



IN VITRO AFFINITY OF *MACROPHOMINA PHASEOLINA* (TASSI) GOID. TO VARIOUS CROP SPECIES

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ABSTRACT

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An *in vitro* study was conducted to evaluate the affinity and relative pathogenicity of *Macrophomina phaseolina* to twenty agriculturally important crop species. Five-day-old cultures of *M. phaseolina* grown on PDA (30 ± 1 °C) were used to pre-colonize Petri plates; surface-sterilized seeds (10 seeds/plate; 4 replicates per crop, $n = 40$) were placed equidistantly around the colony and incubated in darkness at 30 ± 1 °C for six days. Seed and seedling responses were scored on a 0–5 scale and a Virulence Index (VI) was computed following. Based on virulence crops were grouped into high (VI 4.6-4.9), moderate (VI 3.0-4.4) and low (VI 2.2-2.6) virulent categories. Pulses and oilseeds (e.g., sesame, groundnut, green gram, chickpea, redgram, black gram) and some cereals (maize) showed the highest susceptibility (VI 4.6- 4.9), while tobacco, rice and tomato exhibited the least susceptibility (VI 2.2–2.6). The results demonstrate the broad host range and strong affinity of *M. phaseolina* for pulses and oilseeds under the tested *in vitro* conditions and highlight the need for crop-specific management strategies.

KEYWORDS: *Macrophomina phaseolina*, virulence index, seed infection, host affinity, *in vitro* screening.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. is a cosmopolitan, necrotrophic soil-borne fungus that causes charcoal rot, root rot and seedling blight in a wide range of crops, leading to significant yield losses under warm and dry conditions. Its polyphagous nature and long-lived microsclerotia in soil make it a challenging pathogen to manage. Understanding host affinity across crops helps to prioritize management and breeding efforts. *In vitro* seed-colonization assays using standardized severity scales and virulence indices offer a rapid approach to compare relative susceptibility among species (Manici *et al.*, 1995; Cervantes-Garcia *et al.*, 2003; Beas-Fernandez *et al.*, 2006). The present study evaluated the affinity of *M. phaseolina* to 20 crop species representing pulses, oilseeds, cereals, cucurbits and solanaceous crops under controlled *in vitro* conditions and quantified pathogenic impact using a Virulence Index.

MATERIAL AND METHODS

The present investigation was taken at SV Agricultural College, Tirupati on *in vitro* affinity of *M. phaseolina* towards different crop species.

Fungal isolate and culture preparation

A virulent isolate of *Macrophomina phaseolina* was

maintained on Potato Dextrose Agar (PDA). Five-day-old cultures grown at 30 ± 1 °C was used for all inoculations following Manici *et al.* (1995) and Cervantes-Garcia *et al.* (2003).

Selection of plant species

Twenty crop species were selected based on reported or suspected susceptibility to *M. phaseolina*. The list (common name and scientific name) is presented in Table 1.

Surface sterilization of seeds

Seeds were surface-sterilized with 1 per cent sodium hypochlorite (NaOCl) for 1-2 min, rinsed three times with sterile distilled water and blotted dry on sterile filter paper prior to inoculation.

Inoculation procedure

PDA plates were inoculated centrally with a 5 mm disc of five-day-old *M. phaseolina* culture and incubated at 30 ± 1 °C for 48 h to allow pre-colonization. Ten surface-sterilized seeds of each species were placed equidistantly around the fungal colony on each plate. Four replicate plates per species (total $n = 40$ seeds per species) were maintained. To aid seed germination, 2 ml sterile distilled water was added to each plate prior to incubation. Plates were incubated in complete darkness

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at 30 ± 1 °C for six days (Manici *et al.*, 1995; Beas-Fernandez *et al.*, 2006).

Disease severity assessment

On day seven, seed and seedling responses were assessed using a 0–5 scale (Manici *et al.*, 1995; Cervantes-Garcia *et al.*, 2003) where: 0 = healthy seed and seedling; 1 = discoloration of seed portion in contact with mycelium; 2 = seed integuments colonized by mycelium and microsclerotia but seedling healthy; 3 = seed coat unaffected but seedling infected; 4 = both seed integuments and seedling infected; 5 = seed infected and failed to germinate.

Calculation of Virulence Index

Virulence Index (VI) for each species was calculated following Beas-Fernandez *et al.* (2006):

$$\text{Virulence Index} = \frac{\text{No. of infected seeds} \times \text{Rating scale}}{\text{Total no. of seeds}}$$

Statistical analysis

Mean VI values were computed for each species. Standard error of mean (SEm $\pm$), critical difference (CD at $P = 0.05$) and coefficient of variation (CV) were calculated.

RESULTS AND DISCUSSION

Overall affinity pattern

In vitro screening revealed marked variation in *M. phaseolina* affinity among the 20 crops tested. Based on calculated Virulence Indices, crops were classified into three groups: Highly virulent (VI 4.6-4.9), Moderately virulent (VI 3.0-4.4) and Low virulent (VI 2.2-2.6). The summary of results is presented in Table 2.

Several pulses and oilseeds like sesame, groundnut, green gram, chickpea, redgram and black gram along with maize and brassica and cucurbits displayed the highest virulence (VI 4.6-4.9). Most of these crops exhibited seed integument invasion and seedling infection or failure to germinate (scores 4-5). This strong affinity corroborates with earlier reports that *M. phaseolina* is particularly aggressive on pulses and oilseeds under favorable conditions (e.g., warm, dry environment) and is capable of causing severe seedling blight and establishment failure. Production of cell wall-degrading enzymes (CWDEs) and phytotoxins likely contribute to rapid tissue maceration and seed death in these hosts (Ramos *et al.*, 2016; Degani *et al.*, 2023).

Table 1. Crop species screened for affinity to *M. phaseolina* (n = 20)

S. No.	Crop	Scientific name	S. No.	Crop	Scientific name
1.	Sesame	<i>Sesamum indicum</i>	11.	Groundnut	<i>Arachis hypogaea</i>
2.	Sorghum	<i>Sorghum bicolor</i>	12.	Chickpea	<i>Cicer arietinum</i>
3.	Sunflower	<i>Helianthus annuus</i>	13.	Tomato	<i>Solanum lycopersicum</i>
4.	Maize	<i>Zea mays</i>	14.	Chilli	<i>Capsicum annuum</i>
5.	Cotton	<i>Gossypium hirsutum</i>	15.	Tobacco	<i>Nicotiana tabacum</i>
6.	Castor	<i>Ricinus communis</i>	16.	Rice	<i>Oryza sativa</i>
7.	Wheat	<i>Triticum aestivum</i>	17.	Ladyfinger	<i>Abelmoschus esculentus</i>
8.	Redgram	<i>Cajanus cajan</i>	18.	Mustard	<i>Brassica nigra</i>
9.	Mungbean	<i>Vigna radiata</i>	19.	Watermelon	<i>Citrullus lanatus</i>
10.	Urdbean	<i>Vigna mungo</i>	20.	Cucumber	<i>Cucumis sativus</i>

Table 2. Seed infection severity (scale and number of seeds infected) and Virulence Index (n = 40 seeds per crop)

S. No.	Crop	Disease severity scores (No. of seeds /40)	Virulence index	Virulence group
1.	Sesame	4 (4), 5 (36)	4.9	Highly virulent
2.	Groundnut	4 (8), 5 (32)	4.9	Highly virulent
3.	Maize	4 (5), 5 (35)	4.9	Highly virulent
4.	Green gram	4 (4), 5 (36)	4.9	Highly virulent
5.	Chickpea	4 (8), 5 (32)	4.8	Highly virulent
6.	Redgram	4 (8), 5 (32)	4.8	Highly virulent
7.	Brassica	4 (8), 5 (32)	4.8	Highly virulent
8.	Cucumber	4 (8), 5 (32)	4.8	Highly virulent
9.	Chilli	4 (12), 5 (28)	4.7	Highly virulent
10.	Black gram	4 (16), 5 (24)	4.6	Highly virulent
11.	Cotton	4 (13), 5 (27)	4.4	Moderately virulent
12.	Bhendi (Lady's finger)	4 (24), 5 (16)	4.4	Moderately virulent
13.	Sunflower	3 (12), 4 (16), 5 (12)	4.0	Moderately virulent
14.	Wheat	0 (9), 5 (31)	3.9	Moderately virulent
15.	Sorghum	0 (8), 4 (12), 5 (20)	3.7	Moderately virulent
16.	Castor	0 (10), 4 (10), 5 (20)	3.5	Moderately virulent
17.	Watermelon	2 (20), 3 (10), 5 (10)	3.0	Moderately virulent
18.	Tobacco	0 (19), 4 (1), 5 (20)	2.6	Low virulent
19.	Paddy (Rice)	0 (5), 1 (10), 2 (5), 3 (5), 4 (10), 5 (5)	2.5	Low virulent
20.	Tomato	0 (16), 1 (2), 3 (6), 4 (12), 5 (4)	2.2	Low virulent
SEm $\pm$		0.1		
CD (P = 0.05)		0.28		
CV		4.8%		

Cotton, bhendi, sunflower, wheat, sorghum, castor and watermelon fell into the moderate virulence category (VI 3.0-4.4). Symptoms were often less severe with partial seed coat invasion or seedling infection suggesting partial tolerance or structural/biochemical defenses reducing pathogen progress. Differences in host cell wall composition, seed coat thickness or preformed inhibitors may account for reduced colonization in these crops (Kaur *et al.*, 2019; Hameed *et al.*, 2022).

Tobacco, rice and tomato exhibited the lowest virulence indices (VI 2.2-2.6), generally showing discoloration at the seed portion in contact with mycelium or limited seedling symptoms (scores 1-2). Such low susceptibility could reflect effective early defense responses (hormonal signaling involving salicylic acid, jasmonic acid or ethylene) or physical barriers that limit fungal ingress (Radadiya *et al.*, 2021). These crops may be useful models for dissecting resistance mechanisms and identifying candidate genes for breeding.

Interactions between pathogen, host and environment

The pronounced host variation noted here is consistent with the recognized isolate-dependent pathogenicity of *M. phaseolina*. Reports from diverse regions indicate variability in aggressiveness on specific hosts (Rathore *et al.*, 2024; Lokesh *et al.*, 2020). Similarity between studies for example, reports of limited maize infection by some isolates versus high virulence on maize in the present study underline the influence of isolate genotype, host genotype and experimental conditions on observed pathogenicity (Ramos *et al.*, 2016; Rathore *et al.*, 2024).

Implications for management

High VI values for economically important crops such as sesame and groundnut underscore the need for integrated management. Due to longevity of microsclerotia in soil, crop rotation alone may reduce but not eliminate inoculum. Biological control agents (e.g., *Trichoderma* spp., *Bacillus* spp.) and the deployment of resistant genotypes appear promising components of integrated strategies (Khamari and Hashmi, 2019; Singh *et al.*, 2022). Crops categorized in the low virulence group may possess effective defense mechanisms and further investigation of these mechanisms could provide useful leads for breeding durable resistance.

In vitro affinity assay demonstrated that *Macrophomina phaseolina* shows high affinity for pulses and oilseeds (VI 4.6-4.9), moderate affinity for cereals and cucurbits (VI 3.0-4.4) and low affinity on tobacco, rice and tomato (VI 2.2-2.6) under the tested conditions. These results highlight the broad host range and aggressive potential of *M. phaseolina*, particularly on pulses and oilseeds, and provide a basis for prioritizing crops for management, surveillance and breeding for resistance.

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