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Morpho-Molecular Characterization of *Pyricularia grisea* and Multilocational Evaluation of Blast Resistance in a Elite Panel of Finger Millet Genotypes in Karnataka

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ABSTRACT

Blast caused by *Pyricularia grisea* causes significant yield loss in finger millet ecosystem. This study was undertaken to identify, characterize the finger millet blast pathogen. The pathogen was isolated by standard spore drop technique and morphologicaly confirmed. Upon molecular characterization and phylogenetic analysis the pathogen was identified as *Pyricularia grisea.* Among all the diseases management aspects the host plant resistance is the major strategy for disease control. Thirty two elite genotypes were screened for blast disease at two locations in Karnataka *viz.,* Bangalore and Mandya. The Mandya location recorded highest diseases severity of leaf blast, neck blast and finger blast. Out of the various genotypes assessed for resistance against multiple blasts. Six genotypes namely GE 4994, GE 5879, GE 4871, GE 5000, GE 5103 and GE 5772 found resistance against all three types of blasts. So these genotypes can be used as the potential donor parents in resistance breeding programme.

Keywords : Blast, *Pyricularia grisea*, Screening, Molecular characterisation

FINGER millet (*Eleusine coracana* L.) is a millet cultivated widely across India and Africa serving as a staple food for a substantial part of the population in these regions (Devi *et al.,* 2014). Finger millet is abundant in carbohydrates 72.6 g, energy 336 kcal and 11.5g of total dietary fibre per 100g of edible portion. It is also rich source of calcium (350mg) and vitamins like thiamin (0.42mg) and riboflavin (0.19mg) (Kumar *et al.,* 2016). In addition to providing energy, finger millet plays a crucial role in addressing micro-nutrient and protein deficiencies, commonly known as 'hidden hunger'. This issue affects approximately half of the global population, particularly impacting women and preschool children across African and Southeast Asian nations (Underwood, 2000). Alarmingly high rates of protein deficiency-related malnutrition are also prevalent in the Indian subcontinent (Prasad, 2010). The United State national academics have called it as a potential 'Super Cereal' since it is highly nutritious than other popular cereals (Anonymous, 1996).

The cultivation and yield of finger millet face significant impediments from a wide spectrum of biotic and abiotic stresses. These challenges are poised to intensify with the ongoing global climate change. Notably, among the biotic stress, the blast disease incited by *Pyricularia grisea* stands as a critical factor inducing considerable yield loss in finger millet. Blast affects the finger millet at all stages of growth, starting from seedling to grain formation. Leaf blast, neck blast and finger blast are the three kinds of blasts observed on finger millet. The disease is characterized by the emergence of small lesions on

the leaves, neck and fingers. On the leaves, these lesions take on an eye-spot shape, broader in the center and tapering at both ends. They have a greyish center with a dark brown margin. Initially, the leaves display chlorosis, but as the disease progresses, these lesions rapidly enlarge and merge, leading to the drying of leaves. The pathogen also infects the neck area, causing neck rot. When the fingers are affected, the seeds shrivel, become deformed, and turn chaffy (Jeevan *et al.,* 2021).

Finger millet is predominantly grown by small and marginal farmers who often cannot afford costly chemical control methods. Identifying resistant genotypes could lead to the development of resistant varieties, significantly reducing cultivation expenses for these farmers. The present study was planned to evaluate superior genotypes in the finger millet against *M. grisea* to identify new and diverse sources of blast resistance.

Material and Methods

Isolation of Mono-conidial Isolates of *M. grisea*

Infected tissue sample showing typical symptoms of blast disease was collected from ZARS, GKVK, Bengaluru plot and blast infected tissue were cut into small bits. These bits were surface sterilized in 1 per cent sodium hypochlorite solution for 30 seconds and rinsed with sterile distilled water thrice. Further isolation was done using method given by Raja shekara *et al*. (2017). After the fungal growth, a small loop of the culture was taken from the colonies and placed on a clean glass slide containing a drop of lacto phenol. The slide was observed under low and high power magnification for the presence of conidia of fungus.

Molecular Characterization of *M. grisea* Isolates

Fungal DNA of all the isolates was isolated from the established pure culture by using standard CTAB (Cetyl Trimethyl Ammonium Bromide) protocol with slight modification and genomes of the *M. grisea* isolates were amplified in PCR using ITS 4 and ITS 5 primers and sequencing was done. Based on the sequence, phylogenetic analysis was carried out.

Collection and Sources of Finger Millet Germplasm

A set of 32 finger millet genotypes sourced from various regions in Asia and Africa was obtained from the (AICRP on small millet) project coordinating unit in Bengaluru. Instead of a broad array, a more focused selection was made, concentrating on the most promising genotypes identified in previous study (Annonymous, 2019). This deliberate selection aimed to deepen our understanding of these genotypes across diverse geographical locations. Additionally, the set includes several superior varieties cultivated in India. This selection encompasses four resistant genotypes (KMR 204, GPU 28, KMR 340, GE 4449) and four susceptible checks (KMR 301, PR 202, FM 3001, UM). Detailed information regarding the type and origin of genetic material is outlined in Table 1.

Experimental Layout

The genotypes were evaluated in the *kharif* season of 2021 at two locations, comprising ICAR-AICRP on

Table 1

Source of finger millet genotypes used in this experiment

Type	Source	Genotype name
Germplasm	India	GE 5879, GE 5771, GE 5825, GE 5800, GE 5155, GE 5812, GE 5816, GE 6024
	Africa	GE 4994, GE 4879, GE 5126, GE 5000, GE 5772, GE 4911, GE 4796, GE 4866, GE 4997, GE 4861, GE 4722, GE 5112, GE 4837, GE 4907, GE 4998, GE 5103,
Checks	India (R)	KMR 204, GPU 28, KMR 340, GE 4449
	India (S)	KMR 301, PR 202, FM 3001, Uduru Mallige

Small Millets, project coordinating unit in Bengaluru and Zonal agricultural Research Station at V.C. Farm, Mandya. The seeds of each genotypes were sown in two rows of 3m length in randomized block design with two replications. After every five entries two rows of susceptible check was repeated to facilitate the disease development. Seedlings were thinned at 15 days after emergence keeping 15 plants per row. Standard crop management practices were used to raise the crop.

Artificial Inoculation of Fungal Inoculum

The pure culture of *P. grisea,* isolated from blast-affected finger millet plants at specific locations, was employed to artificially infect the test genotypes grown in corresponding fields. This culture was mass multiplied on rice straw extract agar and the modified slide culture method was utilized to induce sporulation, yielding the necessary quantity of conidia. Adjusting the spore suspension to a concentration of 1×10^5 spores/ml using a hemocytometer, tween 20 served as the dispersing agent. Inoculation occurred by spraying the adjusted solution 30 days after sowing to induce leaf blast and at the pre-anthesis stage to incite neck and finger blast effectively. Maintaining humidity for disease development involved periodic water spraying, excluding rainy days.

Data Collection

The assessment of leaf, neck and finger blast was done using standard protocols, employing a rating scale to categorize test entries (Hariprasanna *et al.,* 2022). Leaf blast evaluation was conducted at the seedling stage (40 DAS). Neck blast and finger blast incidence were recorded during the crops dough stage and presented as a percentage. For each genotype within every replication, 20 consistently developed tillers were randomly selected. The count of peduncles showcasing characteristic blast lesions on the neck determined the extent of neck infection (Plate 2). These same selected tillers were utilized to tally both the total number of fingers and those affected by blast, assessing the severity of finger blast. A finger was considered infected if it displayed visible blast-induced lesions (Table 2, 3 & 4).

Results and Discussion

Isolation of Mono-conidial Isolates of *M. grisea*

The pathogen was isolated by following spore drop technique (lesion-print). A bit (6 mm) of mycelial mat with spores from growing colony was removed and suspension was prepared from that single spore isolation was done. Microscopic analysis of the culture revealed the presence of pyriform conidia that were two-septate, three-celled, hyaline to brown and bore

Table 2

Standard leaf blast scoring scale and reaction groups

Score	Description	Disease Reaction
1	Small, brown, pinhead size specks without sporulating centre	Highly Resistant (HR)
2	Small (1-2 mm) roundish to elongated, necrotic grey spots with a distinct brown margin covering up to 5% leaf area	Resistant (R)
3	Typical blast lesions (e”3 mm) with sporulating center, covering 6-10 % of the leaf area	Resistant (R)
4	Blast lesions covering 11-20% leaf area	Moderately Resistant (MR)
5	Blast lesions covering 21-30% leaf area	Moderately Resistant (MR)
6	Blast lesions covering 31-40% leaf area	Susceptible (S)
7	Blast lesions covering 41-50% leaf area	Susceptible (S)
8	Blast lesions covering 51-75% leaf area	Highly Susceptible (HS)
9	Blast lesions covering >75% leaf area & plant dead	Highly Susceptible (HS)

TABLE 3

Standard neck blast scoring scale and reaction groups

Score	Description	Disease Reaction
1	<1% plants infected with neck blast	Highly Resistant (HR)
2	1-5% plants infected with neck blast	Resistant (R)
3	6-10% plants infected with neck blast	Resistant (R)
4	11-20% plants infected with neck blast	Moderately Resistant (MR)
5	21-30% plants infected with neck blast	Moderately Resistant (MR)
6	31-40% plants infected with neck blast	Susceptible (S)
7	41-50% plants infected with neck blast	Susceptible (S)
8	51-75% plants infected with neck blast	Highly Susceptible (HS)
9	>75% plants infected with neck blast	Highly Susceptible (HS)

TABLE 4

Standard finger blast scoring scale and reaction groups

Score	Description	Disease Reaction
1	<1% plants infected with finger blast	Highly Resistant (HR)
2	1-5% plants infected with finger blast	Resistant (R)
3	6-10% plants infected with finger blast	Resistant (R)
4	11-20% plants infected with finger blast	Moderately Resistant (MR)
5	21-30% plants infected with finger blast	Moderately Resistant (MR)
6	31-40% plants infected with finger blast	Susceptible (S)
7	41-50% plants infected with finger blast	Susceptible (S)
8	51-75% plants infected with finger blast	Highly Susceptible (HS)
9	>75% plants infected with finger blast	Highly Susceptible (HS)

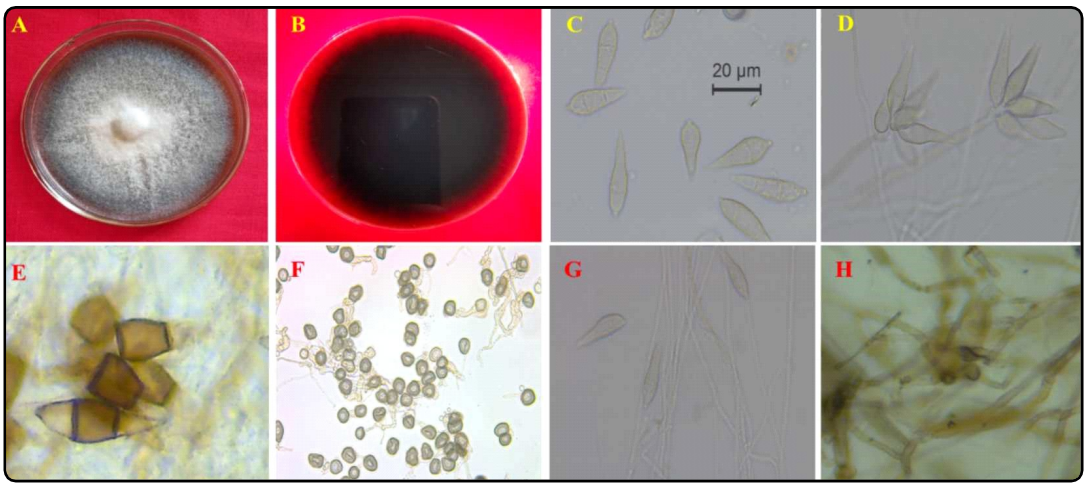

Fig. 1 : Morphological characteristics of *Pyricularia grisae* A) Upper view, B) lower view of culture plate, C) Hyaline conidia, D) Conidiophore, E) Matured conidia , F) Appressoria, G) Hyaline mycelium and H) Coloured mycelium

basal appendage at the point of attachment to the conidiophore. On rice straw extract agar, colony was whitish to greyish with black colour pigmentation on reverse side of the Petri plate (Fig. 1). The isolate was identified as *M. grisea* by comparing its colony and conidial characteristics to the original descriptions provided by Saccardo (1880) and Hebert (1971).

Molecular Characterization of *M. grisea* Isolates

As the morphological characteristics were inadequate to distinguish *M. grisea* from other *Magnaporthe* species, molecular studies were conducted for all the isolates by using fungal universal barcode region (ITS 4 and 5). Fungal DNA was isolated by CTAB method and purified, PCR amplification was done by using ITS primer. PCR primer pair yielded specific PCR products of approximately 550 bp. PCR product was gel eluted by using Medauxin® gel extraction kit and sequencing was performed.

Sequence Alignment and Phylogenetic Analysis

All the sequences were aligned along with other reference sequences of *Magnaporthe* species and the phylogenetic tree was constructed with maximum likelihood approach by using MEGA X software. Analysis of the ITS sequences revealed that the isolate (FMP 2) demonstrated the highest percentage of identity with *Magnaporthe grisea* (Fig. 2) by forming separate clade with *M. grisea* isolates which were already deposited in the NCBI.

Reaction of Finger Millet Genotypes Against Blast

Leaf blast : Among the two locations evaluated, the Mandya location recorded the highest mean disease severity of leaf blast (3.58G) and Bengaluru recorded 1.82G (Fig. 3). Upon classification based on disease grade, none of the genotypes were highly resistant. Whereas, 25 genotypes were resistant, three genotypes were moderately resistant, one genotype (KMR 301) was susceptible and remaining three genotypes were highly susceptible (Table 4).

Neck blast : The highest severity of neck blast was recorded in Mandya location (12.42%) and 5.19 mean percentage disease severity was recorded at Bengaluru

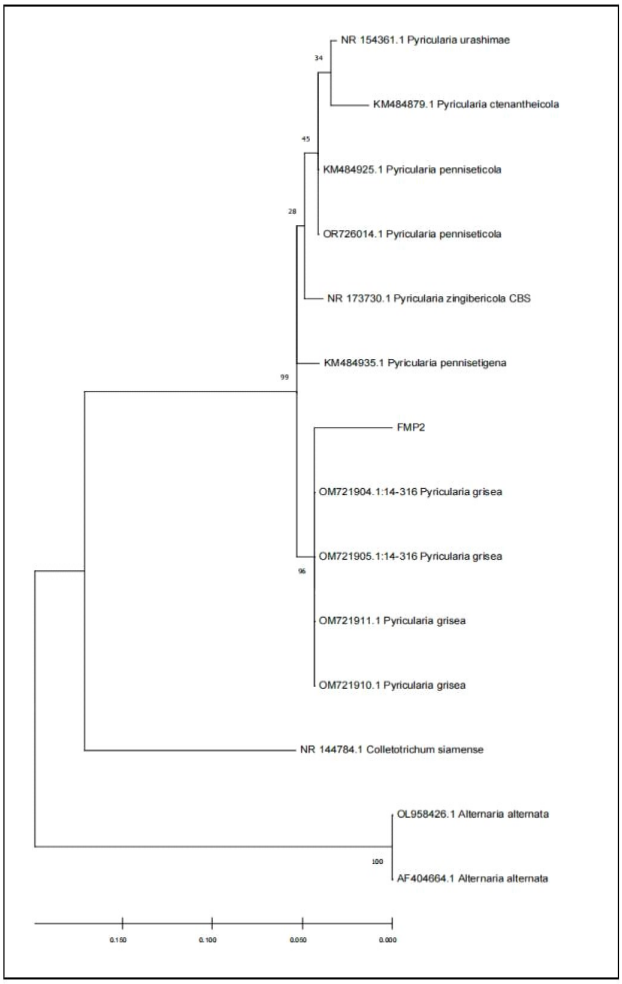

Fig. 2 : Evolutionary analysis of Internal Transcribed [(ITS) region sequences] aligned with other related sequences of *Pyricularia* sp. accessed from GenBank

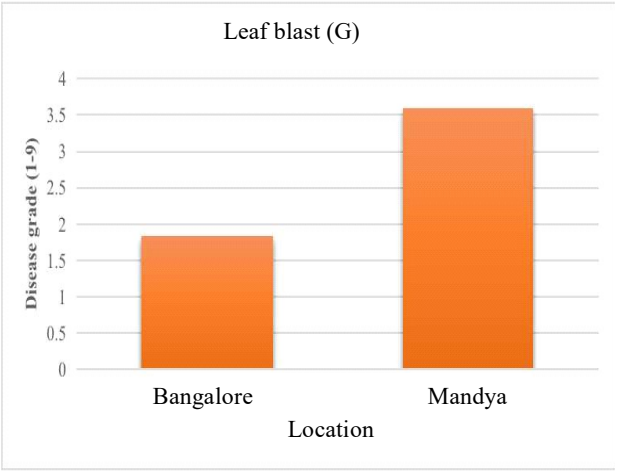

Fig. 3 : Leaf blast incidence at experimental locations

TABLE 5

Leaf, neck and finger blast severity of elite finger millet genotypes across locations

Genotypes	Leaf Blast			Neck Blast			Finger Blast		
	Bangalore	Mandya	Mean	Bangalore	Mandya	Mean	Bangalore	Mandya	Mean
GE 4994	0.96	2.25	1.61	4.65	0.94	2.80	10.73	6.65	8.69
GE 5879	0.49	5.2	2.85	0	8.75	4.38	7.56	11.82	9.69
GE5771	1	2.66	1.83	2.41	13.92	8.17	23.13	16.62	19.88
GE 5825	1.48	5.25	3.37	4.77	20.16	12.47	5.6	21.6	13.60
GE 4879	1.5	2	1.75	2.25	2.83	2.54	6.4	7.56	6.98
GE 5126	1.97	2.96	2.47	5.3	7.24	6.27	24.56	36.98	30.77
GE 5800	1.96	3.92	2.94	2.64	6.27	4.46	15.34	9.55	12.45
GE 5000	1.02	2.73	1.88	1.14	4.03	2.59	4.34	5.68	5.01
GE 5155	0.99	2.31	1.65	0	3.81	1.91	12.35	8.5	10.43
GE 5772	1	1.67	1.34	0	7.24	3.62	10.24	8.96	9.60
GE 4911	2	2.66	2.33	1.22	4.45	2.84	16.45	26.39	21.42
GE 4796	2.03	3.73	2.88	7.66	5.52	6.59	33.17	79.9	56.54
GE 4866	0.99	2.31	1.65	6.86	0.94	3.90	25.67	16.03	20.85
GE 4997	0.98	2.29	1.64	5.35	7.93	6.64	45.56	85.81	65.69
GE 5812	1.02	2.38	1.70	4.51	25.23	14.87	24.24	10.73	17.49
GE 4861	1.5	2.33	1.92	1.38	3.02	2.20	23.21	7.47	15.34
GE 4722	1.5	4.66	3.08	4.91	17.43	11.17	38.12	35.63	36.88
GE 5112	2	3.33	2.67	2.78	3.57	3.18	21.23	18.17	19.70
GE 4837	0.5	2.33	1.42	3.26	3.46	3.36	31.12	20.68	25.90
GE 4907	1.5	2.67	2.09	1.33	2.12	1.73	26.66	17.49	22.08
GE 4998	0.96	3.21	2.09	2.68	4.26	3.47	56.23	61.35	58.79
GE 5816	0.97	3.58	2.28	5.78	11.11	8.45	77.77	89.42	83.60
GE 5103	1.49	2.66	2.08	7.25	3.46	5.36	2.91	2.85	2.88
GE 6024	0.99	5.25	3.12	2.23	12.91	7.57	4.88	74.46	39.67
KMR 301 (SC)	5.01	7.01	6.01	31.24	29.84	30.54	34.26	75.76	55.01
PR 202 (SC)	5.91	8.22	7.07	22.56	39.99	31.28	33.56	38.98	36.27
FM 3001 (SC)	5.87	8.16	7.02	17.03	51.51	34.27	20.78	89.52	55.15
UM (SC)	6.14	8.86	7.50	11.87	71.89	41.88	31.57	59.22	45.40
KMR 204 (RC)	0.99	1.65	1.32	2.01	1.5	1.76	11.23	29.29	20.26
GPU 28 (RC)	1.5	2.34	1.92	3.38	5.64	4.51	12.24	24.4	18.32
KMR 340 (RC)	1	1.66	1.33	4.54	1.68	3.11	13.45	25.51	19.48
GE 4449 (RC)	1.02	2.37	1.70	1.59	4.45	3.02	11.59	29.05	20.32
Loc. Mean	1.82	3.58		5.19	12.42		22.38	32.88	
C.D. (5%)	0.15	0.29		0.51	1.28		0.57	3.48	
C.D. (1%)	0.2	0.38		0.68	1.71		0.76	4.63	
C.V. (%)	5.17	4.94		5.73	6.23		6.69	6.31	

Note : SC- Susceptible check and RC-Resistance check

TABLE 6

Rainfall, relative humidity and temperature of the experimental location in *kharif* 2021

Locations	Rainfall (mm)				RH (%)				Temperature (°C)			
	July	August	September	October	July	August	September	October	July	August	September	October
Bangalore	171.6	211.4	142	361	90.25	89.03	89.73	90.16	28.03-18.68	28.37-18.75	27.97-19.00	28.05-18.69
Mandya	84.3	67.1	149.2	222.5	92.24	88.66	90.70	91.37	28.37-19.20	29.17-19.51	29.9-19.63	29.53-19.33

TABLE 7

Grouping of finger millet genotypes into different reaction groups for leaf blast

Disease grade	Reaction of genotype	Genotype	Frequency
1	Highly resistant	Nil	0
1.1-3	Resistant	GE 4994, GE 5879, GE 5771, GE 4879, GE 5126, GE 5800,GE 5000, GE 5155, GE 5772, GE 4911, GE 4796, GE 4866, GE 4997, GE 5812, GE 4861, GE 5112, GE 4837, GE 4907,GE 4998, GE 5816,GE 5103, KMR 204, GPU 28, KMR 340, GE 4449	25
1-5	Moderately resistant	GE 5825, GE 4722, GE 6024	3
5.1-6	Moderately susceptible	Nil	0
6.1-7	Susceptible	KMR 301	1
>7	Highly susceptible	PR 202, FM 3001, UM	3

(Fig. 4). Upon classification based on disease score, none of the genotypes were highly resistant and highly susceptible. 25 genotypes were found resistant.

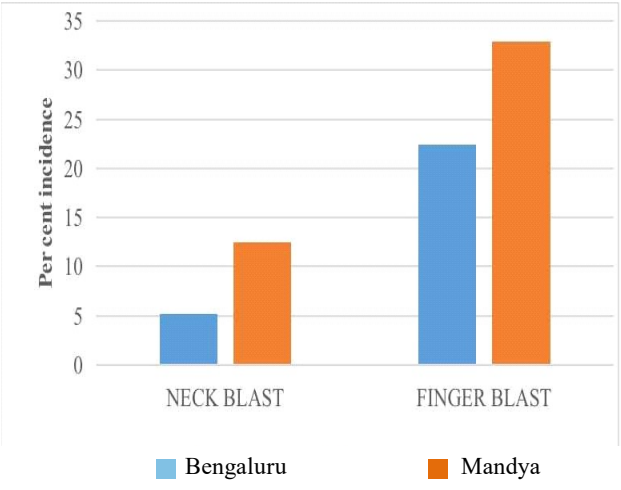

Fig. 4 : Neck and finger blast incidence at experimental locations

Whereas, three genotypes were moderately resistant and four genotypes were susceptible (Table 8).

Finger blast: Mandya location has recorded highest disease severity of about 33.88 percentage. Whereas, Bengaluru location has recorded 22.38 percentage of disease severity (Fig. 4). Based on disease score, none of the genotypes were highly resistant. Whereas, six genotypes were resistant, 15 genotypes were moderately resistant, five genotypes were found susceptible and remaining six genotypes were highly susceptible for finger blast (Table 9).

The susceptible checks were found to be highly susceptible to the blast disease that ensures that there is sufficient blast inoculum present at the test regions.

Genotypes with Multiple Blast Resistance

Out of the various genotypes assessed for resistance against multiple blasts. Six genotypes namely GE

TABLE 8

Grouping of finger millet genotypes into different reaction groups for neck blast

Disease grade	Reaction of genotype	Genotype	Frequency
<1	Highly resistant	Nil	0
1 to 10	Resistant	GE 4994, GE 5879,GE 5771,GE 4879,GE 5126,GE 5800,GE 5000,GE 5155,GE 5772,GE 4911,GE 4796,GE 4866,GE 4997,GE 4861,GE 5112, GE 4837,GE 4907,GE 4998,GE 5816,GE 5103,GE 6024, KMR 204, GPU 28, KMR 340, GE 4449	25
11 to 30	Moderately resistant	GE 5825, GE 5812,GE 4722	3
30.1 -50	Susceptible	PR 202, FM 3001, UM, KMR 301	4
>50	Highly susceptible	Nil	0

Plate 1 : Field view of the experimental plot

TABLE 9

Grouping of finger millet genotypes into different reaction groups for finger blast

Disease grade	Reaction of genotype	Genotype	Frequency
<1	Highly resistant	Nil	0
1 to 10	Resistant	GE 4994, GE 5879, GE 4879, GE 5000, GE 5103, GE 5772	6
11 to 30	Moderately resistant	GE 5771,GE 5825,GE 5800,GE 5155,GE 4911,GE 4866,GE 5812, GE 4861,GE 5112,GE 4837,GE 4904,KMR 204, GPU 28, KMR 340, GE 4449	15
30.1 -50	Susceptible	PR 202, UM, GE 5126, GE 4722, GE 6024	5
>50	Highly susceptible	GE 4796, GE 4997,GE 4998, GE 5816, KMR 301, FM 3001	6

a) **b)** **c)**

Plate 2 : Symptomatology of a) leaf blast, b) neck blast and c) finger blast

4994, GE 5879, GE 4871, GE 5000, GE 5103 and GE 5772 demonstrated resistance against all three types of blasts. Conversely, four genotypes exhibited resistance against leaf and neck blast, but susceptibility to finger blast *viz.,* GE 4796, GE 4997, GE 4998 and GE 5816.

The isolation of *M. grisea* isolate was carried out using spore drop technique. Upon isolation, the pure culture of the *M. grisea* when examined under the microscope it produced septate hyaline hyphae, upon maturity hyphae became darker. The conidia appeared hyaline to brown, with a pyriform shape and two septa, comprising three cells where the central cell was notably broader and darker. On the RSEA medium, the colony exhibited a whitish to greyish colour, with black pigmentation evident on the underside of the Petri plate.

Similar descriptions of hyphae, conidiophore and conidial characters of *M. grisea* have been reported by Shirai (1896), Yaegashi & Udagawa (1978), Klaubauf *et al*. (2014), Sab & Nagaraja (2018) and Shanmugapackiam *et al.* (2019).

Based on morphology it is very difficult to distinguish *Magnaporthe* species. Upon sequencing and molecular analysis using universal primers (ITS 5 & 4), the Bangalore isolate (FMP 1) showed higher similarity by forming separate clade with *M. grisea* isolates which were submitted in NCBI. Puri and Kumar (2018) characterized leaf, neck and panicle derived isolates through ITS amplification. A phylogenetic tree generated from ribosomal DNA-internal transcribed spacer sequences revealed that all the isolates have remarkable similarity with one another.

Thirty two genotypes which included four resistant checks and four susceptible checks were evaluated at two major finger millet growing areas in Karnataka. The Mandya has recorded highest disease severity compared to Bengaluru in all types of blasts *i.e.,* leaf blast, neck blast and finger blast. The Mandya location had a favourable climatic condition like optimum temperature and relative humidity and received optimum rainfall. The relative humidity was above 90 per cent during disease developmental stages. The day temperature was between 28 to 30°C throughout the experimental period (Table 6). This region is

majorly consist of irrigated lands, that helps in buildup of higher relative humidity and damp microclimate helps in disease progression. The blast pathogens primarily spread through airborne conidia. Conditions conducive to the diseases development include day time temperatures ranging from 25 to 30°C accompanied by cooler nights, high relative humidity surpassing 90 per cent, cloudy weather and intermittent rainfall.

Spraying the pathogen inoculum artificially at appropriate growth stages utilizing location-specific isolates will intensify disease severity and significantly reduce the likelihood of evading infection (Babu *et al.,* 2012 and Kumari *et al.,* 2022).

Among 32 genotypes evaluated, 25 genotypes were found resistant to the leaf and neck blast, whereas six genotypes were resistant to all three types of blast. Many studies were done to find out resistant genotypes for the blast disease. The differential reaction of the genotypes attributed to its diverse geographical origin having different genetic composition (Das *et al.,* 2021). There are many studies which elucidated the importance of identification of elite blast resistance genotypes (Mantur *et al.,* 2001, Patro *et al.,* 2013 & Das *et al.,* 2021 and Ranganatha *et al.,* 2022). There are many resistant varieties like GPU 28, GPU 45 and GPU 48 were released for cultivation and it is likely that resistance may break down owing to development of new pathotypes of *Magnaporthe grisea* These kind of study will pave ways for development of new diseases resistance varieties where these genotypes can be used as the donors for disease resistance.

The morphological and molecular characterization gave insights into pathogens proper classification as well as the role of different pathogen structures. It is crucial to identify consistent sources of resistance across various environmental conditions as this holds immense importance for effectively incorporating them into breeding programs. The genotypes GE 4994, GE 5879, GE 4871, GE 5000, GE 5103 and GE 5772 exhibited exceptional multiple disease resistance, which has the potential and desirable traits to be included in the future disease resistance breeding programme.

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