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# Characterization of *Magnaporthe grisea* associated with Finger millet leaf blast disease in Tamil Nadu

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**Abstract**
A survey was conducted for the collection of leaf blast disease in Finger millet growing different regions of Tamil Nadu. The maximum disease incidence (50-60%) was observed in Dharmapuri, Krishnagiri and Salem districts followed by Coimbatore and Dindigul (20-30%). Ten isolates of leaf blast disease were collected and isolated Oat Meal Agar Medium. These isolates are screened for pathogenicity test in pot culture under glass house conditions. The maximum disease incidence was exhibited in TNFGM 4 Dharmapuri (R. Gopinathanpatti) isolate followed by Salem and Krishnagiri. The leaf blast disease was caused by *Magnaporthe grisea* and it was confirmed by molecular technique using ITS 1 and 4 universal primer.

**Keywords:** Finger millet, Blast, Pathogenicity, Molecular characterization, ITS

## 1. Introduction
Finger millet (*Elusine coracana*) is a most important millet crop widely grown by subsistence farmers in Africa and Asia. It is mostly considered as a poor man's crop and it also grown under low input levels with more yielded (Negi *et al*., 2015) [5]. Unfortunately, it is severely affected by blast disease which is caused on leaves, fingers and neck region. The disease is very destructive as it cause both reproductive and vegetative stages of the finger millet crop. The disease is enhanced with optimum temperature is 20 °C to 25 °C and relative humidity up to 90%. Therefore, the annual yield loss is up to 50% due to the blast disease (Jadhao *et al*., 2020) [3]. In India, finger millet is majorily grown and consumed in Karnataka, Andhra Pradesh, Tamil Nadu, Odisha, Maharashtra. In Tamil Nadu, finger millet is majorily growing in Dharmapuri, Krishnagiri, Vellore, Salem, Thiruvannamalai districts (Kumar *et al*., 2020). In this study, we have studied and observed the major finger millet growing areas and blast disease severity in Tamil Nadu districts.

## 2. Materials and Methods
### 2.1 Collection of blast infected plant samples
The survey was conducted during kharif season in different ragi growing regions of Tamil Nadu and also assessed the percent disease incidence. The freshly collected leaf blast samples were kept in clean polythene bag and stored under refrigerator at 4 °C for further use of isolation of pathogen.

### 2.2 Isolation of pathogen
Leaf blast samples are cut into small pieces and surface sterilized with 0.1 per cent mercuric chloride solution after wash with sterile distilled water twice and transferred to petri plates containing Oat Meal Agar Medium (OMA). After 4 days for obtaining monoconidial isolate, a dilute spore suspension was prepared in sterilized distilled water and plated onto 0.8% water agar in Petri plates. After 15 days of incubation at 26 ± 1 $^0$C, single germinating conidium was marked under a microscope and transferred to fresh Petri dish containing OMA medium and then the plates were incubated at 26 ± 1 $^0$C for 10 days to get monoconidial isoloates (Ou, 1985).

### 2.3 Pathogenicity test

#### 2.3.1 Preparation of spore suspension and inoculation

Five to ten discs of the blast pathogen were transferred to conical flasks containing sterilized *Paspalum distichum* weed stem bits. After five to ten days, the spore suspension was prepared by adding sterile distilled water to the conical flasks containing blast culture and this was mixed well and filtered through muslin cloth. The spores were collected in conical flasks and adjusted the concentration at 1 X $10^6$ conidia / ml and tween 20 was added at 0.2 ml / litre. Then the suspension was sprayed onto the 15 days old crop. These plants were tightly covered by polyethylene sheet and sprinkled with water to maintain high humidity for rapid infection by the pathogen. For each isolates three replication was maintained. The symptom development was observed after seven days and graded on a 1-9 scale based on lesion size. The pathogen was re-isolated for confirmation of their fungal characters to prove the Koch's postulate for each isolate. (Radjacommare *et al*., 2004) [8]. Percent disease incidence was calculated based on the formula given below,

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves graded}} \times \frac{100}{\text{Maximum grade}}$$

### 2.4 Molecular characterization of *M. grisea*

#### 2.4.1 Isolation of total genomic DNA isolation of *M. grisea*

Total genomic DNA was isolated from *M. grisea* as described by Anjum *et al*., (2016) [1]. Finally, the isolated DNA was re-suspended in 50 µl of distilled water or 1X TE buffer and stored at -20°C for further use. To verify the quality of isolated DNA, 2.5µl of total DNA solution was resolved in the 1% agarose gel electrophoresis.

#### 2.4.2 Sequencing of ITS region of the rDNA

A PCR was performed in a total volume of 50 µl using Emerald Amp® GT PCR master mix using genomic DNA of *M. grisea* as a template. The intermediate 5.8S ribosomal gene along with ITS1 and ITS2 regions were amplified using the primers ITS1 and ITS4. Initial denaturation was for 4 minutes at 94 °C, followed by 35 cycles of 1 minute at 94 °C, 1 minute at 55 °C and 1 minute at 72° C with a final 7 mins extension at 72° C. The PCR products were resolved by electrophoresis in 1% agarose gel and products were sequenced at Barcode Bioscience, Bangalore.

**The Primers used for amplification of ITS region were**

ITS1 - 5′ TCCGTAGGTGAACCTGCGG 3′ (forward primer)
ITS4 - 5′ TCCTCCGCTTATTGATATGC 3′ (reverse primer)

#### 2.4.3 Sequencing of ITS and identification of *M. grisea* by bioinformatics analysis

The obtained DNA sequences were trimmed at 5'and 3' region where the sequencing chromatogram is not clear. Then DNA sequence, in which clear chromatogram obtained was made in Fasta format. This was used as input sequence (Query sequence) in nucleotide blast analysis program at NCBI database. The output data retrieved from the bioinformatics were analysed and the organism showing major score was considered as the closely related species to the test fungus used in the study.

### 2.5 Statistical analysis

The data were statistically analysed using the SPSS (Statistical Package for the Social Sciences) version 16.0. (Gomez and Gomez, 1984) [2]. Data were subjected to analysis of variance (ANOVA) at significant levels ($P< 0.05$) and means were compared by Duncan's Multiple Range Test (DMRT).

## 3. Results and Discussion

### 3.1 Collection and isolation of blast disease in ragi

Survey conducted in major finger millet growing areas of Tamil Nadu and collected totally 10 leaf blast infected samples from 5 districts. Leaf blast disease is severely affected in Dharmapuri and Tiruvannamalai districts and evaluated their percent disease incidence (Table 1). The diseased samples were isolated in Oat Meal Agar medium and analyzed the cultural and morphological characters for different isolates of pathogen (Table 2). Shanmugapackiam *et al*., (2019) [9] reported that 24 blast infected samples were collected from major finger millet growing areas of Tamil Nadu. The pathogen was isolated from blast infected leaf portion and then the pathogen was purified based on single spore technique. The culture was maintained in oat meal agar medium under refrigerator condition for further use.

### 3.2 Pathogenicity test

The finger millet variety Paiyur 2 was moderately susceptible for *M. grisea* and it was suitable for pathogenicity test in pot culture conditions. Fifteen days old seedlings were used for pathogenicity test. The pathogen was mass multiplied in weed stem bits. The conidia were harvested in weed stem bits by using sterile water and mix with adjuvant tween 20. The spore suspension was sprayed on seedling by using hand atomizer and covered with polythene bag for maintained the temperature and humidity. The symptoms were observed one week after spraying. Among the tested isolates, TNFGM 4 incited severe disease incidence at 64%
followed by TNFGM 7 56% (Table 3). The pathogen was re-isolated and its characters were studied and compared with original culture. Thus, *M. grisea* isolate TNFGM 4 were used in the further studies.

### 3.3 Identification of *M. grisea* by molecular technique

It was reported that the finger millet blast disease has been caused by *Magnaporthe grisea* (Prakash *et al*., 2019) [7]. Thus, the first and the foremost step in this study was the identification of the *Magnaporthe* pathogen at species level.
Initially, the virulent isolate of *M. grisea* isolate TNFGM4 was observed macroscopically and microscopically. Though, *M. grisea* was confirmed by morphological and cultural characters at genus level. In the present study, its identity at species level needs to be confirmed by molecular technique. ITS sequence analysis is one of the commonly used molecular methods for the identification of fungi at species level.
DNA from *Magnaporthe* spp. were isolated using CTAB method. Single band of intact genomic DNA was visualised on the agarose gel. ITS region of *M. grisea* isolate TNFGM4 was amplified with primers ITS 1 and ITS 4 using a thermo cycler and the products produced were visualised as a single band in agarose gel strained with ethidium bromide. The size of the PCR fragments was approximately 560 bp length (Fig 1).

**Table 1:** Geographical details of Finger millet blast isolates collected from different region of Tamil Nadu

| Isolate no | District | Place of collection | Latitude | Longitude |
|---|---|---|---|---|
| TNFGM 1 | Dindigul | Puthupatti | 10.15° N | 78.15°E |
| TNFGM 2 | Krishnagiri | Kuttapatti | 12.41°N | 78.22°E |
| TNFGM 3 | Krishnagiri | Malaiyandhalli | 12.43°N | 78.24°E |
| TNFGM 4 | Dharmapuri | R. Gopinathanpatti | 12.07°N | 78.20°E |
| TNFGM 5 | Dharmapuri | Chinnaperamanoor | 12.13°N | 77.97°E |
| TNFGM 6 | Coimbatore | TNAU | 11.01°N | 76.92°E |
| TNFGM 7 | Salem | Kuruchi | 11.73°N | 78.43°E |
| TNFGM 8 | Salem | Kankattiala | 11.82°N | 78.41°E |
| TNFGM 9 | Salem | Aladipatti | 11.80°N | 78.37°E |
| TNFGM 10 | Salem | Pudupalayam | 11.64°N | 77.90°E |

**Table 2:** Morphological characteristics of the isolates of *M. grisea*

| S. No | Isolate code | Colour on PDA media | Colour of vegetative growth | Colony Texture |
|---|---|---|---|---|
| 1 | TNFGM1 | Black | Greyish brown | Smooth surface |
| 2 | TNFGM2 | Black | Greyish brown | Smooth surface |
| 3 | TNFGM3 | Black | Slight greyish brown | Rough surface |
| 4 | TNFGM4 | Brown | Greyish white | Rough surface |
| 5 | TNFGM5 | Brown | Greyish white | Rough surface |
| 6 | TNFGM6 | Black | Slight greyish brown | Smooth surface |
| 7 | TNFGM7 | Brown | Greyish white | Rough surface |
| 8 | TNFGM8 | Black | Blackish white | Rough surface |
| 9 | TNFGM9 | Black | Slight greyish white | Rough surface |
| 1 | TNFGM10 | Brown | Greyish white | Rough surface |

**Table 3:** Pathogenicity potential of *M. grisea* isolates

| S. No | Isolate code | Per cent Disease Index* % |
|---|---|---|
| 1 | TNFGM1 | 34.33$^{e}$ |
| 2 | TNFGM2 | 25.00$^{g}$ |
| 3 | TNFGM3 | 29.66$^{f}$ |
| 4 | TNFGM4 | 64.00$^{a}$ |
| 5 | TNFGM5 | 50.00$^{d}$ |
| 6 | TNFGM6 | 30.66$^{f}$ |
| 7 | TNFGM7 | 56.00$^{b}$ |
| 8 | TNFGM8 | 52.33$^{cd}$ |
| 9 | TNFGM9 | 55.00$^{bc}$ |
| 10 | TNFGM10 | 49.66$^{d}$ |

*Mean of six replications
The treatment means are compared using Duncan Multiple Range Test (DMRT)
In a column, mean values followed by a common letter (s) are not significantly different (P=0.05)

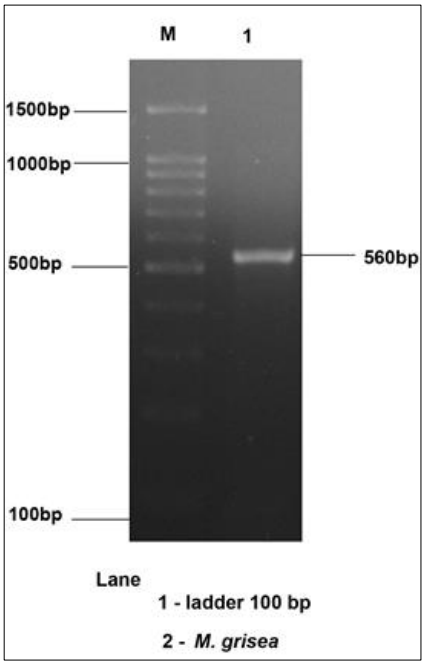


**Fig 1:** Identification of *M. grisea* by molecular technique

## 4. Conclusion

The present study was under taken to find out the leaf blast severity in finger millet crop growing areas in Tamil Nadu. To identified the virulent isolate and the fungus was confirmed by molecular characterization of leaf blast disease by using ITS universal primers.

## 5. Acknowledgement

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