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SCREENING OF SESAME (*SESAMUM INDICUM* L.) GENOTYPES AGAINST CHARCOAL ROT (*MACROPHOMINA PHASEOLINA*) USING *IN VITRO* AND *IN VIVO* METHODS

K. Akshaya[1*], V. Bharathi[2], Y. Bharathi[3] and M. Madhavi[4]

[1]Department of Seed Science and Technology, Seed Research and Technology Centre (SRTC), PJTAU, Rajendranagar, Hyderabad-500030, Telangana, India
[2]Department of Plant Pathology, Seed Research and Technology Centre (SRTC), PJTAU, Rajendranagar, Hyderabad-500030, Telangana, India
[3]Department of Genetics and Plant Breeding, Agriculture Research Station (PJTAU) Tandur, Telangana, India
[4]Department of Plant Pathology, Agricultural college (PJTAU), Palem, -509215, Telangana, India
*Corresponding author E-mail: sonyakshaya62@gmail.com
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ABSTRACT

Charcoal rot, caused by *Macrophomina phaseolina*, is a major fungal disease constraining sesame (*Sesamum indicum* L.) productivity, particularly under high temperature and moisture stress conditions. Identification of host resistance offers a sustainable and environmentally safe strategy for managing this soil and seed borne pathogen. The present study evaluated thirty-one sesame genotypes for resistance to *M. phaseolina* using both *in vitro* (paper towel) and *in vivo* (greenhouse pot culture) screening methods. Analysis of variance revealed significant differences among genotypes for germination percentage, seedling vigour indices (SVI-I and SVI-II), seed rot, seedling infection, mortality and percent disease incidence (PDI), indicating substantial genetic variability in disease response. *In vitro* screening effectively differentiated genotypes based on germination and infection levels, with JCS-3751 and JCS-4805 recording the highest germination (87–89%) and lowest infection (5.33–7.66%), followed by moderately resistant genotypes such as JCS-3287, JCS-4806, and GT-10. Under greenhouse conditions, these genotypes also exhibited higher germination and lower PDI (6.66–8.66%), confirming stable resistance across both screening methods. In contrast, susceptible genotypes including JCS-4983, JCS-4977, JCS-4962 and Swetha showed poor germination, severe seed rot and high seedling mortality under pathogen stress. The strong agreement between *in vitro* and *in vivo* evaluations validates the reliability of the paper towel method as a rapid and cost-effective preliminary screening approach for identifying resistant germplasm. The resistant genotypes, particularly JCS-3751 and JCS-4805 represent valuable sources for developing charcoal rot–tolerant sesame cultivars through host resistance breeding programs.
Keywords: *Sesamum indicum*, *Macrophomina phaseolina*, host resistance, germination, *in vitro* screening, greenhouse evaluation, charcoal rot.

Introduction

Sesame (*Sesamum indicum* L.), popularly known as “Till” and revered as the “Queen of Oilseeds,” is one of the oldest cultivated oilseed crops valued for its high nutritional and economic importance. It contains about 50% oil, 23% protein and 15% carbohydrates, making it a preferred crop across diverse agro-climatic regions ranging from semi-arid tropics to temperate zones (Raikwar and Srivastava, 2013). India ranks as the largest producer of sesame globally (FAO STAT, 2014). During 2023–24, sesame was cultivated over 15.23 lakh hectares, producing 8.02 lakh tonnes with an average productivity of 527 kg ha^{-1}. In Telangana, the crop covered 0.14 lakh hectares with 0.11 lakh tonnes of production and a productivity of 756 kg ha^{-1}, mainly in the districts of Jagtial, Nirmal, Warangal, and Nizamabad (www.indiastat.com, 2023–24).

Sesame is prone to several diseases, among which stem and root rot (charcoal rot) caused by *Macrophomina phaseolina* is the most destructive, affecting the crop at all growth stages and leading to significant yield losses (Deepthi *et al*., 2014). The pathogen survives as sclerotia in soil and crop residues, thriving under high temperature and drought conditions that are common in rainfed sesame fields (Chattopadhyay and Kalpana Sastry, 1998).

Host plant resistance offers the most effective, eco-friendly, and sustainable means of managing this disease. Therefore, the present investigation was undertaken to evaluate thirty-one sesame genotypes under artificial soil infestation with *M. phaseolina* in pot culture (greenhouse conditions) to identify resistant sources. In addition, *in vitro* screening using the paper towel method was employed as a rapid and efficient approach to assess genotype resistance to charcoal rot, facilitating early selection for breeding programs aimed at developing resistant varieties.

Materials and Methods

Collection of stem & root rot samples and sesame genotypes

Sesame plants showing characteristic symptoms of root and stem rot were collected from the Project Coordinating Unit, AICRP on Sesame, Jagtial, PJTSAU, Telangana. The disease was identified by the sudden wilting of plants, particularly after the flowering stage, and was observed to affect various parts of the plant including leaves, petioles, branches, stems, roots, pods, and seeds. Infected roots exhibited rotting and decay, eventually leading to complete wilting of the plant.

For screening purposes, thirty sesame genotypes along with the cultivated variety Swetha (total 31 genotypes) were collected from the same unit. These genotypes were evaluated for their resistance response to *M. phaseolina*, the causal organism of root and stem rot. The screening was carried out through both *in vitro* (paper towel method) and *in vivo* (sick soil/pot culture) techniques to assess disease resistance and related seed quality parameters comprehensively.

S.No	Sesame Genotypes		
1.	JCS-3751	17.	JCS-3287
2.	JCS-4805	18.	JCS-4862
3.	JCS-4824	19.	JCS-3888
4.	JCS-3993	20.	JCS-4982
5.	JCS-4021	21.	JCS-3879
6.	JCS-4830	22.	JCS-3202
7.	JCS-214	23.	JCS-4024
8.	JCS-4981	24.	JCS-4836
9.	JCS-4987	25.	JCS-4806
10.	JCS-4983	26.	GT-10
11.	JCS-4859	27.	JCS-3603
12.	JCS-2454	28.	JCS-4835
13.	JCS-4962	29.	JCS-3976
14.	JCS-1020	30.	AT-378
15.	JCS-4977	31.	Swetha
16.	JCS-4370		

Isolation and Purification of *Macrophomina phaseolina*

The fungus *M. phaseolina* was isolated from sesame plants showing typical root and stem rot symptoms. Diseased portions of roots and stems were washed in running tap water, cut into small pieces, and surface sterilized with 0.1% sodium hypochlorite for 30 seconds. The bits were then rinsed thrice with sterile distilled water to remove any traces of the sterilant and aseptically transferred onto sterilized Potato Dextrose Agar (PDA) plates. The plates were incubated at 28 ± 1°C for three days to allow fungal growth. The emerging colonies were purified using the hyphal tip technique (Rangaswami, 1975), and pure cultures were maintained under aseptic conditions for further studies.

Mass Multiplication of *Macrophomina phaseolina*

The test pathogen *M. phaseolina*, maintained on PDA slants, was mass multiplied using sorghum grains as a solid substrate. For this, 100 g of sorghum grains were washed thoroughly, soaked overnight in 250 ml of water, and then surface-dried on sterilized filter paper for 4–6 hours. The grains were autoclaved at 121.6°C and 1.05 kg/cm² pressure for 20 minutes to ensure sterilization. After cooling, each flask was inoculated with 2–3 discs from a 7-day-old culture of *M. phaseolina* and incubated at 28 ± 1°C in a BOD incubator. The flasks were shaken daily to promote uniform fungal colonization. After two weeks, the fully colonized sorghum grains served as the inoculum for mixing with sterilized soil in pot culture experiments.

Pathogenecity test of *M. phaseolina*

The pathogenicity of *Macrophomina phaseolina* isolate was assessed using the sick pot method under greenhouse conditions on the susceptible sesame cultivar Swetha. The results confirmed that all tested isolates of *M. phaseolina* were pathogenic and capable of inducing characteristic symptoms of root and stem rot lesions in sesame plants and this variety was carried out for further study.

Typical symptoms such as wilting, root discoloration, and stem rot were observed 30–35 days after sowing. The affected seedlings were carefully uprooted, and the pathogen was successfully re-isolated on PDA medium. The morphological characteristics of the re-isolated cultures matched the original cultures used for inoculation, thereby fulfilling Koch's postulates and confirming the pathogenicity of the isolates. These findings align with previous studies (Thirunarayanan *et al*., 2020), which also reported significant pathogenicity of *M. phaseolina* isolates on sesame.

Seed inoculation/seed infusion technique by paper towel method (*in vitro*)

In vitro screening of thirty-one sesame (*Sesamum indicum* L.) genotypes against *M. phaseolina* was conducted using the paper towel method (Thiyagu *et al*., 2007). Seeds were surface sterilized with 1% sodium hypochlorite for 2 minutes, rinsed twice with sterile distilled water, and then rolled over a 7-day-old *M. phaseolina* culture grown on PDA for 15 minutes to ensure uniform mycelial adhesion (Tabassum *et al*., 2014). One hundred inoculated seeds of each genotype were placed equidistantly on moist paper towels, covered with another layer, and incubated at 25 ± 1°C with 95% relative humidity. Moisture was maintained by daily misting with sterile water. Un-inoculated seeds served as the control. After 14 days, observations on germination percentage, seed rot, seedling infection, and seedling vigour indices (SVI-I and SVI-II) were recorded following ISTA guidelines. The presence of *M. phaseolina* was confirmed through re-isolation from infected seedlings. This method enabled effective screening of sesame genotypes for resistance and susceptibility under controlled conditions. Genotypes were classified based on the disease rating scale developed by Nene e*t al.* (1981) and Pandey *et al*. (2020).

SCORE	DESCRIPTION	REACTION
1	No Infection	Immune
>1 to ≤3	A few small lesions covered roots (5% of the root tissue affected)	Resistant
>3 to ≤5	Clear and small lesions on the roots, new roots free from infection	Moderately resistant
>5 to ≤6	Root lesions are moderate; new roots are free from infection	Moderately susceptible
>6 to ≤8	Many lesions are found on roots, new roots unaffected	Susceptible
>8 to 9	Roots with severe infection and discoloration	Highly susceptible

Observations recorded as following

Germination percentage (%)

$$\text{Germination percentage}(\%) = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

Seedling Vigour Index (SVI- I and SVI-II)

Ten normal seedlings from each replication were randomly selected to measure total seedling length (cm), including root and shoot. These seedlings were oven-dried at 100°C for 24 hours to record seedling dry weight (mg). Seedling Vigour Index I and II were calculated as per Abdul Baki and Anderson (1973) using germination percentage, seedling length, and dry weight to assess overall seedling vigour.

SVI I = Seedling length (cms) x Germination (%)

SVI II = Seedling dry weight (mg) x Germination (%)

Percent seed rot (%)

$$\text{Seed rot}\% = \frac{\text{Number of rotted seeds}}{\text{Total number of seed}} \times 100$$

Percent seedling infection (%)

$$\text{Seedling infection}\% = \frac{\text{Number of seedlings infected by Macrophomina sp.}}{\text{Total number of seeds}} \times 100$$

Soil Inoculation by Sick Pot Method (*In vivo* Screening)

For in vivo screening, disinfected plastic pots (5 kg capacity) were filled with sterilized soil and inoculated with *Macrophomina phaseolina* mass-multiplied on sorghum grains at 20 g per kg of soil (Bedawy *et al*., (2019). The experiment was conducted under greenhouse conditions at SRTC, Rajendranagar during Rabi 2024–25. Thirty-one sesame genotypes were sown with 15 seeds per pot in four replications, and pots were arranged in a randomized complete block design (RCBD). Disease severity was recorded at 15, 30, 45, and 60 days after inoculation using a 1–9 rating scale (Bedawy and Mohamed, 2018). Control pots containing sterilized, pathogen-free soil were maintained for comparison with the same genotypes.

Disease scale used for evaluation of disease resistance in sesame genotypes

Disease scale	Reaction
1-10	Resistant
11-20	Moderately Resistant
21-30	Moderately Susceptible
31-50	Susceptible
51-100	Highly susceptible

Observations recorded as following

Percent seed germination (%)

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seedlings}}{\text{Total number of seeds sown}} \times 100$$

Percent seed rot (%)

In this present study seed rot percent is calculated as mentioned above

Percent seedling mortality (%)

In this present study seedling mortality is calculated by using the formula

$$\text{Seedling mortality (\%)} = \frac{\text{Number of dead seedlings}}{\text{Total number of planted seedlings}} \times 100$$

Percent disease incidence (%)

The percent disease incidence is calculated as

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

Results and Discussions

The analysis of variance revealed significant differences among genotypes (Factor A), between treatments (Factor B) and their interaction (A × B) for germination percentage, Seedling vigour index-I (SVI-I), Seedling vigour index-II (SVI-II), Seed rot percentage, Seedling infection percentage, seedling mortality (%), percent disease incidence (PDI) indicating that the genotypes differed considerably in their response to pathogen infestation.

Screening of genotypes through seed infusion technique by using paper towel method under *in vitro*

Seed Germination (%)

Germination percentage of thirty-one sesame genotypes under *Macrophomina phaseolina* infection (treated) and control (un-inoculated) conditions is presented in Table 1. The overall mean germination of untreated seeds (81.00%) was higher than that of treated seeds (64.00%) showing a 21% reduction due to pathogen infection. Under treated conditions, germination ranged from 46.00% (JCS-4983) to 89.00% (JCS-3751) while in control it varied from 71.00% (JCS-4977) to 91.66% (JCS-3751)**.** Among the genotypes, JCS-3751 (91–89%; 2.19% reduction) and JCS-4805 (91–87%; 4.40% reduction) recorded the least decline indicating strong resistance. Moderate reductions were noted in JCS-3202 (9.40%)**,** GT-10 (8.88%)**,** JCS-4806 (11.00%), JCS-3287 (13.00%), JCS-214 (15.00%)**,** JCS-3976 (18.00%) and JCS-4021 (20.00%) showing moderate resistance. Severe reduction in germination was recorded in JCS-4983 (41.00%), JCS-4977 (36.00%), swetha (30.00%), JCS-4024 (32.00%)**,** JCS-4862 (31.00%) and JCS-4982 (32.00%) showing susceptible response. Germination decreased under *M. phaseolina* infection because the pathogen colonizes the seed surface and internal tissues causing seed rot and weakening the embryo (Sharma *et al*., 2013). These results are in accordance with Sodji *et al*. (2025) evaluated 23 cowpea genotypes for resistance to *M. phaseolina* and observed considerable variation in germination percentage**.** Resistant genotypes including IT10K-837-1, IT11K-61-82 etc. maintained high germination (70.00–76.67%) while moderately resistant genotypes ranged 63.33–69.00%. Moderately susceptible genotypes such as IT07K-303-1, IT14K-1682-3 and IT13K-1070-2 showed 50.00–62.00% and susceptible genotypes including IT14K-2030-2 and IT14K-2113-4 showed low germination (31.67–49.00%).

Seedling Vigour Index-I (SVI-I)

The influence of *M. phaseolina* infection on Seedling Vigour Index-I (SVI-I) among thirty-one sesame genotypes revealed marked variation between treated and control conditions. Under inoculated conditions SVI-I ranged from 477 (JCS-4977) to 1348 (JCS-4805) whereas in the un-inoculated control, it varied from 880 (JCS-3993) to 1654 (JCS-4805). The overall mean SVI-I of treated seeds (836) was notably lower than that of the control (1146) indicating a substantial reduction in seedling vigour due to pathogen infection. Genotypes such as JCS-4805, JCS-3751, JCS-3202, JCS-4830 and GT-10 maintained relatively higher SVI-I values under inoculated conditions reflecting better tolerance and metabolic stability against *M. phaseolina*. In contrast, JCS-4977, JCS-4983, Swetha, JCS-4024 and JCS-4862 exhibited severe reductions in SVI-I suggesting high susceptibility and impaired seedling growth due to

pathogen-induced root and shoot decay. The minimal decline in vigour among resistant genotypes indicated efficient physiological and biochemical defense responses enabling sustained seedling growth under pathogen stress. These results are in consistent with the observations of Jacquet *et al*. (2023) in wild soybean (*Glycine soja*), where resistant accession PI 507794 maintained significantly higher seedling vigour under *M. phaseolina* infection, whereas susceptible accessions exhibited substantial reductions in seedling vigour confirming the strong association between disease resistance and seedling growth performance.

Seedling Vigour Index-II (SVI-II)

The Seedling Vigour Index-II (SVI-II) of thirty-one sesame genotypes showed marked variation under *M. phaseolina* infection (Table 1). In treated seeds, SVI-II ranged from 353 (JCS-4977) to 1001 (JCS-4805), while in control it ranged from 686 (JCS-4824) to 1201 (JCS-4805). The mean SVI-II decreased from 861 (control) to 624 (treated) indicating a strong adverse effect of the pathogen on seedling vigour. Genotypes JCS-4805 (1201–1001), JCS-3751 (1193–978), JCS-3202 (926–827), JCS-4806 (931–786), JCS-3603 (919–671) and GT-10 (878–780) maintained higher vigour under infection, showing tolerance to *M. phaseolina*. In contrast, JCS-4977 (772–353), JCS-4983 (773–382), Swetha (999–464) and JCS-4024 (916–432) exhibited severe reduction indicating susceptibility. These results align with Mahmoud *et al*. (2024), who reported higher seedling dry weight in resistant sesame genotypes than susceptible genotypes, confirming SVI-II as a reliable indicator for charcoal rot resistance screening.

Seed rot percentage

Significant variation in seed rot percentage was observed among the sesame genotypes with *M. phaseolina* infection (Table 2). Under treated conditions, seed rot ranged from 4.66% (JCS-4805) to 26.66% (JCS-4983), with an overall mean of 17.24%, while in control it ranged from 4.33% to 9.40% (mean). The minimum seed rot was recorded in JCS-4805 (4.66%), JCS-3751 (5.33%), GT-10 (8.66%), JCS-3202 (12.00%) and JCS-4830 (12.33%) indicating resistance. Genotypes showing a moderate increase in seed rot with infection included JCS-2454, JCS-214, JCS-4859, JCS-3287, JCS-1020 and JCS-4370, reflecting partial resistance. The maximum seed rot was noted in JCS-4983 (26.66%), JCS-4982 (25.66%), JCS-4024 (24.00%), JCS-4862 (23.33%), JCS-4977 (21.66%) and Swetha (22.00%) indicating high susceptibility. Overall, genotypes which are showing resistance response showed only marginal variation between treated and control conditions, while susceptible ones exhibited severe seed rot and poor seedling establishment with pathogen stress. In sesame, resistant genotypes show lower seed rot due to stronger biochemical defenses and antifungal compounds that inhibit *M. phaseolina*, while susceptible genotypes exhibit higher seed rot because of weaker defenses and rapid pathogen colonization (Yan *et al*., 2021; Mahmoud & El-Bramawy, 2024; Ahmed *et al*., 2023).

Seedling Infection (%)

Seedling infection among sesame genotypes with *M. phaseolina* treated ranged from 5.66% (JCS-3751) to 32.66% (JCS-4983), with an overall mean of 16.73% (Table 2). Genotypes showing minimal infection included JCS-3751 (5.66%), JCS-4805 (7.66%), JCS-4806 (9.00%), GT-10 (10.00%) and JCS-3202 (10.66%) categorized as resistant. Genotypes with moderate infection were JCS-4859 (13.00%), JCS-214 (14.33%), JCS-3287 (15.66%) and JCS-1020 (16.00%) reflecting intermediate tolerance. Highly susceptible genotypes included JCS-4983 (32.66%), JCS-4977 (31.33%), Swetha (31.00%), JCS-4962 (30.66%) and JCS-4982 (23.33%). Based on infection levels, genotypes with less than 10% infection were classified as resistant (R), 10–20% as moderately resistant (MR) and above 30% as susceptible (S). Overall, JCS-4805, JCS-3751, GT-10, JCS-4806 and JCS-3202 were identified as resistant with stable and low seedling infection. These results are in accordance with Sodji *et al*. (2025), who reported similar variation in seedling infection among cowpea genotypes under *M. phaseolina* stress.

The *in vitro* screening of sesame genotypes using the paper towel method revealed considerable variability in their response to *M. phaseolina*. Among the 31 genotypes evaluated, five genotypes (JCS-3751, JCS-4805, JCS-3202, JCS-4806 and GT-10) exhibited a resistant reaction, maintaining high germination and minimal infection. Fourteen genotypes (JCS-4824, JCS-3993, JCS-4830, JCS-214, JCS-4859, JCS-2454, JCS-1020, JCS-3287, JCS-3888, JCS-3879, JCS-3603, JCS-4835, JCS-3976 and AT-378) were categorized as moderately resistant, showing intermediate levels of infection. Seven genotypes (JCS-4021, JCS-4981, JCS-4370, JCS-4862, JCS-4982, JCS-4024 and JCS-4836) were moderately susceptible, whereas five genotypes (JCS-4987, JCS-4983, JCS-4962, JCS-4977 and Swetha) were highly susceptible, exhibiting significant seed rot and seedling infection. These findings are in accordance with Thiyagu *et al*. (2007) reported significant variability in resistance among sesame genotypes against *M. phaseolina* using paper towel method.

In this present study, sesame genotypes screened using the *in vitro* paper towel method showed variation in response to *M. phaseolina* consistent with previous findings. Pandey *et al*. (2020) reported 9 resistant mungbean genotypes out of 43 screened, with IPM99-125, EC693368 and EC693369 showing the lowest disease scores. Similarly, Zangui *et al*. (2025) observed variation among sesame genotypes using the paper towel assay, and Bairwa *et al*. (2023) found differential pathogen aggressiveness in sesame, where AUMP-1 was most virulent and AUMP-2 least in paper towel and sick pot assays.

Screening of sesame genotypes under green house conditions (*in vivo*)

Sesame genotypes were evaluated against *Macrophomina phaseolina* in the greenhouse using pot culture to assess germination, seed rot, seedling mortality and disease incidence. Controlled greenhouse conditions reduced environmental variability allowing reliable comparison. Considerable variation in genotype responses enabled their classification into resistant, moderately resistant, moderately susceptible and susceptible categories.

Germination (%)

Under greenhouse pot culture, seed germination of sesame genotypes decreased with *M. phaseolina* infection compared to control (Table 3). In treated seeds, germination ranged from 33.00% (JCS-4977) to 79.00% (JCS-3751), while control seeds ranged from 62.00% (JCS-4806) to 85.00% (JCS-3751) with overall means of 48.00% (treated) and 78.00% (control). The highest germination under infection was recorded in JCS-3751 (79.00%) and JCS-4805 (76.00%), while JCS-4859 (60.00%), JCS-3287 (59.00%), JCS-4021 (57.00%) and GT-10 (55.00%) showed moderate germination. The lowest germination was observed in JCS-4977 (33.00%), JCS-4983 (35.00%), and JCS-1020 (37.00%). Maximum reduction occurred in JCS-4977, JCS-4983, JCS-1020, JCS-3202, and Swetha indicating susceptible response, whereas genotypes such as JCS-3751 and JCS-4805 maintained higher germination reflecting resistance.

Seed rot (%)

The per cent seed rot among the sesame genotypes under pot culture conditions ranged from 6.66% (JCS-3751) to 28.88% (JCS-3202) in treated seeds, while control seeds recorded lower seed rot ranging from 4.22% (JCS-3993) to 13.33% (JCS-4806, JCS-4962, JCS-4983). The overall mean seed rot was 15.90% in treated and 8.03% in control seeds (Table 4.1.3).

Among the treated seeds, the genotypes JCS-3751 and JCS-4824 (6.66%) showed similarity in seed rot per cent and was found the lowest among all the genotypes evaluated. The increase in seed rot was observed in the genotypes JCS-4805 (8.66%) and the other genotypes which were on par with respect to seed rot percentage were JCS-4370, JCS-4836, JCS-4859, JCS-3287, JCS-4024 (11.11%) indicating a higher degree of resistance to *Macrophomina* infection showing lesser superiority over the genotypes JCS-3751 and JCS-4824. In contrast, the genotype JCS-3202 (28.88) exhibited the maximum per cent seed rot, followed by JCS-4021, JCS-4830, JCS-4977, JCS-4983, and GT-10 (22.22). Seed rot was minimum in the control seeds, with reference to most of genotypes JCS-3993 (4.22%), JCS-4824 (4.44%), JCS-3751 (5.44%), JCS-4805(6.22%), JCS-4370, JCS-4836, JCS-3976, JCS-4024, JCS- 214, JCS-4981 and Swetha (6.66%). The mean seed rot percentage in treated seeds was significantly higher than in control, demonstrating the adverse impact of *M. phaseolina* infection on seed health and viability. Genotypes JCS-3751, JCS-4805, maintained low seed rot in both treated and control conditions, may be considered resistant whereas those with higher seed rot above 20% i.e in JCS-4977 and JCS-4983 indicating susceptible. These results are in accordance with El-bramawy *et al*., 2006 in sesame resistant genotypes consistently showing low seed rot percentages ranging from approximately 1–6%**.** In contrast, susceptible genotypes exhibited high seed rot incidence above 25% with *M. phaseolina* stress.

Seedling mortality (%)

Seedling mortality among sesame genotypes under *M. phaseolina* infection varied widely, ranging from 6.66% in genotypes such as JCS-3751, JCS-4824, JCS-4962, JCS-3287, JCS-3879, JCS-4835 and GT-10 to 24.44% in genotypes JCS-1020, JCS-3603 and JCS-3976, with an overall mean of 15.91%. In control seedlings, mortality was lower, ranging from 0.00% (JCS-2454, JCS-4835) to 17.77% (JCS-4977, JCS-4981) with overall mean 7.68%. Moderate mortality was observed in JCS-214, JCS-4806 and JCS-4981 (22.22%), indicating intermediate susceptibility. The markedly higher mortality in treated seedlings highlights the severe impact of *M. phaseolina* on seedling establishment and vigour. Genotypes with lower mortality demonstrated stronger resistance and can be prioritized for breeding programs, while susceptible genotypes showed substantial loss can be used as checks. These results are consistent with Farooq *et al*. (2019), who reported considerable variation in sesame genotypes under *M. phaseolina*

infection, with line 87008 showing the lowest seedling mortality (1–10%), reflecting resistance to charcoal rot.

Percent Disease Incidence (PDI)

Macrophomina phaseolina infection caused wide variation in disease incidence among sesame genotypes, with PDI ranging from 6.66% (JCS-3751) to 33.33% (JCS-4962, JCS-4024), while control seedlings recorded lower PDI of 4.44%–11.11% with mean of 21.93% in treated and 5.02% in control seedlings (Table 4). Genotypes showing minimal PDI included JCS-3751 (6.63%), JCS-4805 (6.67%), JCS-214 and GT-10 reflecting resistant response, whereas highly susceptible genotypes were JCS-4977, JCS-4983, JCS-4024, Swetha and JCS-4962 exhibiting PDI >30%. Moderate PDI (22.22%–24.44%) was observed in genotypes like JCS-4824, JCS-3993, JCS-4981, JCS-4836, and JCS-3202. The higher PDI in treated seedlings confirms the adverse effect of *M. phaseolina* and genotypes with consistently lower PDI such as JCS-3751 and JCS-4805 can serve as potential sources for breeding resistant sesame varieties. The results are consistent with trends observed in germination, seed rot and seedling mortality. Elmerich *et al*. (2022) also showed considerable variation in blackgram genotypes against *M. phaesolina* in their studies, recording none of the screened genotypes showed 0% disease incidence and demarcated the genotypes with less than 10 % disease incidence were classified as resistant.

Evaluation of sesame genotypes under greenhouse conditions showed considerable variation in their tolerance to *M. phaseolina*. Among the genotypes tested, JCS-3751 and JCS-4805 demonstrated strong resistance, with healthy seedlings and minimal disease symptoms. A group of thirteen genotypes (JCS-4021, JCS-214, JCS-4859, JCS-2454, JCS-4370, JCS-3287, JCS-4862, JCS-3888, JCS-4806, GT-10, JCS-3603, JCS-3976 and AT-378) exhibited moderate resistance, characterized by reduced disease levels and moderate seedling survival. Nine genotypes (JCS-4824, JCS-3993, JCS-4981, JCS-1020, JCS-4982, JCS-3879, JCS-3202 and JCS-4836) were moderately susceptible, showing noticeable disease symptoms and partial seedling mortality. Seven genotypes (JCS-4987, JCS-4983, JCS-4962, JCS-4977, JCS-4024, JCS-4835 and Swetha) were highly susceptible, with increase in disease levels. These observations highlight the differential response of sesame genotypes to charcoal rot under greenhouse conditions and provide valuable information for selecting genotypes with stable resistance for future breeding efforts. These results are in consistent with the findings of Thiyagu *et al*. (2007) reported similar variation in disease response among sesame genotypes in controlled environments.

Similar findings have been reported in sesame, soybean and mungbean. Ghias *et al.* (2021) evaluated 52 sesame genotypes against *M. phaseolina*, classifying L-7 as resistant (DI 8.21%), Black Til and TH-6 as moderately resistant (DI 11.39–12.31%), 23 genotypes as moderately susceptible (DI 22–30%), and 95006-2 and 97005 as susceptible (DI 30–31%). Ezhilarasi and Meena (2019) observed 16 of 24 advanced sesame breeding lines as moderately susceptible using the sick pot assay. In mungbean, Avanija *et al*. (2023) reported 6 resistant, 15 moderately resistant, 18 susceptible and 8 highly susceptible genotypes under polyhouse conditions. Similarly, Mishra *et al*. (2021) screened soybean germplasm, identifying 24 lines as moderately resistant (DI 1.1–10%) and 46 lines as moderately susceptible (DI 10–20%), confirming the effectiveness of early screening methods for selecting resistant genotypes.

Screening sesame genotypes against *M. phaseolina* using both *in vitro* (paper towel method) and *in vivo* (greenhouse pot culture) showed minimal variation between methods, confirming the reliability of *in vitro* screening as a rapid preliminary test. Both approaches effectively distinguished genotypes into categories from resistant to highly susceptible. Genotypes JCS-3751 and JCS-4805 exhibited consistent resistance in both methods and can be used as potential sources for breeding programs. Pathogen recovery tests from stem bits further confirmed resistance (fig .10), with no recovery in resistant genotypes and maximum recovery in susceptible ones including JCS-4977, JCS-4983, JCS-4987, Swetha and JCS-4962 which can serve as differential checks in future varietal trials and breeding studies.

Conclusion

Thirty-one sesame (*Sesamum indicum* L.) genotypes were evaluated for resistance to *Macrophomina phaseolina* using both *in vitro* (paper towel) and *in vivo* (greenhouse) screening methods. Analysis of variance revealed significant genotype responses for germination, seedling vigour index (SVI-I and SVI-II), seed rot, seedling infection, mortality and percent disease incidence (PDI), confirming substantial genetic variability in disease response. The *in vitro* assay effectively differentiated genotypes and showed high concordance with greenhouse evaluations, demonstrating its reliability as a rapid and efficient preliminary screening tool. JCS-3751 and JCS-4805 were consistently resistant across both methods, while JCS-214, JCS-2454, JCS-3287, JCS-

3603, JCS-3888, JCS-3976, JCS-4859 and AT-378 were moderately resistant. JCS-4981, JCS-4982 and JCS-4836 exhibited moderate susceptibility, whereas JCS-4987, JCS-4983, JCS-4962, JCS-4977 and Swetha remained highly susceptible in both assays. The results validate the use of the paper towel method (*in vitro*) as a dependable approach for large-scale screening, complementing greenhouse and field evaluations in sesame breeding programs.

Table 1: Effect of *Macrophomina phaseolina* on germination (%) and seedling vigour-(SVI-I and SVI-II) of Sesame Genotypes

		Germination (%)			SV-I			SV-II		
S.no	**Genotype**	**Treated**	**Control**	**Mean A**	**Treated**	**Control**	**Mean A**	**Treated**	**Control**	**Mean A**
1.	JCS-3751	89.00	91.00	90.00	1345	1531	1438	978	1193	1085
2.	JCS-4805	87.00	91.00	89.00	1348	1654	1501	617	779	698
3.	JCS-4824	69.00	81.00	75.00	808	992	900	640	686	663
4.	JCS-3993	69.00	81.00	75.00	841	880	861	640	686	663
5.	JCS-4021	60.00	82.00	71.00	764	911	837	516	722	619
6.	JCS-4830	73.00	84.00	79.00	1051	1216	1133	802	928	865
7.	JCS-214	67.00	82.00	75.00	926	1237	1082	709	950	829
8.	JCS-4981	64.00	77.00	70.00	804	1027	915	538	751	645
9.	JCS-4987	60.00	76.00	68.00	736	969	852	551	713	632
10.	JCS-4983	46.00	78.00	62.00	503	1043	773	382	777	580
11.	JCS-4859	69.00	80.00	74.00	861	984	923	670	741	706
12.	JCS-2454	71.00	85.00	78.00	929	1111	1020	662	775	718
13.	JCS-4962	55.00	76.00	66.00	690	1044	867	500	792	646
14.	JCS-1020	67.00	77.00	72.00	884	1025	954	669	785	727
15.	JCS-4977	48.00	75.00	62.00	477	1066	772	353	804	579
16.	JCS-4370	61.00	81.00	71.00	845	1191	1018	598	836	717
17.	JCS-3287	65.00	82.00	73.00	877	1104	991	652	873	762
18.	JCS-4862	55.00	80.00	67.00	595	1132	863	437	853	645
19.	JCS-3888	69.00	79.00	74.00	1011	1298	1155	739	935	837
20.	JCS-4982	51.00	76.00	63.00	642	1112	877	521	860	690
21.	JCS-3879	59.00	83.00	71.00	792	1165	978	561	845	703
22.	JCS-3202	75.00	81.00	78.00	1054	1199	1126	827	926	876
23.	JCS-4024	52.00	77.00	64.00	570	1247	909	432	916	674
24.	JCS-4836	58.00	77.00	68.00	822	1153	988	683	862	772
25.	JCS-4806	73.00	84.00	79.00	1003	1251	1127	786	931	859
26.	GT-10	77.00	84.00	80.00	1013	1168	1091	780	878	829
27.	JCS-3603	72.00	83.00	78.00	959	1274	1116	671	919	795
28.	JCS-4835	55.00	80.00	67.00	621	1083	852	455	835	645
29.	JCS-3976	48.00	76.00	62.00	772	1021	897	598	805	701
30.	AT-378	61.00	78.00	69.00	745	1079	912	561	824	693
31.	Swetha	58.00	84.00	71.00	622	1370	996	464	999	732
	Mean B	64.00	81.00		836	1146		624	861	
	Factors	Factor (A)	Factor (B)	(A X B)	Factor (A)	Factor (B)	(A X B)	Factor (A)	Factor (B)	(A X B)
	C.D.)	4.10	1.04	5.80	139.872	35.52	N/S	50.49	12.82	71.41
	SE(d)	2.07	0.52	2.93	70.581	17.92	99.81	25.48	6.47	36.03
	SE(m)	1.46	0.37	2.07	49.908	12.67	70.58	18.01	36.03	25.48
	CV	**4.87 %**			**12.09%**			**5.80%**		

Note: Seedling vigour index-I (SVI-I), Seedling vigour index-II (SVI-II)

Table 2: Effect of *Macrophomina phaseolina* on Seed Rot (%), Seedling Infection (%), and Disease Reaction in Sesame Genotypes

S.No	Genotype	Seed Rot (%)			Seedling Infection (%)			Disease Reaction	Disease Scale
		Treated	Control	Mean A	Treated	Control	Mean A		
1.	JCS-3751	5.33	4.33	4.83	5.67	4.00	4.83	R	>1 to ≤3
2.	JCS-4805	4.67	4.33	4.50	7.67	4.33	6.00	R	>1 to ≤3
3.	JCS-4824	14.33	9.33	11.83	16.33	9.67	13.00	MR	>3 to ≤5

4.	JCS-3993	14.00	9.00	11.50	16.00	9.67	12.83	MR	>3 to ≤5
5.	JCS-4021	20.33	8.67	14.50	20.33	9.00	14.17	MS	>5 to ≤6
6.	JCS-4830	12.33	7.67	10.00	14.33	7.67	11.00	MR	>3 to ≤5
7.	JCS-214	14.33	8.67	11.50	18.00	9.00	13.50	MR	>3 to ≤5
8.	JCS-4981	17.00	11.33	14.17	18.00	11.67	14.83	MS	>5 to ≤6
9.	JCS-4987	19.67	12.00	15.83	30.33	11.33	15.83	S	>6 to ≤8
10.	JCS-4983	26.67	10.33	19.50	32.67	11.67	18.17	S	>6 to ≤8
11.	JCS-4859	14.67	10.00	12.33	16.33	9.67	13.00	MR	>3 to ≤5
12.	JCS-2454	15.67	7.00	11.33	12.67	7.67	10.17	MR	>3 to ≤5
13.	JCS-4962	20.67	11.67	16.17	30.67	11.67	17.67	S	>6 to ≤8
14.	JCS-1020	14.33	11.33	12.83	18.00	11.33	14.67	MR	>3 to ≤5
15.	JCS-4977	21.67	11.67	17.17	31.33	13.67	18.50	S	>6 to ≤8
16.	JCS-4370	20.33	9.67	15.00	18.67	9.00	13.83	MS	>5 to ≤6
17.	JCS-3287	17.33	8.67	13.00	16.67	8.33	12.50	MR	>3 to ≤5
18.	JCS-4862	23.33	10.00	16.67	21.67	9.33	15.50	MS	>5 to ≤6
19.	JCS-3888	14.00	10.33	12.17	16.33	10.00	13.17	MR	>3 to ≤5
20.	JCS-4982	25.67	12.00	18.83	23.33	11.67	17.50	MS	>5 to ≤6
21.	JCS-3879	21.33	8.67	15.00	15.67	5.00	10.33	MR	>3 to ≤5
22.	JCS-3202	12.00	9.33	10.67	10.67	9.00	10.33	R	>1 to ≤3
23.	JCS-4024	24.00	11.67	17.83	20.67	15.67	18.17	MS	>5 to ≤6
24.	JCS-4836	21.33	11.33	16.33	15.33	14.00	14.67	MS	>5 to ≤6
25.	JCS-4806	12.00	7.67	9.83	9.00	12.33	10.67	R	>1 to ≤3
26.	GT-10	8.67	7.33	8.00	10.00	7.00	8.50	R	>1 to ≤3
27.	JCS-3603	14.00	8.33	11.17	14.00	12.67	13.33	MR	>3 to ≤5
28.	JCS-4835	22.67	9.00	15.83	18.33	15.67	17.00	MR	>3 to ≤5
29.	JCS-3976	15.67	10.33	13.00	19.33	10.00	14.67	MR	>3 to ≤5
30.	AT-378	21.67	11.67	16.67	14.67	13.33	14.00	MR	>3 to ≤5
31.	SWETHA	22.00	8.33	15.17	31.00	7.67	13.83	S	>6 to ≤8
	Mean B	17.25	9.41		16.76	10.09			
	Factors	Factor (A)	Factor (B)	(A X)	Factor (A)	**SE(d)**	**SE(m)**		
	C.D.)	0.34	0.08	0.49	0.33	0.16	0.11		
	SE(d)	0.17	0.04	0.24	0.16	0.04	0.03		
	SE(m)	0.12	0.24	0.17	0.11	0.23	0.16		
	CV	**2.25%**			**2.27%**				

Note: Resistant (R), Highly resistant (HR), Moderately resistant (MR), Susceptible (S), Moderately susceptible (MS)

Table 3: Effect of *Macrophomina phaseolina* on germination (%) and seed rot of Sesame genotypes under greenhouse conditions (*in vivo*)

		germination (%)			**Seed Rot (%)**		
S.No	**Genotype**	**Treated**	**Control**	**Mean A**	**Treated**	**Control**	**Mean A**
1.	JCS-3751	79.00	85.00	82.00	6.67	5.44	6.56
2.	JCS-4805	76.00	84.00	80.00	8.67	6.22	7.44
3.	JCS-4824	49.00	82.00	62.00	6.67	4.44	5.56
4.	JCS-3993	49.00	82.00	65.00	17.78	4.22	10.00
5.	JCS-4021	57.00	80.00	66.00	22.22	8.89	15.56
6.	JCS-4830	46.00	79.00	59.00	22.22	8.89	15.56
7.	JCS-214	42.00	77.00	60.00	24.45	6.67	15.56
8.	JCS-4981	42.00	69.00	52.00	13.33	6.67	8.89
9.	JCS-4987	44.00	73.00	58.00	13.33	8.89	11.11
10.	JCS-4983	35.00	76.00	48.00	22.22	13.33	21.11
11.	JCS-4859	60.00	80.00	70.00	11.11	6.67	5.56
12.	JCS-2454	55.00	84.00	66.00	15.56	8.89	12.22
13.	JCS-4962	40.00	74.00	55.00	15.56	13.33	14.45
14.	JCS-1020	37.00	77.00	55.00	13.33	6.67	8.89
15.	JCS-4977	33.00	79.00	45.00	22.22	8.89	27.78
16.	JCS-4370	53.00	77.00	63.00	11.11	6.67	5.56
17.	JCS-3287	59.00	79.00	67.00	11.11	6.67	15.56
18.	JCS-4862	48.00	69.00	55.00	20.00	6.67	11.11
19.	JCS-3888	51.00	73.00	62.00	22.22	8.89	14.45

20.	JCS-4982	44.00	73.00	56.00	13.33	11.11	12.22
21.	JCS-3879	50.00	77.00	63.00	17.78	6.67	13.89
22.	JCS-3202	37.00	75.00	53.00	28.89	11.11	20.00
23.	JCS-4024	37.00	76.00	53.00	11.11	6.67	6.67
24.	JCS-4836	46.00	84.00	62.00	11.11	6.67	7.78
25.	JCS-4806	44.00	62.00	52.00	20.00	13.33	16.67
26.	GT-10	55.00	80.00	65.00	22.22	6.67	13.33
27.	JCS-3603	42.00	72.00	56.00	15.56	8.89	12.22
28.	JCS-4835	44.00	82.00	59.00	13.33	8.89	11.11
29.	JCS-3976	47.00	75.00	61.00	11.11	6.67	7.78
30.	AT-378	53.00	74.00	66.00	17.78	6.67	12.22
31.	Swetha	41.00	73.00	62.00	11.11	6.67	8.89
	Mean B	48.00	77.00		15.91	8.04	
	Factors	Factor(A)	Factor(B)	(A X B)	Factor(A)	Factor(B)	(A X B)
	C.D.)	1.22	0.31	1.72	0.18	0.09	0.06
	SE(d)	0.61	0.15	0.87	0.04	0.02	0.01
	SE(m)	0.43	0.87	0.61	0.26	0.13	0.09
	CV	1.80			**2.23**		

Table 4: Effect of *Macrophomina phaseolina* on seedling mortality (%), percent disease index (PDI), and disease reaction of sesame genotypes under greenhouse conditions (*in vivo*)

S.No	Genotype	Seedling mortality			PDI			Disease Reaction
		Treated	Control	Mean A	Treated	Control	Mean A	
1.	JCS-3751	6.67	4.22	4.45	6.67	4.45	5.55	R
2.	JCS-4805	8.89	4.22	5.56	6.67	4.45	7.78	R
3.	JCS-4824	15.56	8.89	12.22	26.67	4.45	18.89	MS
4.	JCS-3993	11.11	6.67	8.89	22.22	6.67	14.45	MS
5.	JCS-4021	11.11	6.67	8.89	13.33	4.45	11.11	MR
6.	JCS-4830	11.11	6.67	11.11	22.22	4.45	22.22	MS
7.	JCS-214	22.22	8.89	15.56	11.11	6.67	8.89	MR
8.	JCS-4981	22.22	17.78	20.00	22.22	6.67	17.78	MS
9.	JCS-4987	11.11	8.89	10.00	31.11	8.89	20.00	S
10.	JCS-4983	11.11	6.67	25.56	31.11	11.11	28.89	S
11.	JCS-4859	11.11	8.89	21.11	17.78	11.11	12.22	MR
12.	JCS-2454	13.33	0.00	6.67	15.56	6.67	14.45	MR
13.	JCS-4962	11.11	6.67	7.78	33.33	4.45	21.11	S
14.	JCS-1020	24.44	8.89	16.67	24.44	6.67	17.78	MS
15.	JCS-4977	22.22	17.78	13.33	31.11	11.11	28.89	S
16.	JCS-4370	17.78	6.67	15.56	17.78	6.67	12.22	MR
17.	JCS-3287	11.11	6.67	7.78	17.78	6.67	16.67	MR
18.	JCS-4862	15.56	13.33	14.45	15.56	11.11	16.67	MR
19.	JCS-3888	11.11	6.67	8.89	15.56	6.67	14.45	MR
20.	JCS-4982	20.00	11.11	15.56	22.22	4.45	15.56	MS
21.	JCS-3879	11.11	4.22	8.89	20.00	6.67	14.45	MS
22.	JCS-3202	11.11	6.67	13.33	24.44	6.67	16.67	MS
23.	JCS-4024	15.56	6.67	11.11	33.33	11.11	25.56	S
24.	JCS-4836	20.00	10.00	10.00	24.44	8.89	20.00	MS
25.	JCS-4806	22.22	13.33	18.89	15.56	4.45	10.00	MR
26.	GT-10	11.11	6.67	7.78	11.11	4.45	10.00	MR
27.	JCS-3603	24.44	13.33	18.89	17.78	4.45	10.00	MR
28.	JCS-4835	11.11	0.00	5.56	31.11	8.89	23.33	S
29.	JCS-3976	24.44	11.11	22.22	17.78	6.67	11.11	MR
30.	AT-378	8.89	6.67	8.89	20.00	11.11	12.22	MR
31.	Swetha	17.78	13.33	15.56	31.11	6.67	13.33	S
	Mean B	15.91	7.69		21.93	5.02		
	Factors	Factor(A)	Factor(B)	(A X B)	Factor(A)	Factor(B)	(A X B)	
	C.D.)	0.30	0.07	0.42	1.14	0.29	1.62	
	SE(d)	0.15	0.03	0.21	0.57	0.14	0.81	

	SE(m)	0.10	0.02	0.15	0.40	0.10	0.57	
	CV	**2.01**			**6.31**			

Note: Resistant (R), Highly resistant (HR), Moderately resistant (MR), Susceptible (S), Moderately susceptible (MS)

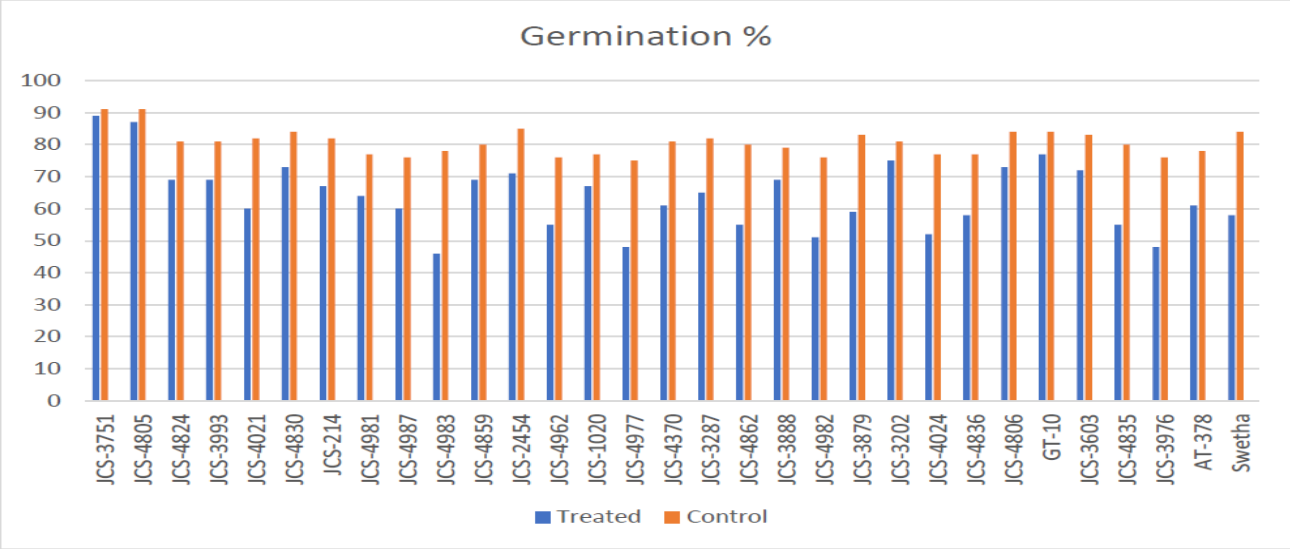

Fig 1: Graph representing percentage of germination in treated and control genotypes

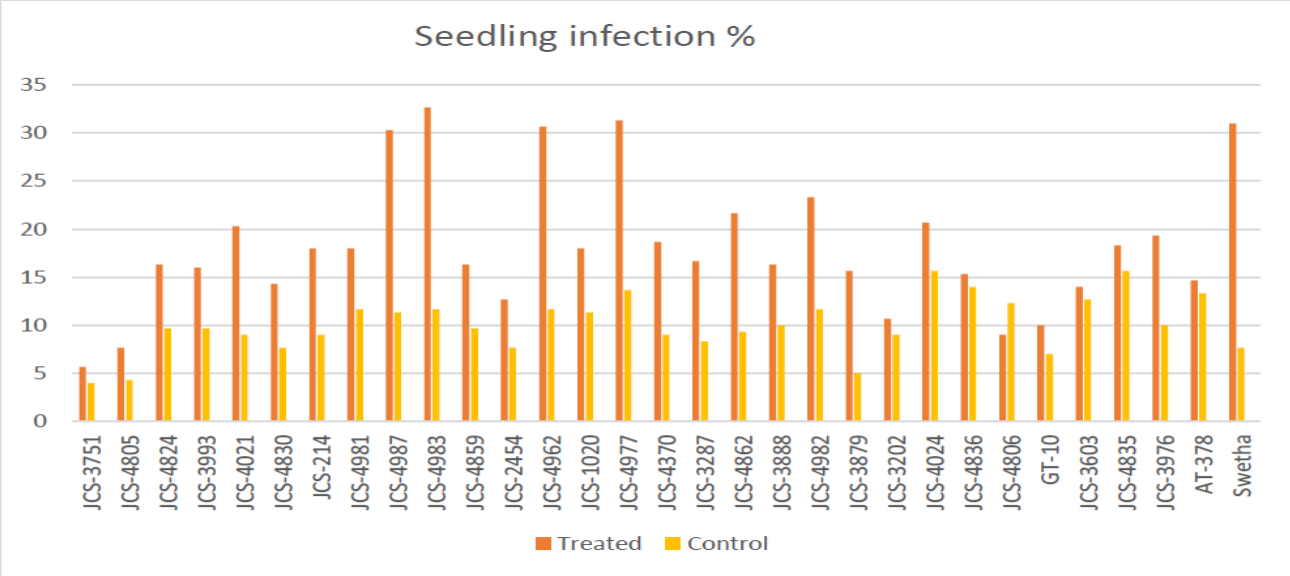

Fig 2: Graph representing percentage of seedling infection in treated and control genotypes

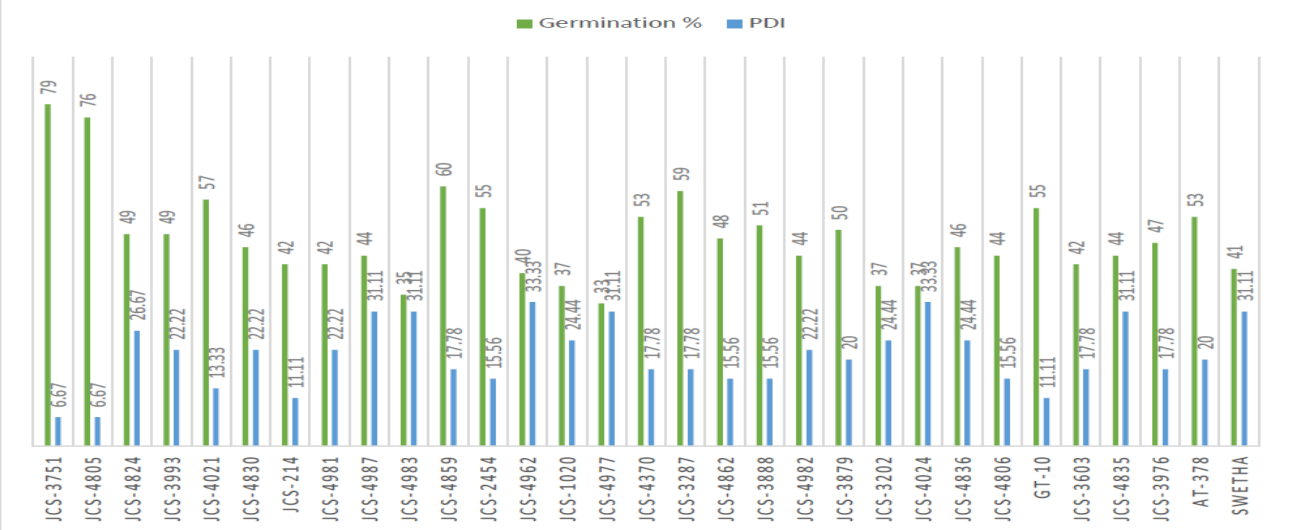

Fig. 3 : Graph representing percentage of germination and PDI in treated genotypes

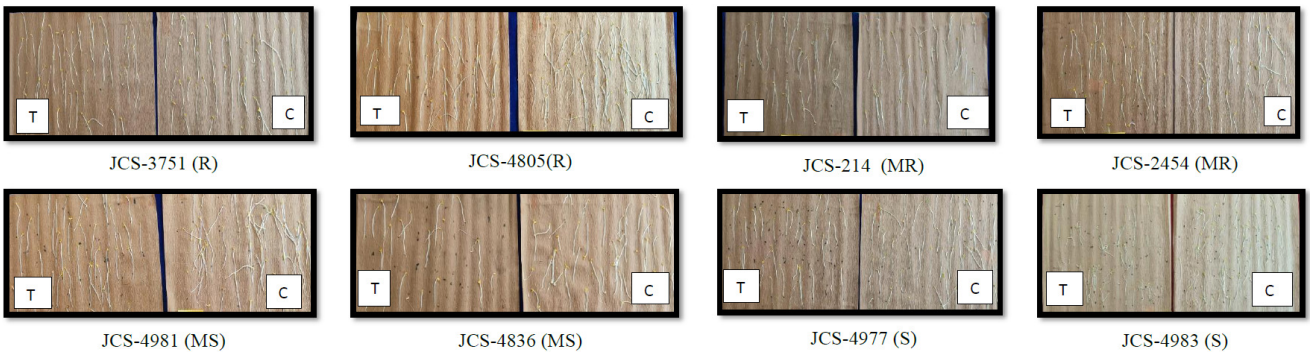

Fig. 4: Screening of sesame genotypes by artificial inoculation of pathogen (Paper towel method) (T-Pathogen treated, C-control)

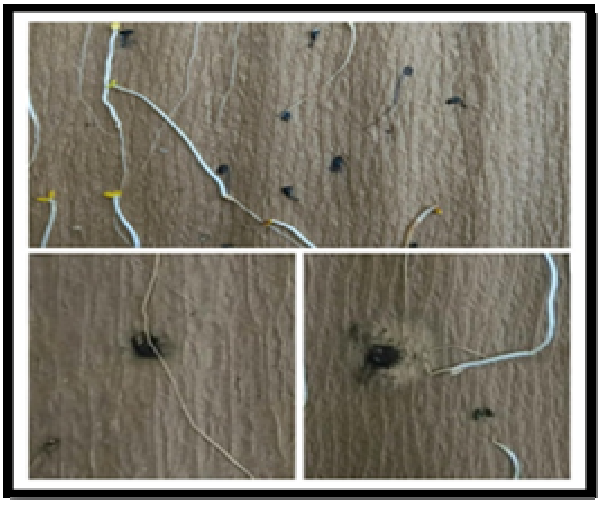

Fig. 5: Seed rot of artificially inoculated sesame seeds

Fig. 6 : Seedling infection of artificially inoculated sesame seeds

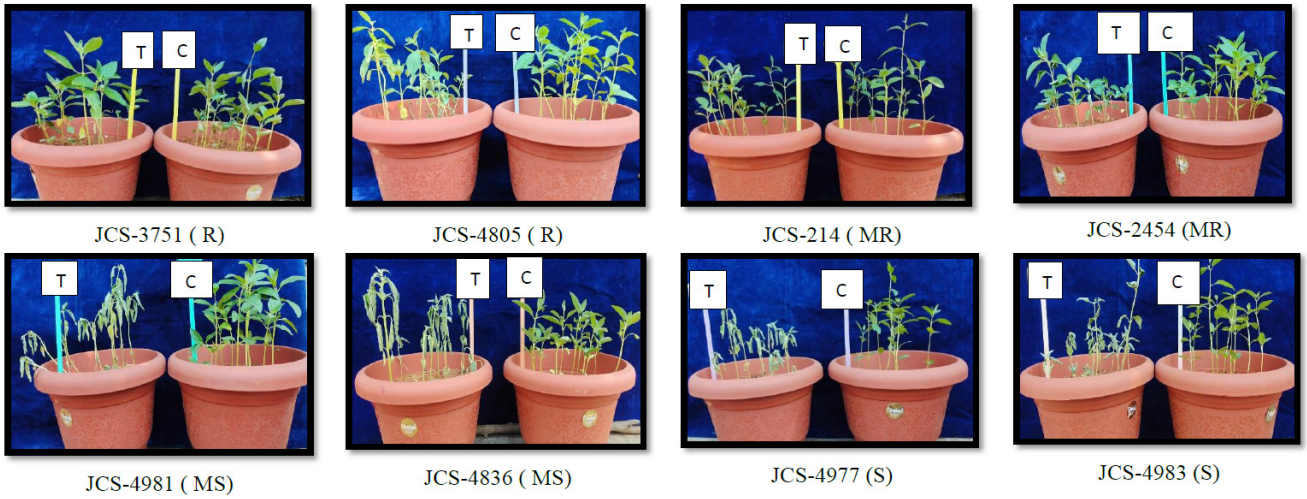

Fig. 7: Screening of sesame genotypes by artificial inoculation of pathogen (*in vivo*) (T-pathogen treated, C-control)

Fig. 8 : Seedling mortality in treated

Fig. 9 : Overall view of screening of sesame genotypes under (*in vivo*)

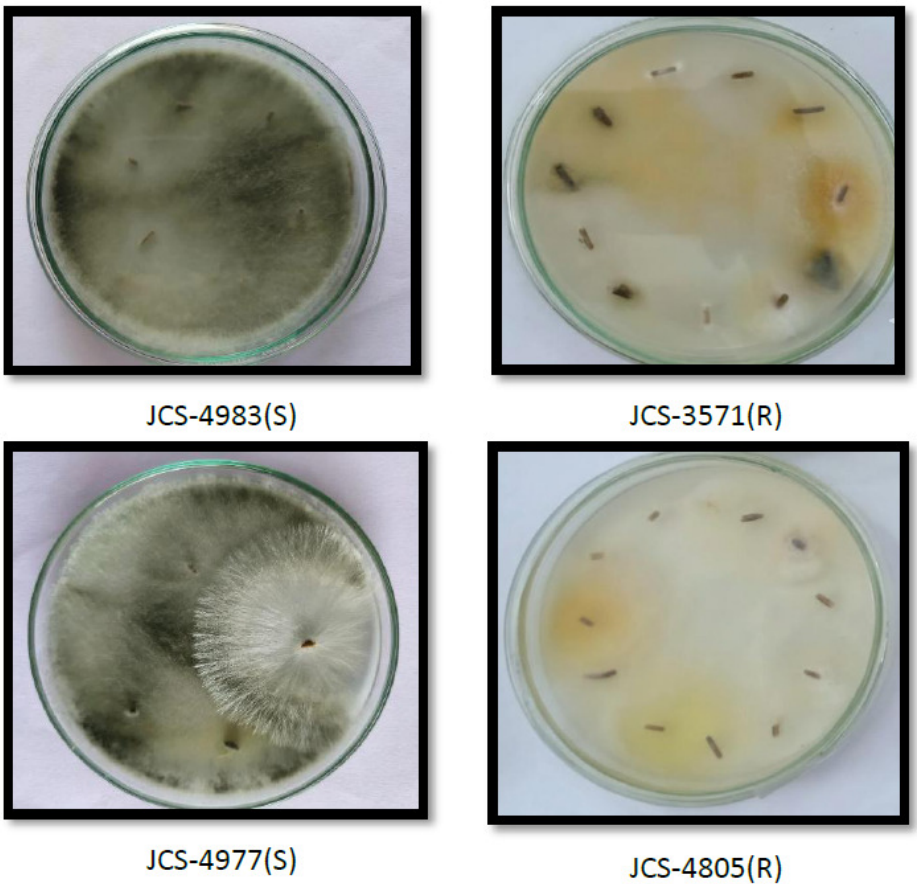

Fig. 10 : Tested for re-occurrence of pathogen in susceptible and resistant genotypes

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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