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MORPHOLOGICAL CHARACTERIZATION AND MOLECULAR IDENTIFICATION OF S. MELONGENA L. AND D. STRAMONIUM L

Malik Abdul Rahim[*1], Aftab Iqbal[2], Abdul Maajid Khokhar[3], Sabaht Nazir[4]

*[*1,4]Department of Botany, Government Graduate College of Science, Wahdat Road, Lahore.*
[2]Kauser Abdulla Malik, School of Life Sciences, Forman Christians College University, Lahore.
[3]Department of Public Health, Riphah International University Faisalabad, 44000, Pakistan.

[*1]28060malikabdulrahim@gmail.com

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Corresponding Author: *
Malik Abdul Rahim

Abstract

Two important plant Solanum melongena L. and Datura stramonium L. belonging to family Solanacae were taken due to medicinal and genetical significance purpose. Eggplant (S. melongena L.) is an important crop in terms of economic and genetic significance in tropical and subtropical regions. Eggplant has antioxidants like vitamins A and C, which help protect your cells against damage. It's also high in natural plant chemicals called polyphenols, which may help cells do a better job of processing sugar if you have diabetes. The parts of plant D. stramonium L. are utilized as a part of people's medication for their anti-asthmatic, anti-spasmodic, and anti- parkinsonian properties. D. stramonium has been utilized broadly in medication. The juice of its fruit is applied to the scalp, to treat dandruff and falling hair. An accurate identification of plants is essential to the safety, efficacy, and effectiveness of herbal remedies. By using DNA barcoding, we identified two locally grown plants. To elucidate the variation at molecular level this study was done by DNA barcoding. An analysis of DNA barcoding was conducted to elucidate the variation at the molecular level. With DNA barcoding, species can be identified quickly and accurately by using short, standardized gene regions. As part of our present research, DNA was extracted, gel electrophoresed, PCR amplification, DNA sequenced, and analyzed. Using conventional PCR techniques, genomic DNA was extracted from plant leaves samples and amplified. Plant species were compared and differentiated using short sequence diversity in matK and rbcL genes of plastid genomes. Purified and sequenced samples of both plants were then obtained. Based on NCBI database blasting, all samples show high similarity to homologs.

INTRODUCTION

The Solanaceae or "nightshade" family is an economically important group with remarkable diversity. Shrubs or perennial to annual herbs, rarely trees, rosette-forming or ephemerals, sometimes with tuberous or gemmiferous roots, or with tubers or stolons; stems occasionally with heteroblastic growth or with cauline spines (Barboza et al., 2016). S. melongena also known as "Eggplant" belongs to the Solanaceae family and the genus Solanum (Sukprasansap et al., 2019). S. melongena is perennial to annual herb in the family of Solanaceae. It is an annual, biennial or more commonly perennial plants, succulents in Sclerophylax. The shrubs are 0.5m–6m tall or small trees ranges from 5–10 m tall. The roots

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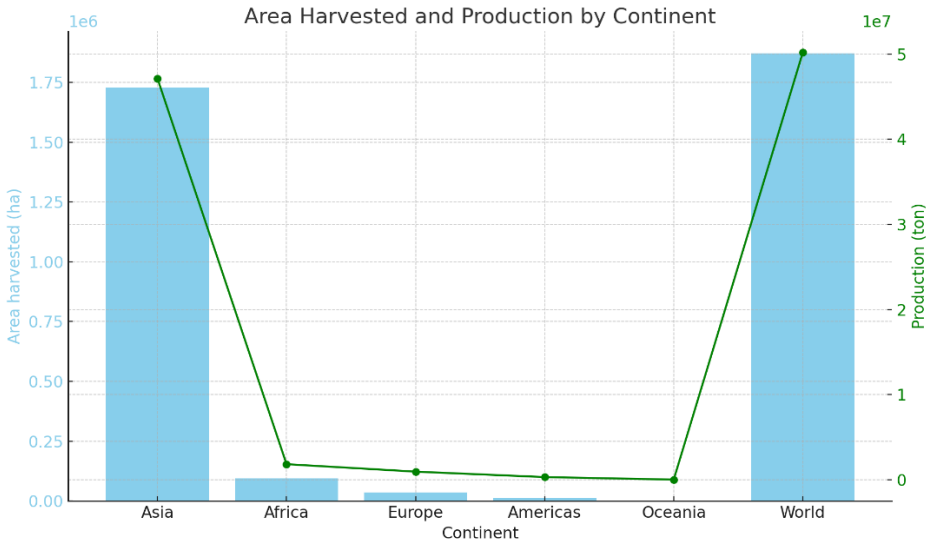
may be gemmiferous, tubers, stolons or rhizomes. Rarely greatly reduced (Barboza et al., 2016). It is an economically important vegetable crop of tropical and subtropical zones and their cultivars produce wide fruit diversity with different shapes, sizes and colors (San José et al., 2013)

S. melongena is one of the common plants which is growing all around the world, especially in Asian countries, the Middle East, and around the Mediterranean basin (Cericola et al., 2013; Daunay, 2008). In Pakistan, it is a popular vegetable that is grown in both tropical and subtropical regions all year long. The crop is cultivated on small family farms or gardens and is also a good source of income for resource-limited farmers in Pakistan (Shafique et al., 2021).



Graph 1: The graph compares the area harvested and production levels across different continents, highlighting the dominance of Asia in both metrics.

The graph compares the area harvested (in hectares) and production (in tons) across different continents. It highlights how much land is dedicated to agriculture and the resulting production. Asia leads both in area harvested and production, significantly outpacing other continents. Africa, with a smaller area harvested, shows relatively high production levels, indicating a more efficient use of land for agricultural output. Europe and Americas have lower figures compared to Asia but still contribute significantly to global production. Oceania, with the smallest area harvested, also produces the least in terms of agricultural output. The World category aggregates the data, offering a global perspective on agricultural scale and productivity. The graph emphasizes the variations in agricultural efficiency and the overall impact of each continent on global food production.

The Leaves of S. melongena are alternate which are mostly in pairs and represents the opposite in the inflorescence. They are usually simple, entire, pinnatifid to deeply dissected or compound and exstipulate (Barboza et al., 2016). The flowers are perfect and rarely functioning as unisexual in dioecious or andromonoecious plants. The Perianth is merous and calyx is actinomorphic or rarely zygomorphic. The corolla is actinomorphic or zygomorphic along with 2 fertile mobile stamens in lateral or dorsal position (Barboza et al., 2016).

S. melongena also has known as aubergine and it is an important source of fiber, minerals (iron, calcium, potassium, magnesium, sodium, zinc, and phosphorus), vitamins C, thiamin, niacin, B6, B12, A, E, D, and K (Gürbüz et al., 2018). S. melongena has been used in the treatment of several diseases, including asthma, bronchitis, diabetes, arthritis, and hypercholesterolemia (Magioli & Mansur, 2005). It is also used in clinical practice due its phenolic and alkaloid contents (Daunay, 2008).

S. melongena have also shown many health benefits. Several studies have been reported the pharmacologic aspects of eggplant, such as anti-oxidant (Kaur &

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Kapoor, 2002), anti-inflammatory (Im et al., 2016), antibacterial (Gubarev et al., 1998), antifungal (Das et al., 2010), antidiabetic (Qonita et al., 2013), antihypertensive (Yamaguchi et al., 2019), anti-obesity (Scorsatto et al., 2019), hepatoprotective (Komara et al., 2015) and hypolipidemic properties (Audu et al., 2014).

Vegetables are very important due to their considerable nutritional value (Shaheen et al., 2016) but most of the vegetable crops are known for their high sensitivity to salt and heat stress (Shaheen et al., 2016). Eggplant is an important summer vegetable of Pakistan exposed to high temperature stress, during hottest months (Faiz et al., 2020).

1.1. Morphology and Ecology of Datura

1.2. Stramonium L.

D. stramonium L. is an herbaceous annual plant that can grow up to 1.5 meters, and in rich soil, it may reach 6 feet. The plant has a thick stem, smooth leaves with short, curved hairs, and exudes a strong, unpleasant odor, especially when the leaves are crushed. The plant blooms in late spring, with large, attractive flowers that open at night and attract night-flying moths. The flowers have a white or pale violet corolla and produce a strong scent.

The root system consists of a long, thick, whitish taproot, and the plant has an erect, green or purple stem with forked branching. The seeds are small, kidney-shaped, and dark-colored. D. stramonium has various uses, including its psychoactive effects, and is traditionally used in herbal remedies, especially for asthma and pain relief. It has also been used in different cultures for religious and spiritual purposes. The plant is known for producing alkaloids like atropine, hyoscamine, and hyoscine, which have medicinal properties.

Datura is also used for its potential in metal accumulation and biomass production. While the plant has been historically used for its pharmacological effects, it is also known for its psychoactive impact, attracting some for recreational use. It is considered holy in some cultures, particularly in Nepal, and has been part of traditional medicine in various parts of the world.

2. Materials and Methods

This study was conducted at the Plant Molecular Biology Research Lab, Department of Botany, Government College of Science (GCS), Wahdat Road, Lahore, from May 2022 to September 2022.

2.1 Selection of Plant Species

S. melongena L. (eggplant) and D. stramonium L. (jimson weed), both from the Solanaceae family, were selected due to their medicinal, health, and economic benefits.

2.2 Selection of Plant Parts

Leaves from both plants were selected for DNA extraction due to their soft nature, which allows for easier cell content extraction, ultimately resulting in higher DNA yield for PCR and sequencing.

2.3 Sample Collection

Various visits were made to botanical gardens and other locations in Punjab (e.g., GCS Lahore, Changa Manga, Kasur, and Pakpattan) to collect fresh, newly grown leaves. These leaves were transported in polythene zipper bags and packed in cotton boxes to the laboratory for further processing.

2.4 Sample Preparation

The plant leaves were thoroughly washed with distilled water, followed by a 70% ethanol wash to remove dust and contaminants. After washing, the leaves were dried at room temperature and cleaned with filter paper to remove any remaining water. The midrib and petiole were then removed to avoid carbohydrates, and the leaves were cut into fine pieces. Finally, the cut leaves were wrapped in aluminum foil and stored at -20°C for further experiments.

2.5 Sterilization of Instruments

All necessary instruments (e.g., pestle and mortar, Eppendorf tubes, falcon tubes) were cleaned with methylated spirit after washing with tap water. They were then sterilized in an autoclave at 121°C and 15 psi for 30 minutes and transferred to a laminar air chamber for use.

2.6 DNA Extraction

DNA extraction was carried out using the Doyle and Doyle method with minor modifications.

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Approximately 100 mg of leaf tissue was ground in liquid nitrogen, and 1.2 mL of 3% CTAB buffer was added to the sample. The protocol was followed as described (Doyle and Doyle, 1990).

2.7 Preparation of Stock Solutions

Stock solutions for various reagents used in DNA extraction were prepared as follows: 10 g of CTAB was dissolved in 100 mL of distilled water to prepare a 10% CTAB solution. To prepare a 5M sodium chloride (NaCl) solution, 29.22 g of NaCl was dissolved in 100 mL of distilled water. For a 2M Tris-Base solution, 24.29 g of Tris-Base was dissolved in 100 mL of distilled water. A 10% polyvinyl pyrrolidone (PVP) solution was made by dissolving 10 g of PVP in 100 mL of distilled water. Finally, for a 0.5M EDTA solution, 18.61 g of EDTA was dissolved in 100 mL of distilled water.

2.8. DNA Extraction, Amplification, and Sequencing Protocol

The CTAB extraction buffer working solution was prepared by combining appropriate volumes of stock solutions, with β-mercaptoethanol added just before use. The 50X TAE buffer was prepared by dissolving Tris-Base, acetic acid, and EDTA in distilled water, and the 1X TAE buffer was prepared by diluting the 50X TAE buffer with deionized water. DNA isolation was performed by crushing 1.5-2 cm of newly developed leaves in liquid nitrogen and adding 1000 µL of CTAB extraction buffer. After mixing with chloroform: isoamyl alcohol (24:1) and centrifuging, the supernatant was collected, followed by another round of extraction. The aqueous phase was mixed with isopropanol and stored overnight at -20°C. DNA was then pelleted by centrifugation, resuspended in distilled water, and stored at -20°C. The quality of genomic DNA was assessed using agarose gel electrophoresis, with a 1% agarose gel prepared by adding 0.25 g of agarose to 25 mL of 1X TAE buffer, heating, and adding 5 µL of ethidium bromide. The DNA sample was mixed with 4 µL of 6X gel loading dye and loaded into the gel for electrophoresis, which was carried out at 80V for 30 minutes. The gel was then visualized under a UV trans illuminator. PCR amplification of the rbcl gene was conducted using specific primers in a Bio-Rad Thermocycler, with an initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C, annealing at 52°C, and extension at 72°C, with a final extension step at 72°C for 10 minutes. PCR products were analyzed by electrophoresis on a 1% agarose gel with a DNA ladder for size comparison. Once amplification was confirmed, the products were sent to CELEMICS BTSeq™ in Seoul, Korea, for sequencing. This methodology ensures reliable and reproducible results in the identification of S. melongena and D. stramonium using DNA barcoding techniques.

3. Results

3.1 Morphological Characteristics of Solanum melongena

S. melongena L., commonly known as brinjal, aubergine, or guinea squash, belongs to the Solanaceae family. It is an annual herb that thrives in both tropical and subtropical regions. While it is found worldwide, including Pakistan, it is native to Asia and Africa. The flowering period spans from April to September, during the summer months.

The stem of S. melongena is round, yellowish-green, and densely covered with short, white hairs. The leaves are simple, ovate in shape, and range from 10-23 cm in length and 9-15 cm in width. They are moderately pubescent, with 4-7 pairs of primary veins and a lobed margin. The flowers are white to mauve or purple and range from 2.5-5 cm in diameter. The fruit is a berry, globular to ovoid, and can grow between 3-20 cm in length, with a smooth pericarp that varies in color from green to purple and maroon upon maturity. The root system is deep, helping the plant withstand dry weather.

3.2 Morphological Characteristics of D. stramonium

D. stramonium L., also known as common thorn apple, jimson weed, or devil's trumpet, is an annual herbaceous plant from the Solanaceae family. It is commonly found in disturbed areas, agricultural lands, and roadsides. Native to North America, it is distributed across Pakistan, India, China, Europe, and West Asia. The flowering period occurs from January to June.

The stem of D. stramonium is cylindrical, solid, and sometimes woody at the base, with young twigs covered in simple hairs. The leaves are simple,

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alternate, and vary in size, with margins that are irregularly toothed. The flowers are large, white or purple, and have a narrow corolla tube that is 6-10 cm long. The fruit is a sub-globose capsule, thorny, and measures 3-6 cm long, containing numerous seeds. The root system is a taproot with many secondary white roots.

3.3 Molecular Analysis

DNA was extracted from the leaves of S. melongena and D. stramonium following the CTAB protocol, with liquid nitrogen used to enhance the extraction process. Agarose gel electrophoresis confirmed the presence of genomic DNA, which was then subjected to PCR amplification using rbcl primers. Gel electrophoresis was performed to confirm the amplification of the desired product. Finally, next-generation sequencing was conducted by sending the PCR products to CELEMICS BTSeq™ in Seoul, Korea, and phylogenetic analysis was performed using BLAST and MEGA X tools.

3.4 Total Genomic DNA Isolation

DNA was successfully extracted from both plant species, and its concentration was confirmed using agarose gel electrophoresis.

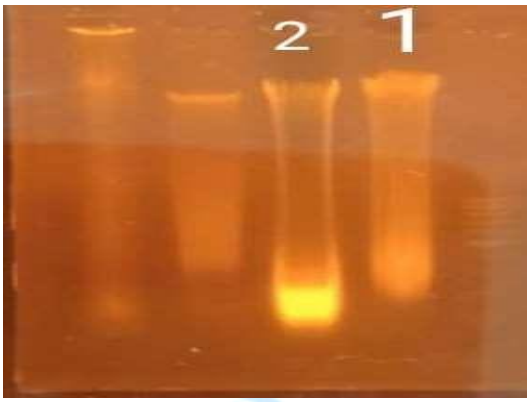

Figure 3.1: Gel electrophoresis of genomic DNA of S. melongena and D. stramonium (lanes 1 and 2) shows the presence of DNA.

3.5 Polymerase Chain Reaction (PCR)

The extracted DNA was amplified using specific forward and reverse primers. The amplified product was approximately 1000 bp, as confirmed by comparing its size with a 100 bp DNA ladder on a 1% agarose gel.

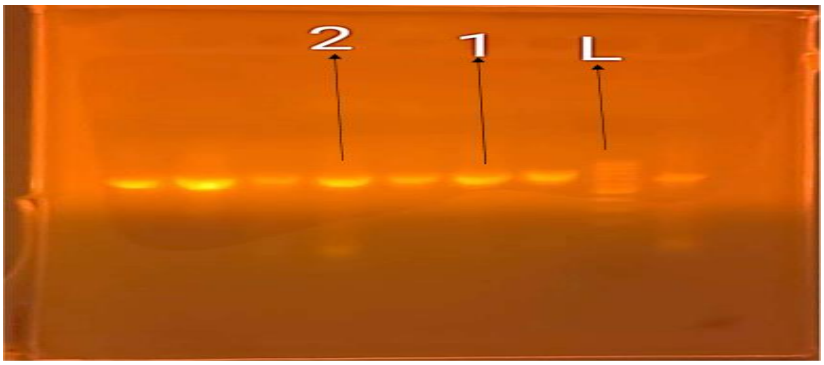

Figure 3.2: PCR amplification of tested samples (lanes 1 and 2) with rbcl forward and reverse primers. The bands, approximately 1000 bp in size, are clearly visible.

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3.6 DNA Sequence

The sequencing of S. melongena and D. stramonium was carried out, with the following sequences obtained:

DNA Sequence of Solanum melongena:

CCACAAACAGAAACTAAAGCAAGTGTTGGA TTCAAAGCTGGTGTTAAAGAGTACAAA... (Full sequence available in the document)

DNA Sequence of D. stramonium:

TAAGAGACAGTACCCGCAGTTGCATTCAAG TAATGTCCTTTAATTTCACCTGTTTCAG...
(Full sequence available in the document)

3.7 DNA Sequence Analysis

The DNA sequence of S. melongena was compared with similar sequences from other plant species, and likewise for D. stramonium. The sequence analysis showed high similarity with known species, confirming the accuracy of the DNA extraction and sequencing process.

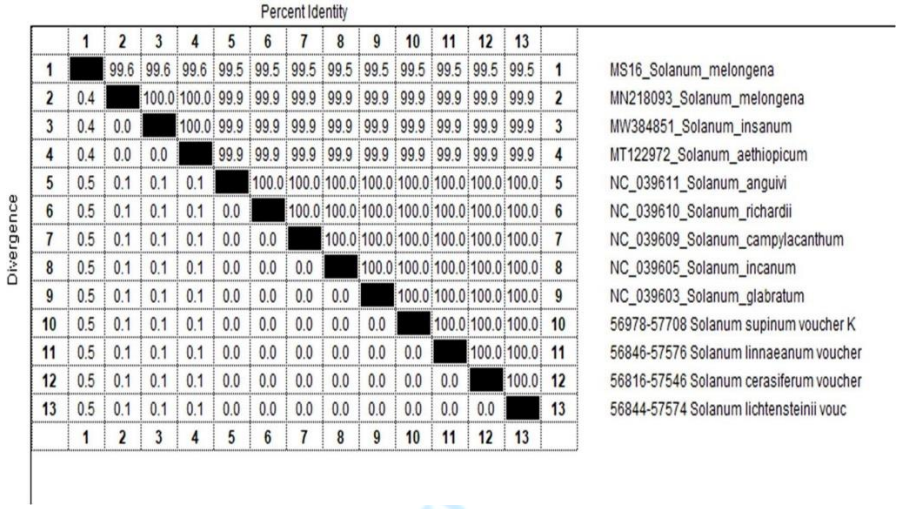

Figure 3.3: DNA sequence analysis of S. melongena with similar sequences from plant species.

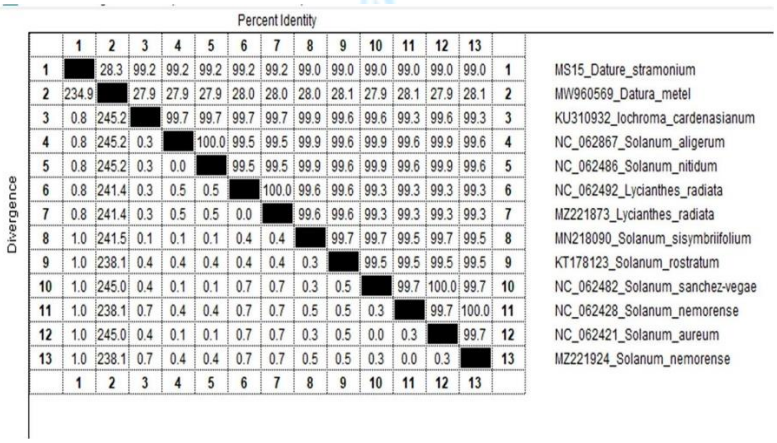

Figure 3.4: DNA Sequence analysis of Datura stramonium with similar sequences of plant species

3.8 Phylogenetic Analysis

A phylogenetic tree was constructed based on the DNA sequences of S. melongena and D. stramonium, using BLAST and MEGA X tools to analyze the genetic relationship between these species and other related plants.

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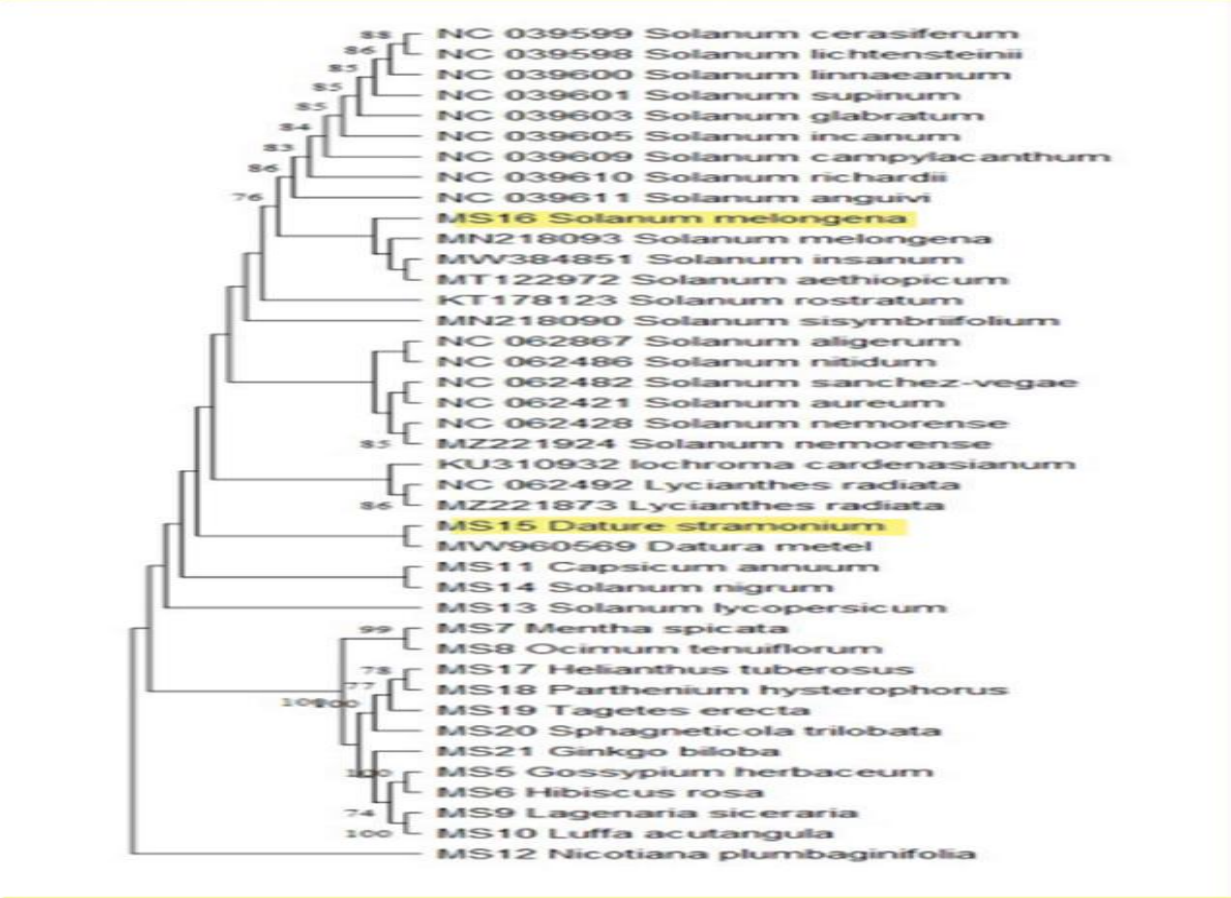

Figure 3.5: Phylogenetic analysis tree depicting the relationship of S. melongena and D. stramonium with other plant species.

These results confirm the successful molecular characterization of S. melongena and D. stramonium, supporting their identification and classification through DNA barcoding techniques.

4. Discussion

The Solanaceae or "nightshade" family is an economically important group with remarkable diversity. S. melongena and D. stramonium are two important members of this family. This variety of eggplant, also called brinjal, is an economically significant vegetable crop growing in tropical and subtropical regions. It is a popular vegetable that is grown in both tropical and subtropical regions all year long. The crop is cultivated on small family farms and is a good source of income for resource-limited farmers in Pakistan. D. stramonium is also a yearly weed of gardens, roadsides, and other waste places or developed land. It is broadly naturalized in warmer countries throughout the world, and it is very common in Pakistan, frequently showing up in open, aggravated spots, roadsides, pastures, livestock enclosures, agronomic and vegetable yield fields, plantations, vineyards, jettison banks, and bothered, unmanaged ranges (Niño-Medina et al., 2017).

It also has known as aubergine and it is an important source of fiber, minerals (K, Fe, Ca, Na, Mg. Zinc and P), vitamins C, thiamin, niacin, B6, B12, A, E, D, and K. Several studies have reported the pharmacologic aspects of eggplant, such as anti-oxidant, anti-inflammatory, antibacterial, antifungal, anti-diabetic, antihypertensive, anti-obesity, hepato-protective and hypolipidemic properties (Audu et al., 2014).

Vegetables are very important due to their considerable nutritional value but most of the vegetable crops are known for their high sensitivity to salt and heat stress. Eggplant is an important summer

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vegetable of Pakistan exposed to high temperature stress, during hottest months (Faiz et al., 2020).

D. stramonium is smooth, aside from a slight softness on the more youthful parts, which are secured with short, bended hairs, which tumble off as development continues. The Chinese utilize the blooms of D. stramonium in homegrown arrangements. Middle Easterners in Africa used to smoke the dried leaves, blossoms, and seeds in hookahs as a solution for asthma and influenza. Today, individuals smoke the dried leaves and seeds due to their opiate impacts and to mitigate asthma.

D. stramonium is a metal-tolerant plant and is being utilized to consider Zn^{2+} accumulation. Psychoactive impacts of Datura attract the youngsters to it. Oil produced from D. stramonium seeds is utilized to regrow hair, for treating gloom, and in India, people utilize it as an offering for master Shiva. In some regions of world, it is very sacred to the people for their divine love.it very contagious when inhaled or taken orally. The dose may cause death to adults for taking 15-100 g of leaf or 15-25 g of the seeds of it.

DNA barcoding methodologies are being increasingly applied not only for scientific purposes but also for diverse real-life uses. DNA barcoding has had a major impact on biodiversity science. The elegant simplicity of establishing massive scale databases for a few barcode loci is continuing to change our understanding of species diversity patterns, and continues to enhance human abilities to distinguish among species. Capitalizing on the developments of next generation sequencing technologies and decreasing costs of genome sequencing, there is now the opportunity for the DNA barcoding concept to be extended to new kinds of genomic data.

Conclusion

This study successfully characterized the morphological and molecular traits of S. melongena and D. stramonium, two important medicinal plants from the Solanaceae family. Morphological analysis revealed distinct features such as the leaf shape, flower characteristics, and fruit structure that differentiate these species. Furthermore, molecular analysis confirmed the presence of high-quality genomic DNA, which was successfully amplified and sequenced using PCR and next-generation sequencing. The DNA sequences obtained for both plants were compared with similar plant species, providing clear identification. The phylogenetic analysis also established the genetic relationships of these species with related plants. Overall, this study demonstrates the effectiveness of combining traditional morphological identification with modern DNA barcoding techniques for the accurate identification and classification of medicinal plants. The results contribute to the growing database of plant DNA barcodes and support further research.

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