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Exploring the Genetic Diversity of *Macrophomina phaseolina* isolates in Soybean Crops: A Study from Madhya Pradesh

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ABSTRACT: *Macrophomina phaseolina* is a soil-borne pathogen causing significant yield losses in soybean crops, particularly in Madhya Pradesh, India, where soybean is a major agricultural commodity. This study aims to explore the genetic diversity of *M. phaseolina* isolates collected from infected soybean plants across various regions of Madhya Pradesh. A total of 12 isolates were collected from symptomatic plants displaying signs of charcoal rot. Molecular analysis was conducted using molecular markers such as Random Amplified Polymorphic DNA (RAPD) to assess genetic variability among the isolates. The results elucidated substantial genetic diversity among the isolates, signifying that *M. phaseolina* populations within this geographical area exhibit a high degree of heterogeneity. Phylogenetic analysis categorized the isolates into discrete clusters, implying the existence of multiple strains with differing pathogenicity levels. This genetic variability may enhance the pathogen's capability to acclimatize to diverse environmental conditions and host resistance mechanisms, thereby complicating disease management efforts. The genetic diversity of *M. phaseolina* is integral to formulating effective control strategies, which encompasses the cultivation of resistant soybean varieties and the execution of targeted disease management interventions. This genetic variation may contribute to the pathogen's ability to adapt to different environmental conditions and host resistance mechanisms, making disease management more challenging. The genetic diversity of *M. phaseolina* is crucial for developing effective control strategies, including the breeding of resistant soybean cultivars and the implementation of targeted disease management practices. This study provides valuable insights into the population structure of *M. phaseolina* in Madhya Pradesh, highlighting the need for region-specific approaches to manage charcoal rot in soybean crops.

Keywords: *Macrophomina phaseolina*, genetic diversity, soybean, Madhya Pradesh, charcoal rot, molecular markers

INTRODUCTION

Soybean

The soybean (*Glycine max*) constitutes a crop of considerable global significance, recognized for its protein quality that is analogous to that of animal-based sources, thus establishing it as a critical nutrient provider (Hartman et al., 2011). This crop is positioned among the top ten commodities traded on an international scale (Anonymous, 2013). Throughout the production year 2018-19, the United States emerged as the principal producer with an output of 120.5 million tonnes, succeeded in rank by Brazil, Argentina, China, and India (SOPA, 2020). In the Indian context, soybean cultivation predominantly occurs under rainfed conditions during the Kharif season, with Madhya Pradesh accounting for over 50% of both the total cultivated area and production (Anonymous, 2018). The central region encompassing Madhya Pradesh, Maharashtra, and Rajasthan holds the reins for 92-93% of India's soybean farming terrain and yield (Anonymous, 2019). As an essential global pulse, soybean (*Glycine max*) serves a pivotal function in delivering both protein and oil for the nutritional demands of humans and livestock (USDA, 2020). However, production faces daunting challenges from diseases like charcoal rot, which is triggered by the soil-

dwelling fungus *Macrophomina phaseolina* (Wrather et al., 2001; Chaudhary, 2017; Mengistu et al., 2007). As the degree of susceptibility is contingent upon a variety of factors, encompassing environmental conditions, local pathogen strains, and the genetic makeup of each variety. Nevertheless, a considerable number of commercial soybean varieties exhibit varying levels of susceptibility to these isolates. Although certain soybean varieties may demonstrate partial resistance, a complete absence of fully resistant varieties in the market persists. Breeding initiatives are currently in progress aimed at cultivating varieties with enhanced resistance to *M. phaseolina*; however, the intricate nature of the disease and its interplay with environmental variables complicates the precise quantification of the number of varieties that are significantly susceptible.

Mechanisms of *Macrophomina phaseolina*

Macrophomina phaseolina, the causal agent of charcoal rot disease in plants, utilizes several mechanisms to infect and colonize its host plants. Understanding these mechanisms is essential for developing effective strategies to manage this pathogen.

Production of Cell Wall Degrading Enzyme

Macrophomina phaseolina secretes various cell wall degrading enzymes such as pectinases, cellulases, and hemicellulases. These enzymes help in breaking down the plant cell wall, facilitating penetration and colonization of the host tissues (Jogaiah *et al*., 2018).

Production of Phytotoxins

The fungus produces Phytotoxins like oxalic acid, which can contribute to the pathogenesis of charcoal rot disease by causing cellular damage and weakening the host defense responses (Vaishnav *et al*., 2019).

Induction of Plant Defense Suppression

Macrophomina phaseolina has been shown to suppress plant defense responses, such as oxidative burst and defense-related gene expression, enabling successful colonization of host tissues (Islam *et al*., 2017).

Manipulation of Plant Hormone Signaling

The fungus can modulate plant hormone signaling pathways, particularly auxin and ethylene, to promote disease development and colonization of host tissues (Chittem *et al*., 2015).

Formation of Melanized Structures

Melanized structures formed by *Macrophomina phaseolina* within host tissues can aid in survival and persistence, contributing to the chronic nature of charcoal rot disease (Mengistu *et al*., 2019).

Genetic Diversity and Population Structure

Characterizing the genetic diversity and population structure of *M. phaseolina* isolates from sorghum fields is essential for understanding the evolutionary dynamics of the pathogen and its adaptation to different environments. Molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs) will be employed to assess the genetic variability among isolates. Population genetic analyses, including cluster analysis, principal component analysis (PCA), and genetic distance estimation, will be performed to elucidate the population structure and genetic relationships among *M. phaseolina* isolates (Pearson *et al*., 1984).

Virulence and Pathogenicity

The virulence and pathogenicity of *M. phaseolina* isolates on sorghum will be evaluated through controlled inoculation experiments under greenhouse and field conditions. Sorghum genotypes with contrasting levels of resistance will be inoculated with representative *M. phaseolina* isolates, and disease progression will be monitored over time. Pathogenicity assays will include assessments of disease severity, symptom development, and histological analyses of infected tissues. The virulence spectrum of *M. phaseolina* isolates will be determined by testing their pathogenicity on a panel of sorghum genotypes with diverse genetic backgrounds (Pearson *et al*., 1984).

Disease life cycle

The disease life cycle of *M. phaseolina* is favoured by dry soils and annual temperatures between 28 ºC and 35 ºC. Under these conditions, microsclerotia begin to germinate and produce germ tubes that penetrate plant epidermal cell walls or through natural openings (Dhingra and Sinclair, 1978; Bressano *et al*., 2010). During the early stages of infection, hyphae grow intercellularly and then intercellularly through the xylem in vascular tissues, where it can form microsclerotia that interfere with plant functions of transporting water and nutrients, resulting in disease symptoms, such as wilting and premature leaf death (Francl *et al*., 1988; Gupta and Chauhan, 2005; Khan, 2007). After harvest, the disease cycle starts over with the fungus surviving in the soil and soybean crop debris, generally as microsclerotia (Short *et al*., 1980).

Disease symptoms

Charcoal rot symptoms mainly appear during hot and dry conditions and can be observed in soybean at all stages, although above-ground symptoms are more prevalent during reproductive stages of soybean development and are characterized by stunted growth, leaf chlorosis, early maturation, and incomplete pod filling (Hartman *et al*., 1999). Moreover, symptoms in the soybean fields can be easily recognized by premature yellowing in scattered patches, which can increase under severe disease conditions (Gupta *et al*., 2012).

Microsclerotia form in the vascular tissues and in the pith, and can result in plant wilting and flagging of branches as a result from the blockage of water flow (Hartman *et al*., 1999). Microsclerotia can grow aboveground and are first visible in stem nodes. As the disease progresses, it can infect and penetrate pods and grains. The belowground symptom, if the infection starts through the roots, is mainly a reddish-brown discoloration of the vascular elements (Gupta *et al*., 2012).

Molecular markers

DNA markers have played an important role in plant genetics and breeding for decades. The first application of DNA polymorphism applying DNA markers in plant genotyping was described by Michelmore *et al*. (1991), using Random Amplified Polymorphic DNA (RAPD). Although the RAPD technique had been valuable in the construction of genetic linkage maps, it is complicated by use of hybridizations and radioactivity, is time consuming, and limited by the number of available probes (Bernatzky and Tanksley, 1986).

MATERIAL AND METHODS

The current study, elaborated a comprehensive investigation that included a survey of charcoal rot, isolation and purification of the pathogen (Chitrabhanu *et al*. 2021) pathogenicity tests, morphological and physiological variability studies, pathogen prevalence, and molecular identification using RAPD analysis. Laboratory experiments were conducted in the Department of Biological Sciences at Rani Durgavati University, Jabalpur (M.P.), with pot experiments carried out in a greenhouse. The materials and methods used are detailed below.

Procurement of Samples

Sterilized tools were employed between sampling sites or individual plants by immersing them in a disinfectant solution, such as 70% ethanol, to prevent the transmission of pathogens. From this perspective, symptomatic plant parts—such as stems, roots, and leaves—exhibiting clear signs of infection were collected for analysis (Frederiksen & Odvody, 1990). During the study period, samples were selected from a minimum of 10 to 20 plants per sampling site or field, depending on the size of the area and the uniformity of disease distribution. The plant samples were in clean, labeled bags or containers to prevent physical damage during transport. Keep samples cool and avoid extended exposure to sunlight or heat, especially during transit to the laboratory. Once the samples were reach the laboratory, process them promptly to prevent deterioration or loss of diagnostic value. Depending on the purpose (e.g., pathogen isolation and DNA extraction) were store samples appropriately (refrigeration, freezing or drying) until further analysis (Islam *et al*., 2017).

Purification of Pathogen

Pure culture of fungus *M. phaseolina* was obtained by hyphal tip method on SDA medium. For this, hyphal tips were obtained from culture slants after 96 hours of incubation and one ml of suspension was spread in petriplates containing 20 ml sterilized agar medium after 12-24 hours of inoculation, the germinating spores were observed and transferred to SDA slant and kept in BOD for further growth. The confirmed pure cultures were observed under microscope and the stock cultures maintained on SDA slants and stored in refrigerator at 4°C for further studies. A total of 12 isolates of *M. phaseolina* were isolated and subjected to examine the further experiments work.

Macroscopic and Microscopic Study

The morphological study was primary identification method then after microscopic and macroscopic observation of fruiting body and conidia done by using slide culture techniques. **Macroscopically**: the fungus produces dark, microsclerotia that are visible to the naked eye in infected plant tissues, often appearing as black streaks or patches on the stem or roots. The plant tissues show signs of necrosis, and in severe cases, the plant may wilt and die, especially under hot and dry conditions (Mihail, 1989).

Microscopically: *M. phaseolina* is characterized by the presence of abundant, small, spherical to oblong microsclerotia, typically 50-100 µm in diameter, which are darkly pigmented due to melanin deposits. The fungus also forms hyaline, septate hyphae that can be observed under a light microscope. The conidia are single-celled, hyaline and cylindrical to oblong, measuring about 20-30 µm in length (Wyllie, 1989).

Slide Culture Technique

The microscopic examination of fungal isolates were done by using slide culture bed techniques (Vaishnav *et al*., 2019) with the help of Trinocular microscope (Metzer) microscopy. To prepare a glass slide for microscopy, place a cover slip over the fungal growth on the slide. This helps to prevent dehydration and provides a clear surface for observation. For the identification of fungal isolates, disinfected bed chambers were prepared. A drop of PDA media was set inside the wet chamber on a glass slide and a circle brimming with inoculums was inoculated on it and moist chamber petri-plate was incubated at 22±1ºC in fungal incubator. Examine the glass slides under a Trinocular microscope by using cotton blue and mounted in one drop of Lactophenol blue, saw under magnifying lens observations of fungal isolates. Further identification rely on mycelia color, fruiting body, hyphal structure, conidia and spore formation at various magnifications (10 to 100X). Based on various parameters facilitated by microscope (Metzer) features mycelial growth pattern, spore advancement and examination were achieved as described by (Dhingra *et al*., 2022).

Quantification of DNA

The concentration and purity of the extracted DNA are determined using a UV-Vis spectrophotometery which was measured absorbance at 260 nm (for DNA concentration). The DNA concentration is typically expressed in nanograms per microliter (ng/µL). A purity ratio (A260/z 1.8-2.0) indicates high-quality DNA suitable for downstream applications. Additionally, DNA can be quantified using a (Shimanzu 2600) UV-vis Spectrophotometric method, such as the Qubit spectrophotometry, which is more sensitive and accurate for low-concentration samples.

RAPD Fingerprinting

DNA from *Macrophomina phaseolina* isolates was amplified by the RAPD method using 13 randomly selected 10-mer random oligonucleotide primers from Operon kit (OPJ-07). The PCR were carried out in a total volume of 25 µl reaction mixture containing 0.2 mM of each dNTPs, 50 mol. primer 2.5 µl of 10 × PCR buffer (100 mM, Tris-HCl, pH 8.3, 250 mM KCl, 15 mM $MgCl_2$)-1U of Taq DNA polymerase (Bangalore Genei; India), and 50 ng genomic DNA in H_2O. The PCR was carried out for 5 min of initial denaturation at 94°C, followed by 40 cycle of denaturation at 95°C for 1 min, annealing at 35°C for min and extension at 72°C for 2 min, with a final extension at 72°C for 5 min.

All the amplifications were repeated three times to check the reproducibility of the banding pattern. The PCR amplified products were run on 1.5% agarose gel in 1 TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) containing 0.5 mg/ ml Ethidium bromide, at constant voltage (5 V/cm) and visualized under UV transilluminator.

RESULTS AND DISCUSSION

In the present study investigations were undertaken to considering the economic significance of soybean charcoal rot disease, determine status, role of factors in disease development and management through integrating approaches. The results so obtained were presented herewith. The pathogen was adeptly isolated from infected soybean specimens utilizing selective PDA media or relevant isolation methodologies customized to the expected pathogen type (*M. phaseolina*). The pure cultures of the isolated pathogen were secured through the implementation of single-colony isolation methodology. *Macrophomina phaseolina* (Tassi) Goid was derived from the specimens assembled in the examined soybean cultivation locales of Indore district in Madhya Pradesh, where their characteristic attributes, comprising colony coloration, colony diameter, sclerotial color and morphology, as well as the dimensions of the sclerotia, were meticulously evaluated. Reflecting these characteristics, twelve isolates were classified as (I1, I2, I3……to I12) in alignment with their respective local cultural types. The isolated pathogen was cultivated on specialized growth media under rigorously regulated laboratory conditions, encompassing temperature, humidity, and variations in light exposure or darkness. The below-listed characteristics studied for cataloging above 12 isolates on the basis of Macroscopic and Microscopic observations.

Effect of various culture medium on growth of *M. phaseolina*

Medium	**Radial growth (mm)***	
	After 72 HAI	**After 120 HAI**
Potato dextrose agar	71.67	90.00
Czapek's dox agar	70.00	80.00
Sabrouds dextrose agar	63.00	80.23
Corn Meal Agar	65.00	82.00
Water Agar	1	1
S.Em±	1.19	1.23
CD (P=0.05)	3.37	3.48

Table 1. Effect of various culture medium on growth of *M. phaseolina* at 25±1°C

* Average of three replications; HAI= Hours after inoculation

Effect of five solid media, viz., Corn Meal Agar, Potato Dextrose Agar, Czapek's Dox Agar, and Water Agar on radial growth of *M. phaseolina* were studied and observations have been presented in **Table 1** and shown in

Figure 1. The radial growth was maximum on potato dextrose agar (90.00 mm) which was statistically at par with Czapek's dox agar (80.00 mm) and Sabrouds dextrose Agar (87.00 mm). This was followed by corn meal agar (65.67 mm). No radial growth was obtained in water agar, respectively. Data presented in **Table 1** clearly indicate that potato dextrose agar and Czapek's dox agar medium were best for radial growth *M. phaseolina.*

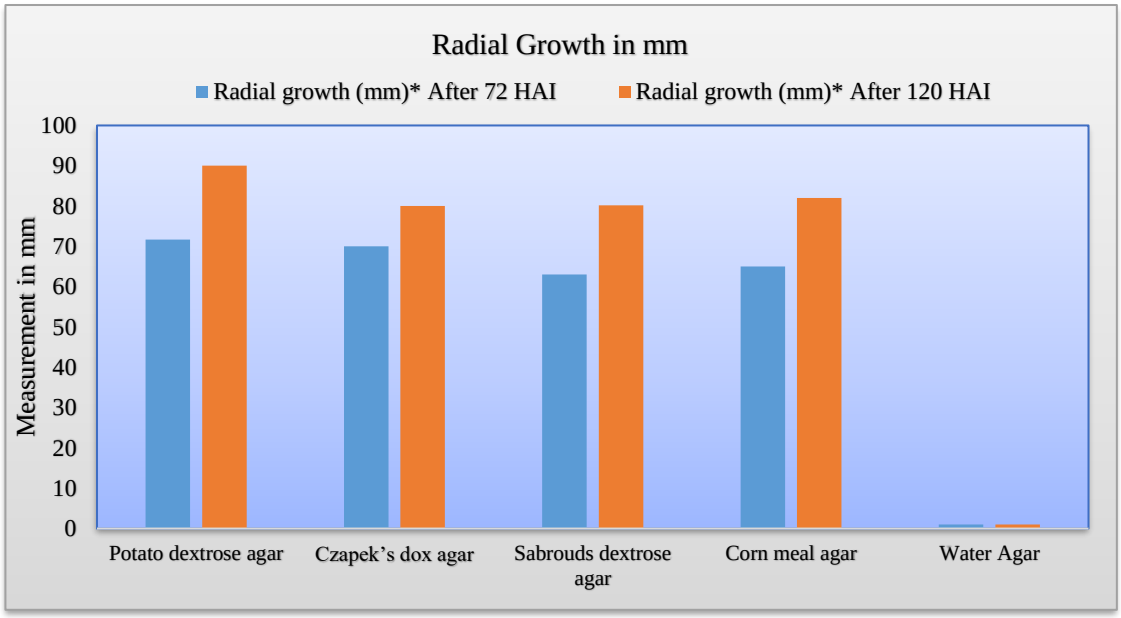


Figure 1. Effect of various culture medium on growth of *Macrophomina phaseolina* at 30±1°C.

Sclerotial growth

On the basis of microscopic observations in **Table 2** and **Figure 2**, it reveals that sclerotia was brown to black in colour, round to irregular in shape, size ranging between 47-141 µm in all the cultures of *M. phaseolina*. Most of the isolates of *M. phaseolina* were produced black coloured sclerotia except I_3 and I_5 isolates which were brown coloured. The shapes of sclerotia were classified in three groups that were round, ovoid and irregular. Round shaped sclerotia recorded in isolates I_2 and I_5, ovoid shaped sclerotia were found in I_1 and I_3 where's, irregular shaped sclerotia were found in I_4, I_6 and I_7 isolates.

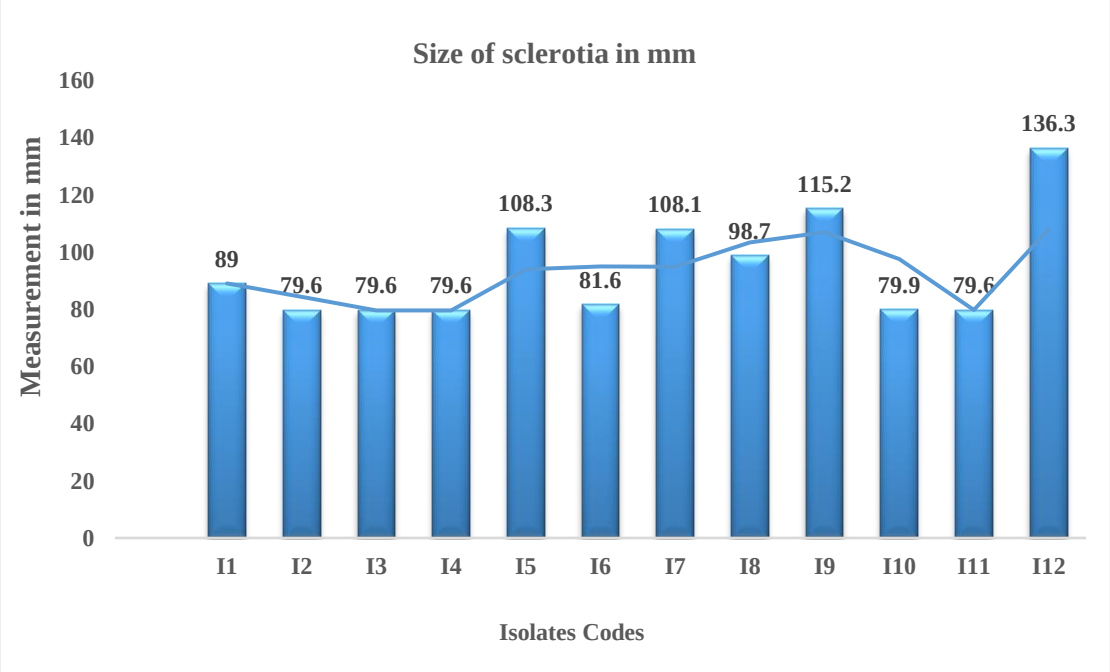
Table 2. Variation in abundance, growth, colour, shape and size of sclerotia of *M. phaseolina* isolates.

Isolates	Abundance	Colour	Shape	Length of sclerotia (µm)*	Width of sclerotia (µm)*	Size of sclerotia (µm)*
I_1	++	Black	Ovoid	94.0	84.0	89.0
I_2	+++	Brown	Round	84.6	74.6	79.6
I_3	+++	Black	Round	84.6	74.6	79.6
I_4	++++	Black	Ovoid	84.6	74.6	79.6
I_5	++	Brown	Irregular	112.8	103.8	108.3
I_6	+++	Black	Round	84.6	78.6	81.6
I_7	++	Black	Ovoid	112.8	103.4	108.1
I_8	++	Black	Round	103.4	94.0	98.7
I_9	+	Black	Irregular	122.2	108.2	115.2
I_{10}	++	Brown	Round	84.6	75.2	79.9
I_{11}	++++	Black	Ovoid	84.6	74.6	79.6
I_{12}	+	Black	Irregular	141.0	131.6	136.3

* Average of three replications;

The sclerotia size on the basis of microscopic observations, it varied from 94 x 84 µm in length and width. Where's I_{12} found to have largest sclerotial size (141.0 x 131.6 µm) followed by I_9 (122.2 x 108.2 µm), I_5,I_7 (112.8 x 103.4 µm), I_8 (103.4 x 94.0 µm), I_6 I_3 (84.6 x 78.6 µm). The smallest sclerotial size was found in I_{10} (84.6 x 75.2 µm). Largest size of sclerotial was recorded in isolate I_{12} (148.5 µm) and the smallest size sclerotia was found in isolate namely I_2 (68.9 µm).

Figure 2. Variation in abundance, colour, shape and size of sclerotia of *M. phaseolina* isolates.



Moreover, Gavali Manaji *et al*., (2017) obtained eleven isolates of *M. phaseolina* incitant of charcoal rot of *Sorghum bicolor* from different agro-ecological areas of solapur districts in Maharashtra. It's varied in their cultural characteristics & pathogenic behavior. On the basis of colony colour, isolates were divided into four groups *i.e.* blackish grey, grey, blackish in center periphery creamish and grayish white. The average radial growth of different 11 isolates of *M. phaseolina* ranged from 79.11 to 90.00 mm in 7 days after incubation. North Solapur isolates produced highest number of sclerotia (143.8 sclerotia per 9 mm disc and 40.4 per microscopic 10X field) while Mohal isolates produced minimum number of sclerotia (57.0 sclerotia per 9 mm disc & 16.4 per microscopic 10X field). Mohal and Pandharpur isolates produced largest size of sclerotia (28.6-39.6µm and 30.6-38.5µm), whereas Akkalkot produced smallest size of sclerotia (15.2-16.6µm). On the basis of sclerotia morphology, isolates were categorized in two group one with oblong shape having irregular edges & other are being round with regular edges.

Examine the Pathogenicity and its different stages of life cycle.

To prove pathogenicity of *Macrophomina phaseolina* isolated from infected soybean root. The *M. phaseolina* artificially inoculated in seed and soil, under pot greenhouse condition. The inoculated seeds were sown in pot and inoculated soil filled in pot and soybean seed sown. The first signs of plant death are withering and drying leaves, which were then progressively followed by root rot. Brown to black lesions could be seen on the sick plant's roots. *M. phaseolina* (Tassi) Goid was isolated from a plant that had been intentionally inoculated, same as original strain depicted. The fungal pathogen was examined using soil and seed inoculation methods seed inoculation techniques had the highest disease incidence rate of 54.76 per cent followed by soil inoculation techniques. Minimum of 73.33 per cent germination was reported using the seed inoculation method. Blotter paper technique as described by Nene *et al*., (1981), for *Rhizoctonia bataticola. (Macrophomina phaseolina).* To conduct the pathogenicity test of soybean given (JS 95- 60) in **Table 3 Figure3**.

Table 3. Pathogenicity of *M. phaseolina* with Soybean.

Inoculation technique	Germination (%)*	(%) Disease incidence
Seed inoculation	73.33	54.76
Soil inoculation	76.67	48.14
Control	86.67	00

* Average of three replications

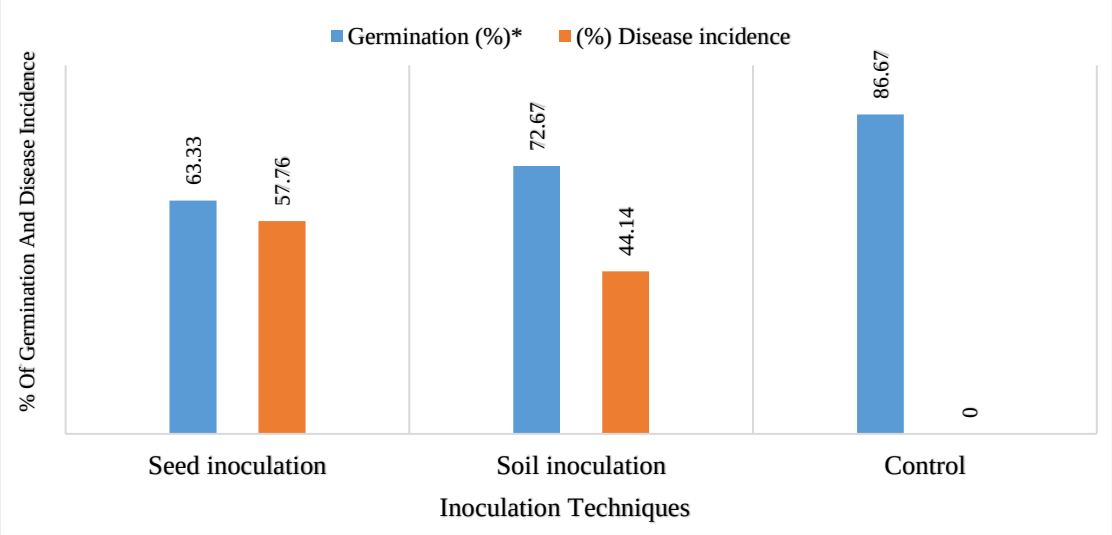


Figure 3. Pathogenicity of *M. phaseolina* with soybean.

Study Genetic Diversity of *Macrophomina phaseolina* by RAPD Analysis.

Random amplified polymorphic DNA technique is used for the evolutionary changes and genetic variation within a group. RAPD is PCR based very unique technique that doesn't require any specificity with their primer requirements. It is frequently operated method to determine the differences in between developed fusants and parental string by mean of polymorphism. In this present study RAPD technique was used to check similarity and dissimilarity of *Macrophonia phaseolina*. This procedure was completed with three major steps including genomic DNA isolation, PCR amplification with random primers and resolving the bands using agarose gel electrophoresis. Finally produced band patterns were read for their polymorphism by the presence (+) and absent (-) scoring bands. Highly purified genomic DNA was isolated using LETS method and lysis/homogenization technique (Sandhu *et al*., 2005).

Isolated genomic DNA was estimated for their quantity and quality using spectrophotometer at 260nm and 280nm and resolved with 1.5 % of agarose gel in horizontal electrophoresis and was visualized under gel documentation as shown in **Figure 4**.

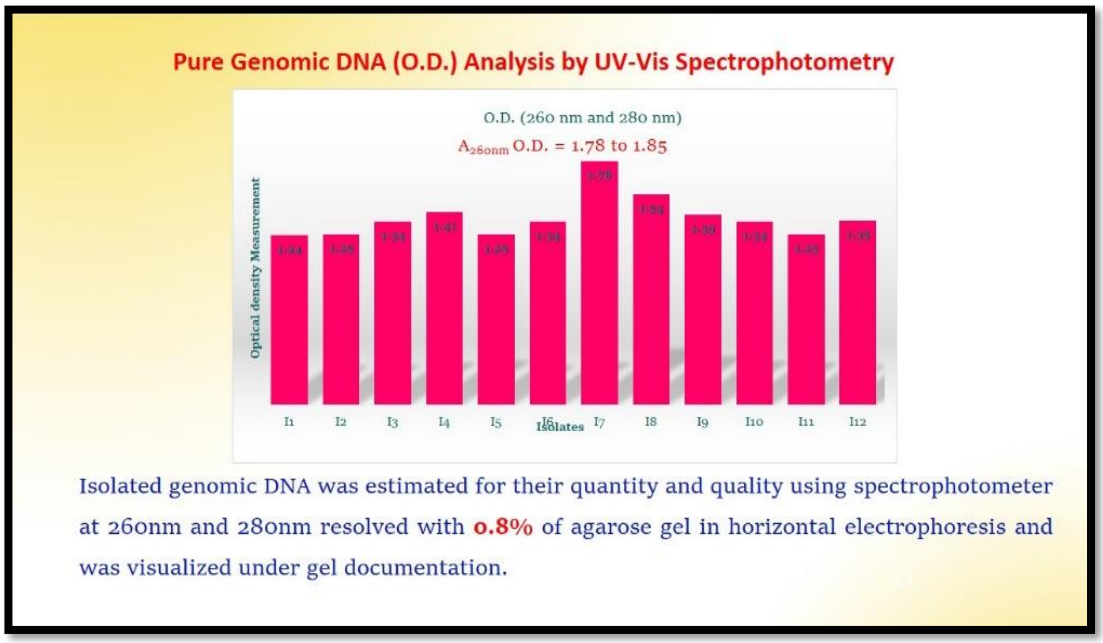


Figure 4: Observation of O.D. from different fungal isolates.

For PCR-based RAPD analysis, random arbitrary decamer primers OPJ-7 were selected for the PCR amplification with all 12 isolates and two parental DNA templates, because it produces bands for all sample templates with 76 to 80% band producing rate. **Figure 5** showed RAPD band pattern for genomic DNA template from 12 investigated samples amplified using OPJ-7 decamer arbitrary primer that was produced. According to the RAPD band pattern arrangements OPJ-7 produced recognizable band patterns for all 12 samples and showed polymorphic band patterns. Similarly OPJ-7 was able to produce bands only for three samples including I_9 and showed monomorphic band pattern. According to the band produced by OPJ-7 arbitrary primer, I_7 was closest with (*Macrophomina phaseolina*) and was closer to I_4. Combinational similarity found in between I_1, I_2 & I_{12} with their both parental strains considerably. This result shows the significant distinguish in between parental strains.

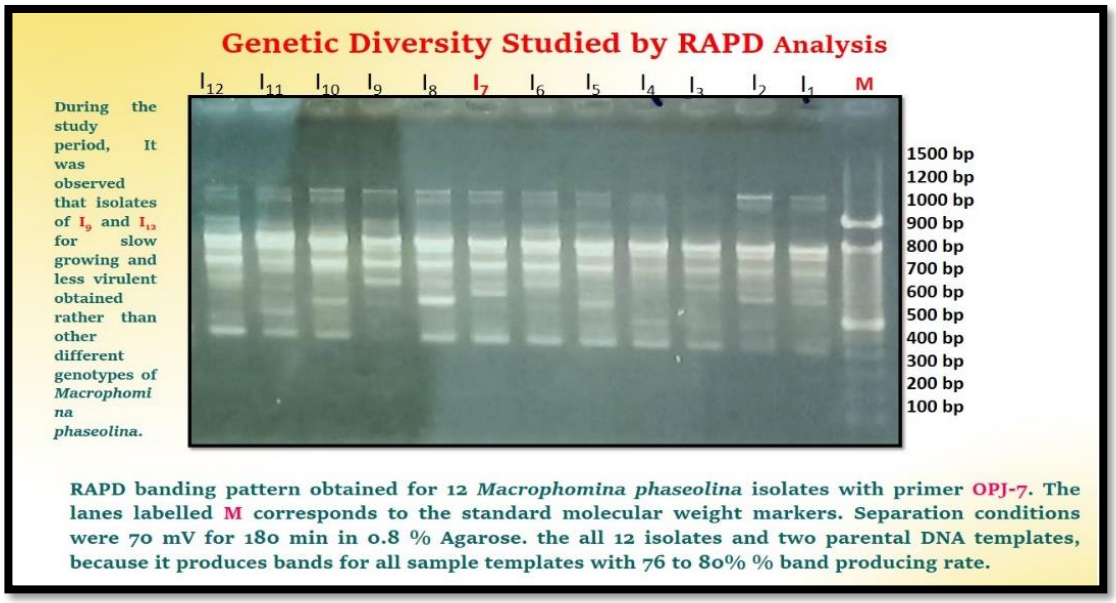


Figure 5: A depicted image of RAPD Band Analysis.

In the present study revealed RAPD analysis based identification of *M. phaseolina* isolated from soybean. Different inoculation screening methods revealed the presence of complete resistance (31 to 50% disease incidence) to *M. phaseolina* in the present set of soybean genotypes. However, cultivation of resistant elite genotype MP#7 (Gadarwara Isolate) could be recommended for soybean production in disease problematic areas after multi-location field trials as shown in **Figure 6**. Host resistance may be the best alternative for cost-effective and eco-friendly management of the disease. The resistant genotypes identified in the present studies could also be utilized as resistant donors for developing resistant varieties after successful field trials.

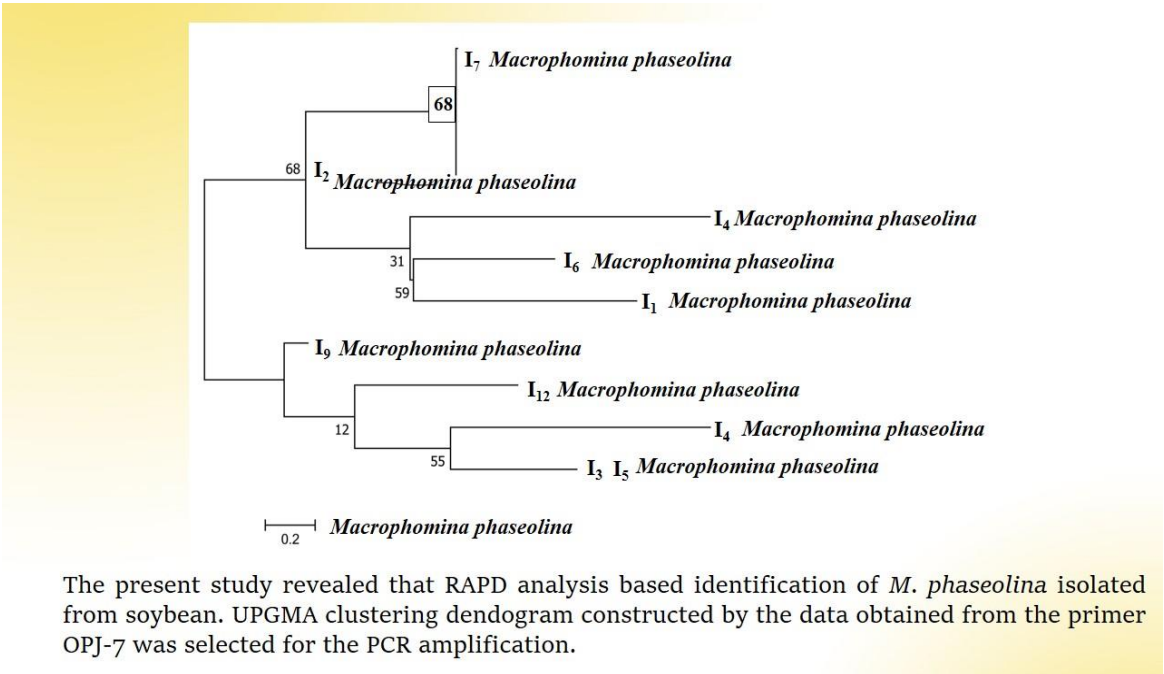


Figure 6: A Phylogenetic root constructed by Mega Software (V 2.0)

CONCLUSION

In this scientific study, *Macrophomina phaseolina* was successfully isolated and identified from soybean plants exhibiting symptoms of infection, offering important new information about its function as the cause of charcoal rot. By using a methodological approach that included surface sterilization and then incubation on Potato Dextrose Agar (PDA) medium supplemented with antibiotics, the fungal pathogens were effectively isolated while the bacterial contamination was decreased. The identity of *M. phaseolina* was confirmed by analyzing its peculiar sclerotia production and unique mycelial growth, which are acknowledged as fundamental traits of the fungus. These findings were also confirmed by additional purification and morphological analyses, and the pathogenicity evaluations determined the isolates' virulence. The inoculated soybean plants showed clear signs of illness, confirming *M. phaseolina* ability to cause charcoal rot in the right circumstances. The study emphasizes how important precise identification and testing are to the development of it is frequently operated method to determine the differences in between developed fusants and parental string by mean of polymorphism. In this present study RAPD technique was used to check similarity and dissimilarity of *Macrophomina phaseolina*. This procedure was completed with three major steps including genomic DNA isolation, PCR amplification with random primers and resolving the bands using agarose gel electrophoresis. Finally produced band patterns were read for their polymorphism by the presence (+) and absent (-) scoring bands. Highly purified genomic DNA was isolated using LETS method and lysis/homogenization technique (Sandhu *et al*., 2005).

Random arbitrary decamer primers OPJ-7 were selected for the PCR amplification with all 12 isolates and two parental DNA templates, because it produces bands for all sample templates with 76 to 80% band producing rate. Figure 11 showed RAPD band pattern for genomic DNA template from 12 investigated samples amplified using OPJ-7 decamer arbitrary primer that was produced. According to the RAPD band pattern arrangements OPJ-7 produced recognizable band patterns for all 12 samples and showed polymorphic band patterns. Similarly OPJ-7 was able to produce bands only for three samples including I_9 and showed monomorphic band pattern. According to the band produced by OPJ-7 arbitrary primer, I_7 was closest with (*Macrophomina phaseolina*) and was closer to I_4. Combinational similarity found in between I_1, I_2 & I_{12} with their both parental strains considerably. This result shows the significant distinguish in between parental strains.

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