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Assessment of soybean genotypes for charcoal rot disease resistance and agronomic performance

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Abstract
The Indian soybean field impacted a lot by catastrophic disease called charcoal rot, causal organism is *Macrophomina phaseolina* (Tassi). In 2020, seven genotypes of soybean were evaluated for their charcoal rot resistance in hotspot region, Amravati, Maharashtra. The seven genotypes underwent field evaluation in 2020 and 2021, targeting on disease reaction and agronomic traits. The evaluations employed Percent Disease Incidence (PDI), Area under Disease Progress Curve (AUDPC), and grain yield as key criteria. Among the genotypes, AMS-1001 demonstrated the lowest PDI (2.14) and AUDPC (8.37) values in 2020, along with the highest grain yield. These findings underscore the potential of AMS-1001 as a high-yielding, charcoal rot resistant genotype, highlighting its suitability for inclusion in breeding programs aimed at enhancing soybean productivity and disease resilience in India.

Keyword: Charcoal rot, *Macrophomina phaseolina*, resistant, soybean

Introduction
Soybean is the world's most significant leguminous crop, offering 25% of edible oil and 2/3 of the protein in cattle feed (Agarwal *et al*., 2013) [1]. India secures seventh rank in the global edible oil market; yet, imports account for fifty percent of its edible oil requirement. India ranks sixth in terms of soybean production, but the yields from soybeans grown were significantly lower in comparison to United States and Brazil where it is widely grown mainly because of rainfalls being used as source throughout growing season along with biotic constraints which are various types including pests, diseases as well; hence they require enough attention both before after planting season to ensure good growing conditions for better harvests and commercialization (Agarwal *et al*., 2013) [1].

India's most devastating soybean disease, with almost 77% lower yield due to *Macrophomina phaseolina* (Tassi) called as charcoal rot disease (Sharma *et al*., 2014) [13]. Despite occurrence of drought-like conditions, this disease has hindered considerable harvest even with irrigation water. Charcoal rot infection causes the mortality of soybean plants during their maturity periods (Mengistu *et al*., 2018) [15]. The basis for diagnosis in soy plants include dark grey/black discoloration at the bottom stem and root, and there being numerous black microsclerotia (Luna *et al*., 2017 and Amrate *et al*., 2020) [9, 2]. *Macrophomina phaseolina* is necrotrophic fungus that has many host species. Most of these species are grown for commercial purposes including soybean, Sorghum, Maize, cotton etc. The most effective and resilient treatment for this disease is genetic resistance (da Silva *et al*., 2019). Nonetheless, the slow pace of progress in charcoal rot resistance breeding and genomics can be linked to the following: quantifiable plant-host resistance, the variability of pathogenicity, and multidimensional pathways for pathogenicity (Gupta *et al*., 2012) [7].

Although artificial screening takes less time, field-based screening should not be ignored because it is the actual environment in which the crop is cultivated. The Area Under Disease Progress Curve (AUDPC) has commonly used for expressing levels of horizontal resistance, as well as for integrating plant growth stages with respect to pathogen development timelines under field conditions (Jeger *et al*., 2001) [8].

In India this disease is so prevalent, but there are few reports. Selecting for stress resistance along with grain yield increases that few superior cultivars reported.

However, its effect on yield in terms of grain was shown to be positive sometimes though more often than not negative depending on which genotype was used among other factors (Smith *et al*., 2018) [15]. In addition, it was reported that a few of tolerant soybean genotypes were infected by charcoal rot without having their yields reduced (Smith *et al*., 2018) [15]. Hence, this implies that when dealing with charcoal rot in soybeans, close attention should be paid to the seed quality consideration.

Breeders frequently priorities various qualities through the selection process along with yield performance. The genetic base for soybean varieties bred in India is extremely narrow. The genetic basis of traditional phenotypic diversity studies, however, can be very variable, while that of molecular diversity analysis is more reliable and uniform. In breeding programs, genotypes showing genetic variability have been used as breeding lines (Maughan *et al*., 1996 and Thompson *et al*., 1998) [10, 16]. Given the background described above, present study identified and describe those soybean varieties that yielded more output despite being resistant to charcoal rot based fungal infections.

Materials and Methods
In 2020, charcoal rot resistance was tested for 7 soybean genotypes at hot spot condition, Regional Research Centre in Amravati, India. The randomised block design with three blocks experimental design was used. All the seven genotypes were selected for next year's screening based on disease resistance and enhanced agronomy. The genotypes were planted in three rows of nine metres each; one susceptible check Tams-38 was repeated within the blocks. The conditions were determined according to the percentage occurrence of these diseases. Also, a disease scoring range 0–9 (Table 1) using Percent Disease Incidence (PDI) scale at reproductive R7 stage (Twizeyimana *et al*., 2012) [17] was employed. The range observed for the susceptible checks showed variations from just being able to resist some levels of the pathogen (resistant) to full blown symptoms (susceptible) hence signifying very high level susceptibility in this field experiment.

Table 1: A disease assessment scale for determining the severity of charcoal rot disease

Grade/Scale	Disease severity	Status	Symptoms
0	0%	Highly resistant	No pathogen attack
1	1–9%	Resistant	Plant resistant against infection and no disease symptoms
3	10–24%	Moderately resistant	Smaller number of sclerotia, only inside the stem
5	25–49%	Moderately susceptible	Sclerotia formed inside the stem, and external stem discoloration also appeared
7	50–74%	Susceptible	Large number of sclerotia formed inside the stem and outer discoloration also severe
9	75% and above	Highly susceptible	Whole plant becomes wilt and severe inner and outer stem infection occurs, with pycnidia formation in the host

Source: Siddique *et al*., 2021

On-field Screening
The seven genotypes in 2020, which were retested in the field for disease reaction and enhanced agronomic performance, were reassessed in 2021 at same sick plot. The PDI, Area Under Disease Progress Curve and grain production per sick plot were assessed in seven genotypes and with susceptible check TAMS-38. The RBD was adopted with three replications each during experiment. To increase disease pressure and minimize disease escapes, additional infestation was required in the sick plot. This was achieved by using mass-multiplied charcoal rot to make more soil sick. Before planting, sorghum mass media culture was grown and added to sick soil (10g per genotype in each replicate). Prior to enormous growth of the pathogen, ITS PCR (Twizeyimana *et al*., 2012) [17] was employed to confirm the pathogenicity of the isolate.

$$AUDPC = n-1\sum i=1\ [(y_i + y_{i+1})/2][t_{i+1} - t_i]$$

Where,
y_i = per cent incidence of charcoal rot at i th observation,
t_i = time (days) at i th observation, and
n = number of observation.

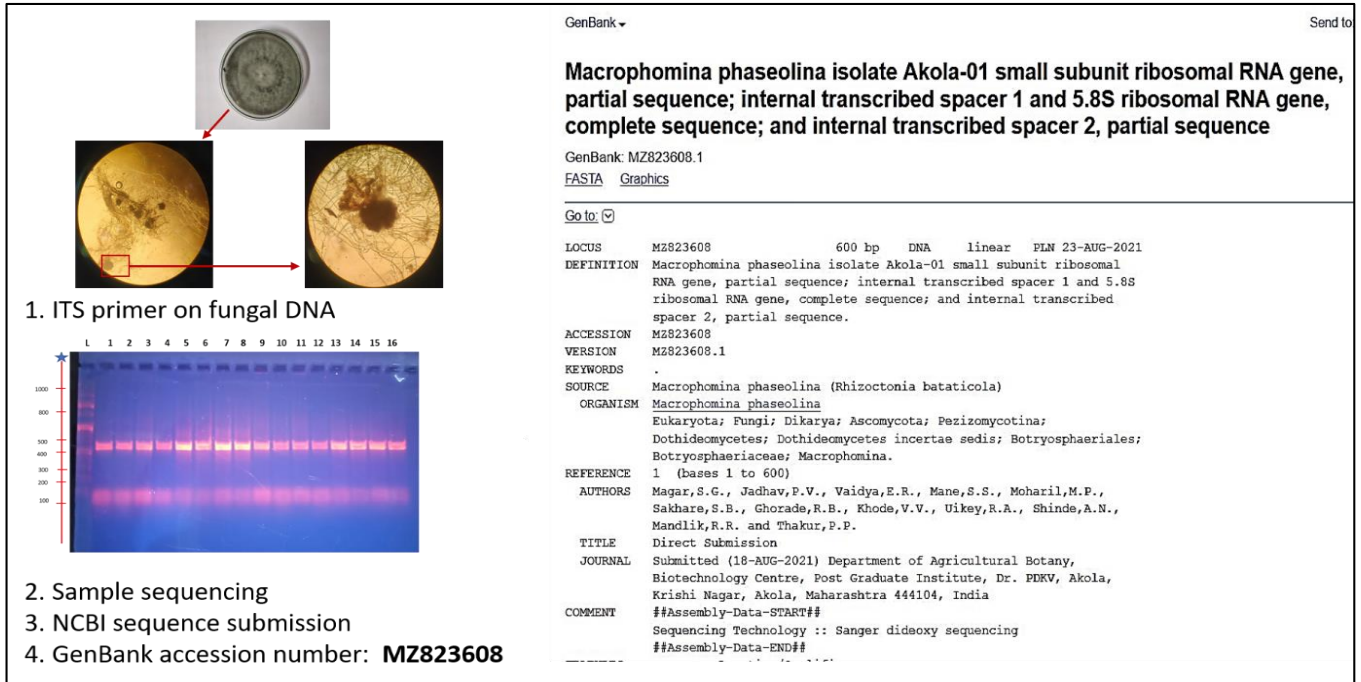## Molecular characterization of *Macrophomina phaseolina*
The study involved collecting soil from a charcoal rot disease-affected soybean plot in RRC for soybean at Amravati and extracting genomic DNA from *M. phaseolina*.

PCR was conducted using ITS1/4 primers sixteen isolates of *M. phaseolina* from diverse hosts and ecological conditions were chosen for ITS region sequencing. The DNA fragments were extracted and purified using the Prepagene kit from BioRad. The extracted fragments of PCR products were sequenced and homology search was done then successfully submitted sequence to NCBI.

Results
Molecular characterization and confirmation of *Macrophomina phaseolina*.
The *Macrophomina phaseolina* was isolated from soil samples obtained from diseased soybean fields experiencing charcoal rot disease using serial dilution method. Identification of the organism was confirmed under the microscope by lactophenol cotton blue staining. The isolated 16 samples from soil specimens in which genomic DNA was extracted by DNAzol method, with subsequent ITS1/4 PCR primer mediated DNA amplification was done. the sequencing was done by outsourcing it. The sequences were compared and analyzed for homology against sequences in the GenBank and EMBL databases. The resulted sequence exhibited 96% identity with several annotated *Macrophomina phaseolina* ITS sequences, predominantly aligning with the sequence ID MT645816.1 in the NCBI database. The sequence data was officially submitted to the NCBI and assigned the GeneBank accession number MZ823608 (Fig 1).

Fig 1: Molecular characterization and confirmation of *Macrophomina phaseolina*

Evaluation of Soybean Genotypes Centered on Disease Reaction Categories along with PDI, AUDPC and yield

The assessment of seven soybean genotypes for disease resistance and agronomic performance across two years, 2020 and 2021, revealed distinct reactions cantered on disease severity. Among the seven genotypes categorized as high resistant (HR), AMS-MB-05-18 demonstrated consistently low levels of disease incidence, as indicated by the lowest values for Percent Disease Incidence (PDI) and Area Under Disease Progress Curve (AUDPC) in both years, coupled with high grain yields. Conversely, highly susceptible (HS) genotypes such as AMS-77 and the check variety TAMS-38 exhibited significantly higher disease incidence and lower yields compared to the other genotypes. Moderately resistant (MR) genotypes, including JS-335, AMS-MB-05-19, and AMS-100-39, displayed intermediate levels of disease incidence and yield, while the moderately susceptible (MS) genotypes AMS-38-24 and TAMS-38 check exhibited higher disease incidence and moderate yields. AMS-1001 had notably little incidence of the disease and the highest grain yields and this illustrated its potential for increasing soybeans resistance to diseases when cultivated, hence the need for further improvement and use it in future breeding programs aimed at improving soybean disease resistance. Based on positive performance, all genotypes were chosen for the next experiment. In 2020 and 2021, the genotypes underwent extensive testing for Plant Disease Index (PDI), Area Under Disease Progress Curve (AUDPC), and grain yield. In 2020, PDI ranged from 2.14 (AMS-1001) to 71.67 (AMS-77), with average mean of 30.87. According to AUDPC the values ranged from 8.37 (AMS-1001) to 806.70 (AMS-77) with an average of 333.15 mean. Grain yield ranged between 415.00 g (AMS-1001) and 17.00 g (AMS-77) and average mean grain yield was 210.43 g. Genotype AMS-1001 was found to show lowest PDI followed by AMS-MB-05-18. Genotype AMS-1001 was observed to reveal lowest AUDPC followed by AMS-MB-05-18. Highest grain yield was recorded in the genotype AMS-1001 followed by AMS-MB-05-18 (Table 2). During the year 2021, the PDI was distributed across a range of 0.14 to 50.67, noting the lowest disease index at AMS-1001 (0.14) and highest at AMS-77 (50.67) with mean average of 29.92. Peak AUDPC manifested in AMS-77 registering at 834.43 compared to 3.34 found in AMS-1001 with average mean of 364.23. AMS-1001(513.00 g) had the highest grain yield during the period of study while AMS-77 (14.00 g) represents the lowest on the opposite spectrum of productivity with average mean grain yield was 232.49 g. AMS-1001 genotype was recorded lowest PDI followed by AMS-MB-05-18. AMS-1001 genotype was recorded to be lowest AUDPC followed by AMS-MB-05-18. Grain yield was observed high in the AMS-1001 genotype followed by AMS-MB-05-18 (Table 2).

Table 2: Mean performance of seven soybean genotypes with respect to PDI, AUDPC and Grain yield

S.N.	Genotype	2020			2021			Disease Reaction*
		PDI	AUDPC	Yield	PDI	AUDPC	Yield	
1	JS-335	11.33	93.23	256.23	12.21	81.23	287.66	MR
2	AMS-MB-05-18	3.43	9.37	391.67	2.45	3.45	421.76	R
3	AMS-MB-05-19	12.34	78.23	296.23	13.31	75.26	308.76	MR
4	AMS-100-39	15.35	98.32	256.32	16.71	89.36	268.76	MR
5	AMS-1001	2.14	8.37	415.00	0.14	3.34	513.00	HR
6	AMS-38-24	50.35	668.70	42.00	53.67	734.43	42.00	MS
7	AMS-77	71.67	806.70	17.00	50.67	834.43	14.00	HS
	TAMS-38	80.32	902.30	9.00	90.23	1092.34	4.00	S-Check
		30.87	333.15	210.43	29.92	364.23	232.49	

ANNOVA

The genotypic effects of three traits analyzed in 2020 and 2021 turned out to be significant at $p < 0.001$ during within-year ANOVA. Analysis of variance revealed substantial effects on these three variables from genotype, environment, and the G×E interaction (ANOVA) ($p < 0.001$). In PDI, the genotypic effect (87.72%) was responsible for the majority of the the variance, follow by the G×E interaction impact (2.49%) and environmental factor (1.32%). In AUDPC, the genotypic impact makes for 73.6 percent of the overall variance, follow by the G×E interaction impact (5.63%) and the environmental factor (1.42%). The genotypic factor accounted for 75.22% of grain yield variation, followed by the G×E interaction impact (4.29%) and the environmental factor (1.07%).

Discussion

Charcoal root was considered one of the most serious fungal infections in India, causing economic loss to the farmers, yet few trials have been conducted in Maharashtra vidharbha region to identify charcoal rot resilient with high yield genotypes specifically. This study ambitions to address this gap by considering grain yield and resistance reactions under severe disease pressure to identify such genotypes. Aboveground charcoal rot symptoms typically first appear at the R5 stage. Studies have shown that *M. phaseolina* colonization of soybeans is limited during the vegetative and early reproductive stages, peaking between the R5 growth satge and R7 growth stages (Mengistu *et al*., 2011) [12]. As a result, in the current study, AUDPC was measured at the reproductive stage and PDI was measured at the R7 stage, which is considered the optimal growth stage for assessing charcoal rot plant resistance. According to ANOVA analysis, greater genotypic variability and heritability indicate a stronger sensitivity to selection in the variables studied. The major portion of the total variance is attributed to the gene effects with substantial G × E interaction implying increased response of any variable that is changed. Genotype influence must be sustained regardless of the source. According to our results, the AMS-1001 genotype is the most suitable one for the further gene identification using transcriptomics to determine charcoal rot resistance, whereas AMS-MB-05-18 would be the best parent in high disease pressure conditions for breeding other lines with higher yields. These genotypes are able to give high harvest and protect against charcoal rot. Other traits that can add to high yield like seed weight at 100, plant height, nodal number, branching intensity and harvest index must be considered in further studies for identifying yield contributing traits under disease pressure and for researching this relationship between yield and yield-related traits and disease indices for high disease stress conditions, as researched in the case of fungal resistance in sorghum (Diatta *et al*., 2019) [6]. These identified genotypes, which exhibit both charcoal rot resistance and high yield can serve as breeding lines in developing multiple disease-resistant soybean varieties (Aruna *et al*., 2021). By crossing these varieties using deliberate breeding procedures will almost certainly result in soybeans that are resilient to charcoal rot and maybe other diseases that destroy these plants. This approach can significantly boost soybean output in India by reducing crop losses due to diseases while also fostering greater yield consistency. Furthermore, developing such resilient cultivars ensures that soybeans are grown in a sustainable manner while also increasing their availability for domestic as well as global commerce, therefore improving food security.

Conclusion

In the current investigation, AMS-1001 has been identified as the genotype with the greatest resistance to charcoal rot disease, whereas AMS-MB-05-18 had the highest yield. This implies that the two varieties conveyed the qualities for obtaining high productivity in soybean farming methods because they combine resistance against this particular type of ailment and ensure enough produce was gotten out of them at the same time aiming at solving immediate problems while improving future efficiency among Indian farmers.

Acknowledgment

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