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ISOLATION AND ITS SEQUENCING-BASED IDENTIFICATION OF *MACROPHOMINA PHASEOLINA* IN SOYBEAN (*GLYCINE MAX*)

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ABSTRACT

Macrophomina phaseolina, the causal agent of charcoal rot, is a major pathogen of soybean (*Glycine max*), leading to severe yield losses in hot and dry conditions. This study aimed to isolate and characterize *Macrophomina phaseolina* using morphological and molecular tools. Isolates from a charcoal rot-infected field were purified via serial dilution on PDA, with typical microsclerotia observed microscopically. DNA was extracted and amplified using ITS1/ITS4 primers. Sequence analysis confirmed identity via NCBI-BLAST, and the representative strain was deposited in GenBank (Accession: MZ823608). Histopathological assays further revealed distinct pathogenic behaviors in susceptible and resistant soybean genotypes. These findings confirm isolate identity and contribute to understanding *Macrophomina phaseolina* pathogenesis for future disease management and diagnostic development.

Key words : Characterization, ITS Sequencing, *Macrophomina phaseolina*, NCBI-BLAST, Soybean.

Introduction

Soybean (*Glycine max* L.) is a vital leguminous crop cultivated worldwide for its high protein and oil content. However, its productivity is threatened by over 100 documented diseases, among which charcoal rot caused by the soil-borne fungal pathogen *Macrophomina phaseolina* is a major concern, particularly under hot and drought-prone conditions (Hartman *et al*., 2011). This disease has emerged as a significant constraint to soybean cultivation, especially in India, where yield losses due to *Macrophomina phaseolina* can exceed 70% under stress conditions (Reznikov *et al*., 2018; Ansari, 2010). *Macrophomina phaseolina* has an exceptionally broad host range, affecting more than 500 plant species, including agronomic crops such as soybean (Kaur *et al*., 2012; Kumar and Dubey, 2023).

The pathogen primarily survives as microsclerotia in infected seeds and plant debris, remaining viable in the soil for up to four years (Sarr *et al*., 2014; Chavan *et al*., 2019; Liu *et al*., 2022). Under favourable soil temperatures (30–33°C), these microsclerotia germinate and initiate infection by producing hyphae that invade soybean roots, disrupting vascular functions and ultimately causing wilting, necrosis, and plant death (Chaudhary *et al*., 2023; Gupta *et al*., 2012; Mengistu *et al*., 2013). Charcoal rot symptoms typically appear after flowering (R1 stage) and intensify during seed development stages (R5 to R7), complicating early diagnosis and limiting effective control measures at later growth stages (El-Araby *et al*., 2003). The disease is characterized by root and stem discoloration, wilting, and the formation of black, dusty microsclerotia within vascular tissues (Mengistu *et al*., 2007; Mengistu *et al*., 2011). Following infection, the fungus completes its lifecycle by returning to the soil through plant residue, thereby serving as a primary source of inoculum for subsequent cropping cycles (Luna *et al*., 2017; Kumar and Dubey, 2023; Kaur *et al*., 2012; Smith *et al*., 2014). Accurate identification of *Macrophomina*

phaseolina is vital for disease management. The ITS region of rDNA is widely used as a fungal barcode, but primer bias can affect amplification accuracy (Bellemain *et al*., 2010). Primers like ITS1-F, ITS1, and ITS5 favor basidiomycetes, while ITS2, ITS3, and ITS4 target ascomycetes, including *Macrophomina phaseolina*. ITS sequencing enables precise identification and phylogenetic analysis. Given its destructive nature, especially under climate stress (Meyer, 1974; Sarr *et al*., 2014), molecular diagnostics are essential for resistance breeding and sustainable crop management. Therefore, the present study aims to isolate and identify *Macrophomina phaseolina* from soybean using morphological observations and ITS-based sequencing, contributing to the molecular epidemiology and early detection of charcoal rot disease.

Materials and Methods

Soil samples were collected from a charcoal rot symptomatic soybean field in Amravati, India (20.9374°N, 77.7795°E). Ten grams of each sample were suspended in 90 mL sterile buffer to obtain a 10^{-1} dilution; serial tenfold dilutions were prepared up to $10^{-}6$ (Lakhran **et al**., 2018). Aliquots (0.1 mL) of each dilution were spread onto Potato Dextrose Agar (PDA) plates, prepared as follows (Sreedevi *et al*., 2011): peeled potatoes were boiled, filtered, and adjusted to pH 7.0; 20 g dextrose and 15 g agar were added per liter; media were autoclaved at 121 °C for 15 min and poured into Petri dishes. Plates were incubated at 28 ± 2°C for 7 days. Typical grey black colonies bearing microsclerotia were subculture to obtain pure isolates, which were confirmed morphologically using lactophenol cotton blue mounting (Almomani *et al*., 2013).

Mass Multiplication

A pure culture of *Macrophomina phaseolina* was grown on sterilized Jowar seeds for 21 days at 28 °C in the dark to produce inoculum for downstream assays. Seeds colonized by fungal mycelium and microsclerotia were air dried and used for in vitro soil infestation.

Genomic DNA extraction

Sixteen *Macrophomina phaseolina* isolates were cultured on PDA for 7 days. Mycelial mats were harvested, frozen in liquid nitrogen, and ground in 50 µL lysis buffer (50 mM Tris–HCl pH 7.2, 50 mM EDTA, 3% SDS) supplemented with 3 µL β mercaptoethanol. After incubation at 65°C for 60 min, lysates were extracted twice with phenol–chloroform–isoamyl alcohol (25:24:1) and centrifuged; DNA in the aqueous phase was precipitated with cold isopropanol and 3 M ammonium acetate, washed in 70% ethanol, air dried, and resuspended in TE buffer. Samples were treated with RNase A (10 mg mL^{-1}) at 37°C for 1 h, re extracted with phenol–chloroform, precipitated with isopropanol and 95% ethanol, then stored at –20°C. DNA concentration and purity were assessed spectrophotometrically (Jiang *et al*., 2015).

PCR amplification

The internal transcribed spacer (ITS) region was amplified using primers ITS1 (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) (White *et al*., 2013). PCR reactions (50 µL) contained: 5 µL 10× buffer, 1 U Taq DNA polymerase, 160 µM dNTPs, 50 pmol each primer, 50 ng template DNA, and nuclease free water. Cycling conditions were: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min; final extension at 72 °C for 10 min. Amplified products (~600 bp) were digested overnight at 37 °C with a restriction enzyme (10 U mL^{-1}) in a 20 µL reaction, then separated on a 2% agarose gel (6 V cm^{-1}, 6 h) in 1× TBE, stained with ethidium bromide and visualized under UV light.

ITS Sequencing and Database submission

Eight representative isolates from diverse hosts were selected for ITS sequencing. PCR products were purified (Prepagene Kit, Bio Rad) and sequenced by Eurofins Scientific. Resulting sequences were compared to GenBank and EMBL databases using BLASTn. A consensus sequence from the soybean derived isolate (Akola 01) was submitted to NCBI GenBank (Accession No. MZ823608; submitted 23 August 2021).

In vitro Histopathological assay

Soybean seedlings at V6 stage (unrolling of six trifoliolates) were uprooted and their roots immersed in a fungal suspension (10 g colonized jawar seeds per 100 mL sterile water). Ten plants per genotype were sampled daily from 3 to 14 days' post inoculation. Roots were sectioned (2–3 mm), softened in 0.5 M NaOH for 3 min, washed, and stained with lactophenol cotton blue. Fungal colonization and microsclerotia development were examined under light microscopy (Hemmati *et al*., 2018).

Results and Discussion

Macrophomina phaseolina harbors a diverse array of pathogenicity associated genes, underscoring the importance of molecular characterization to confirm its identity. The isolation of *Macrophomina phaseolina* from soil sample gathered from a soybean field affceted with charcoal rot disease. It was successfully achieved using the serial dilution and potato dextrose agar (PDA) method.

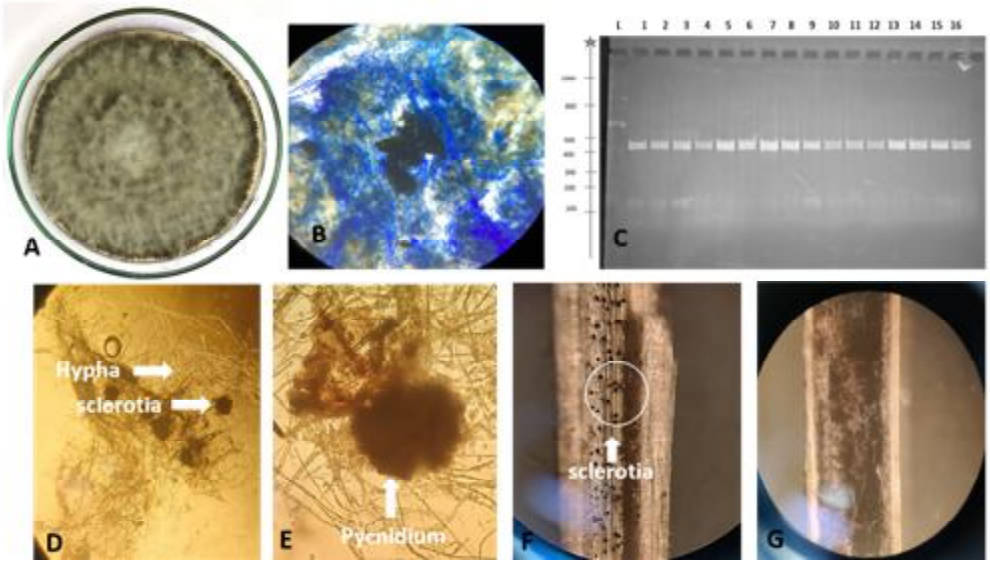

Fig. 1 : Characterization of *Macrophomina phaseolina* (*Mp*) based on morphological and molecular charateristics.

This method also employed by An and Kim (2023). The colonies of *Macrophomina phaseolina* grown on potato dextrose agar after 7 days of incubation at 28°C is demonstrated in Fig. 1 (A). The morphological characteristics were documented and confirmed the isolated fungus under a microscope using lactophenol cotton blue mounting solution. The aerial hyphal network of *Macrophomina phaseolina*, visualized through cotton blue staining, is illustrated in Fig. 1 (B). The pattern identified in this investigation was aligned with the observations made by Dell'Olmo *et al*. (2022). Genomic DNA of *Macrophomina phaseolina* was extracted using the DNAzol technique (Amrate *et al.*, 2023). Fig. 1(C) illustrates the PCR amplification of genomic DNA from 16 *Macrophomina phaseolina* isolates using ITS1/ITS4 primers, yielding a consistent amplicon of approximately 430 bp. Fig. 1 (D-E) present light micrographs showcasing the presence of globus pycnidium and sclerotia, alongside hyphae. Figs. 1(F–G) show longitudinal sections of soybean stems infected with *Macrophomina phaseolina*, revealing abundant sclerotia and a dense, web-like hyphal network predominantly localized on the inner vascular tissues. The results from present study supports the conclusions outlined by Hemmati *et al.* (2018) on post penetration events. The evidence gathered in this investigation corresponds with the conclusions reached by Bhuiyan *et al*. (2015), Reznikov *et al*. (2018) and Kouadri *et al*. (2023).

The DNA extracted from the samples underwent PCR amplification using ITS1/4 primers. The selection of these primers was aimed at specifically amplifying the DNA region of *Macrophomina phaseolina*, facilitating species identification, as previously described by Bellemain *et al*. (2010) and Nahberger *et al*. (2020). Subsequent to PCR, the amplified DNA fragments underwent restriction digestion and were then separated by gel electrophoresis (Fig. 1C). Following this, sequencing of the PCR products was carried out. The obtained sequences of the ITS PCR products were then compared and analyzed for similarity against sequences stored in both GenBank and EMBL databases, as referenced (Bellemain *et al*., 2010; Nahberger *et al*., 2020). The outcomes of these analyses are illustrated in the flowchart provided in Fig. 2. In this analysis, the ITS region was sequenced utilizing the ITS1/4 primer, and the resulting sequences were deposited in GenBank under the accession. BLASTn analysis of Akola strain *Macrophomina phaseolina* revealed that the sequence exhibited 96.59% identity with several annotated *Macrophomina phaseolina* ITS sequences, predominantly aligning with the sequence ID OM421976.1 in the NCBI database followed by sequences of OM341626.1 and OR240847.1. Thus, confirming the identity of *Macrophomina phaseolina*. The sequence data was officially submitted to the National Centre for Biotechnology Information (NCBI) and assigned the GeneBank accession number MZ823608 (Table 1). Similar results were reported by Pandey *et al*. (2020).

Behavior of *Macrophomina phaseolina* under *in-vitro* screening condition

The histopathological examination yielded valuable insights into the pivotal stage of interaction between plants and *Macrophomina phaseolina*. Throughout the study, captivating video footage captured the live movement of the fungus as it began penetrating the soybean root with its hyphae and then progressed within the root structure. The video footage (https://youtube.com/shorts/

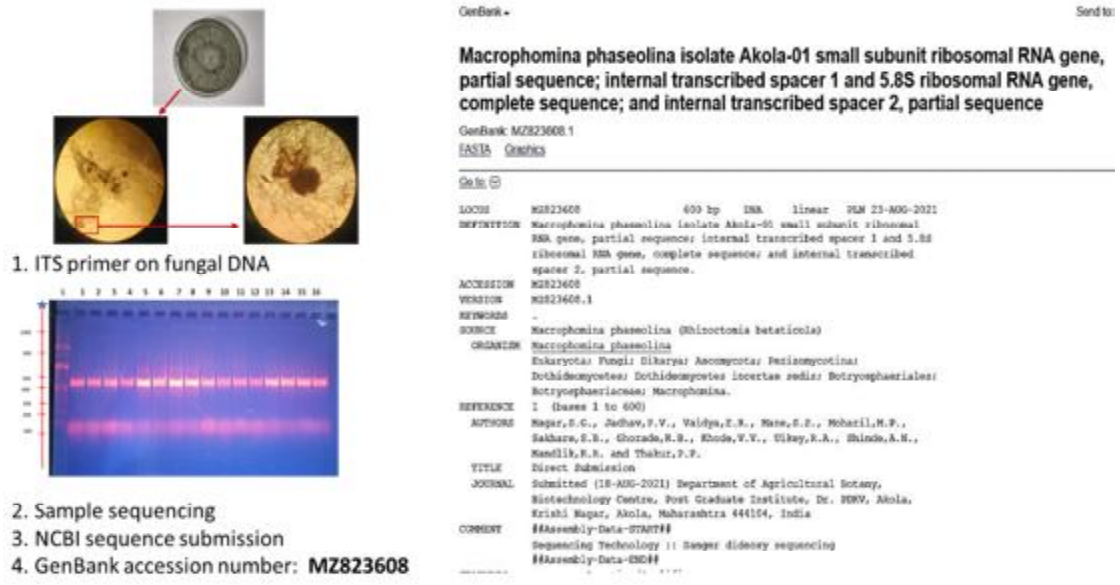

Fig. 2 : ITS region sequencing for molecular confirmation of *Macrophomina phaseolina*.

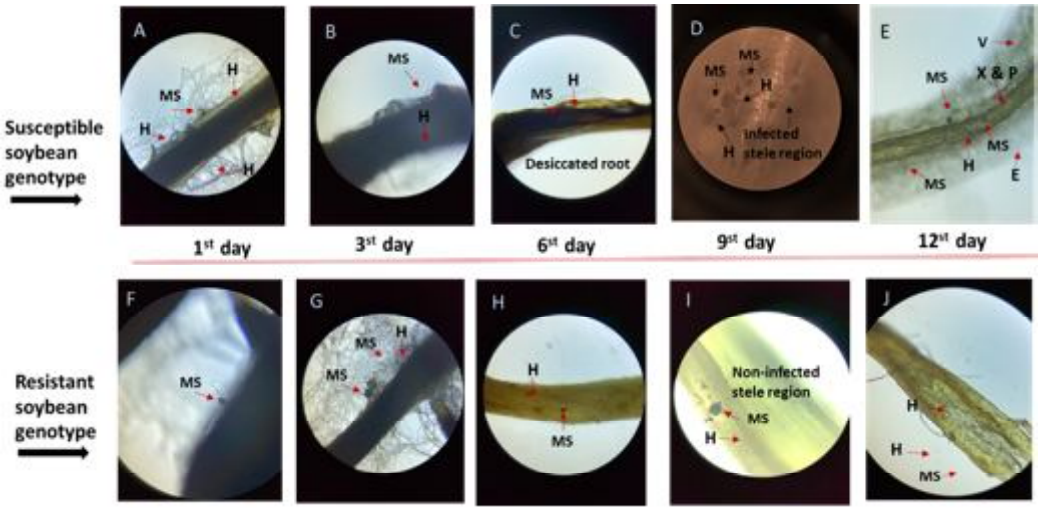

Fig. 3 : Progression of charcoal rot symptoms in susceptible (TAMS-38) and resistant (Swarna Soya) soybean genotypes. MS: Microsclerotia; H: Hyphae; X & P: Xylem and Phloem; V: Vessels; E: Epidermis.

t1iT5Hic2_M) unveiled that the fungus *Macrophomina phaseolina* required approximately 3-4 hours to establish a successful foothold on the soybean root. Examination of susceptible and resistant genotype roots at various time points post-inoculation (1, 3, 6, 9 and 12 days) revealed pathogen infiltration within intercellular spaces, resulting in significant root tissue damage and eventual plant death (Fig. 3). As the infection developed, the susceptible genotype was the only one to show signs of microsclerotia penetration and multiplication (Fig. 3 A), while the resistant genotype remained unaffected (Fig. 3 F). This was a noteworthy first finding made on the third day of the infection. The susceptible genotype experienced adverse effects in the following days, such as root dehydration and necrotic signs by the sixth day in the second observation, which showed the advancement of the disease (Fig. 3 B-C). Multiple microsclerotia invasions inside the susceptible genotype's root material resulted in browning by the third monitoring day, which was the ninth day (Fig. 3 D). In contrast, Fig. 3 G-I showed that the resistant genotype Suvarn soya had a distinct area that was infected with very few microsclerotia invading. By the time of the fourth observation on the twelfth day, there was a clear distinction between the two genotypes. At that point, microsclerotia completely encased the inner tissue of the susceptible genotype, covering the sections involved in the transportation of nutrients and water (Fig. 3 E). Interestingly, the pathogen had no effect on the resistant plant (Fig. 3 J). These results highlight the susceptible genotype's vulnerability to disease and highlight the function of hyphae and microsclerotia development in *Macrophomina phaseolina* pathogenicity.

Table 1 : Molecular characteristics of *Macrophomina phaseolina* sequence.

Source	Soybean infected field
Organism	*Macrophomina phaseolina*
Molecular Type	Genomic DNA
Isolate	Akola-01
Host	*Glycine max*
Database Cross-Reference	taxon:35725
Sequence ID	MZ823608.1
Sequence	1 ccacaccgggggggtttcaaaggggggggggcgggggggggcgactcaactcgtaatgatt 61 cctctaggtggaactgcggaaggatcattaccgagttgattcgggctccgtcccggtcct 121 cccacccttgtatacctacctctgttgctttggcgggccgcggtcttccgcggccgccc 181 cccgattttgggggggtggctagtgcccgccagaggactatcaaactccagtcagtaaacg 241 ttgcagtctgaaaaaaatattaaataaactaaaactttcaacaacggatctcttggttct 301 ggcatcgatgaagaacgcagcgaaatgcgataagtaatgtgaattgcagaattcagtgaa 361 tcatcgaatctttgaacgcacattgcgccccttggtattccgggggggcatgcctgttcga 421 gcgtcatttcaaccctcaagctctgcttggtattgggcaccgtcctttgcgggcgcgcct 481 caaagacctcggcggtggcgtcttgcctcaagcgtagtagaatacacctcgcttccggag 541 cgtagggccgtcgccacgccaggacgttccttcctgttacttttcctcgaaggttgcggt

BLASTN : Sequences alignment

Description	Scientific name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Test sequence of *Macrophomina phaseolina* isolate from Akola								
1. *Macrophomina phaseolina* isolate Akola-01 small subunit ribosomal RNA gene	*Macrophomina phaseolina*	1109	1109	100%	0.0	100.00%	600	MZ823608.1
Sequences alignment match with database reference sequences								
Description	**Scientific name Score**	**Max Score**	**Total Cover**	**Query**	**E value Ident**	**Per. Len**	**Acc.**	**Accession**
1 *Macrophomina pseudophaseolina* isolate CRb6 small subuni tribosomal RNA gene, partial sequence	*Macrophomina pseudophase-olina*	915	915	92%	0.0	96.59%	686	OM421976.1
2 *Macrophomina phaseolina* isolate CRb7 small subuni tribosomal RNA gene, partial sequence	*Macrophomina pseudophase-olina*	915	915	92%	0.0	96.59%	693	OM341626.1
3 *Macrophomina phaseolina* isolate Mp10 small subunit ribosomal RNA gene	*Macrophomina phaseolina*	909	1076	92%	0.0	96.42%	702	OR240847.1

The present study, resembling the approaches of Hemmati *et al*. (2018) and Siddique *et al*. (2021), employs histopathological analysis to understand the infection dynamics of *Macrophomina phaseolina* in soybean plants. Both studies investigate the responses of various genotypes, including susceptible and resistant ones, to the infection, uncovering distinctions in root colonization and tissue degradation, akin to present study.

Conclusion

The present study successfully confirmed the identity of *Macrophomina phaseolina* through molecular characterization using ITS1/ITS4 primers. BLASTn analysis of the amplified ITS region showed 96%

sequence identity, and the representative isolate from Akola was submitted to GenBank (Accession No. MZ823608), ensuring reliability for subsequent experimental analyses. Histopathological investigations using hydroponic and rhizotron systems revealed early root colonization by *Macrophomina phaseolina* within 3–4 hours post-inoculation. The susceptible soybean genotype exhibited pronounced microsclerotia development, tissue colonization, and root necrosis. In contrast, the resistant genotype 'Swarna Soya' showed limited fungal invasion, indicating effective defense responses. The sequenced isolate offers a valuable genomic reference for the development of diagnostic molecular markers, enabling early detection and supporting resistance breeding programs aimed at mitigating charcoal rot in soybean.

References

Almomani, F., Alhawatema M. and Hameed K. (2013). Detection, identification and morphological characteristic of *Macrophomina phaseolina*: The charcoal rot disease pathogens isolated from infected plants in Northern Jordan. *Arch. Phytopathol. Plant Protect.*, **46(9)**, 1005-1014.

Amrate, P.K., Shrivastava M.K., Bhale M.S., Agrawal N., Kumawat G., Shivakumar M. and Nataraj V. (2023). Identification and genetic diversity analysis of high-yielding charcoal rot resistant soybean genotypes. *Scientific Reports*, **13(1)**, 1–13.

An, S.H. and Kim H.T. (2023). Establishment of Pathogenicity Test Method for *Macrophomina phaseolina* causing Soybean Charcoal Rot. *Res. Plant Dis.*, **29(1)**, 1–10.

Ansari, M.M. (2010). Integrated management of charcoal rot of soybean caused by *Macrophomina phaseolina* (Tassi) Goid. *Soybean Res.*, **8**, 39-47.

Bellemain, E. Carlsen T., Brochmann C., Coissac E., Taberlet P. and Kauserud H. (2010). ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiol.*, **10**, 1-9.

Bhuiyan, M.A.H.B., Groom T., Nicolas M.E. and Taylor P.W.J. (2015). Histopathology of *S. tanaceti* infection in pyrethrum leaf lamina. *Australasian Plant Pathology*, **44(6)**, 629–636.

Chaudhary, H., Kumar P. and Dubey R.C. (2023). Mechanism of disease development in host plants by *Macrophomina phaseolina*. *Ecobiology, Pathology and Management*, 133–154.

Chavan, S.V., Jadhav P.V., Mane S.S. and Nichal S.S. (2019). Morphological characterizations of soybean genotypes in response to charcoal rot disease under sick plot conditions. *J. Pharmacog. Phytochem.*, **8(5)**, 989-992.

Dell'Olmo, E., Tripodi P., Zaccardelli M. and Sigillo L. (2022). Occurrence of *Macrophomina phaseolina* on Chickpea in Italy: Pathogen Identification and characterization. *Pathogens*, **11(8)**, 842.

El-Araby, M.E., Kurle J.E. and Stetina S.R. (2003). First Report of Charcoal Rot (*Macrophomina phaseolina*) on Soybean in Minnesota. *Plant Disease*, **87(2)**, 202-202.

Gupta, G.K., Sharma S.K. and Ramteke R. (2012). Biology, Epidemiology and Management of the Pathogenic Fungus *Macrophomina phaseolina* (Tassi) Goid with Special Reference to Charcoal Rot of Soybean (*Glycine max* (L.) Merrill). *J. Phytopathol.*, **160(4)**, 167–180.

Hartman, G.L., West E.D. and Herman T.K. (2011). Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests. *Food Security*, **3**, 5-17.

Hemmati, P., Zafari D., Mahmoodi S.B., Hashemi M., Gholamhoseini M., Dolatabadian A. and Ataei R. (2018). Histopathology of charcoal rot disease (*Macrophomina phaseolina*) in resistant and susceptible cultivars of soybean. *Rhizosphere*, **7**, 27–34.

Jiang, L., Wu J., Fan S., Li W., Dong L., Cheng Q., Xu P. and Zhang, S. (2015). Isolation and characterization of a novel pathogenesis-related protein gene (GmPRP) with induced expression in soybean (*Glycine max*) during infection with Phytophthora sojae. *PLoS ONE*, **10(6)**, 129932.

Kaur, S., Dhillon G.S., Brar S.K., Vallad G.E., Chand R. and Chauhan V.B. (2012). Emerging phytopathogen *Macrophomina phaseolina*: biology, economic importance and current diagnostic trends. *Crit. Rev. Microbiol.*, **38(2)**, 136-151.

Kouadri, M.E.A., Bekkar A.A. and Zaim S. (2023). Morphological, molecular and pathogenic characterization of *Macrophomina pseudophaseolina* the causal agent of charcoal rot disease on lentil (*Lens culinaris*) in Algeria. *Physiolog. Mole. Plant Pathol.*, **128**, 102-143.

Kumar, P. and Dubey R.C. (Eds.) (2023). *Macrophomina phaseolina*: ecobiology, pathology and management. Elsevier, PP. 109-120.

Lakhran, L., Ahir R.R., Choudhary M. and Choudhary S. (2018). Isolation, purification, identification and pathogenicity of *Macrophomina phaseolina* (Tassi) goid caused dry root rot of chickpea. *J. Pharmacog. Phytochem.*, **7(3)**, 3314-3317.

Liu, J., Dong L., Duan R., Hu L., Zhao Y., Zhang L. and Wang X. (2022). Transcriptomic Analysis Reveals the Regulatory Networks and Hub Genes Controlling the Unsaturated Fatty Acid Contents of Developing Seed in Soybean. *Front. Plant Sci.*, **13**, 876371.

Luna, M.P.R., Mueller D., Mengistu A., Singh A.K., Hartman G.L. and Wise K.A. (2017). Advancing our understanding of charcoal rot in soybeans. *J. Integrated Pest Manage.*, **8(1)**, 8.

Mengistu, A., Arelli P., Bond J., Nelson R., Rupe J., Shannon G. and Wrather A. (2013). Identification of soybean accessions resistant to *Macrophomina phaseolina* by field screening and laboratory validation. *Plant Health*

Progress, **14(1)**, 25.

Mengistu, A., Ray J.D., Smith J.R. and Paris R.L. (2007). Charcoal rot disease assessment of soybean genotypes using a colony-forming unit index. *Crop Sci.*, **47(6)**, 2453–2461.

Mengistu, A., Smith J.R., Ray J.D. and Bellaloui N. (2011). Seasonal progress of charcoal rot and its impact on soybean productivity. *Plant Dis.*, **95(9)**, 1159–1166.

Meyer, W.A. (1974). Factors Affecting Charcoal Rot of Soybean Seedlings. In *Phytopathology*, **64(6)**, 845.

Nahberger, T.U., Kraigher H. and Grebenc T. (2020). PCR primers comparisons for a successful Tuber spp. DNA region amplification in routine identifications. *Folia Biologica et Geologica*, **61(2)**, 229–238.

Pandey, A.K., Burlakoti R.R., Rathore A. and Nair R.M. (2020). Morphological and molecular characterization of *Macrophomina phaseolina* isolated from three legume crops and evaluation of mungbean genotypes for resistance to dry root rot. *Crop Protection*, **127**, 104962.

Reznikov, S., Vellicce G.R., Mengistu A., Arias R.S., Gonzalez V., Lisi V., De MARÍA Gabriela, G. Carla MARÍA LOURDES, R. Pardo E.M., Castagnaro A.P. and Ploper L.D. (2018). Disease incidence of charcoal rot (*Macrophomina phaseolina*) on soybean in north-western Argentina and genetic characteristics of the pathogen. *Canadian J. Plant Pathol.*, **40(3)**, 423–433.

Sarr, M.P., Ndiaye M., Groenewald J.Z. and Crous P.W. (2014). Genetic diversity in *Macrophomina phaseolina*, the causal agent of charcoal rot. *Phytopathologia Mediterranea*, **53(2)**, 203-376.

Siddique, S., Shoaib A., Khan S.N. and Mohyuddin A. (2021). Screening and histopathological characterization of sunflower germplasm for resistance to *Macrophomina phaseolina*. *Mycologia*, **113(1)**, 92–107.

Smith, D., Chilvers M., Dorrance A., Hughes T., Mueller D., Niblack T. and Wise K. (2014). Charcoal rot management in the north central region. University of Wisconsin Extension Bulletin A, PP. 4037.

Sreedevi, B., Charithadevi M. and Saigopal D.V.R. (2011). Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina*. *J. Agricult. Technol.*, **7(3)**, 623-635.

White, T.J., Bruns T., Lee S.J.W.T. and Taylor J. (2013). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: A guide to Methods and Applications*, **18(1)**, 315-322.