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RESEARCH ARTICLE

Molecular and pathotype diversity of *Magnaporthe oryzae* isolates in NEH region of India and its implications on rice resistance breeding

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Abstract

Rice blast, caused by filamentous pathogen *Magnaporthe oryzae*, is a major peril to rice production, particularly in the North Eastern Hill (NEH) region of India. The dynamics of 24 *M. oryzae* isolates collected from different geographical locations within the NEH region using 43 SSR markers and pathotyping 17 rice monogenic lines revealed significant genetic diversity. The highest polymorphic information content value of 0.90 was observed for marker mgm285. Five distinct groups were identified with isolate LB 3 collected from upland field in Meghalaya distinct from the rest of the isolates. Significant genetic distinction between isolates from upland and lowland fields, as well as differentiation of tissue infection were observed suggesting the adaptive nature of pathogen and the involvement of specific virulence factors. The highest dissimilarity (0.90) was found between NB 1 and NoB 1 isolates. The virulence frequency varied in the range of 35–76%. The pathotyping of isolates using monogenic lines identified 8 pathotypes, of which pathotype 1 (LB 5 and LB 11) from lowland (Meghalaya) was the most virulent, while pathotype 8 (LB 1) from Nagaland was the least virulent. The data revealed that lowland isolates were more heterogeneous than upland isolates, which had intermediate virulence. In order to prevent future disease outbreaks and to strategically and sustainably breed blast-resistant rice cultivars for NEH region, the profiling *M. oryzae* isolates is needed along with additional research to unravel the mechanism of host-pathogen interaction via inclusive molecular approaches.

Keywords Genetic diversity · Isolates · Pathotyping · Rice blast · SSR markers

Introduction

Magnaporthe oryzae is a virulent phytopathogen responsible for the pernicious rice blast disease, often referred to as “rice cancer” due to its catastrophic ramifications on global rice production. Its widespread occurrence across temperate and tropical regions, spanning all rice-growing ecosystems, illustrates its remarkable adaptability to diverse heterogeneous and fluctuating environmental conditions, alongside its expansive host spectrum (Hosahatti et al. 2021). *M. oryzae* infects over 50 grass species, including key crops such as rice, wheat, barley, rye, finger millet and pearl millet (Devanna et al. 2022). The severity of the disease is primarily influenced by conducive environment, host susceptibility and virulence of the fungal strains involved. Yield losses attributed to this infection are substantial, typically ranging from 10 to 30%, with severe outbreaks causing losses as high as 80–100% (Chuwa et al. 2015; Sakulkoo et al. 2018). The genome of *M. oryzae* harbors numerous repeat and transposable elements, which contribute to genomic variability

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through processes such as gene duplication, chromosomal rearrangement and mutation (Gray 2000; Kito et al. 2002). This genomic flexibility facilitates the rapid evolution of new strains, altering the pathogen's virulence spectrum and driving the continuous emergence of novel pathogenic races capable of interacting with specific host genotypes, ultimately leading to resistance breakdown in certain cultivars (Yoshida et al. 2016). Unravelling the mechanisms underlying the frequent outbreaks, investigation of genotypic and phenotypic diversity within *M. oryzae* populations in specific geographical regions is essential. Pathogenic differences among individual isolates have long served as critical indicators of variation within natural pathogen populations (Palanna et al. 2023). Furthermore, distinct races of *M. oryzae* characterized by differing virulence profiles, have been identified based on their ability to infect specific rice cultivar (Hao et al. 2021).

The utilization of molecular markers in pathogen population genetics has yielded epidemiological insights with a level of precision that was previously unattainable. These markers offer advantages such as environmental stability, high reproducibility, and cost-effectiveness. Over the years, identification of more than 500 quantitative trait loci (QTLs) for blast resistance, with approximately 102 blast resistance (R) genes mapped, 38 of which have been cloned and functionally characterized (Wang et al. 2017; Zhao et al. 2018; Zheng et al. 2016; Zhu et al. 2016). To date, more than 40 avirulence (*Avr*) or effector genes have been mapped in *M. oryzae*, of which 18 have been cloned and characterized (Dong et al. 2015; Ray et al. 2016; Wu et al. 2015; Liu et at. 2024).

Attempts had been made to understand the pathogenic diversity using numerous different types of markers to decipher its evolutionary roots, host-pathogen interaction for strategic disease management. Hamer et al. (1989) discovered the presence of family of dispersed repetitive DNA sequences within the *M. oryzae* genome, which have since become valuable tools for assessing genetic diversity within and between populations of the rice blast pathogen. Subsequently, Levy et al. (1991) explored the relationship between virulence and distinct genetic lineages. With the advent of comparative genomics from different isolates, such as Ina 68 (Yoshida et al. 2009), Guy11 (Soanes et al. 2012), and 98−06 (Dong et al. 2015), it has become evident that genotypic characterization of field isolates is essential to elucidate the molecular basis of virulence, as isolate-specific genomic regions have been identified (Dong et al. 2015; Xue et al. 2012). Therefore, the deployment of blast-resistant cultivars necessitates monitoring race dynamics and avirulence gene composition within *M. oryzae* populations, as it is not practical to track all races simultaneously (Fang et al. 2018). The most effective and economically sustainable strategy for controlling blast disease remains the cultivation of varieties that carry major blast resistance genes (Zhu et al. 2000). However, for this approach to be effective, the cultivars must encounter *M. oryzae* strains harbouring the corresponding avirulence genes. Nevertheless, resistance conferred by major genes may be rapidly overcome due to the evolutionary plasticity of *M. oryzae* population, often leading to the emergence of new virulent races shortly after deployment of resistant cultivars (Huang et al. 2014).

This challenge is particularly acute in the North Eastern Hill (NEH) region, where rice cultivation spans 3.238 million hectares (Laitonjam et al. 2022), and the crop is highly vulnerable to blast disease due to congenial environmental conditions, such as rainfed uplands and relatively low temperatures. Yield losses from leaf and neck blast, ranging between 40% and 47%, are a common occurrence among rice cultivars in this region (Raj et al. 2024). Consequently, there is an urgent need for research aimed at identifying the prevalent blast races through pathotyping and molecular characterization. Thus, this study focused on identifying and characterizing isolates of *M. oryzae* prevalent in the NEH region and subsequently, selecting genotypes resistant to most of the virulent races, if not all. This will be crucial in developing durable resistance breeding strategies leading to increased and stable rice productivity in the region.

Materials and methods

Collection of *M. oryzae* isolates and establishment of single conidial cultures

Samples of infected rice plant parts (leaves, panicle, and nodes) were obtained from different rice ecosystem of NEH Region comprising of upland and lowland rice (Table 1). After collecting the infected samples (Fig. 1a) were kept in filter paper and then brought to the laboratory and kept in the fridge at 4 °C until they were further processed. *M. oryzae* isolates were cultured on an oatmeal agar medium using standard methods after incubating the infected samples in moisture chambers at room temperature for 24 h following established protocol (Hayashi et al. 2009). The cultures were then incubated at 25 ± 1 °C for 10–12 days. Gradually, followed by the establishment of monoconidial culture which was obtained by the preparation of spore suspensions from the above-mentioned cultures. The spore suspensions were prepared in sterilized distilled water with 0.5 ml of the suspension, inoculated on 3% water agar plate and incubated at room temperature for 2–3 days. Using a dissecting microscope, a single conidium was picked with the help of a sterilized needle and inoculated on a plated oatmeal

Table 1 List of *M. oryzae* isolates collected from different geographical regions of North Eastern States

Sl. No	State	Code Name	Details of Sample	Field	No. of locations
1	Nagaland	LB 1	Medziphema	Lowland	1
2	Manipur	LB 2	Lamphel	Upland	2
3	Meghalaya	LB 3	S5 NEHU	Upland	2
4	Meghalaya	LB 4	Ioro Epyo	Upland	2
5	Meghalaya	LB 5	Mynri	Lowland	3
6	Meghalaya	LB 6	IRBL20-IR24	Lowland	3
7	Meghalaya	LB 7	Chakhao Amubi	Lowland	3
8	Meghalaya	LB 8	CAU R1	Lowland	3
9	Meghalaya	LB 9	RCPL116	Lowland	3
10	Meghalaya	LB 10	IRBL Z5CA	Lowland	3
11	Meghalaya	LB 11	IRBLSH B	Lowland	3
12	Meghalaya	LB 12	C101 A51	Lowland	3
13	Meghalaya	LB 13	IRBLZFU	Lowland	3
14	Meghalaya	LB 14	IRBL TA2Pi	Lowland	3
15	Meghalaya	LB 15	IRBL ZT-T	Lowland	3
16	Meghalaya	LB 16	IRBL TA-KI	Lowland	3
17	Meghalaya	LB 17	Sahbhagi Dhan	Lowland	3
18	Meghalaya	LB 18	Balwai	Lowland	3
19	Meghalaya	NB 1	White Mohar	Lowland	3
20	Meghalaya	NB 2	Mynri-NB	Lowland	3
21	Meghalaya	NB 3	Chakhao Poireiton	Lowland	3
22	Meghalaya	NB 4	SMS	Lowland	3
23	Meghalaya	NoB 1	LR 68	Lowland	3
24	Meghalaya	NoB 2	LR 18	Lowland	3

agar containing streptomycin (10 mg/ 250 ml of media) on which filter paper disks were placed at the periphery of the plate, sealed and incubated at 25 ± 1 °C for 10–12 days. The monoconidial cultures were preserved using the filter paper disks taken out from the cultures and kept in petri plates (Fig. 1b). The petri plates were kept in a desiccator till the disks dried and then stored at -20 °C until further use.

Isolation of genomic DNA and PCR amplification

Genomic DNA was isolated from the monoconidial cultures using the DNeasy Plant Mini Kit^R from Qiagen^R and stored at 4 °C till PCR was completed. The isolates were genotyped using previously reported SSR primers (Kaye et al. 2003; Zheng et al. 2008). A total of 43 SSRs (Table 2) were selected (a minimum of two per chromosome) for ascertaining diversity (Yumnam et al. 2012). PCR was performed in 10 µl volume containing 100–150 ng of genomic DNA, 0.5 U *Taq* DNA polymerase (Sigma, India), 10 picomoles of each forward and reverse primer, 25 mM dNTPs (Sigma, India), 1.0 µl of 10X buffer (Sigma), 25 mM $MgCl_2$ (Sigma, India), 5% DMSO (Sigma, India) using the same PCR cycle as reported previously (Zheng et al. 2008). The amplified products were resolved using 3% agarose gel and 100 bp DNA ladder as size marker.

Preparation of plant material for inoculation and disease scoring

Racial determination was done following the new international system of differentiating *Magnaporthe* races with reference to Lijangxintuanheigu (LTH) monogenic lines (Hayashi and Fukuta 2009). Seventeen monogenic lines procured from division of genetics, IARI, New Delhi were used for this purpose. Five to ten seeds of the distinct monogenic lines were sown in individual pots filled with potting mixture soil (3 parts) and farm yard manure (one part). The pots were kept in the greenhouse (28–30 ºC with 80–90% relative humidity) for 12 days (2–3 leaf stage), to prepare the plants for inoculation. Inoculation was done at 21 days (3–4 leaf stage) after germination. Conidial suspension was prepared by scraping the sporulating mycelia (5–7 days old; grey mycelial appearance) with 10–15 ml of deionized water. The mycelial and conidial suspension thus obtained

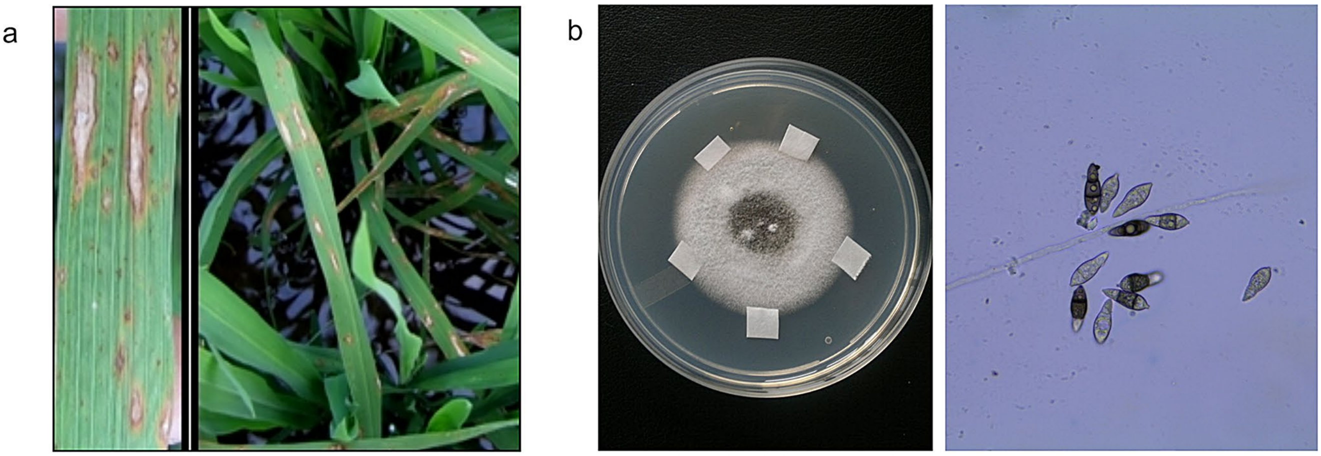

Fig. 1 Pictorial presentation of (**a**) leaf blast symptoms (**b**) monoconidial culture of *M. oryzae* isolate

Table 2 Summary of 43 SSR markers utilized in the study with chromosomal localization and sequence

Sl. No.	Marker	Chromosome position	Sequence (5’-3’)
1	mgm12	1	F: TGATTGCGTGATCCTTTCAG R: CTCTTTTCGCCAAGATCGAC
2	mgm35	1	F: GTTGAATTACCTTTCGGACTGG R: AAGGACTTTGCTCAGACCGTAG
3	mgm109	5	F: GAGATGCTGGCCAAGAATTG R: GGCCAGAACCCATTATCTCC
4	mgm125	6	F: CGCGGTAATGGAAGCTACA R: AGCGAGCTGTGTTGACCTTT
5	mgm127	6	F: ACGAACCCCTGGAATTTCTT R: TATAACGCCCAGGTGAGTCC
6	mgm134	6	F: TCGACGATAGGGTCTTCCTG R: CGATACCCTTCTCCGTCAAA
7	mgm142	7	F: CTGATGGTATTTCCGCGTCT R: CTCTGCCCTCCTTGTTTTCC
8	mgm170	5	F: GCGATTGGGACCAATAAGTG R: GGGCCTCACTCCGTAAATTG
9	mgm177	5	F: TGACTCGACCTGACATCTGC R: TTCCTGGGACTGTTTCATGG
10	mgm188	2	F: TGGGAAGTCGATAGTCAGGAA R: TGCACGATTAGCTGGTGAAG
11	mgm192	2	F: GGAGGGCGTCACTGTACCTA R: ATGAGGCATGTACCCCAAAA
12	mgm246	4	F: CCGGATGTCACCTACCAACT R: CCTTGTTTTCCCCCTGTGTA
13	mgm248	5	F: CAAGGCTGGTATCCAAGAGG R: CTTGAGGAGGTCGTCGATGT
14	mgm254	5	F: CCTGGGTCAGGTACATATCAAA R: AGCGGACTAGCTTGACTGGA
15	mgm266	6	F: TGTGGTGGGTGATCTTGTTG R: ATTCCCGGCGAGAGAGATT
16	mgm285	3	F: GCCACTCACACAAACACCAC R: AGCGCTGTGATGAGGAGAAT
17	mgm286	7	F: CGGCTGTGGTTTAACGATTT R: CCATCAGGATCCATGAACAC
18	mgm334	3	F: GACCCTGGTGGTAGGAGTGA R: TCTTATCGTTGCAGCCAATG
19	mgm352	4	F: CAGGAGATGCACTGCACATT R: CAAGCAATCAAGCAGCAAAA
20	mgm365	4	F: TCTATCGTGCCTCCTTGTCC R: ATGACGACATCCCAGTGGTT
21	mgm402	6	F: CCAATCTGGGGGCCTAATAA R: CGAACAGACGAGCAAACAAA
22	mgm423	1	F: AGGGCAATGGTGTGTGTGT R: TTGCTTGCCATAGCAGTTTG
23	mgm437	3	F: GCCCCTCAATAGATCGTCAA R: ACTGCGGCATTTTAACCTGT
24	mgm7	1	F: TATTGTCGTTGGCGAGTTTG R: GGCTGCACTGGGAAGATAGA
25	mgm15	1	F: GAAACCATCCGCAAAGATG R: ACCACAACGGCTGCTACG
26	mgm33	1	F: GGGAATGAGAATGATGAGGAAG R: TTGTTTTCCGCTGTGCTAAATA
27	mgm37	unknown	F: GGCCTGATCCTCACAAAAAG R: GATTCGCTTCTATGCCCTTG
28	mgm39	unknown	F: CCTTGTTTTCCCCCTGTGTA R: TATATTGCGACCCGTTCCTG
29	mgm41	2	F: CAATTGGTGGGAGGAAGAAA R: GAGCCCGGCGTTAACTAATA

Table 2 (continued)

Sl. No.	Marker	Chromosome position	Sequence (5’-3’)
30	mgm43	2	F: TGCATGAAGCTGATTTGCTC R: TTTGACTCTCGCTCCCTCTC
31	mgm45	2	F: ATCGCTGGGACCAAGTCAAA R: GAACCCTCCGTGCACCTTAG
32	mgm59	2	F: CAGGCTCGATTCGCTTCTAT R: GACCTGAGCATGCGGACTAT
33	mgm61	3	F: GAACCGACGACAACACATGA R: GGCGAGTACAAGAACCCAAC
34	mgm63	3	F: CCTTGTTTTCCCCCTGTGTA R: AGTGGCTGTTTCGGTAGCAC
35	mgm77	5	F: CACGGAACTGCATTGTAACG R: GCGGCTAACTGGCAACTATC
36	mgm81	3	F: TGACAATGAAACCAGGTCAGA R: CCTAAAAATTTCCGCCTCCT
37	mgm83	3	F: AGAACGCCAATGGAGGAAG R: GCACACCGTCGTCTATACCC
38	mgm87	4	F: GTCCACCGCTTAAACACTGC R: CTCCACTCGCTATGCACGTA
39	mgm93	1	F: GGGACAGGGTGAGAGAAGTG R: AAGCCCTCTTCTCAACGACA
40	mgm99	4	F: TCCCAGTACTTGCCCATCTC R: ATCTCATATCCGTCGGTCGT
41	mgm107	4	F: GCAAGCTCAAGATGATGCTG R: TAGCCAGACTGGTGGTGATG
42	mgm109	5	F: GAGATGCTGGCCAAGAATTG R: GGCCAGAACCCATTATCTCC
43	mgm125	6	F: CGCGGTAATGGAAGCTACA R: AGCGAGCTGTGTTGACCTTT

was filtered through a double-layered muslin cloth, and the resultant conidial-suspension served as inoculum (Bonman et al. 1986). The conidial count adjusted to 5×10^6 conidia per mL (using hemocytometer) was mixed with two drops of surfactant (Tween 20; 0.02%) before spray inoculation (by glass atomizer) on seedlings (Sharma et al. 2005). The inoculated plants were incubated in the dark using a black cloth for next 24 h, with relative humidity of 80–90% and temperature maintained at 24^0C. Relative humidity was maintained using water-soaked jute gunny bags. After the initial 24 h of dark incubation, the plants were transferred to bright illumination, and agronomic practices were followed. Disease scoring was done based on lesion length and given a score of 0–5 based on standard scale (Mackill and Bonman 1992). Plants showing scores 1–3 were considered resistant, and 4–5 were rated as susceptible. The virulence frequency of the isolates was also estimated using the formula; Virulence frequency (%) = No. of susceptible genotypes/ Total no. of genotypes x 100 (Hayashi et al. 2009).

Statistical analysis

DNA extracted from all the 24 lines (Table 1) were subjected to gel electrophoresis using 43 SSR markers (Table 2). Amplified bands were scored according to the desired expected band size. The scored molecular data were further used to analyze the major allele frequency, number of alleles, gene diversity, and PIC values using PowerMaker Software, version 3.25 based on the formula $PIC = 1-\sum (P_i)^2$ where, P_i depicts the proportion of samples carrying the i^{th} allele (Botstein et al. 1980; Liu and Muse 2005) (Table 3). Microsoft excel was used to calculate the means of blast disease scores and heterozygosity for the marker data. For genetic diversity analysis, pairwise genetic distance was computed using Ward’s coefficient in DARwin software (Perrier and Jacquemoud-Collet 2006), version 6.0. Dendrogram was constructed using the molecular data in R statistical software (v4.1.2; R Core Team 2021) using package pvclust (Suzuki and Shimodaira 2006) and bootstrapping was performed with 1000 replication using boot package (Canty and Ripley 2022) in R. Additionally, mantel test was performed to determine the correlation between virulence phenotypic and genotypic data between different isolates using R statistical software (v4.1.2; R Core Team 2021) using package vegan (Oksanen et al. 2020).

Table 3 Allelic profile of 24 *M. oryzae* isolates using 43 SSR markers

Marker	Major Allele Frequency	Allele No.	Gene Diversity	Heterozygosity	PIC
mm107	0.375	6	0.771	0	0.741
mgm246	0.167	10	0.885	0	0.874
mgm134	0.708	4	0.469	0	0.439
mgm177	0.792	3	0.351	0	0.322
mm109	0.208	7	0.840	0	0.820
mm39	0.208	11	0.875	0	0.862
mgm142	0.542	4	0.604	0	0.541
mgm334	0.250	8	0.851	0	0.834
mm33	0.250	11	0.869	0.25	0.856
mgm365	0.250	9	0.847	0	0.830
mgm35	0.208	11	0.868	0	0.854
mm83	0.792	2	0.330	0	0.276
mm99	0.250	7	0.792	0	0.760
mgm402	0.417	9	0.771	0	0.750
mm77	0.208	9	0.865	0	0.850
mgm437	0.375	8	0.799	0	0.790
mm93	0.333	4	0.712	0	0.656
mm37	0.417	4	0.694	0	0.640
mm61	0.542	3	0.601	0	0.533
mm63	0.250	6	0.795	0	0.764
mgm423	0.375	7	0.785	0	0.760
mgm286	0.292	10	0.844	0	0.830
mgm285	0.167	13	0.903	0	0.900
mgm352	0.583	5	0.608	0	0.570
mm125	0.333	7	0.757	0	0.718
mgm192	0.208	9	0.872	0	0.860
mgm125	0.333	9	0.786	0.042	0.760
mgm248	0.250	11	0.864	0.917	0.850
mm87	0.375	6	0.743	0	0.703
mm43	0.479	5	0.678	0.042	0.631
mgm188	0.292	7	0.802	0	0.775
mgm12	0.208	8	0.840	0	0.820
mm45	0.458	5	0.694	0	0.650
mm15	0.458	8	0.743	0	0.722
mgm127	0.333	6	0.757	0	0.720
mgm254	0.500	5	0.663	0	0.620
mm81	0.375	6	0.771	0	0.741
mgm266	0.375	11	0.810	0	0.793
mm41	0.458	9	0.739	0.083	0.715
mgm109	0.500	19	0.734	0.333	0.726
mm59	0.667	4	0.503	0	0.456
mm7	0.833	4	0.294	0.042	0.279
mgm170	0.625	4	0.559	0	0.518
		313			
Mean	0.396	7.279	0.729	0.040	0.700

Results

Molecular diversity of blast isolates

A total of 24 isolates of *M. oryzae* collected from different locations of the NEH region of India were analyzed using 43 SSR markers (Fig. 2).

For diversity analysis, 43 primers were used (Table 2). Highest PIC value was observed for marker mgm285 followed by mgm246 and mm39, while the marker mm83 resulted in lowest PIC value followed by mm7 and mgm177, respectively (Table 3). The total number of alleles was 313 and the average was 7.27. The marker mgm109 showed the highest allele number (19), followed by mgm285 and the marker with lowest allele number (2) was mm83 followed by markers mgm177 and mm61. The highest genetic diversity was represented by marker mgm285 which was about 90% followed by mgm246 which constituted 88.5% of genetic diversity whereas the marker mm7 showed the lowest genetic diversity of about 29% followed by mm83 with genetic diversity of about 33%.

Mantel test with a correlation coefficient r value of 0.2 signifies a weak association between the two matrices.

According to the dendrogram, the *M. oryzae* isolates were grouped into five clusters (Fig. 3).

Cluster II comprised the largest group with 9 isolates. The isolate LB 3 (S5 NEHU) from Umjalynshir of Meghalaya formed a separate group (Cluster I) from the rest of the 23 isolates. The upland isolate LB 2 (Lampel) from Imphal and LB 4 from Sawkilo of Meghalaya were grouped in separate groups (Cluster III and Cluster IV). While nodal blast isolates NoB 1 (LR 68) and NoB 2 (LR 18) isolates from Umiam, Meghalaya, were also grouped in separate Cluster V and Cluster I, respectively. The isolates for neck blast (NB 2, NB 3) lesions from Umiam, Meghalaya, were grouped together in Group V. While other neck blast isolates (NB 1 and NB 4) also from Umiam, Meghalaya were placed in separate groups (Group IV and Group II, respectively). The dendrogram also showed that the isolate LB 4 which was collected from the upland field was grouped along with the isolate from Umiam, Meghalaya collected from lowland field and was not categorized in the same group as that of LB 3 which was also collected from upland field of Meghalaya. The highest genetic distance (0.90) was found to be between NB 1 and NoB 1 followed by genetic distance of 0.89 between LB 5 and LB 3; LB 3 and LB 18; LB 13 and NoB 2; LB 10 and NB 4. The lowest genetic distance (0.25) was found to be between LB 10 and LB 12 followed by the genetic distance (0.28) between LB 7 and LB 8 (Table 4).

Furthermore, for pathotyping, 20 *M. oryzae* isolates (Table 1) from different sites of NEH region, were tested on 17 rice monogenic lines, resulting in 8 pathotypes based on the reaction types (Table 5). Out of the 24 isolates, 4 isolates namely NB 2, NB 4, NoB 1 and NoB 2 were not included for pathotyping (samples from similar locations with similar molecular data were treated as duplicates and hence, removed). Pathotype 1 comprises of isolates LB 5 and LB 11 collected from Umiam and Umroi area of Meghalaya were

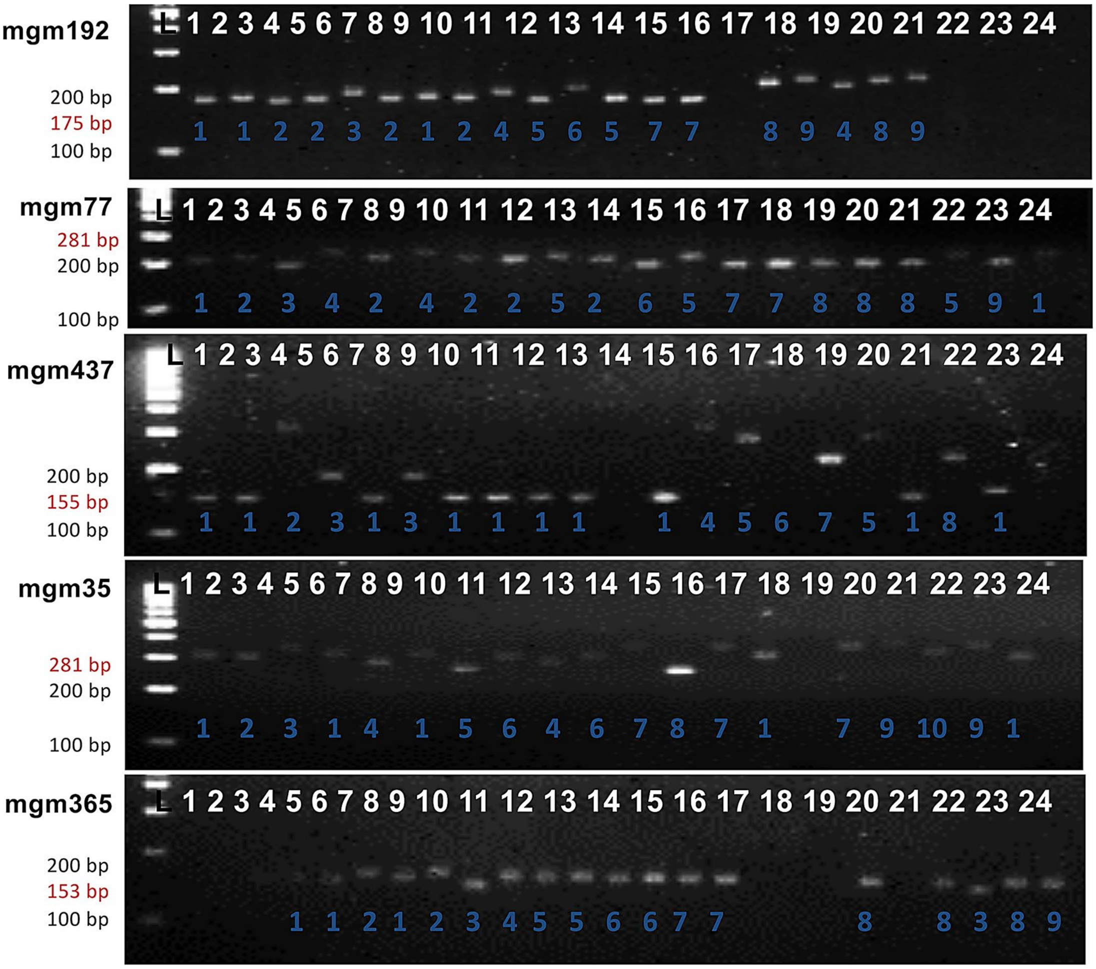

Fig. 2 Representative gel electrophoresis picture illustrating polymorphic bands for SSR markers mgm192, mgm77, mgm437, mgm35 and mgm365. L: Ladder (100 bp); Numbers in red represent the expected band size, numbers in white on the top represent the isolates as listed in Table 1, while numbers in blue indicate the alleles

the most virulent strains as they could infect 13 monogenic lines out of 17 used in the study. Pathotype 8 isolated from Nagaland was found to be the least virulent as it could infect only 5 lines followed by pathotype 7 from Umiam area of Meghalaya which could infect 6 lines out of 17 monogenic lines used. About 29% of the pathotypes could infect 10 lines, 18% of the pathotypes infected 8 lines and 11 lines each whereas 12% of the pathotypes could infect 9, 12, 13 and 6 monogenic lines and 6% could infect only 5 lines. The virulence frequency of *M. oryzae* isolates was given by the number of genotypes infected by an individual isolate and ranged from 35 to 76%. The most virulent isolates were LB 11 (IRBLSH B) and LB 5 (Mynri) infecting 13 out of 17 monogenic lines followed by isolates LB 13 and LB 14 which were virulent on 12 lines. However, the least virulent isolate was LB 1 (Medziphema) infecting only 5 lines, followed by isolates LB 8 and LB 18 which infected 6 lines. Results also revealed that isolates LB 3 from Umjalynshir and LB 2 from Imphal each collected from upland rice fields not only showed similar virulence on the monogenic lines but also with other 2 isolates collected from lowland fields, whereas the isolate LB 4 from Umiam, Meghalaya which was also collected from an upland genotype infected 9 monogenic lines. The data also showed that *M. grisea* isolates from neck blast infected samples i.e., NB 1 and NB 3 from Umiam, Meghalaya also caused leaf blast and were virulent to 10 and 11 monogenic lines, respectively. The isolates which were least virulent as well as highly virulent were found to be from lowland fields whereas those from the upland field were found to be intermediate virulent.

Discussion

Rice blast disease has been a great concern for the farming community and researchers across the globe. Climate change impacts the ecosystem including pathogens, resulting in genetic variation, virulent pathotype evolution, wider

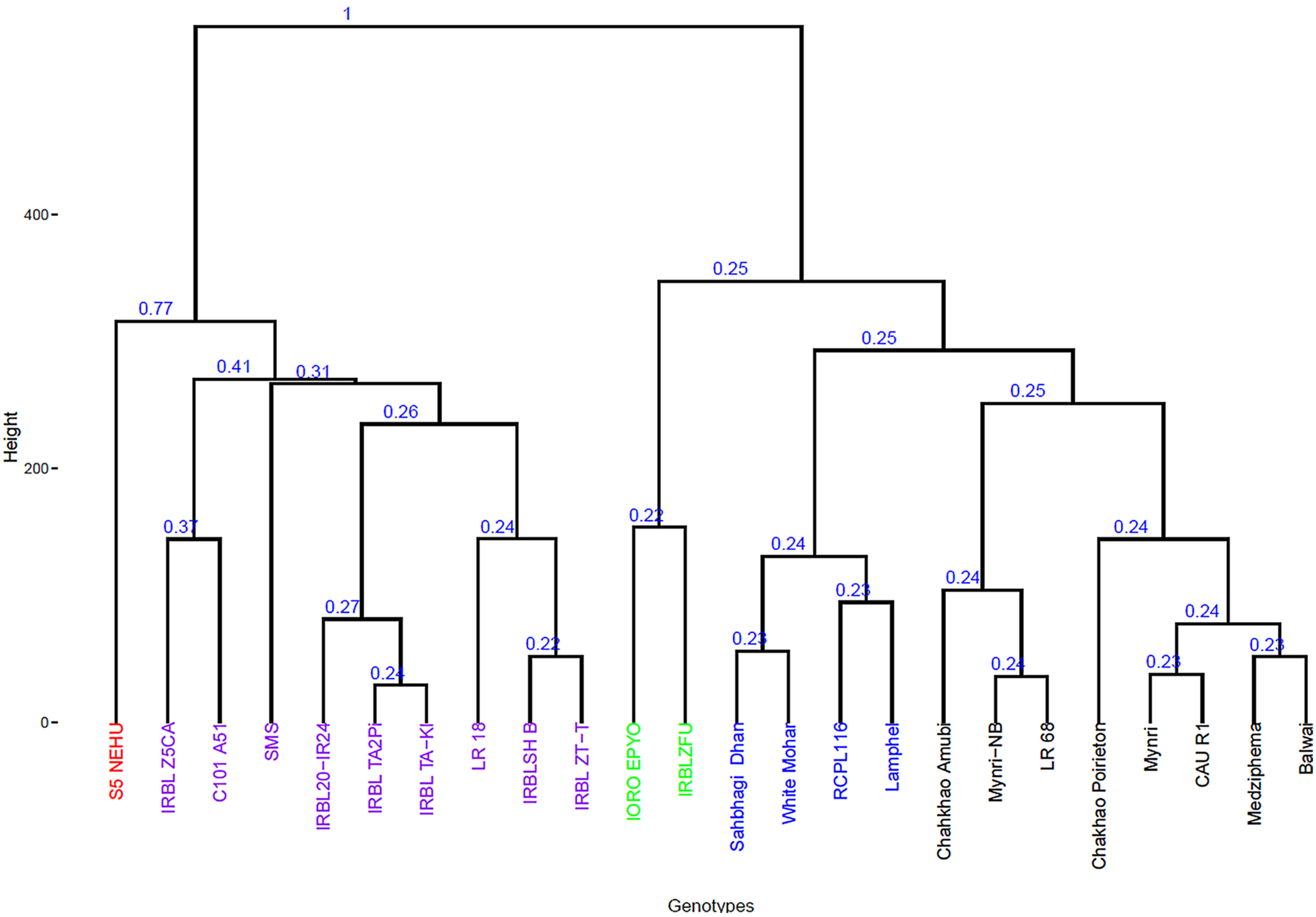

Fig. 3 Dendrogram displaying cluster analysis of *M. oryzae* isolates. Hierarchical clustering where height on the y-axis represents the distance or dissimilarity at which clusters are merged computed based on the Ward D^2 method. The height reflects the squared distance between centroids of merged clusters. The values on the branches (e.g., 0.77, 0.25) are bootstrap values, indicating the stability of the clusters based on 1000 bootstrap iterations. Different colours represent different clusters e.g., Cluster I (red), Cluster II (purple), Cluster III (green), Cluster IV (blue), Cluster V (black)

host range and host shifts (Morris and Moury 2019). Therefore, pathotyping of *Magnaporthe* spp. is crucial to gain insights into the pathogen's diversity, evolution, and interaction with host. Hence, this study focused on understanding the pathotypes prevailing in the NEH region for strategic breeding of rice cultivars with durable resistance against virulent strain of *M. oryzae*. *Magnaporthe oryzae* isolates collected from Meghalaya, Mizoram and Nagaland revealed significant variations in pathogen profiles across different rice ecosystems (upland and lowland fields). SSR marker analysis of blast isolates revealed significant genetic diversity, with marker mgm285 exhibiting the highest PIC value, making it the most informative marker. Markers mgm285 and mgm109 exhibit significant polymorphism and can be used as robust markers for mapping and genetic variation studies. Diversity assessment is essential for understanding the pathogen's adaptability and potential evolution of new pathogenic races. High level of genetic diversity was also reported for blast isolates from different rice growing states of Chhattisgarh, Odisha, Karnataka and Telangana using SSR markers (Aravind et al. 2022; Jagadeesh et al. 2020; Yadav et al. 2019). Previous studies have reported that the pathogenicity varies under different agroecological conditions and across monogenic lines, suggesting the existence of different virulent strains of the pathogen. For instance, the black rice landrace of Manipur exhibited higher blast infestation as compared to other lines/varieties (Debnath et al. 2024).

High genetic variance among isolates originating from different geographical locations was observed, as LB 3 was genetically diverse from the rest of the isolates. Similar findings were observed among isolates from different locations of Karnataka and eastern India (Kumar et al. 2019; Jagadeesh et al. 2020). Jagadeesh et al. (2020) and Tuan et al. (2020) also found association of groups/clades with geographical boundaries. Additionally, genetic similarity within the leaf blast isolates (LB 1, LB 2, LB 5) despite having different geographical origins, could be due to similar

Table 4 Pairwise genetic distance matrix indicating the genetic dissimilarity of *M. oryzae* isolates using Ward's coefficient

OTU	LB12	LB 8	NNB 3	LB 7	LB4	LB 14	LB 16	LB 10	LB 15	LB 6	LB 11	LB 13	LB 2	NNoB 2	NoB 1	LB 1	LB 5	NB 2	LB 9	LB 3	LB 17	NB 4	NB 1
LB 18	0.85	0.33	0.85	0.42	0.80	0.83	0.78	0.85	0.83	0.78	0.82	0.88	0.72	0.85	0.85	0.66	0.46	0.76	0.80	0.89	0.69	0.76	0.46
LB 12	0.00	0.76	0.81	0.83	0.75	0.73	0.76	**0.25**	0.73	0.76	0.67	0.77	0.80	0.87	0.87	0.71	0.83	0.80	0.75	0.84	0.71	0.87	0.66
LB 8		0.00	0.87	0.28	0.80	0.83	0.76	0.80	0.78	0.78	0.78	0.83	0.69	0.78	0.83	0.66	0.39	0.78	0.82	0.82	0.69	0.80	0.32
NB 3			0.00	0.89	0.82	0.75	0.78	0.88	0.77	0.80	0.88	0.81	0.76	0.80	0.58	0.74	0.88	0.53	0.84	0.88	0.65	0.69	0.83
LB 7				0.0	0.8	0.81	0.78	0.85	0.83	0.78	0.82	0.88	0.65	0.80	0.76	0.73	0.46	0.83	0.82	0.82	0.79	0.78	0.44
LB 4					0.0	0.64	0.64	0.72	0.73	0.41	0.61	0.75	0.85	0.82	0.78	0.82	0.83	0.87	0.72	0.77	0.80	0.78	0.83
LB 14						0.00	0.30	0.78	0.53	0.66	0.61	0.62	0.71	0.84	0.83	0.71	0.88	0.78	0.68	0.82	0.78	0.69	0.71
LB 16							0.00	0.80	0.55	0.64	0.61	0.59	0.61	0.82	0.80	0.65	0.86	0.78	0.75	0.73	0.75	0.73	0.67
LB 10								0.00	0.78	0.71	0.63	0.82	0.80	0.81	0.82	0.75	0.87	0.87	0.72	0.84	0.70	0.89	0.78
LB 15									0.00	0.73	0.39	0.59	0.71	0.82	0.85	0.73	0.83	0.74	0.82	0.75	0.73	0.57	0.74
LB 6										0.00	0.70	0.80	0.73	0.87	0.81	0.73	0.83	0.81	0.78	0.77	0.69	0.76	0.76
LB 11											0.00	0.70	0.74	0.82	0.84	0.70	0.85	0.82	0.73	0.81	0.72	0.69	0.77
LB 13												0.00	0.74	0.89	0.87	0.82	0.88	0.85	0.77	0.73	0.73	0.78	0.73
LB 2													0.00	0.75	0.66	0.53	0.88	0.68	0.70	0.68	0.48	0.78	0.69
NoB 2														0.00	0.64	0.80	0.87	0.80	0.81	0.79	0.82	0.78	0.81
NoB 1															0.00	0.78	0.88	0.56	0.82	0.82	0.78	0.72	**0.90**
LB 1																0.00	0.76	0.64	0.70	0.75	0.59	0.82	0.64
LB 5																	0.00	0.86	0.85	0.89	0.81	0.81	0.37
NB 2																		0.00	0.85	0.87	0.66	0.74	0.83
LB 9																			0.00	0.88	0.77	0.85	0.73
LB 3																				0.00	0.79	0.77	0.83
LB 17																					0.00	0.82	0.71
NB 4																						0.00	0.74

OTU: Operational taxonomic unit; LB: Leaf Blast; NB: Neck blast; NoB: Nodal blast isolates

Table 5 Pathotype classification of blast isolates based on Monogenic line reaction

Blast Isolates	Monogenic Lines																	Pathotypes
	C101 A51	IRBL sh-B	IRBL TACP1	IRBL Kh-K3	IRBL ta-CT2	C101 LAC	IRBL 20-IR24	IRBL ta2-Pi	IRBL Ks-F5	IRBL zt-T	IRBL ta-K1	IRBL t-K59	IRBL b-B	IRBL 9-W	IRBL z5-CA	DHMAS	IRBL 5-M	
LB 1	R	R	R	R	R	R	R	R	R	S	S	R	S	S	R	R	R/S	Pathotype 8
LB 8	S	S	R	R	S	R	R	R	R	R	R	R	R/S	S	R	R	S	Pathotype 7
NB 3	R	R	R	S	R	S	S	R	R	S	R	R/S	R	R	R	R	S	Pathotype 7
LB 3	R	S	R	S	R	R	R	S	R	R	S	R/S	R/S	S	S	R	R	Pathotype 6
LB 2	R	S	R	S	R	R	S	R	S	R	S	R	S	S	R	R	R/S	Pathotype 6
LB 6	R	S	S	R/S	S	R	R	R	R	R	R/S	R	R/S	S	R	R	R/S	Pathotype 6
LB 17	R	S	S	S	R	R	R	S	R	S	R/S	R	S	S	R	R	S	Pathotype 5
LB 4	R	S	R	R/S	S	S	S	R	R	R	S	R/S	R	R	S	R	S	Pathotype 5
LB 10	S	S	R	S	R	R	S	S	S	S	S	R	S	R	R	R	R/S	Pathotype 4
LB 7	R	S	S	S	S	R	R/S	S	R	S	R	R	R/S	R	S	R	S	Pathotype 4
LB 16	S	S	S	S	S	S	R	R	R	R	R	R/S	S	S	R	R	S	Pathotype 4
LB 15	R	R	S	S	S	R	S	S	S	R	R/S	R	S	S	S	R	R	Pathotype 4
NB 1	R	R	S	S	S	S	S	R	R	S	R/S	R/S	R	S	R	R	S	Pathotype 4
LB 9	S	S	S	S	S	R	S	S	S	R	R	R/S	R	S	R	R	S	Pathotype 3
LB 12	R	S	S	R	R	R	S	S	S	R	S	R	S	S	S	S	S	Pathotype 3
NB 3	S	S	R	S	S	S	R	R	S	S	S	R/S	R	S	R	R	R/S	Pathotype 3
LB 13	S	S	S	R	S	R	S	S	R	S	S	R/S	S	S	S	R	R	Pathotype 2
LB 14	S	S	S	S	S	R	S	S	R	R	R/S	R/S	S	R	S	S	R	Pathotype 2
LB 11	R	S	S	S	S	R	S	S	S	S	S	R	S	S	S	R	S	Pathotype 1
LB 5	R	S	S	R	S	S	R/S	R	S	R	S	R/S	S	S	S	S	S	Pathotype 1

ecological niche, pathogen migration, gene flow or adaptive nature of the pathogen (Gladieux et al. 2018; Thierry et al. 2022). Kumar et al. (2019) also reported the existence of high genetic diversity among blast isolates and simultaneously, genetic similarity among blast isolates having different geographical origins. Isolates originating from different tissues like node (NoB 1, NoB 2) vs. neck (NB 2, NB 3) also showed high degree of genetic similarity suggesting that these isolates either belong to the same genetic lineage or have similar adaptative mechanisms. This was evident from the findings of Sheoran et al. (2021) as 17 genomic loci resulted in genetically identical with no or minor nucleotide polymorphism for *M. oryzae* causing leaf-blast and panicle-blast. Furthermore, the separation of other neck blast isolates (NB 1 and NB 4) into different groups (Group III and Group IV) indicates substantial genetic variation within neck blast isolates suggesting the existence of multiple pathogenic races within the population, each potentially exhibiting different virulence profiles and host interactions (Bentham et al. 2021; Sheoran et al. 2021).

Isolate LB 4 from an upland field in Meghalaya clustered together with lowland isolates from the same region, but not with LB 3 which was also collected from an upland field, suggesting that variation in virulence and genetic relatedness in *M. oryzae* are under the influence of pathogen diversity and environmental conditions (Yoshida et al. 2016). The genetic distance indicates that the pathogen population is highly diverse and heterogenous. Variability in virulence as assessed using monogenic lines suggests heterogeneous nature of the *M. oryzae* population and complex nature of the pathogen (Thierry et al. 2022). High level of virulence (pathotype 1) indicates that this pathotype has evolved mechanisms to overcome multiple resistance genes (Kito et al. 2002), making blast disease management in the NEH region challenging. However, sequence analysis using *MAT1* gene-specific primer revealed genetically homogenous population of *M. oryzae* from Karnataka (Chittaragi et al. 2022), suggesting the influence of genetic and environmental conditions on pathogenicity. The observation that both highly virulent and least virulent isolates were predominantly from lowland fields, while those from upland fields exhibited intermediate virulence, indicates that environmental factors, such as moisture levels and temperature, might affect the pathogen's ability to express virulence (Qiu et al. 2022). It was evident that genetic polymorphism was not impacted by just virulence polymorphism (Emani et al. 2024) and that climatic variation was linked with geographic distribution of *M. oryzae* groups (Thierry et al. 2020). The environment could influence the pathogen's evolutionary pressures, leading to the selection of different virulence traits as observed across different parts of India and the world. For instance, Sheoran et al. (2021) observed varying degrees of pathogenicity among 21 isolates collected from Kashmir. Other studies include detection of six pathotypes from Malaysia when checked across 22 IRRI-bred blast resistance lines (Misman et al. 2021); 99 isolates categorized into 62 races from Kenya (Fukuta et al. 2019), 206 isolates from northern China grouped into 175 races (Wang et al. 2024).

Conclusion

In conclusion, the present study suggests that the blast isolates prevailing in the North Eastern region are virulent to many monogenic lines. Neck blast isolates are genetically distinct from leaf blast isolates and would serve as a basis of future studies on molecular genetics. The resistant and moderately resistant cultivars identified can be used directly as donor parents in rice breeding or can be used for identification of race-specific/broad spectrum sources of resistance against blast. Continuous surveys of virulent races would help in predicting and forecasting disease and the distribution of resistant varieties, for preventing disease epidemics. Focus should be more in studying large number of isolates and understanding the mechanism of host-pathogen interaction at molecular level using advanced genetic tools (sequencing, CRISPR-Cas9) and breeding methods (gene pyramiding, MAS, MAB) for mitigating blast disease.

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Author contributions MR, WT conceived and supervised the project. WT supervised the molecular work. YS, MR and WT interpreted the data. YS and BL wrote the original draft and WT and MR edited the manuscript. All authors read and approved of the final manuscript.

Declarations

Ethics approval and consent to participate Ethics approval and consent to participate are not applicable for this manuscript. The manuscript does not contain any studies with human or animal subjects.

Competing interests Authors declare that there is no conflict of interest.

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