

Analysis of genetic diversity and population structure of *Magnaporthe grisea*, the causal agent of foxtail millet blast using microsatellites

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Abstract

Foxtail millet blast caused by *Magnaporthe grisea* is becoming a severe problem of foxtail millet farming in all over-regions of India. Information on the genetic diversity and population structure of foxtail millet infecting *M. grisea* is essential for developing effective management strategies, including the breeding of blast-resistant cultivars. We analyzed thirty-two *M. grisea* isolates from ten foxtail millet-growing districts in Tamil Nadu, India for genetic diversity using twenty-nine microsatellite or Simple Sequence Repeat (SSR) markers. ~~analysed thirty-two *M. grisea* isolates from ten foxtail millet growing districts in Tamil Nadu, India for genetic diversity using twenty-nine microsatellite or Simple Sequence Repeat (SSR) markers in this study. A total of 103~~In total, 103 alleles were identified, with a mean of 3.55 alleles/locus. Gene diversity ranged from 0.170 to 0.717, while major allelic frequencies ranged from 0.344 to 0.906. The polymorphism information content (PIC) value was identified as 0.155 to 0.680, with an average value of 0.465. Population structure analysis of genomic data sets revealed two major populations (SP1 and SP2) with different levels of ancestral admixture among the 32 blast isolates. The phylogenetic analysis classified the isolates into three major clusters. Among the three clusters, cluster one recorded 14 isolates, cluster two consisted of 16 isolates, and cluster three had 2 isolates. Analysis of molecular variance (AMOVA) showed high genetic variation among individuals and less among populations. Principal coordinate analysis (PCoA) showed a 27.16% genetic variation among populations. The present study provides the first report on the genetic diversity and population structure of the foxtail millet infecting *M. grisea* population in Tamil Nadu, which could be useful in developing blast-resistant foxtail millet cultivars.

Keywords: Foxtail millet, diversity, blast, population structure, microsatellites, phylogenetics

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1. INTRODUCTION

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is one of the most important climate-resistant nutri-cereals plants that comprise a vast array of nutritional properties such as protein, starch, fiber, minerals, and antioxidants. It is widely cultivated around the world (Sharma & Niranjana, 2018). The cultivation and consumption of small millet are increasing in ~~India~~India. The agro-industry is now focusing ~~to increase the productivity of small millet~~on increasing the small millet productivity by implementing appropriate management strategies against yield-limiting factors (Sharma & Niranjana, 2018).

The ascomycetous fungus *Magnaporthe grisea* (T.T. Herbert) M.E. Barr (anamorph: *Pyricularia grisea* (Cooke.) Sacc.) causes blast disease in various cereals and millets worldwide (Noman et al., 2022; Aravind et al., 2022). The disease has become a significant obstacle in cultivating foxtail millet, particularly in Karnataka, Tamil Nadu, Andhra Pradesh, Rajasthan, Madhya Pradesh and Chhattisgarh in India (Sharma et al., 2014). The pathogen has a hemibiotrophic and heterothallic lifestyle (Koeck et al., 2011), which affects different parts of the plant, including leaves, leaf sheaths, nodes, necks, heads or panicles at all stages of plant growth (Wilson & Talbot, 2009). Initially, the disease manifests itself as a small water-soaked lesion, after which the spots turn dark green with a central light green to greyish area. Fully developed spots have a brown to dark brown margin with a white to grey center. During the life cycle, the pathogen produces thin-walled, three-celled, pear-shaped conidia with a protruding hilum at the tips of the conidiophores, which cause and spread disease from one plant to another (Klaubauf et al., 2014).

~~It is known that Blast~~blast pathogens develop resistance to fungicides (Suzuki et al., 2010). Therefore, the best way to control this disease is to breed resistant cultivars. However, the pathogen can rapidly evolve ~~new pathogenic races which to~~ overcome the ~~host~~plant resistance ~~genes~~as different strains and pathotypes are present in the *Magnaporthe* populations in the field (Sharma et al., 2014; Kim et al., 2019). Therefore, it is necessary to analyze the genetic diversity among the *Magnaporthe* populations in a particular region to understand the mechanism of the breakdown of resistance in blast-resistant cultivars (Rieux et al. 2011).

Previous studies have demonstrated genetic diversity among blast pathogens in ~~a variety of food~~ crops, including rice (Aravind et al., 2022), wheat (Noman et al., 2022), finger millet (Takan et al., 2012), and pearl millet (Sharma et al., 2021). However, no studies have been conducted on the genetic structure of foxtail millet infecting *Magnaporthe* populations in India. Although the

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pathogen is variable, it is very host-specific ~~in relation to~~ foxtail millet (Sharma et al., 2014). Improving our understanding of the genetic diversity within and among populations of blast pathogens at the field level is critical to formulating effective management strategies, including the development of blast-blast-resistant cultivars of foxtail millet. ~~Among the various tools and methods used. To determinestudy~~ the genetic diversity of plant pathogens, microsatellites or simple sequence repeats (SSRs) are ~~the most~~ widely used marker system ~~to study the genetic diversity or evolutionary aspects of organisms~~ (Rieux et al., 2011; Li et al., 2021). The microsatellites or SSRs are tandemly repeated DNA sequences present throughout the eukaryotic genome. ~~In addition, SSR markers have several properties superior to other marker systems, which include being relatively~~ Compared to other marker systems, SSRs are inexpensive (Schoebel et al., 2013), highly polymorphic, multi-allelic, ~~highly~~ reproducible, and user-friendly (Nowicki et al., 2022). Several SSR markers (Kaye et al., 2003) and minisatellite markers (Li et al., 2007) are developed already for *M. grisea*. We aimed to analyze the extent of genetic variability and population structure in field populations of foxtail millet infecting *M. grisea* in Tamil Nadu using SSR markers. ~~that we discussed in this study. This is the first report of its kind up to our knowledge on the genetic structure of foxtail millet infecting M. grisea.~~

2. MATERIALS AND METHODS

2.1. Survey, collection, and isolation of *M. grisea*

A detailed roving survey was conducted in major foxtail millet growing areas (hills and plains) in Tamil Nadu, India during the rainy season between 2017 and 2018 ~~to assess the severity of blast disease~~. Blast severity was measured on a 1-9 scale (Sharma et al., 2014) and expressed as a Percent Disease Index (PDI) using the formula $PDI = (\text{sum of individual ratings/no. of leaves assessed} \times \text{maximum disease grade value}) \times 100$ (Wheeler, 1969; Amoghavarsha et al., 2022). Plant samples (leaves and leaf sheaths) infected with the blast were collected from the farmers' fields. Collected samples were air-air-dried, bagged, labelled, and stored under refrigerated conditions at 4°C for the isolation of pathogens. The pathogen was isolated using the standard tissue isolation procedure (Tuite, 1969) with Potato Dextrose Agar (PDA) medium under aseptic conditions at 25±2°C. Mycelium growing from infected plant tissue was identified based on its morphological and cultural characteristics. The fungus was then purified by a single spore isolation technique (Ricker & Ricker, 1936) and the purified isolates were stored on PDA slants for further study.

2.2. DNA extraction

Each isolate was inoculated into a 100 ml Erlenmeyer flask containing 20 ml of potato dextrose broth and incubated at 25 ± 2°C. After 7 days of incubation, the mycelia were harvested and immediately ground in liquid nitrogen to a fine powder for DNA extraction with CTAB buffer, following the protocol described by Murray & Thompson (1980) with minor modifications (Jagadeesh et al., 2018) using equal volumes of supernatant and a mixture of phenol-chloroform-isoamyl alcohol to precipitate and isolate the DNA from other impurities. The DNA was verified quantitatively and qualitatively using a Nano-Drop Spectrophotometer (Thermo scientific, City, State) and then diluted to a working concentration of 50 ng/μl and stored at -20°C for later use.

2.3. PCR assays

2.3.1. ITS rDNA amplification

All the isolates were subjected to PCR amplification using a pair of universal primers, ITS1 and ITS4 (White et al., 1990). Primer sequences were synthesized by Eurofins Genomics India (Bangalore, India). The PCR was performed in 20 μl reaction volume with 2.0 μl template DNA (50 ng/μl), 10.0 μl master mix, 2.0 μl forward primer, 2.0 μl reverse primer, and 4.0 μl sterile double-distilled water. The reaction mixture was centrifuged briefly to thoroughly mix the

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components of the cocktail. Amplification was performed in a thermal cycler (C1000 Touch™ Thermal Cycler, Bio-Rad Laboratories, Inc., Singapore). Thermal cycling conditions were achieved consisting of 35 cycles, initial denaturation at 94°C for 5 min, denaturation at 94°C for 1min, annealing at 55°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 5 min. The PCR products were subjected to agarose (1%) gel electrophoresis in 0.5X Tris-Borate-EDTA buffer at 110V. A 100 bp DNA molecular ladder (MEDOX Biotech, Chennai, India) was used to estimate the size of the amplicon. After electrophoretic separation, the gel was read under the gel documentation system (Bio-Bio-Rad, USA) for a more detailed analysis.

2.3.2. Microsatellite genotyping

~~A total of twenty-nine~~ Twenty-nine microsatellite loci (SSRs) distributed across seven *M. grisea* chromosomes were used ~~in this study~~ (Table S1) to analyze genetic diversity and population structure as described by Kaye et al. (2003) and Adreit et al. (2007). These primer sequences were synthesized at Eurofins Genomics India (Bangalore, India). PCR amplification was performed in 20 µl of reaction mix in a 0.5 microcentrifuge tube using 10 ng of template DNA, 0.2 mM of dNTPs, 0.4 µM of primers, 1.5 mM MgCl₂, 1X Taq buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3) and 1U Taq DNA polymerase (DreamTaq, Thermo Scientific, USA). Conditions for PCR were initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 45 s, and extension at 72°C for 45 s, with the final extension for 10 min at 72°C. For marker scoring, the PCR products were separated by electrophoresis on a 2.5% agarose gel stained with ethidium bromide. After electrophoretic separation, the gels were ~~analysed-analyzed~~ under the gel documentation system (Biorad, City, State, USA). All PCR reactions for each primer were repeated at least twice to confirm the data scored.

2.4. Bioinformatics and computational and statistical analyses

The percent disease index of foxtail millet blast and observations on the conidial morphology of *M. grisea* were subjected to analyses of variance (ANOVA). The averages were compared by the Tukey test ($p < 0.05$) in the Statistical Package for the Social Sciences (SPSS), version 21.0.

The amplified PCR products were sequenced at M/S Eurofins Genomics Bangalore, India for ~~double-pass~~ double-pass DNA sequencing using the universal primers (ITS1 and ITS4) mentioned above. The DNA sequences ~~obtained in this study~~ were subjected to phylogenetic analysis. A neighbor-joining tree (Saitou & Nei, 1987) was constructed with MEGA v 6.1 software

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to study divergence patterns, and a 1000-repetition bootstrap analysis was performed to support nodes in clusters (Tamura et al., 2011).

Basic computational and statistical analyses such as polymorphic information content (PIC), gene diversity, major allele frequency, and heterozygosity were analyzed using Power Marker version 3.25 (Liu & Muse, 2005). Pairwise F-statistics, Nei genetic distance, analysis of molecular variance (AMOVA), and principal coordinate analysis (PCoA) were performed using the package, namely GenAlEx v. 6.502 (Peakall & Smouse, 2006). PIC values measure the significance of a given DNA marker. The PIC value for each SSR locus was measured as described by Anderson et al. (1993). An unweighted neighbor-joining tree was constructed based on the simple matching dissimilarity matrix of SSR markers genotyped across the *M. grisea* isolates as implemented in the DARwin v. 5.0.157 program (Perrier & Jacquemoud-Collet, 2006). Population structure between *M. grisea* isolates was analyzed with the software package STRUCTURE (Pritchard et al., 2000) in the revised version 2.3.4. This approach makes use of multilocus genotypes to infer the fraction of an isolate's genetic ancestry that belongs to a population for a given number of populations (K). The optimum number of populations (K) was selected after five independent runs of a burn-in of 50,000 iterations followed by 50,000 iterations for each value of K (testing from K = 2 to K = 10). The program STRUCTURE HARVESTER was used to determine the peak value of delta K according to the method described by Evanno et al. (2005).

3. RESULTS

3.1. Survey and assessment of blast severity in foxtail millet

The prevalence of foxtail millet leaf blast was noticed in all surveyed areas (plains and hills) in Tamil Nadu. Interestingly, the occurrence of sheath blast_s was noticed only in the Kunnur village, Salem District. The severity of leaf blast disease ~~were~~ ranged from 10.34 to 72.19 PDI. The highest incidence was observed in cv. CO(Te)7 (72.19 PDI) in Athiyandal village, Tiruvannamalai District, followed by a local variety from a farmer's field in Kunnur village, Salem District, which had a PDI of 70.16. The minimum PDI (10.34) was observed in the cv. CO(Te)5 in Madurai. Sampling locations were marked on the map of Tamil Nadu using ARC GIS software, and the PDI or blast severity was given in three different ~~colours~~ according to its range. The green ~~colour~~ represents a lesser incidence of the disease in the Salem, Madurai, and Virudhunagar districts. The yellow ~~colour~~ indicates a moderate disease ~~indidence-incidence~~ in the Thoothukudi, Dindigul, Erode, Salem, Namakkal, and Dharmapuri districts. The highest incidence of the disease was recorded in Vellore, Tiruvannamalai, Dindigul, and Virudhunagar districts and is marked in red (Figure 1; Table 1). At the time of the survey, the leaf blast was observed by the type of lesion, which was characterized by a spindle-shaped lesion with a dark brown border with a grey center, and the sheath blast symptom was observed as a spindle-shaped lesion with a dark brown border with a white center on the sheath (Figure 2a,b).

3.2. Morphological characterization

~~In all, A total of~~ 32 isolates of *M. grisea* were isolated from the diseased samples (31 from leaf and 1 from sheath) and designated as TNFxm1 to TNFxm32 (Table 1). All isolates were classified based on morphological and conidial characteristics variations, namely colony ~~colour~~, surface appearance, size, shape, and ~~colour~~ of the conidia. Colony ~~colour~~ varied as greyish brown (15), slightly greyish brown (3), creamish white (1), white (4), greyish white (4), blackish white (3), and greyish black (2). Similarly, most of the isolates were smooth (19) and some were rough (13) in colony appearance (Figure S1; Table 2). In all the isolates, the shape of the conidia was typically pyriform with a rounded base, narrow apex, 2 septa, 3 ~~celled cells~~, and the middle cells were broader than the adjacent cells in all the isolates,. Some of them were very long and narrow, while others were quite wide. The length and width of the conidia of different isolates of *M. grisea* were measured (Figure S2; Table 2). The size of the conidia ranged from 20.06 µm to 37.76 µm in length and 6.88 µm to 11.93 µm in width. All isolates varied significantly in terms of conidial

size. The blast isolates TNFxm7 and TNFxm20 had the longest conidia of 37.76 µm and 33.83 µm, respectively. The shortest conidial length was observed in isolates TNFxm23 (20.06 µm) and TNFxm6 (21.48 µm). The highest conidial width was observed in isolates TNFxm26 and TNFxm27 at 11.93 µm and 10.77 µm, respectively.

3.3. ITS-DNA barcoding and phylogenetic analysis

ITS-based primers amplified the expected amplicon size of 560 bp (Figure S3). All the sequences obtained from this study by partial gene sequencing showed more than 99% sequence similarity to GenBank sequences (*M. grisea*) deposited with the NCBI. Accession numbers obtained from the NCBI GenBank for each isolate are listed in Table 1.

The phylogenetic analysis revealed that all 32 *M. grisea* isolates were divided into two groups (Group I and Group II). The two isolates (MN017168 and MN028778) from Virudhunagar District were only clustered separately and formed group I. Group II consists of thirty isolates with a further subdivision into subgroup I and subgroup II. Subgroup I was formed with eight isolates from Tiruvanamalai (MN017167), Salem (MW534814, MW535244, MW504790, MW535295, MW535961 and MW496127) and Namakkal (MW497613) districts. The remaining twenty-two isolates from Dindigul (MT053476, MK990557, MN028779, and MN017169), Thoothukudi (MN028776), Madurai (MT043762 and MT053462), Dharmapuri (MT043764), Vellore (MT043805), Erode (MW497608 and MW534750), Salem (MW534813, MW534870, MW535067, MW535174, MW494605, MW498280, MW504997, MW504990, and MW494316) and Namakkal (MW494589 and MW535771) districts were grouped altogether and formed subgroup II (Figure 3).

3.4. SSR genotyping and cluster analysis

All 32 isolates were genotyped with 29 SSR markers distributed throughout the *M. grisea* genome. A total of 103 alleles were detected among the 29 SSR markers, ranging from 2 (Pyrms 67-68, Pyrms 107-108, and Pyrms 533-534) to 6 (Pyrms 607-608) alleles with an average of 3.55 alleles per locus. The major allele frequency ranged from 0.344 (Pyrms 83-84) to 0.906 (Pyrms 533-534), with a mean of 0.605. Genetic diversity ranged from 0.170 (Pyrms 533-534) to 0.717 (Pyrms 657-658) with a mean value of 0.517. The polymorphism information content (PIC) of the markers had an average value of 0.465 and varied from 0.155 to 0.680. The maximum value of PIC was observed for marker Pyrms 37-38, while the minimum value was observed for marker Pyrms 533-534. The observed mean heterozygosity was 0.007517 with a range of 0.00 to 0.094.

Among the markers used, only 5 (Pyrms 37-38, Pyrms 39-40, Pyrms 99-100, Pyrms 427-428, and Pyrms 453-454) showed a heterozygosity value greater than zero, while 24 had zero values (Table 3). The unrooted unweighted neighbour Joining method was used to estimate the genetic distance and dissimilarity index of 32 *M. grisea* isolates. For the unrooted tree, isolates were classified into three major clusters (Figure 4). Among the three clusters, cluster one (red colour) recorded 14 isolates, while cluster 2 (blue colour) consisted of 16 isolates, and cluster 3 (green colour) had 2 isolates.

3.5. Population structure analysis

For genetic structure estimation, a Bayesian clustering approach was followed by taking probable subpopulations (K), and a higher delta K value using STRUCTURE 2.3.6 software. The maximum plateau of *adhoc* measurement of ΔK was K=2 with a ΔK value of 219.8 (Table S2). The population structure was analyzed for genetic relatedness among 32 *M. grisea* isolates collected from ten different districts of Tamil Nadu, India. Based on an ancestry threshold of >70%, all 32 isolates were classified into two major populations and considered pure, while <70% were considered admixture (AD). Among the 32 isolates, 13 isolates collected from Salem and Namakkal districts were classified as subpopulation 1 (SP1), and 16 isolates collected from Tiruvanamalai, Dindigul, Virudhunagar, Thoothukudi, Madurai, Dharmapuri, Vellore, Madurai, Erode and Salem districts were classified as subpopulation 2 (SP2), while 3 isolates (TNFxm6, TNFxm17, and TNFxm18) were of admixture type with a major genetic component of two subgroups (Table 4). The population shows admixture, suggesting that there is a gene flow between these populations. The fixation index (F_{st}) values of the two populations were 0.4296 for SP1 and 0.2021 for SP2. The highest divergence in allele frequency between populations was observed in SP1 and SP2 (0.1077) based on the net nucleotide distance calculated using point estimates of P. The mean distance (expected heterozygosity) between individuals in the panel population was 0.2036 and 0.2800 in response to SP1 and SP2, respectively (Figure 5a,b; Figure S4a-c).

3.6. Analysis of molecular variance (AMOVA)

The two populations, i.e., subpopulation 1 (13), and subpopulation 2 (16), along with the admixtures (3) generated from structure analysis, were analyzed to determine genetic variation among and within populations. In the AMOVA analysis, maximum variation (79%) was found among individuals, while minimum variation (20%) was noticed between the populations and

261 within individuals (1%) (Figure 6; Table S3). The deviation from Hardy-Weinberg's prediction
262 was performed using Wright's F-statistics. The F_{is} and F_{it} values for all 29 loci were found to be
263 0.984 and 0.987 ($r = <0.001$), respectively, while F_{st} between populations was 0.202. The NM
264 value of assumed subpopulations was noted as 0.989. The highest pairwise genetic Nei distance
265 was observed between SP1 and AD (0.404), followed by SP1 and SP2 (0.362) and SP2 and AD
266 (0.307). Furthermore, the biplot generated from the principal coordinate analysis (PCoA) showed
267 that the first two components account for 100% of the variations (Figure 7a,b). The scatterplot was
268 developed from the PCoA analysis and displayed across isolates on the first two axes. The PCoA
269 1 biplot captured 18.03% of the variation, while PCoA 2 contributed 9.13% to the total genetic
270 variation, suggesting that the total genetic variation among the populations was 27.16% (Table
271 S4a, S4b).

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4. DISCUSSION

Foxtail millet is grown in both the plain and hilly regions of Tamil Nadu in India. The blast disease caused by *M. grisea* (Sharma et al., 2014), ~~causing~~ caused a vast yield loss. We examined the morphological and genetic variability of 32 *M. grisea* isolates using 29 microsatellite markers (Kaye et al., 2003; Adreit et al., 2007) and universal ITS primers (ITS1 and ITS4) to characterize the genetic diversity and population structure of foxtail millet infecting *M. grisea*. We demonstrated the genetic analyses of foxtail millet blast populations, which can be used to develop blast-resistant foxtail millet cultivars.

In the present study, the severity of the blast was assessed in various foxtail millet growing areas in Tamil Nadu during the rainy season between 2017 and 2018. The highest incidence of the disease was observed in the Vellore, Tiruvannamalai, Dharmapuri, and Dindigul districts of Tamil Nadu. Similarly, Sharma et al. (2014) also noticed the occurrence of blast incidence during the rainy season in 2008 in different foxtail millet growing tracts in Telangana and Andhra Pradesh. We isolated the causative agent of foxtail millet blast from the infected leaf and sheath samples collected at surveyed fields using a PDA medium and purified by the single spore isolation technique. Other reports demonstrating that a similar single-spore isolation technique was used to isolate and purify *Magnaporthe* from rice, wheat, finger millet, and foxtail millet using different media (Sharma et al., 2014; Yadav et al., 2019) were discussed and corroborated ~~to~~ by our study.

The present study demonstrates a variation in mycelium colour and texture variation among the 32 fungal isolates collected in Tamil Nadu. This confirms previous reports by Panda et al. (2017) and Sahu et al. (2018), who classified the rice blast isolates collected from different parts of Chhattisgarh and Odisha in India into different groups based on colony colour and texture. The present study shows the variation among the 32 isolates of *M. grisea* in terms of morphological and conidial characteristics. However, there is no significant difference between the colony morphology and conidial characters concerning the collection period and geographical distribution of the isolates.

Sequencing of the ITS region of rDNA has become the universal barcode system for identifying fungal taxonomy at the species level (Paloi et al., 2022). In the present study, all *M. grisea* isolates were amplified using universal primers (ITS1 and ITS4) and the result showed the expected 560 bp amplicon in all isolates. The isolates showed a sequence homology of >99% with *M. grisea*. The results of the present study are consistent with Jagadeesh et al. (2018) who

examined the diversity of 72 isolates of *M. oryzae* collected in Karnataka, India using primers ITS1 and ITS4.

Microsatellites or SSRs are powerful markers used for the population genetic analyses due to their high specificity, polymorphism, and reproducibility (Schoebel et al., 2013; Nowicki et al., 2022). We evaluated 29 SSR markers reported by Kaye et al. (2003) and Adreit et al. (2007) to analyze genetic diversity in the population of foxtail millet infecting *M. grisea* from Tamil Nadu. To the best of our knowledge, this is the first study to investigate the molecular diversity and population structure of 32 isolates of foxtail millet infecting *M. grisea* from Tamil Nadu using microsatellites. The SSR markers used in this study varied widely and the results were generally consistent with previous population genetic studies conducted in India and elsewhere (Yadav et al., 2019). In the present study, 103 alleles were detected using 29 microsatellite markers. The number of alleles per locus ranged from 2 to 6, averaging 3.55 alleles/locus. A variation in the number of alleles was observed between the markers used. Previous studies reported similar results in rice (Aravind et al., 2022), wheat (Noman et al., 2022), finger millet (Takan et al., 2012), and pearl millet (Sharma et al., 2021) infecting populations of *Magnaporthe* in different geographic locations. Some previous studies showed that the high number of alleles per locus resulted in high genetic variation. The high genetic diversity was demonstrated in the present study, confirming previous results of Saleh et al. (2014), while Yadav et al. (2019) reported a low level of genetic diversity among the *M. oryzae* isolates collected in North Eastern India.

Similarly, high levels of polymorphism were found among the 29 microsatellites used in this study. The results agree with Wang et al. (2017), where a high degree of polymorphism was observed when analyzing 457 rice blast isolates collected in the United States using ten SSR markers. Recently, Adhikari et al. (2020) also observed high levels of polymorphism when analyzing 17 pearl millet blast isolates collected in India using SSR markers. However, the a low level of polymorphism has been observed in the rice blast population in Brazil and Japan (Prabhu et al., 2002). Analysis of genetic diversity showed the a high percentage of identical alleles, indicating considerable gene flow between all isolates of *M. grisea* population in Tamil Nadu.

As most foxtail millet cultivation in Tamil Nadu is dominated by a single cultivar CO(Te)7 and no resistant cultivar is grown yet, so there is no selection pressure on the pathogen to induce change. Hence this could be why there is a high percentage of identical alleles between populations of *M. grisea*. The present study undoubtedly shows an excellent diversity of *M. grisea* in the hilly

region and- that most of the variability was within individuals. Our results agree with those of Wang et al. (2017), who analyzed 457 rice blast isolates using the ~~neighbour~~-neighbor-joining method to build an unrooted tree. A total of five major clusters were identified. The observed clusters were mainly related to the sampling period but not to the geographic location of the isolates.

A Bayesian clustering approach was used to analyze the structure of the foxtail millet blast isolates. The structure analysis divided the *M. grisea* isolates into two subpopulations (K=2) with three admixtures. The first subpopulation (SP1) consisted of isolates from the Salem and Namakkal districts of Tamil Nadu. Similarly, the second subpopulation (SP2) consisted of Tiruvannamalai, Madurai, Dindigul, Erode, Virudhunagar, Tuticorin, Dharmapuri, Vellore, and Salem districts in Tamil Nadu. Admixtures (AD) present in isolates can increase genetic diversity. However, the structure analysis could not differentiate the blast isolates based on the geographic location. Similarly, Yadav et al. (2019) identified two subpopulations of leaf and neck blast isolates infecting rice crops and showed limited categorization of blast isolates based on the location. Interestingly, Onaga et al. (2015) categorized the 88 rice blast isolates collected from East Africa into five ancestral genetic clusters (K=5) with Structure V 2.3. Package. Wang et al. (2017) analyzed 457 blast isolates using ten polymorphic microsatellites. They identified 6 genetic clusters (K=6) based on collection period but not geographic location. However, a PCoA analysis was performed from the results obtained from interpreting the structure. The AMOVA analysis was worked out by dividing the genetic diversity within and between the populations of 32 isolates of foxtail millet blast. Variation between these populations contributed 20% of the genetic diversity, while 79% of the genetic variation was present between the individuals; however, one percent of the variation was found within individuals. A higher genetic diversity was found among the blast isolates than in the population. Identically, analysis of molecular variance values for genetic diversity in the pathogen population varied from 78.66% (Wang et al., 2017) to 86.6% (D'Ávila et al., 2016), 88.09% (Onaga et al., 2015) and 98% (Yadav et al., 2019). Our results are consistent with previous reports in which the pathogen population was not differentiated by geographic location (Onaga et al., 2015).

The genetic variation and population structure of *M. grisea* infecting foxtail millet from different regions of Tamil Nadu shows significant genetic variation within populations, similar to those observed in other population studies of *M. grisea* in India and elsewhere. Our results showed

366 that gene flow occurs between regions and has significant implications for foxtail millet growers
367 when virulent or fungicide-resistant strains move between regions. Preventing such a threat from
368 the foxtail millet blast requires proper prediction and forecasting systems. In addition, long-term
369 monitoring is essential to identify the population origin and evolutionary potential of foxtail millet
370 blast isolates in India.

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378 **DATA AVAILABILITY STATEMENT**

379 The data that support the findings of this study are available from the corresponding author
380 upon reasonable request.

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FIGURE LEGENDS

FIGURE 1. Map showing the collection sites of *Magnaporthe grisea* isolates and foxtail millet blast severity in Tamil Nadu.

FIGURE 2. Foxtail millet blast symptoms. (a) Leaf blast: ~~spindle-spindle~~-shaped lesion having a dark brown margin with a grey coloured center, (b) Sheath blast: ~~spindle-spindle~~-shaped lesion having a dark brown margin with the white-white-coloured center.

FIGURE 3. Phylogenetic analysis. Phylogenetic tree constructed based on multiple alignments of nucleotide sequences of *M. grisea* isolates. The tree was generated using the ~~neighbor~~ ~~neighbor~~-joining method with 1000 bootstrap replications and the tree was drawn at a cut-off value of 60% in MEGA 6.1.

FIGURE 4. Cluster analysis.-Neighbor-joining cluster analysis of 32 blast isolates based on 29 SSR markers using DARwin software.

FIGURE 5. Population structure analysis. (a) Values of DK, with its modal value used to detect the true K of the group (K =2). For each K value, at least three independent runs were considered and averaged over the replicates, (b) Population structure of 32 blast isolates based on 29 markers (K = 2) and graph of estimated membership fraction for K = 2. The maximum of *ad hoc* measure ΔK determined by the structure harvester was found to be K = 2, which indicated that the entire population can be grouped into two subgroups. Different colour within the group indicates the proportion of shared ancestry with another group which-that has the same colour with the admixture.

FIGURE 6. AMOVA analysis of foxtail millet infecting *M. grisea* isolates.

FIGURE 7. Principal coordinate analysis (PCoA). (a) PCoA for Nei genetic diversity among the three populations, (b) PCoA of 32 blast isolates in the panel population using 29 molecular markers. The isolates are coloured ~~on the basis of~~based on sub-populations obtained from structure analysis (SP1- yellow, SP2 - green, AD - red).

534 **SUPPORTING INFORMATION LEGENDS**

535 FIGURE S1. Colony morphology of *M. grisea* isolates. Variability in morphology and cultural

536 characters of *M. grisea* isolates on PDA.

537 FIGURE S2. Conidial characteristics of *M. grisea* isolates. Variation in conidia shapes and sizes

538 among the isolates of *M. grisea* was observed under the image analyzer.

539 FIGURE S3. PCR amplification of *M. grisea* using ITS Primers (ITS1 and ITS4).

540 FIGURE S4. Population structure analysis. (a) Histogram distribution of Fst1, (b) Histogram

541 distribution of Fst2, (c) Histogram distribution of alpha.

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