Original Article

Microbial Biosystems 10(2) (2025) 2025.366142
10.21608/mb.2025.366142.1264

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

Identification of *Alternaria tenuissima* on soybeans in the forest-steppe zone of North Kazakhstan and identification of the phytopathogen

Yerlan Utelbayev[1*], Chingiz Kanapin[2], Kazhymurat Mussynov[2], Abdumamat Kochorov[1], Berik Bazarbayev[1], Gaukartas Abysheva[2]

[1]*A.I. Barayev Research and Production Centre for Grain Farming, Shortandy, Kazakhstan.*
[2] *Saken Seifullin Kazakh Agrotechnical University, 62 Pobeda Avenue, Astana, Kazakhstan.*

ARTICLE INFO

Article history
Received 7 March 2025
Received revised 25 April 2025
Accepted 18 May 2025
Available online 1 June 2025

Corresponding Editors
Vasiliev, A.
Sapanov, M.
Abdel-Azeem, A. M.

Keywords
Agronomy,
biocontrol,
BLAST analysis,
disease management,
phytopathology,
TEF-1α gene.

ABSTRACT

From 2014 to 2024, soybeans have been the most popular crop in Kazakhstan's agriculture, especially in the northern grain-growing regions. However, in terms of the yield of this crop, Kazakhstan lags behind the largest-producing countries because of the spread of diseases caused by fungi, which lead to lower yields and seed quality. Therefore, the main objective of the study was to identify fungal pathogens affecting soybean crops in the forest-steppe zone of North Kazakhstan, with a focus on isolating and characterizing strains of the Alternaria genus. To identify the fungal strains, the method of determining the direct nucleotide sequence of the internal transcribed spacer region was used, followed by the determination of the nucleotide identity with sequences deposited in the international GenBank database, as well as the method of constructing phylogenetic trees with nucleotide sequences. Fungal isolates were obtained from infected soybean tissues, cultured on potato dextrose agar, and identified through direct sequencing of the ITS region and partial sequencing of the TEF-1α and Tub2 genes, followed by BLAST analysis and phylogenetic tree construction. As a result of the analyses, the authors identified a phytopathogenic strain of *Alternaria tenuissima*. The analyses revealed that the isolates belonged to *A. tenuissima*, confirmed through both sequence identity and phylogenetic analysis. The identification of *A. tenuissima* as a soybean pathogen provides essential data for the development of effective disease management strategies in Kazakhstan's soybean production.

Published by Arab Society for Fungal Conservation

Introduction

Soybeans are a widespread crop worldwide. Its primary purpose is industrial processing to produce oil with high nutritional and industrial value (Jacob, Karikalan 2021, Kazeem et al. 2023, Brandibur et al. 2024). According to the international technical classification, soybeans belong to oilseeds, and according to biological taxonomy, they are legumes with a high protein content in seeds (33-44%) (Tleulina et al. 2024). Therefore, another important area of its use is the production of high-protein cake and meal for food additives and the needs of livestock production (Arinov et al. 2016; Makulbekova et al. 2017; Mussynov et al. 2017, Zhakyalkov et al. 2023).

In the Republic of Kazakhstan, the main areas of soybean cultivation are concentrated in the south, while

***Corresponding author Email address**: yutelbayev@gmail.com (Yerlan Utelbayev)

the acreage in the northern regions is insignificant (Kenenbayev et al. 2023). Producing soybeans is profitable since their price is several times higher than that of monoculture wheat, which occupies about 80% of the acreage in this grain-growing region (Bureau of National Statistics 2014).

Soybean acreage has shown unstable trends in recent years, with sharp increases and decreases, reflecting ongoing challenges in the industry (Tirono, 2022; Smirnova et al., 2024). This instability points to issues in cultivation practices and highlights the need to improve the phytosanitary condition of crops. One of the key barriers to achieving stable, high-quality yields is the widespread occurrence of soybean diseases (Shumenova et al., 2024)

Research shows that soybeans are vulnerable to a number of serious diseases, many of which can significantly reduce crop yields. Among the most common and harmful are black stem—caused by several types of fungi like *Ascochyta sojaecola*—as well as *Fusarium blight*, downy mildew (*Peronospora manshurica*), white rot (*Sclerotinia sclerotiorum*), and Alternaria blight (*Alternaria tenuis*). Stem blight, caused by *Diaporthe phaseolorum* (var. *caulivora* and var. *sojae*), is also a major concern. On top of these, bacterial and viral infections contribute to a wide range of other diseases that threaten soybean health (Yorinori et al., 2005; Mueller et al., 2016; Utelbayev et al., 2021; Kuldybayev et al., 2023).

Among these diseases, Sclerotinia disease, root rot, and late blight are the most common among leading soybean producers in Canada and China (Lerch-Olson, Robertson 2020; Yang et al. 2020). *Fusarium* blight and root rot, *Alternaria* leaf spot, seed rot, and anthracnose are more often mentioned in soybean regions of Ukraine, Russia, India, and Africa (Kurilova 2010, Hailemariam et al. 2019, Mykhalska et al. 2020, Nataraj et al. 2020, Ouedraogo et al. 2024).

However, there are no comprehensive studies on soybean diseases in Kazakhstan (Kuldybayev et al. 2023). To fill the gap, in 2023-2024, field studies to assess the phytosanitary condition of soybean crops in the forest-steppe zone of North Kazakhstan were conducted.

The primary objective of this study was to investigate the species composition of soybean diseases in the forest-steppe zone of North Kazakhstan and to identify the key pathogens affecting soybean crops.

Materials and Methods

Study area and sampling

According to field surveys conducted during the 2023–2024 growing season in the forest-steppe zone of North Kazakhstan, symptomatic soybean plants (leaves and stems) exhibiting signs of *Alternaria* blight were identified within the fields of Kamenka & D LLP in the Sandyktau district of the Akmola region. These samples were collected for laboratory analysis in accordance with standard phytopathological survey protocols (Agrios, 2005).

Phytopathological analysis and fungal isolation

Fungal isolates of the *Alternaria* genus were obtained from infected soybean leaves and stems exhibiting symptoms of *Alternaria* blight (Fig. 1). The infected plant fragments were surface-sterilized and placed on potato dextrose agar (PDA). After incubation, fungal colonies were examined under a microscope. Morphological identification of *Alternaria* spp. was done based on colony appearance and conidial characteristics under a compound microscope, as described in Simmons (2007).

Fig 1. Soybean leaves showing visible signs of infection caused by *Alternaria* spp.

Molecular characterization and phylogenetic analysis

Genomic DNA was extracted from fungal mycelia using the cetyltrimethylammonium bromide (CTAB) method according to Abdel-Azeem et al. (2019). The DNA concentration and purity were determined using a NanoDrop 1000 spectrophotometer. Absorbance was measured at 260 nm and 280 nm to assess nucleic acid and protein content, respectively.

To identify fungal species, polymerase chain reaction (PCR) was used to amplify two gene regions: TEF-1α and Tub2. For the TEF-1α gene, amplification was carried out using universal primers TEF-1α_F (gcyccygghcaycgtgayttyat) and TEF-1α_R (achgtrccrataccaccratctt). Each 30 µL reaction included 5 µL of DNA template, 1 unit of Taq DNA polymerase (ThermoFisher), 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 10× KCl buffer, and 10 pmol of each primer. The PCR

cycling conditions started with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 58°C for 45 seconds, and 72°C for 1.5 minutes. A final extension was performed at 72°C for 10 minutes.

Amplification of the Tub2 gene followed a similar protocol, using primers TUB2_Bsens (atcacwcactcictiggtggtgg) and TUB2_Brev (catgaagaartgiagacgiggg). The reaction components were the same as for TEF-1α, with the only difference being the annealing temperature, which was set at 55°C.

To check the success of the PCR, the amplified products were run on a 1.5% agarose gel in TAE buffer and stained with ethidium bromide. The DNA bands were then visualized under UV light using a PowerPac electrophoresis system (Bio-Rad). No bands appeared in the negative controls, confirming that there was no contamination in the reactions (Fig. 4).

After amplification, the PCR products were purified using magnetic silica-coated beads, following the method described by Berdimuratova et al. (2020). The DNA bound to the beads in a solution containing 20% polyethylene glycol and 2.5 M NaCl. The beads were washed with 70% ethanol, and the clean DNA was then eluted in either TE buffer or deionized water.

The purified DNA was sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and the sequencing was carried out on an ABI 3730xl DNA Analyzer, following the manufacturer's guidelines (Vegas et al., 2006).

Once the raw sequence data were obtained, they were assembled and cleaned up using SeqMan software (DNASTAR). Low-quality ends and leftover primer sequences were trimmed off. The final cleaned sequences were identified by comparing them with those in the GenBank database using the BLAST algorithm. To strengthen the identification, phylogenetic trees were also built using ITS gene sequences from known reference strains.

Results

The recovered isolates of Alternaria were first examined based on their physical characteristics using the identification key developed by Simmons (2007) and were identified as *A. tenuissima*.

The electropherogram displays the results of PCR amplification for fragments of the Tub2 and TEF-1α genes. Clear, distinct bands were observed for both targets, indicating successful amplification of the expected DNA fragments. The consistency and clarity of these bands confirm the reliability of the PCR process and suggest that the extracted DNA was of good quality (Fig. 2). These results provide a solid foundation for further genetic analysis and species identification

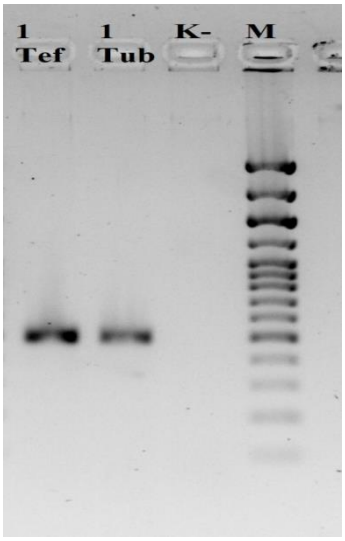

Fig 2. Electrophoregram of PCR amplification products of a fragment of the Tub2 and TEF-1α genes.

To confirm this identification, BLAST analysis was performed on the nucleotide sequences (Table 1). The results showed a high degree of similarity between the sample and several species within the *Alternaria* genus. However, sequence similarity alone is not always enough for precise species identification, as closely related species can share highly conserved regions of their genomes.

Phylogenetic trees based on the TEF-1α and Tub2 gene sequences are shown in figures 3 and 4, respectively. In both analyses, the test specimen clusters on the same branch as *A. tenuissima*, indicating a close evolutionary relationship. This strong genetic similarity supports the conclusion that the specimen most likely belongs to the species *A. tenuissima*.

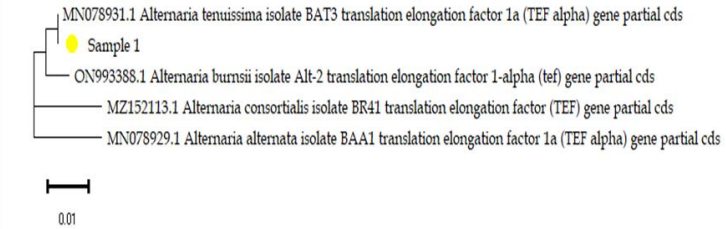

Fig 3. Phylogenetic tree based on the analysis of a fragment of the TEF-1α gene.

The sample clusters analyzed on the same branch as *A. tenuissima* and *Alternaria alternata* in the phylogenetic tree shown in Figure 4. In Figure 3, based

Table 1 Gene fragments, corresponding sequences, BLAST-based identification from GenBank, accession numbers, strain names, and sequence identity percentages.

Name of the gene	Fragment sequence	Identification of nucleotide sequences in an international database (http://www.ncbi.nlm.nih.gov), BLAST algorithm		
		GenBank accession number	Strain name	Matching %
TEF-1α	GGGTCTTACCAGTGGCCTTGGCCTTGGTC TCCTTCTCCCAACCCTTGTACCAGGGGCA GTTGGATGAGGCCTCAATCATGTTGTCAC CGTTGAAACCGGAGATGGGGACGAAGG GAACGTGCTTGGGGTTGTAGCCGACCTT CTTGATGAAGTTGGAGGTCTCCTTGATGA TCTCCTGGTAACGCTCCTCGGACCACTTG GTGGTGTCCATCTTGTTGATGGCAACGAT GAGCTGCTTGACACCGAGGGTGTAAGCG AGGAGAGCGTGCTCACGAGTCTGGCCAT CCTTGGAGATACCAGCCTCGAACTCACC AGTACCGGCGGCAATGATGAGAATAGCG CAGTCGGCCTGGGAGGTACCAGTGATCA TGTTCTTGATGAAAT	KP009004.1	*Alternaria alternata*	100
		JQ672478.1	*Alternaria toxicogenica*	100
		MN078931.1	*Alternaria tenuissima*	100
TUB2	GTACGCTTCTCATCTCCAAGATCCGTGAG GAGTTCCCCGACCGCATGATGGCCACCT ACTCCGTCGTGCCTTCCCCCAAGGTCTCC GACACCGTTGTCGAGCCCTACAACGCCA CACTCTCCATCCACCAGCTGGTTGAGAA CTCAGACGAGACCTTCTGCATTGACAAC GAGGCTCTCTACGACATCTGCATGAGGA CTCTCAAGCTGAACAACCCCTCCTACGG CGACCTGAACCACCTCGTTTCCGCCGTCA TGTCGGGTGTCACTACCTGCCTGCGTTTC CCTGGTCAGCTCAACTCTGACCTGAGGA AGTTGGCCGTCAACATGGTTCCCTTCCCC CGTCTCCACTTCTTCATGA	MT003125.1	*Alternaria alternata*	100
		OM674441.1	*Alternaria arborescens*	99.16

on the TEF-1α gene sequence, the sample appears most closely related to *A. tenuissima*. Similarly, the Tub2 gene analysis in figure 4 also places the sample near both *A. tenuissima* and *A. alternata*, suggesting a close genetic relationship with these species. However, when both gene sequences are analyzed together, the evidence more strongly supports that the sample belongs to *A. tenuissima* (Table 2).

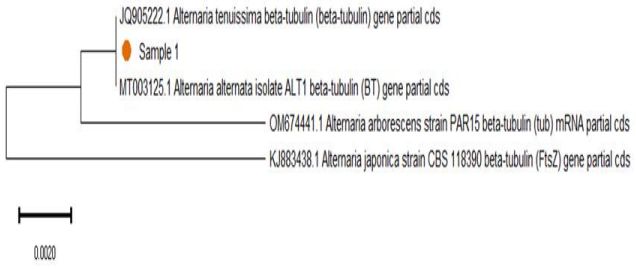

Fig 4. Phylogenetic tree based on a fragment of the TUB2 gene, illustrating the genetic relationship between the studied specimen and other known *Alternaria* species.

Overall, the phylogenetic analysis provides a clearer picture than BLAST alone, confirming that the studied strain is most likely *A. tenuissima* based on its evolutionary placement among closely related species (Saitou & Nei, 1987; Tamura et al., 2021).

Table 2 Summary of strain identification based on sequence analysis of TEF-1α and TUB2 gene fragments.

No.	Taxa	Note
1 TEF-1α	*Alternaria tenuissima*	Determined based on a phylogenetic tree constructed by the neighbor-joining (N-J) algorithm
1 TUB2	*Alternaria tenuissima/Alternaria alternata*	Determined based on a phylogenetic tree constructed by the N-J algorithm

DISCUSSION

The genus *Alternaria* is very adaptable and serves many purposes in different ecosystems. It is well known as a plant pathogen that causes diseases in many crops, but it can also act as a saprobe by eating dead organic matter and as an endophyte by living safely inside plant tissues. Some types of *Alternaria* even act as natural recyclers, breaking down plant waste and putting nutrients back into the soil (Abed et al. 2020, Abo Nouh et al. 2021, Yadav et al. 2021, AL-Ziadi et al. 2024, Mansour et al. 2025, Alzahrani 2025). This ability to change lets *Alternaria* live in a variety of places and interact with plants in both good and bad ways, depending on the situation.

The control of *Alternaria tenuissima*, a phytopathogen affecting soybean (*Glycine max*) and a host of plants, requires a multi-faceted approach integrating chemical, biological, and agronomic measures. The increasing prevalence of *Alternaria* species in Kazakhstan's soybean production underscores the necessity for effective disease management strategies, and this section aims to highlight the current control measures and their effectiveness in mitigating the impact of *Alternaria tenuissima* on crop production.

Chemical control strategies such as the application of fungicides remain a widely acceptable strategy for managing Alternaria infections. For instance, naphthyridine-based oxadiazole derivatives have shown significant antifungal activity against *Alternaria alternata* and may hold potential for broader applications against related pathogens (Domala et al., 2022). A study by Lan et al. (2023) concluded that hymexazol fungicide was able to control *Alternaria tenuissima* leaf spot disease in Luobuma. Their study showed that *A. tenuissima* had a relatively higher sensitivity to the hymexazol fungicide and displayed a 98% inhibition rate. Tang et al. (2023b) in their study concluded that 10% difenoconazole WDG, a triazole, had the best inhibition effect on *A. tenuissima* during in vitro testing. Further conclusion stated that 10% difenoconazole and fludioxonil in a 2:8 ratio exhibited great synergy and resulted in control of *A. tenuissima.* Recent studies has shown that for optimal results chemical methods should be combined a different strategy such as biological or agronomic. Utelbayev et al. (2021) achieved efficient results by combining chemical and Extrasol biofungicide, while Liu et al. (2023) achieved results by combining fungicides and crop rotation practices.

Biological control using antagonistic microorganisms offers an environmentally friendly alternative to chemical fungicides. Narendrakumar et al. (2021) report that chitinase enzymes extracted from Serratia marcescens su05 exhibit significant antifungal activity against *Alternaria alternata*. Some soil bacteria have shown antagonistic effects up to >90% efficiency in controlling the growth of *A. tenuissima* in spring wheat seeds (Tarar et al., 2022; Puchkova & Alekseeva, 2024). In a recent study, Manea et al. (2023) presented results showing the effect of *Trichoderma harzianum* and its combinations in inhibiting the growth of *A. tenuissima.* A study by Abbas et al. (2020) reported on a biochemical approach in controlling *A. tenuissima.* Vicilin, a glycoprotein isolated from chickpeas, displayed an inhibitory effect on the growth of *A. tenuissima* in postharvest fig fruits. Studies such as Carrascal-Hernández et al. (2022) have shown the potential of applying CRISPR/Cas9 and RNAi technologies in controlling the Alternaria fungi. Practices such as silencing the *AbSte7* gene have shown significant effects in reducing pathogenicity.

Cultural practices play a vital role in reducing *Alternaria* infections. Practices like proper irrigation and crop rotation with non-host species, such as cereals or legumes less susceptible to fungal infections, help break the disease cycle (Al-Rawashdeh, 2025). Another strategy is the use of resistant varieties, which has been shown to be the easiest, safest, and cheapest approach to controlling *Alternaria* infections (Nsah et al., 2024).

An integrated disease management system that combines all three approaches will be highly efficient in controlling *Alternaria* infections (Zhang et al., 2022). While fungicides provide immediate disease suppression, the integration of biocontrol agents, resistant cultivars, and cultural practices ensures long-term sustainability. Future studies need to be directed towards understanding the molecular mechanisms of *Alternaria tenuissima* pathogenicity, identifying new biocontrol agents, and strengthening plant resistance using advanced breeding methods (Kone et al., 2023). In addition, farmer education and awareness development on best management practices are also needed to attain successful disease management in agricultural systems.

Conclusions

The Tub2 and TEF-1α genes were amplified and sequenced to study the fungal strain. Using the GenBank database, a BLAST analysis of the resulting sequences showed that they were very similar to a number of species in the Alternaria genus. The strain had a 100% sequence match with the TEF-1α gene in *Alternaria alternata*, *A. tenuissima*, and *A. toxicogenica*. *A. alternata* had a 100% match with the Tub2 gene. The phylogenetic tree made it clear what species the strain belonged to, even though the BLAST data showed that it was very similar to a number of other species. It was

most likely A. tenuissima. Using the Neighbor-Joining (N-J) method for phylogenetic analysis, it was found that the strain is most closely related to A. tenuissima.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

We want to express our special gratitude to the management of the National Center for Biotechnology RGP of the Ministry of Education and Science of the Republic of Kazakhstan for allowing the study to be conducted in the center's laboratories. We also thank A.I. Dzheyranov, Director of the Kamenka & D LLP for providing a test plot and agricultural machinery for fieldwork. The study was conducted within the scientific and technical program "Development of a sustainable farming system in changing climate conditions for various soil and climatic zones of Kazakhstan" (BR22885719).

References

Abbas E, Osman A, Sitohy M. (2020). Biochemical control of *Alternaria tenuissima* infecting post-harvest fig fruit by chickpea vicilin. Journal of the Science of Food and Agriculture. 100(7), 2889–2897. https://doi.org/10.1002/jsfa.10314

Abdel-Azeem A M, Held B W, Richards J E, Davis S L, Blanchette R A. (2019). Assessment of Biodegradation in Ancient Archaeological Wood from the Middle Cemetery at Abydos, Egypt. PLoS One 4(3):e0213753. doi: 10.1371/journal.pone.0213753.

Abed R, Ibrahim N, AL-Khazreje A, Hussein R, Abbas R. (2020). Investigation of fungi and mycotoxins contamination in some herbal slimming mixtures in Baquba city- Iraq. Microbial Biosystems, 5(1), 1-6. doi: 10.21608/mb.2020.30860.1013

Abo Nouh F, Abu-Elsaoud A, Abdel-Azeem A M. (2021). The role of endophytic fungi in combating abiotic stress on tomato. Microbial Biosystems, 6(1), 35-48. doi: 10.21608/mb.2021.91779.1037

Alzahrani, S. (2025). Utilizing endophytic fungus *Alternaria tenuissima* for silver nanoparticles biogenic formation and their antibacterial efficacy. Microbial Biosystems, 10(2), 251-258. doi: 10.21608/mb.2025.398889.1361

AL-Ziadi S, Yass Lahmood W, Abd Alabbas Muhammad D, Rashid Swadi R. (2024). Bio-Production of silver nanoparticles by a Brevibacillus sp. and testing of its inhibitory efficacy on pathogenic fungi. Microbial Biosystems, 9(2), 58-66. doi: 10.21608/mb.2024.296842.1106

Agrios GN. (2005). Plant Pathology (5th ed.). Elsevier Academic Press.

Al-Rawashdeh ZB. (2025). The Environmentally Friendly Practices to Fight Plants' Diseases: Scope Review. Journal of Global Innovations in Agricultural Sciences 13(1), 405–412. https://doi.org/10.22194/JGIAS/25.1505

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. (1990). Basic local alignment search tool. Journal of molecular biology 215(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Arinov K, Musynov K, Shestakova N, Serekpaev A, Apushev A. (2016). Rastenievodstvo. Uchebnik. Izdatelstva Foliant, 584 p.

Berdimuratova K, Amirgazin A, Kuibagarov M, Lutsay V, Mukanov K, Shevtsov A. (2020). Optimization of PCR purification using silica-coated magnetic beads. Eurasian Journal of Applied Biotechnology 1. https://doi.org/10.11134/btp.1.2020.8.

Berdimuratova K, Amirgazin AO, Kuibagarov MA, Lutsay VB, Mukanov KK, Shevtsov AB. (2020). Optimization of PCR purification using silica-coated magnetic beads. Eurasian Journal of Applied Biotechnology 1. https://doi.org/10.11134/btp.1.2020.8

Brandibur T, Manea AM, Boia M, Nișulescu D-D, Blidisel A, Kundnani NR, Popoiu MC. (2024). Parenteral lipid nutrition in congenital gastrointestinal malformation operated cases. Advancements in Life Sciences, 11(4), 918-926. https://als-journal.com/11432-24/

Bureau of National Statistics. (2014). Agency for Strategic Planning and Reforms of the Republic of Kazakhstan. Retrieved from. https://stat.gov.kz/industries/business-statistics/stat-forrest-village-hunt-fish/spreadsheets/?year=2014&name=24484&period=&type=.

Carrascal-Hernández DC, Flórez-López E, Peralta-Ruiz Y, Chaves-López C, Grande-Tovar CD. (2022). Eco-friendly biocontrol strategies of Alternaria phytopathogen fungus: A focus on gene-editing techniques. Agriculture 12(10), 1722. https://doi.org/10.3390/agriculture12101722

Domala R, Butti ARS & Eppakayla L. (2022). Study of antifungal activity of 2-Methyl-3-(5′-aryl/aryloxymethyl-1′,3′,4′-oxadiazol-2′-yl) amino-1,8-naphthyridines. Research Journal of Pharmacy and Technology 15(2), 757–760. https://www.scopus.com/record/display.uri?eid=2-s2.0-85128824407

Doyle, J.J., & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19, 11–15.

Hailemariam M, Tesfaye A, Belachew K, Sileshi Y.(2019). Economic analysis of different fungicides rates for Asian soybean rust (Phakopsora pachyrhizi Syd.) on soybean (Glycine max (L) Merrill) sub-humid tepid to cool mid high land agro-ecology of Ethiopia. International Journal of Agricultural Biosciences 8(5), 251-256.

Jacob S, Karikalan L.(2021). Optimization of combustion characteristics of diesel engine fueled by biofuels and its diesel blends with additive titanium dioxide nano-particles. International Journal of Heat and Technology 39(6), 1973-1978. https://doi.org/10.18280/ijht.390636

Kazeem RA, Fadare DA, Adebayo AS, Ikumapayi OM, Awonusi AA, Dare AA, Okokpujie IP.(2023). Cutting fluids usage and impacts in metal workshops in Ibadan, southwestern Nigeria. International Journal of Sustainable Development and Planning 18(1), 1-16. https://doi.org/10.18280/ijsdp.180101

Kenenbayev S, Yessenbayeva G, Zhanbyrbayev Y, Bekturganov A, Dutbayev Y, Toktay H. (2023). Influence of climate conditions and biofertilizers on soybean yield in Southeastern Kazakhstan. International Journal of Design & Nature and Ecodynamics 18(6), 1391-1398. https://doi.org/10.18280/ijdne.180612

Kone Y, Sangare MB, Dagno K, Niangaly A, Doumbia S, Amadou H & Kourouma M. (2023). Efficacy of several species of fungi bioagents and fungicides against Magnaporthe oryzae in vitro. International Journal of Biological and Chemical Sciences 17(3), 935–949. https://doi.org/10.4314/ijbcs.v17i3.14

Kuldybayev N, Dutbayev Y, Konstantinova O, Borodulin D, Yessimbekova M, Daugaliyeva S, Toishimanov M, Yesserkenov A, Bastaubaeva S, Temreshev I. (2023). Identification and pathogenicity of the soybean root rot pathogen in arid conditions. OnLine Journal of Biological Sciences 23(2), 202-209. https://doi.org/10.3844/ojbsci.2023.202.209

Kurilova DA. (2010). Harmfulness of Fusarium species on soybean depending on a degree of plants affection. Oil Crops 2(144-145), 84-89.

Lan Y, Yan Z, Duan T. (2023). Luobuma leaf spot disease caused by *Alternaria tenuissima* in China. Journal of Fungi 9(11), 1062. https://doi.org/10.3390/jof9111062

Lawrence, D.P., Gannibal, P.B., Peever, T.L., & Pryor, B.M. (2013). The sections of Alternaria: formalizing species-group concepts. Mycologia 105(3), 530–546. https://doi.org/10.3852/12-249

Lerch-Olson ER & Robertson AE. (2020). Effect of coinoculations with Pythium and Fusarium species on seedling disease development of soybean. Canadian Journal of Plant Pathology 42(3), 408-418.

Liu J, Zhang J, He Y, Li Y, Deng H, Jiang Y. (2023). Evaluation and control of *Alternaria tenuissima* causing leaf spots in blue honeysuckle in China. Plant Disease 108(5), 1270–1277. https://doi.org/10.1094/pdis-07-23-1470-re

Makulbekova A, Iskakov A, Kulkarni KP, Song JT & Lee JD. (2017). Current status and prospects of soybean production in Kazakhstan. Plant Breeding and Biotechnology 5(2), 55-66.

Manea NAO, DKhamas NA, AFayyadh NM, Azeem NH. (2023). Record of *Alternaria tenuissima* as a causal pathogen of leaf spots in chard plant in Basrah, Iraq, and its in vitro management. University of Thi-Qar Journal of Agricultural Research 12(2), 238–246. https://doi.org/10.54174/utjagr.v12i2.292

Mansour R, Elshoubaky G, Attia E. (2025). Exploring the antioxidant and cytotoxicity perspective of melanin pigment produced by fungi associated with marine macroalgae, Fayed, Suez Canal, Egypt. Microbial Biosystems, 10(1), 181-194. doi: 10.21608/mb.2025.352914.1229

Mueller DS, Wise KA, Sisson AJ, Smith DL, Sikora EJ, Bradley C & Robertson AE. (2016). A farmer's guide to soybean diseases. In D Mueller (Ed.), pp. 5-21. St. Paul, MN: American Phytopathological Society.

Mussynov KM, Kipshakbaeva AA, Arinov BK, Utelbayev YA & Bazarbayev BB. (2017). Productivity and cooking advantages of lentil grades grown under conditions found in North Kazakhstan. Pakistan Journal of Nutrition 16(11), 843-849.

Mykhalska LM, Sanin OY, Schwartau VV, Zozulia OL & Hrytsev OA. (2019). Distribution of species of Fusarium and Alternaria genera on cereals in Ukraine. Biosystems Diversity 27(2), 186-191.

Narendrakumar G, Namasivayam KR & Saravanan S. (2021). Depiction of chitinase extracted from Serratia marcescens su05 on agriculturally important fungi. Research Journal of Pharmacy and Technology 14(2), 757–760. https://doi.org/10.52711/0974-360X.2021.00425

Nataraj V, Maranna S, Kumawat G, Gupta S, Rajput LS, Kumar S, ... & Bhatia VS. (2020). Genetic inheritance and identification of germplasm sources for anthracnose resistance in soybean Glycine max (L.) Merr.. Genetic Resources and Crop Evolution 67, 1449-1456.

Ngoh Dooh JP, Boydoul FU, Madjerembe A, Tchoupou Tsouala DB, Hawaou Adagoro DB, Kosma P &

Ambang Z. (2020). Inventory of the potato diseases and impact on growth and yield traits in Far North Cameroon. International Journal of Biological and Chemical Sciences 14(8), 2826–2836. https://doi.org/10.4314/ijbcs.v14i8.14

Nsah FS, Kinge TR & Annih MG. (2024). Identification of fungal species isolated from diseased cabbages in the western highlands of Cameroon. International Journal of Biological and Chemical Sciences 18(2), 338–354. https://doi.org/10.4314/ijbcs.v18i2.2

Ouedraogo TMP, Kassankogno AI, Sanon E, Kafando A, Barro S, Nikiema BC, Sohoro H, Wonni I. (2024). Identification of the pathogens responsible for the primary fungal infections linked to soy seeds in Burkina Faso. International Journal of Agricultural Biosciences 13(4), 639-648.

Puchkova E, Alekseeva A. (2024). Soil bacteria from autochthonic microbial communities of Central Siberia in biological protection of wheat from Alternaria tenuissima. Bulletin of KSAU 0(6), 62–68. https://doi.org/10.36718/1819-4036-2024-6-62-68

Saitou N & Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406-425.

Saitou N, Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4(4), 406–425.

Sambrook J, Russell DW. (2001). Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor Laboratory Press.

Shumenova N, Nauanova A, Kazangapova N, Yesmurzayeva A, Bekenova S.(2024). Optimal technological parameters for cultivating Trichoderma consortia isolated from Northern Kazakhstan soils. International Journal of Design & Nature and Ecodynamics 19(4), 1293-1300. https://doi.org/10.18280/ijdne.190421

Simmons EG. (2007). *Alternaria*: An Identification Manual. CBS Biodiversity Series 6.

Smirnova I, Sadanov A, Baimakhanova G, Faizulina E, Tatarkina L.(2024). Phosphate-solubilizing salt-tolerant bacteria to support soybean growth (Glycine max (L.) Merr.) on saline soils. OnLine Journal of Biological Sciences 24(1), 9-18. https://doi.org/10.3844/ojbsci.2024.9.18

Tamura K, Stecher G & Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution. https://doi.org/10.1093/molbev/msab120.

Tamura K, Stecher G, Kumar S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution 38(7), 3022–3027.

Tang X, Ban R, Wu X, Li H, Javed K, Peng L, Ding H. (2023). Identification and fungicide sensitivity of *Alternaria tenuissima* causing leaf spot of *Tetrapanax papyrifer* newly reported in China. Journal of Phytopathology 172(1). https://doi.org/10.1111/jph.13244

Tarar ZH, Ashraf W, Asghar S, Habib F, Shah SAH, Khaliq A, Ul Sher R, Shaheen MK, Majeed T, Tariq H, Sarwar S & Aas MA. (2022). Review on soil fertility and soybean yield improvement by managing micronutrients. Journal of Global Innovations in Agricultural Sciences 10(4), 255–266. https://doi.org/10.22194/JGIAS/10.1019

Tirono M.(2022). The use of a time-changing magnetic field to increase soybean (Glycine max) growth and productivity. International Journal of Design & Nature and Ecodynamics 17(5), 737-743. https://doi.org/10.18280/ijdne.170511

Tleulina Z, Kipshakbayeva G, Ashirbekova I, Amantayev B, Kipshakbayeva A. (2024). Comparative analysis of soybean (Glycine max (L.) Merrill) varieties: Implications for yield and quality. International Journal of Design & Nature and Ecodynamics 19(1), 249-257. https://doi.org/10.18280/ijdne.190127

Tyurina L.E., Tabakov N.A. (2008). Ispolzovanie i pererabotka soi: ucheb. posobie [Use and processing of soybeans: a manual]. Krasnoyar. gos. agrar. un-t, 90 p.

Utelbayev YA, Abysheva GT, Bazarbayev BB, Mussynov KM & Tahsin NT. (2021). Development and spread of diseases in spring camelina (Camelina sativa (L.) Grantz) when using various treatments. OnLine Journal of Biological Sciences 21(4), 288-298.

Utelbayev YA, Abysheva GT, Bazarbayev BB, Mussynov KM, Tahsin NT. (2021). Development and spread of diseases in spring camelina (Camelina sativa (L.) Grantz) when using various treatments. OnLine Journal of Biological Sciences 21(4), 288–298. https://doi.org/10.3844/ojbsci.2021.288.298

Vegas EZS, Nieves B, Araque M, Velasco E, Ruiz J, Vila J. (2006). Outbreak of infection with Acinetobacter strain RUH 1139 in an intensive care unit. Infection Control and Hospital Epidemiology 27(4), 397-404.

Woudenberg JH, Seidl MF, Groenewald JZ, de Vries M, Stielow JB, Thomma BP, Crous PW. (2015). Alternaria section Alternaria: Species, formae speciales or pathotypes? Studies in Mycology 82, 1–21. https://doi.org/10.1016/j.simyco.2015.07.002

Yadav AN, Kour D, Kaur T, Devi R, Yadav A, Dikilitas M, Abdel-Azeem AM, Ahluwalia AS, Saxena AK

(2021). Biodiversity, and biotechnological contribution of beneficial soil microbiomes for nutrient cycling, plant growth improvement and nutrient uptake. Biocatalysis and Agricultural Biotechnology, 33, 2021, 102009, https://doi.org/10.1016/j.bcab.2021.102009.

Yang J, Ye W, Wang X, Ren L, Yao Y, Wang X et al. (2020). An improved method for the identification of soybean resistance to Phytophthora sojae applied to germplasm resources from the Huanghuaihai and Dongbei regions of China. Plant Disease 104(2), 408-413.

Yorinori JT, Paiva WM, Frederick RD, Costamilan LM, Bertagnolli PF, Hartman GE & Nunes Jr J. (2005). Epidemics of soybean rust (Phakopsora pachyrhizi) in Brazil and Paraguay from 2001 to 2003. Plant Disease, 89(6), 675-677. https://doi.org/10.1094/pd-89-0675.

Zhakyalkov RA, Bayazitova KN, Polyak AI, Kassymbekova LN, Mustafina R, Zabolotnykh MV. (2024). Veterinary and sanitary assessment of milk quality in black-and-white cows fed with extruded compound feeds in North Kazakhstan. Advancements in Life Sciences, 11(4), 893-898. https://als-journal.com/11428-24/

Zhang Y, Wang W, Zhang F, Ma Q, Gao S, Wang J, Sun J & Liu Y. (2022). Rapid and non-destructive decay detection of Yali pears using hyperspectral imaging coupled with 2D correlation spectroscopy. International Journal of Agricultural and Biological Engineering, 15(5), 236–244. https://doi.org/10.25165/j.ijabe.20221505.7313