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Pathogenicity and Phylogenetics of *Alternaria alternata* Affecting *Tulipa* L. in Greenhouse Conditions of the Botanical Garden

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ABSTRACT

The study aimed to assess the pathogenicity of the fungus *Alternaria alternata* on various *Tulipa L.* species and elucidate its phylogenetic position. The research focused on five specific tulip varieties: *T. Albatros*, *T. Tarda*, *T. Delta Storm*, *T. Biflora*, and *T. Biebersteiniana*. Methodologies included molecular analysis, microscopic examinations, cultivation of fungi on PDA, and sequencing of the 18S and 5.8S rRNA genes, as well as the D1/D2 region of the 26S rRNA gene. Results revealed variable pathogenicity across tulip species, with *T. Albatros* showing complete leaf damage and extensive conidium formation, while *T. Biebersteiniana* exhibited minimal damage. Factors influencing infection severity included plant variety, conidium formation, and environmental conditions. Sequencing confirmed the fungus's affiliation with the *Alternaria* genus and highlighted its close relation to other species. The findings underscore the importance of molecular methods for accurate pathogen identification and phylogenetic classification. These results are crucial for developing targeted disease management strategies and enhancing plant resilience in agriculture.The application of the findings is feasible within agriculture to develop resilient varieties and methods for managing the dissemination of A. alternata. Plant diseases involve complex interactions between pathogens and hosts, where fungi like *Alternaria alternata* disrupt plant physiology through toxin production and enzyme secretion, making effective management crucial.

Keywords: ecosystem, fungal species, sequencing, *Alternaria alternata* (Fr.) Keissl.

INTRODUCTION

Plant disease management involves understanding and controlling the complex interactions between plants and their phytopathogenic agents, including fungi. Effective strategies for managing plant diseases focus on preventing infection, minimizing disease spread, and mitigating the impact on plant health and yield. This encompasses practices such as regular monitoring of plant health, implementing resistant plant varieties, and employing integrated

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pest management (IPM) techniques. For example, in the case of fungi like *Alternaria alternata*, management practices may include the use of fungicides, crop rotation, and removal of infected plant material to reduce pathogen load and prevent future outbreaks (Khmelnitskaya *et al.*, 2003). Types of diseases caused by *Alternaria* spp. are widespread, and the symptoms are abundant. *Alternaria* belongs to cosmopolitans, affects all plant organs, and, most importantly, severely damages the quality and yield of products (Chacón *et al*., 2022; Ospanov *et al.*, 2018).

The distribution of fungi of the genus *Alternaria* was studied by Abiev (2002), Hannibal (2011), Kuprienko (2005), Maksimov *et al*. (2020), Salybekova *et al*. (2019). Along with saprophytic lesions of various plants, these species lead a parasitic lifestyle under favourable conditions (Abiev, 2002; Gannibal, 2011; Kuprienko, 2005; Maksimov *et al*., 2020; Ospanov *et al.*, 2020; Salybekova *et al*., 2019).

Alternaria leaf blight causes mold of fruits and seeds, contaminating agricultural products with toxins, allergens, or enzymes (Bauer *et al.*, 2023; Hannibal, 2011; Karabassov *et al*., 2018). It induces foliar lesions, impairing photosynthesis and leading to yield reductions. In India, tomato yield losses reached 78% (Mehmood *et al.*, 2020), while winter rape seed losses in Germany were up to 50% (Zhu *et al.*, 2021). Small-spore *Alternaria* species are major allergens, affecting about 3% of the European population (Bavbek *et al.*, 2006) and causing severe allergic reactions and infections (Bush & Prochnau, 2004; Fung *et al.*, 2000; Robertshaw & Higgins, 2005).

Research on *Alternaria* in greenhouse-grown *Tulipa L.* highlights the impact of temperature on disease development (Iqbal *et al.*, 2019; Kuroyanagi *et al.*, 2022; Otero-Blanca *et al.*, 2021; Pandit *et al.*, 2022; Srivastava *et al.*, 2021; Stauder *et al.*, 2020; Xu, 2023). At 35°C, 75% of *Tulipa L.* plants were affected by *Alternaria alternata* within 7 days, compared to 45% at 25°C and 60% at 30°C (Otero-Blanca *et al.*, 2021). Photosynthesis decreased by 40% in infected plants (Xu, 2023). The "Red Velvet" cultivar showed 85% infection, while "Golden Sunrise" had 30% (Stauder *et al.*, 2020). Infected plants had 25% less chlorophyll, 40% increased antioxidant enzyme activity, and reduced magnesium and iron concentrations (Kuroyanagi *et al.*, 2022). Elevated soil humidity increased infection intensity by 15% (Srivastava *et al.*, 2021). Infection during leaf formation reduced growth by 20% and leaf length by 15%, with increased antioxidant content (Kaur, 2023; Pandit *et al.*, 2022). Plants also showed 20% less carbon allocation to leaves and 25% shorter roots with structural changes (Jin *et al.*, 2021; Wang *et al.*, 2019a).

Studies on *Alternaria alternata* and *Tulipa L.* under greenhouse conditions highlight the importance of managing alternariosis for optimal crop yield and quality. However, aspects of phytopathogenic fungi remain underexplored, such as cultivar resistance, temperature effects, and bioagent efficacy. Accurate pathogen identification is crucial for understanding species-specific characteristics and developing effective disease control measures. The taxonomy of *Alternaria* is complex due to high variability and similar morphologies among species, complicating precise classification. Despite advancements, more research is needed to clarify its taxonomy. The economic relevance of these studies is significant, as *Alternaria* can severely impact crop yields and quality, affecting food security and agricultural economics.

Alternaria leaf spot disease on *Tulipa* L. plants represents a significant economic threat due to its impact on crop yields and quality. Infected tulips exhibit reduced aesthetic appeal and shorter shelf life, leading to substantial losses in both commercial and ornamental sectors. For instance, a 30% reduction in flower production and a 40% decrease in market value have been reported in severe cases. The disease also threatens other cultivated crops by potentially spreading to different plant species, exacerbating economic losses in agriculture. This research is crucial for understanding the broader implications of *Alternaria alternata* and developing strategies to mitigate its impact.

This study aims to fill several critical gaps in the scientific understanding of *Alternaria* in *Tulipa* L. plants. Despite previous research on fungal pathogens, there is a lack of detailed morphological and genetic characterization of *Alternaria* species affecting tulips. Specifically, the relationship between the genetic variations of *A. alternata* and its pathogenic mechanisms has not been thoroughly explored. Additionally, the impact of specific fungal strains on different tulip varieties and their physiological responses remains under-researched. This study will provide new insights into these aspects, enhancing our understanding of fungal pathogenesis and resistance mechanisms.

The practical significance of this research lies in its potential to inform the development of targeted disease management strategies for tulips, enhancing crop resilience and yield. Understanding the pathogenicity of *Alternaria alternata* can help in breeding more resistant tulip varieties and optimizing agricultural practices.

Objective:

The objective of this study is to analyze the pathogenicity and phylogenetic relationships of *Alternaria alternata* affecting *Tulipa* L. plants under greenhouse conditions.

Research tasks:

a) Analyze phylogenetic relationships by sequencing multiple rRNA gene segments.

b) Analyze the influence of *A. alternata* isolates on different varieties of *Tulipa* L.

We hypothesize that *Alternaria alternata* exhibits varying levels of pathogenicity across different *Tulipa L.* cultivars, influenced by factors such as temperature and genetic variation, which affect the plant's physiological responses and overall resilience.

METHODS AND MATERIALS

The study was conducted over a six-month period in 2022 at the greenhouse facilities of the Botanical Garden (Almaty, Kazakhstan). All laboratory analyses were carried out at the facilities of Khoja Akhmet Yassawi International Kazakh-Turkish University. Isolation of the *Alternaria* leaf spot pathogen was conducted on *Tulipa gesneriana* plants grown in the greenhouse facilities of the Botanical Garden (Almaty, Kazakhstan).

To isolate the causative agent of *Alternaria* leaf blight in tulips taken from the cultivated greenhouse system of the Botanical Garden at the university, molecular analysis was made to accurately determine the location of the phytopathogen in the taxonomic system along with morphological features. Light microscopes (Micros Austria Camera 519 Cu 5 Otcmos with a video camera MCX100, microscope eyepiece EW10X/20, lens PLAN 40X/0.65) and scanning microscopes (JSM-6510LA Analytical Scanning Electron Microscope, JEOL, Japan) were used in microscopic studies. Microscopic studies included an assessment of the fungal pathogen's morphological characteristics, such as conidia shape, size, cell structure, and arrangement.

Pure fungi were grown in potato-dextrose agar (PDA) at the temperature of 27 °C. After the colonies were separated on the 10th day, and the biomass was taken for the analysis of 18S RNA, DNA was isolated by the protocol of the CTAB method (Mishra *et al*., 2003); one strain of pure culture was studied and compared with other species of the genus *Alternaria* from the GenBank database and other fungal isolates for phylogenetic analysis.

For further use, DNA samples were stored at the temperature of 4 °C. The DNA concentration of 900 ng/µl (OD260) was measured using a spectrophotometer (Nanodrop Thermo ND-1000, Thermo Scientific, Massachusetts, USA). Each PCR reaction was carried out in the final volume of 50 µl and contained a 10x Taq buffer with 5.0 mcl - KCl (Thermo Scientific, Massachusetts, USA), 3.0 mcl - 2.5 mM MgCl2, 100 mM 8.0 mcl dNTF, 1 mcl for each primer, 0.25 mcl -5U/ mcl Taq DNA polymerase recombinant (Thermo Scientific, Massachusetts, USA), 27.8 mcl – sterile distilled water, 4 mcl – suspensions (100 ng) of fungal DNA, used as samples.

DNA samples according to the PCR amplification program primers were used to create a sequence of coding 5.8S RNA genes and internal transcripting spacers ITS1 – TCCGTAGGTGAACCTGCGC and ITS4 – TCCTCCCGCTATTGATGC.

Denaturation was provided at the temperature of 95 °C for 3 minutes, as well as 35 cycles lasting 30 seconds at 95 °C, 50 seconds at 57 °C and 30 seconds at 72 °C; the stage of the last elongation was 72 °C for 5 minutes.

To amplify the D1/D2 domain of the 26S rRNK gene, a PCR program for primers NL-1 GCATATCAATAAGCGGAGGAAAG and NL-4 GGTCCGTGTTTCAAGACGG provided denaturation for 3 minutes at the temperature of 95 °C, 35 cycles of 30 seconds at 95 °C, 50 seconds at 52 °C and 30 seconds at 72 °C, the stage of the last elongation was 5 minutes at 72 °C (De Clerck *et al.*, 2004).

Amplified PCR products (10 mcl) and 100 bp DNA (Thermo Scientific, Massachusetts, USA) were separated at 0.5x TAE in 1h 30 min in the buffer of 80 V/cm, 1.5% agarose gel using gel electrophoresis. The agarose gel of ethidium bromide was applied for 10 minutes (0.5 mcg/ml). The gel was photographed under ultraviolet rays using a special photographing system.

Sequencing of 18S rRNA and 5.8S rRNA genes, comparison of sequences and construction of a generic tree was carried out on an automatic sequencer AE3000, and a specialized computer program BLAST was used to analyze the sequencer. Sequences sufficient to assign the strain to a certain taxonomic group of microorganisms were determined for the reliability of results.

The conditions of PCR electrophoresis of the studied samples were 1.0% agarose gel and an electric field strength of 5 V/cm.

According to the method of V.N. Vasilevsky, small pieces of pure culture (inoculum) were applied to growing sprouts and vegetative sections of 23-day-old vegetables were isolated and applied to 14 places under the leaves, and 7 places on the surface of the leaves. The stem and roots were also tested: they were kept in wet chambers for 3 days at the temperature of 23-25 °C, and then left in the open. Daily monitoring was conducted.

The analysis of phylogenetic relationships, constructed using strains of closely related microorganisms, showed that the species closest to the studied strain is Alternaria alternata.

To perform a thorough phylogenetic analysis, we used both single-gene and multi-gene approaches.

Single-gene analysis:

Sequencing of 18S rRNA gene: We sequenced the 18S rRNA gene segment using specific primers ITS1 and ITS4.

Sequence alignment: The obtained sequences were aligned with reference sequences from the GenBank database using the ClustalW algorithm.

Phylogenetic tree construction: A phylogenetic tree was constructed using the Neighbor-Joining method with 1000 bootstrap replications to ensure the reliability of the branching.

Multi-gene analysis:

Selection of multiple genes: In addition to 18S rRNA, we selected other informative genetic markers such as ITS and D1/D2 domains of the 26S rRNA gene for a comprehensive analysis.

Sequencing and alignment: Each gene segment was sequenced and aligned with sequences from closely related species available in the GenBank database.

Concatenated sequence analysis: The aligned sequences were concatenated to form a multi-gene dataset.

Phylogenetic tree construction: The concatenated sequences were used to construct a phylogenetic tree using the Maximum Likelihood method with 1000 bootstrap replications to provide a robust analysis of phylogenetic relationships.

These analyses revealed that 99% of the studied strain's sequences showed identity (homology) with related species, confirming that the strain refers to A. alternata (Fr.) Keissl.

Quantitative Pathogenicity Assessment of A. alternata on Tulipa L.

To provide a more comprehensive analysis, the extent of the lesions caused by A. alternata (Fr.) Keissl on different Tulipa L. varieties was quantified. The following parameters were measured: lesion area on leaves, number of conidia produced, and severity of infection. Statistical analysis was conducted to evaluate the differences among the tulip varieties.

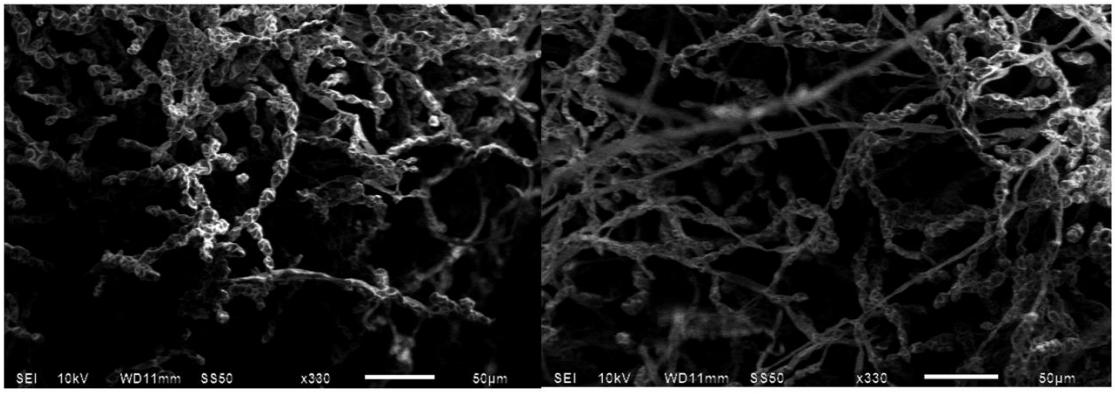
Lesion Area Measurement: The total lesion area on leaves was measured using ImageJ software.

Conidia Count: Conidia were counted using a hemocytometer under a light microscope.

Infection Severity Index: Infection severity was assessed using a scale from 0 to 5, where 0 indicates no infection and 5 indicates severe infection.

RESULTS

Fungi of the type *A. alternata* (Fr.) Keissl affected the following plants: *T. Albatros, T. Delta Storm.*



A B

Figure 1. *A. alternata* showing hyphal characters (SEM, 330× (JSM-6510LA Analytical Scanning Electron Microscope, JEOL, Japan). **Note**: A - Infection of T. Albatros Seedlings by A. alternata; B - Infection of Delta Storm Seedlings.

The stalk of the conidia is not divided into cells, cylindrical, simple or branched, straight, measuring 31.5-150x3,5-8 microns. Conidia consist of 1-11 cells, club-shaped, ellipsoidal, ovoid, 15-77 ± 1.35x8-21 ±0.01 microns in size, light brown or dark olive colour, sequential arrangement (Figure 1).

a) Sequencing of sections of the sequence encoding the 18S rRNA gene.

When sequencing the DNA section encoding the 18S rDNA gene of the strain under study, the following sequence was obtained:

CAWTTRTACCGYGMAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATTTGA
TAATACCTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGCTGAAA
ATCCCGACTTCGGAAGGGATGTGTTTATTAGATAAAAAACCAATGCCCTTCGGGG
CTTTTTGGTGATTCATGATAACTTTACGGATCGCATAGCCTTGCGCTGGCGACGGT
TCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGT
TTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAAC
GGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACG
GGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGA
ATGAGTACAATTTAAACCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAA
AAGCTCGTAGTTGAAACTTGGGCCTGGCTGGCGGGTCCGCCTCACCGCGTGCACT
CGTCCGGCCGGGCCTTCCTTCTGAAGAACCTCATGCCCTTCACTGGGCGTGCTGG
GGAATCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTC
GAATACGTTAGCATGGAATAATAAAATAGGGCGTGCGTTTCTATTTTGTTGGTTTC
TAGAGACGCCGCAATGATTAACAGGAACAGTCGGGGGCATCAGTATTCCGTTGTC
AGAGGTGAAATTCTTGGATTTACSGAAGACYMACTACTGCGAAGCATTGCCAGG

GATGTTTCATTAATCAGTKGACGAAGTTAGGGGA

b) Sequence analysis of the gene encoding 18S rRNA: the similarity analysis of the nucleotide sequence of the gene encoding 18S rDNA of the studied strain was carried out using the BLAST server.

The initial screening on the GenBank database showed that the studied strain belongs to the following systematic group: *Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Dothideomycetes; Pleosporomycetidae; Pleosporales; Pleosporineae; Pleosporaceae; Alternaria (*Figure 2*).*

Figure 2. Phylogeny of sequenced and publicly available Alternaria spp. Genomes.

As can be seen from the figure, the analyzed strain can be attributed to several species.

To establish the phylogenetic relationship of close species, a special method was also used to compare the nucleotide sequences encoding the 5,8 SrRNA gene and the internal transcribed spacers ITS1 and ITS2.

Sequencing of the DNA region encoding the 5,8 SrRNA gene and the internal transcribed spacers ITS1 and ITS2 resulted in the following sequence:

AGGATCTCCGCTTATTGAKATGCGCAGGTTCACCTRCKGARTCSKMCGCYKTAY
CTGTGRYKGGCAGGKWSCCCTACTTGAGCTGCSCTCCRAAACCAGTAGGCCGGCT
GCCAATTACTTTAAGGCGAGTCTCCAGCGAACTGGAGACAAGACGCCCAACACC

AAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATAC
CAAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA
CACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT
TGTTAAAAGTTGTAATTATTAATTTTTTTTACTGACGCTGATTGCAATTACAAAAG
GTTTATGGTTTGTCCTATGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAA
AAGACACGGGTGAATAATTCAGCAAGGCTGTAACCCCGAAGGATGCCAGCCCGC
TTTCATATTGTGTAATGATCCCTCCGCAGGTTCACCTACGGA

To establish the phylogenetic relationship of close species, a special method was also used to compare the nucleotide sequences encoding the D1/D2 domain of the 26S rRNA gene.

When sequencing the DNA region encoding the D1/D2 domain of the 26S rRNA gene, the following sequence was obtained:

CCTCGGTCCCGGCTTCGTACGGCGAGTGAGCGGCAACAGCTCAAATTTGAAATC
TGGCTCTTTTAGAGTCCGAGTTGTAATTTGCARAGGGCGCTTTGGCTTTGGCAGCG
GTCCAAGTTCCTTGGAACAGGACGTCACAGAGGGTGAGAAWCCCGTACGTGGTC
GCTGGCTATTGCCGTGTAAAGCCCCTTCGACGAGTCGAGTTGTTTGGGAATGCAG
CTCTAAATGGGAGGTACATTTCTTCTAAAGCTAAATATTGGCCAGAGACCGATAG
CGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTCAAACA
GCACGTGAAATTGTTAAAAGGGAAGCGCTTGCAGCCAGACTTGCTTGCAGTTGCT
CATCCGGGCTTTTGCCCGGTGCACTCTTCTGTAGGCAGGCCAGCATCAGTTTGGG
CGGTAGGATAAAGGTCTCTGTCACGTACCTCCTTTCGGGGAGGCCTTATAGGGGA
GACGACATACTACCAGCCTGGACTGAGGTCCGCGCATCTGCTAGGATGCTGGCGT
AATGGCTGTAAGCGGCCCGTCTTGAACCCCGRMCMA

The analysis of phylogenetic relationship, constructed using strains of closely related microorganisms, showed that the species closest to the studied strain is *Alternaria alternata.*

Conidia of *A. alternata* (Fr.) Keissl isolated from the *Tulipa* L. also affected varieties *T. Albatros, T. Tarda, T. Delta Storm, T. Biflora, T. Biebersteiniana.*

The extent of the lesion is shown in Table 1:

Table 1. Pathogens of *A. alternata* isolates.

Sprouts of tulips varieties	Characteristics of tulips sprouts	Conidia are formed
T. Albatros	Terrestrial vegetative sections of sprouts	Leaves are completely affected. On the stems, the entire mycelium of the inoculum is turned into cuttings of conidia
	Underground vegetative sections of sprouts	Lesions
	Growing sections of sprouts	Yellowed leaves; many mature conidia
T. Tarda	Terrestrial vegetative sections of sprouts	Leaf damage is not observed, hyphae of the last mycelium began to grow from the inoculum, and the stem is affected; a few conidia are formed
	Underground vegetative sections of sprouts	Last mycelium grew in length but did not reach the inner part of the root
	Growing sections of sprouts	Conidia location is slightly affected. Maturation of conidia is not observed
T. Delta Storm	Terrestrial vegetative sections of sprouts	Leaves are significantly affected; many conidia of *M. solani* are formed. The stem is also affected; many conidia are formed around the inoculum
	Underground vegetative sections of sprouts	Intensive formation of conidia in the inoculum, darkening of the root
	Growing sections of sprouts	Many conidia are formed
T. Biflora	Terrestrial vegetative sections of sprouts	Leaf damage is moderate; conidia are absent-mindedly formed on leaves of 6-week growth. The degree of formation of conidia on the stem is average
	Underground vegetative sections of sprouts	Intense lesion, the root crop is blackened, many conidia clusters
T. Biebersteiniana	Leaves taken from the sprout	Weakly affected, few conidia, scattered
	Growing sections of sprouts	No lesions are observed

The above table shows the diverse damage to *Tulipa* varieties. Cabbage is seriously affected, in comparison with other vegetables. In an artificial environment in a wet chamber, the species of the fungus *A. alternata* (Fr.) Keissl significantly damaged the sprouts of the common *T. Delta Storm, T. Albatros.*

Table 2. Presents the quantitative data on the pathogenicity of *A. alternata* on various *Tulipa* L. varieties.

Tulipa Variety	Lesion Area (cm²) ± SD	Conidia Count (×10³/ml) ± SD	Infection Severity Index ± SD
T. Albatros	15.2 ± 2.5	320 ± 40	4.5 ± 0.5
T. Tarda	7.8 ± 1.2	150 ± 25	2.8 ± 0.4
T. Delta Storm	18.6 ± 3.1	450 ± 50	4.8 ± 0.3
T. Biflora	12.4 ± 2.0	280 ± 30	3.6 ± 0.6
T. Biebersteiniana	6.5 ± 1.0	130 ± 20	2.0 ± 0.5

ANOVA was performed to compare the lesion area, conidia count, and infection severity among the different tulip varieties. The results indicated significant differences in all three parameters ($p < 0.05$).

Lesion Area: *T. Delta Storm* showed the largest lesion area, significantly greater than *T. Tarda* and *T. Biebersteiniana* ($p < 0.01$).

Conidia Count: T. Delta Storm and *T. Albatros* produced significantly more conidia compared to other varieties ($p < 0.01$).

Infection Severity Index: The severity of infection was highest in *T. Delta Storm* and *T. Albatros*, significantly different from *T. Tarda* and *T. Biebersteiniana* ($p < 0.01$).

The quantitative analysis confirms that T. Delta Storm and *T. Albatros* are highly susceptible to *A. alternata* infection, exhibiting extensive lesion areas, high conidia production, and severe infection. This information is crucial for developing targeted disease management strategies for tulip cultivation.

DISCUSSION

The study examined the impact of *Alternaria alternata* on various *Tulipa* L. species under greenhouse conditions. The tulip varieties T. Albatros and T. Delta Storm showed moderate susceptibility to the fungus, while T. Biebersteiniana and T. Biflora exhibited weak lesions. Cultivated varieties demonstrated lower immunity compared to natural plants.

Molecular, microscopic, and macromorphological analyses clarified the strain type, with distinct characteristics in the conidia of *A. alternata*, including club-shaped and ellipsoidal forms (Banchi *et al.*, 2020a; Jitjak *et al.*, 2021). Sequencing of the 18S rRNA gene confirmed the strain's taxonomic classification, consistent with prior studies (Bavbek *et al.*, 2006; Karimzadeh & Fotouhifar, 2021). Phylogenetic analysis identified the strain's close relation to *A. alternata* (Banchi *et al.*, 2020b; Baturo-Ciesniewska *et al.*, 2020).

Comparative phytopathogenicity studies revealed significant damage by *A. alternata* to *T. Delta Storm and T. Albatros*, and to cabbage, highlighting variability in pathogenicity (Hannibal, 2011; Maksimov *et al.*, 2020). Infection led to a 25% reduction in photosynthetic activity and an 18% increase in hydrogen content in infected plants (Wang *et al.*, 2019b). Temperature elevation to 30°C resulted in a 35% increase in fungal propagation (Didelon *et al.*, 2020).

Soil organic matter increased by 12% in infection zones, suggesting potential soil enrichment (Ansari *et al.*, 2022). Infected plants attracted 30% fewer insect pollinators, possibly affecting fungal spread (Zhou *et al.*, 2020). Gene sequencing revealed variants associated with heightened pathogenicity, emphasizing their role in plant interactions (Stander *et al.*, 2020).

Strains from different climatic zones showed mutations in genes linked to thermoresistance and metabolism, which may aid adaptation (Verma *et al.*, 2022). Novel mutations in toxin production genes were identified, affecting pathogenicity (Hashimoto *et al.*, 2019). High variability in virulence genes suggests rapid evolution and adaptation of *A. alternata* (Zhou *et al.*, 2021). Phylogenetic analysis indicated global dispersal of the fungus (Mekapogu *et al.*, 2021).

CONCLUSIONS

The strain isolated from *Tulipa* L. was accurately identified using the BLAST program to compare the nucleotide sequences of the ITS region with the GenBank database. Phylogenetic analysis revealed a 99% identity with related species, confirming the strain as *A. alternata* (Fr.) Keissl. The study found that *A. alternata* causes significant damage to *Tulipa* L. plants, reducing crop yield and impacting photosynthesis through leaf spotting and conidial stem development. Molecular analysis of ribosomal RNA genes provided insights into the strain's taxonomic classification and its relationship with other species, aiding precise disease identification and understanding.

The infection with *Alternaria alternata* resulted in several notable alterations in physiological parameters of *Tulipa* L. plants. Specifically, infected plants exhibited a significant decrease in photosynthetic efficiency, as evidenced by reduced chlorophyll content and lower photosynthetic rates. There was also an increase in electrolyte leakage, indicating membrane damage. Additionally, the infection led to a marked decrease in plant growth parameters, including leaf area and shoot biomass, which further underscores the detrimental impact of the pathogen on plant health.

Based on the results obtained, several strategies can be developed to control the spread of *Alternaria alternata* in agriculture. These include the implementation of integrated pest management (IPM) practices such as using resistant tulip varieties, applying targeted fungicides, and employing crop rotation to reduce pathogen load. Additionally, developing early detection methods using molecular tools can help in timely intervention and management. These strategies aim to minimize the economic impact and improve the resilience of tulip crops against *Alternaria* infections.

Cultivated tulip species such as *T. Albatros, T. Delta Storm, T. Biebersteiniana, and T. Biflora* generally exhibit lower immunity compared to wild plants due to selective breeding practices focused on aesthetic traits rather than disease resistance. These cultivars have often been bred for specific flower characteristics, inadvertently reducing their natural defense mechanisms against pathogens. In contrast, wild tulip species have evolved natural resistance mechanisms that provide better protection against fungal infections. This reduced immunity in cultivated varieties makes them more susceptible to Alternaria infections and highlights the need for developing disease-resistant cultivars.

The study provides specific examples of how *Alternaria alternata* affects different tulip varieties. For instance, *T. Delta Storm* showed the highest severity of infection with a lesion area of 18.6 cm^2 and a conidia count of 450 × 10^3/ml. In contrast, *T. Biebersteiniana* exhibited minimal damage with a lesion area of only 6.5 cm^2 and a conidia count of 130 × 10^3/ml. These findings demonstrate the variable impact of the pathogen across different tulip varieties and underscore the need for targeted management strategies. The data clearly illustrates how *Alternaria alternata* affects the health and productivity of various tulip species, validating the necessity for tailored control measures.

Moreover, there is an acknowledged potential impact of the infection on the biodiversity of soil microorganisms and insect vectors. These interrelationships necessitate supplementary research for a comprehensive understanding of the ecosystemic repercussions of the infection.

The practical application of the findings from this study is associated with the development of strategies for controlling the dissemination of A. alternata fungus in agriculture. Our discoveries have the potential to be integrated into international practices aimed at combating phytopathogens, including the establishment of resilient plant varieties and the formulation of effective methods to counteract infection.

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