemonum pro parte: Solanaceae) is not monophyletic. Botanical Journal of the Linnean Society, 181(2), 199-223.

37. Tegally, A., Jaufeerally-Fakim, Y., & Dulloo, M. E. (2019). Molecular characterisation of Solanum melongena L. and the crop wild relatives, S. violaceum Ortega and S. torvum Sw., using phylogenetic/DNA barcoding markers. Genetic Resources and Crop Evolution, 66, 1625-1634.

38. Asmussen, C. B., Dransfield, J., Deickmann, V., Barfod, A. S., Pintaud, J. C., & Baker, W. J. (2006). A new subfamily classification of the palm family (Arecaceae): evidence from plastid DNA phylogeny. Botanical Journal of the linnean Society, 151(1), 15-38.

39. Ashraf, M., Ahmad, M. S. A., Öztürk, M., & Aksoy, A. (2012). Crop improvement through different means: Challenges and prospects. Crop production for agricultural improvement, 1-15.

40. Bogdanović, S., Mitić, B., Ruščić, M., & Dolina, K. (2006). Nicotiana glauca Graham (Solanaceae), a new invasive plant in Croatia. Acta Botanica Croatica, 65(2), 203-209.

41. De-la-Cruz, I. M., Hallab, A., Olivares-Pinto, U., Tapia-López, R., Velázquez-Márquez, S., Piñero, D., ... & Núñez-Farfán, J. (2021). Genomic signatures of the evolution of defence against its natural enemies in the poisonous and medicinal plant Datura stramonium (Solanaceae). Scientific Reports, 11(1), 882.

42. Furer, V., Hersch, M., Silvetzki, N., Breuer, G. S., & Zevin, S. (2011). Nicotiana glauca (tree tobacco) intoxication— two cases in one family. Journal of medical toxicology, 7, 47-51.

43. Ghimire, B., & Ghimire, B. K. (n.d.). 가지과 Withania somnifera 의 종자 형□. 41(2), 5.

44. Knapp, S., Bohs, L., Nee, M., & Spooner, D. M. (2004). Solanaceae—a model for linking genomics with biodiversity. Comparative and functional genomics, 5(3), 285-291.

45. Li, X., Yang, Y., Henry, R. J., Rossetto, M., Wang, Y., & Chen, S. (2015). Plant DNA barcoding: from gene to genome. Biological Reviews, 90(1), 157-166.

46. Suzuki, J. Y., Matsumoto, T. K., Keith, L. M., & Myers, R. Y. (2014). The chloroplast psbK-psbI intergenic region, a potential genetic marker for broad sectional relationships in Anthurium. HortScience, 49(10), 1244-1252.

47. Tole, T. T., & Saifu, Y. T. (2019). Phytochemical investigation of Withania somnifera and Commiphora kataf. Journal of Pharmacognosy and Phytochemistry, 8(3), 2958-2964.