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Morphological and biochemical variation of rice blast fungus *Magnaporthe oryzae* in Karnataka, India

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Abstract Rice blast caused by *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*) is a destructive disease in all rice growing regions of the Karnataka. The morphological and biochemical variations existing among the field isolates collected from different agro-climatic zones was investigated. The influence of culture media on growth, colony character of *M. oryzae* isolates on two different media *viz.,* potato dextrose agar and oat meal agar was studied. Results revealed that there was a significant variation among the isolates of *M. oryzae* in different parameters such as growth pattern, colour of media and mycelia, texture and sporulation ($P \leq 0.05$). These variations are supported by isozyme analyses which showed significant level of variations among the isolates with 17 polymorphic bands. In UPGMA cluster analysis, the coefficient of similarity ranged from 0.26 to 1. Two distinct clusters were formed among the isolates i.e., from the southern districts (cluster I) and northern district isolates (cluster II). This suggested that there is a correlation among the geographical boundaries with the diverse isozyme profile of the pathogen for its adaptability to the different environmental conditions.

Keywords: Isozyme, *Magnaporthe oryzae*, Rice blast disease, UPGMA

Introduction

Morphological variations are one of the criteria to understand the nature of virulence of this rice blast fungus *Magnaporthe oryzae* B.C. Couch, (anamorph: *Pyricularia oryzae* Cavara) (Couch and Kohn, 2002). Several studies on differential response of *M. oryzae* isolates obtained from rice, finger millet on different media were reported (Khadka *et al*., 2012; Tochinai and Shimamura, 1932; Kumar and Singh, 1995). Analyses of morphological characteristics are very much important to maintain *M. oryzae* in laboratory conditions to sustain its viability, to get high sporulation and to maintain the

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pathogen for further experiments. This is ultimately required for carrying out investigations on assessment of pathogen virulence and genetic variation.

This pathogen is known to grow vigorously on oat meal agar (OMA), potato dextrose agar (PDA) medium at neutral pH and 30 ℃ (Kulkarni and Govindu, 1976; Awoderu *et al*., 1991). The optimum growth and sporulation of *M. oryzae* is favored by high relative humidity and optimum temperature of 26 to 28 ℃ (Perezsendin *et al*., 1982; Teng, 1994). Sun *et al*. (1989) studied the effect of different media on *P. oryzae* growth and sporulation. They have observed luxurious growth of *P. oryzae* on PDA medium compared to all other media. Similarly, Kumar and Singh (1995) studied the growth pattern of *P. oryzae* obtained from rice on different culture media and showed maximum linear growth even on malt agar. The varied colour of the colony of same isolates grown on two different media was observed by Vanaraj *et al*. (2013). Extent morphological variations are indicative of genetic variations also and understanding of such variations is essential for breeding disease resistant cultivars (Sonah *et al*., 2009). With above observations, the growth pattern of *M. oryzae* on different media *viz*., OMA and PDA was studied for the 72 isolates of *M. oryzae* obtained from different geographical locations of Karnataka.

Morphological variations can be further validated using biochemical parameters *viz*., isoenzymes. These are also known as isozymes which are group of enzymes catalyzing the same chemical reaction but having variants due to the difference in their amino acid composition. Accordingly, they show different electrophoretic mobilities depicting protein polymorphisms. All living systems require multiple molecular forms of certain enzymes to maximize their biological functions (Eun, 1996). Isozymes/allozymes are neutral and co-dominant markers formed from gene mutations such as gene duplication, deletion, insertion and epigenetic modifications of gene products (Chen, 2007). Isozyme analysis has many applications to examine the population genetics in plant pathology and mycology. Many mycologists used to resolve taxonomic disputes, identify the unknown fungal taxa and genetic variability in a population through fingerprint pattern of isozymes (Burdon and Marshall, 1983; Micales, 1986). This technique is also useful to distinguish the isolates which are morphologically identical. The capacity of electrophoresis to differentiate fungal species is directly dependent on the amount of genetic variation existing within the population (Burdon *et al*., 1983). Thus, isozyme analysis is one of the most powerful versatile and highly informative tools for understanding the genetic variability in *M. oryzae* populations. The information regarding biochemical variability *viz*., isozyme profiles of *M. oryzae* isolates is not available. One study was reported on isozyme on this pathogen with respect to only peroxidase and polyphenol oxidase by Meena (2006). Isozyme patterns

among other fungi were reported by various researchers. Isozyme pattern was extensively studied in *Rhizoctonia solani* (Chand and Logan, 1983; Guleria *et al*., 2007; Seema, 2013) as biomarkers. Hence, zymograms of certain important enzymes produced by *M. oryzae* during infection such as Catalase, Esterase and Protease were studied for understanding variations in the *M. oryzae* isolates.

Materials and methods

Collection of rice blast disease samples

Collection of rice blast disease samples from different districts of south Karnataka and establishment of pure cultures of blast pathogen isolates were conducted. A total of 171 places were visited during 2012-2014 (kharif season) in different districts of Karnataka, India. The rice blast disease samples were collected from the farmers' fields (101 diseased plots) from different districts of Karnataka including different agro climatic zones (Jagadeesh *et al*., 2018a). Seventy two monoconidial *M. oryzae* isolates were obtained and pure cultures were established and maintained using a simple, reliable and inexpensive isolation procedure developed by us (Jagadeesh *et al*., 2018b).

Culture M. oryzae on different media

The growth pattern and growth rate of all the 72 *M. oryzae* on different media *viz*., oat meal agar (OMA) and potato dextrose agar (PDA) was studied to understand the extent of morphological diversity among these isolates. Five mm diameter mycelial discs of *M. oryzae* taken from 14 day old pre-cultured Petri dishes were inoculated to OMA and PDA plates under aseptic conditions and incubated at 27±1 ℃ for 16 days. Radial growth of the fungus was measured on 8th and 16th day with 3 replications. Morphological growth characters of the fungal isolates were recorded by following a standard procedure as mentioned in link below. (https://nios.ac.in/media/documents/dmlt/Microbiology/Lesson-51.pdf). Standard terminologies were used to describe common colony types.

Statistical analysis

The frequency of growth characteristics such as growth pattern, sector formation, colour of the medium, colour of the mycelium, texture, sporulation and growth rate of 72 isolates of *M. oryzae* grown on two media namely PDA and OMA were analysed using Pearson Chi-Square Test (SPSS software, IBM

Corp, 2015). Relationship among all 72 isolates with respect to different parameters on each medium was analysed separately. Comparative studies among different parameters between two media were also analysed using Phi and Cramer's V test. The growth rates of all the isolates on two different media were compared and grouped using Post Hoc tests.

Testing a hypothesis

This is done as follows: **Ho:** There is no significant difference among/between the growth parameters of *M. oryzae* on OMA and PDA. **H1:** There is significant difference among/between the growth parameters of *M. oryzae* on OMA and PDA

Pathogenicity test

In our investigations, pathogenicity test was carried out to see the virulence spectrum of each of 72 isolates. The top 20 isolates which are showing higher virulence ranging from 6 to 9 were taken up for isoenzyme study. For this purpose, sterilized soil was filled in 25 cm diameter plastic pots. HR12 (susceptible variety) paddy seeds were sown and conidial suspension of *M. oryzae* was sprayed over 10 days old seedlings and disease severity was recorded from the individual plant using standard leaf blast scoring procedure developed by IRRI; 0-5 scale and 0-9 scale (IRRI, 1996) after 72 hr of inoculation. The top 20 isolates which are showing higher virulence ranging from 6 to 9 were taken up for isoenzyme study.

Isoenzyme studies

Protein was extracted from those isolates. The top twenty *M. oryzae* isolates with regard to virulence pattern for the isozymes analyses was chosen. Those *M. oryzae* isolates were grown on corn meal broth for 14 days in 250 ml conical flasks under continuous shaking at 50 rpm at 28 ℃. The fungal mycelia were rinsed twice with the autoclaved sterile distilled water and blot- dried. These mycelial mats were freeze-dried and stored at -20 ℃. Soluble proteins were extracted by grinding 200 mg freeze-dried mycelium with a pestle and mortar in liquid nitrogen to a fine powder and filtered through sterilized miracloth and 10 ml of 0.1 M Tris-HCl with pH 6.8 was transferred to a centrifuge tube. The mixture was thoroughly mixed and centrifuged for 15 min at 15,000 rpm. The supernatant was collected and protein was estimated

according to the Lowry method with bovine serum albumin as standard protein (Lowry *et al*., 1951).

Protein fingerprinting by SDS PAGE

Protein samples were analyzed by 10% SDS-PAGE as described by Laemmli (1970) and modified according to Hames (1995) with the gel dimension of 8.6 × 6.7 × 0.1 cm in a Mini-PROTEAN Tetra Cell vertical gel electrophoresis (Bio-Rad Laboratories, Inc. USA). For each well 10 µl of extract (supernatant) was loaded on a polyacrylamide gel. Electrophoresis was carried out at a constant voltage of 40 V initially for stacking gel and at 100 V for separation gel at room temperature. After electrophoresis, the gels were stained in a mixture of Coomassie brilliant blue R 250 for 2 hrs and destained repeatedly in the destaining solution on gel rocker.

Visualization of isozymes

Isozymes are identified on non-denaturing PAGE through the use of specific stains as described by Gabriel (1971) and Vallejos (1983). Specific enzymes were detected when the gel was immersed in appropriate substrates and cofactors where substrates and other reagent molecules can diffuse into the gel and enzymes can act upon. This can be detected by zones of enzyme activity such as formation of specific colored bands or a zone of clearance.

Evaluation and documentation of isozyme pattern

The relative mobility (Rm) of each band on non-denaturing PAGE was calculated by the using formula given below:

$$\textbf{Relative mobility (Rm)} = \frac{\textbf{Distance travelled by the band}}{\textbf{Distance travelled by the tracking dye}}$$

Sample preparation for isozymes electrophoresis

100 mg of freeze-dried mycelia were homogenized by grinding with a mortar and pestle along with the 2 ml of extraction buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7) and then incubated in ice bath for 1 hr. After incubation extracts were centrifuged at 15,000 rpm for 15 min at 4 ℃. The supernatant was used for the isozyme analysis.

Catalase (CAT): Catalase activity was assayed by incubating the native gels in the 50 ml of 30% H_2O_2 solution for 10 min and then gels were stained in

a solution containing 0.5% (w/v) potassium ferric cyanide and 0.5% (w/v) ferric chloride for 10-15 min until bands appeared. The gels were then rinsed 2-3 times with autoclaved distilled water, afterwards fixed in 5% acetic acid.

Esterase (EST): Presence of esterase was assayed by incubating the native gels in a solution containing sodium dihydrogen phosphate: 2.8 g, disodium hydrogen phosphate: 1.1 g, fast blue RR: 0.2 g, alpha-naphthyl acetate 0.03 g dissolved in 200 ml of distilled water. The enzyme reaction was stopped by adding a mixture of methanol: water: acetic acid: ethyl alcohol in the ratio 10:10:2:1.

Protease (PRO): For detection of protease activity, 1% (w/v) casein dissolved in 25 mM phosphate buffer (pH 7.2) was incorporated into the separating gel and stained by immersion in a 1% (w/v) solution of amido black dissolved in methanol: acetic acid: water in the ratio of 30:10:60 for 30 min. The gel was destained in methanol: acetic acid: water 40:10:50, until bands appeared.

Results

Culture of M. oryzae

Cultural diversity among the *M. oryzae* isolates on different media was observed. Results of growth characteristics of all the 72 *M. oryzae* isolates on two culture media *viz*., OMA and PDA are given in Figure 1 & 2 and Table 1.

Frequency table for the growth of M. oryzae on OMA

In the above study, with respect to growth characteristics of *M. oryzae* on OMA, P-value was typically less than 0.05 ($P \leq 0.05$) for the parameters such as growth pattern, colour of media and mycelia, texture and sporulation which indicated a strong evidence against the null hypothesis. Hence, results revealed that there was a significant difference among the growth parameters of *M. oryzae* on OMA, except for the sector formation which showed P-value greater than 0.05 (> 0.05) indicating that there was no significant difference among the 72 isolates regarding this parameter.

Measure of strength between growth characteristics of *M. oryzae* on two different media *viz.,* OMA and PDA was analyzed using Phi and Cramer's V test. It indicated that there was a significant difference among these two parameters. Growth patterns, sector formation, colour of media and sporulation parameters showed P-value greater than 0.05 (> 0.05) indicating that there was no significant difference among these parameters between the media.

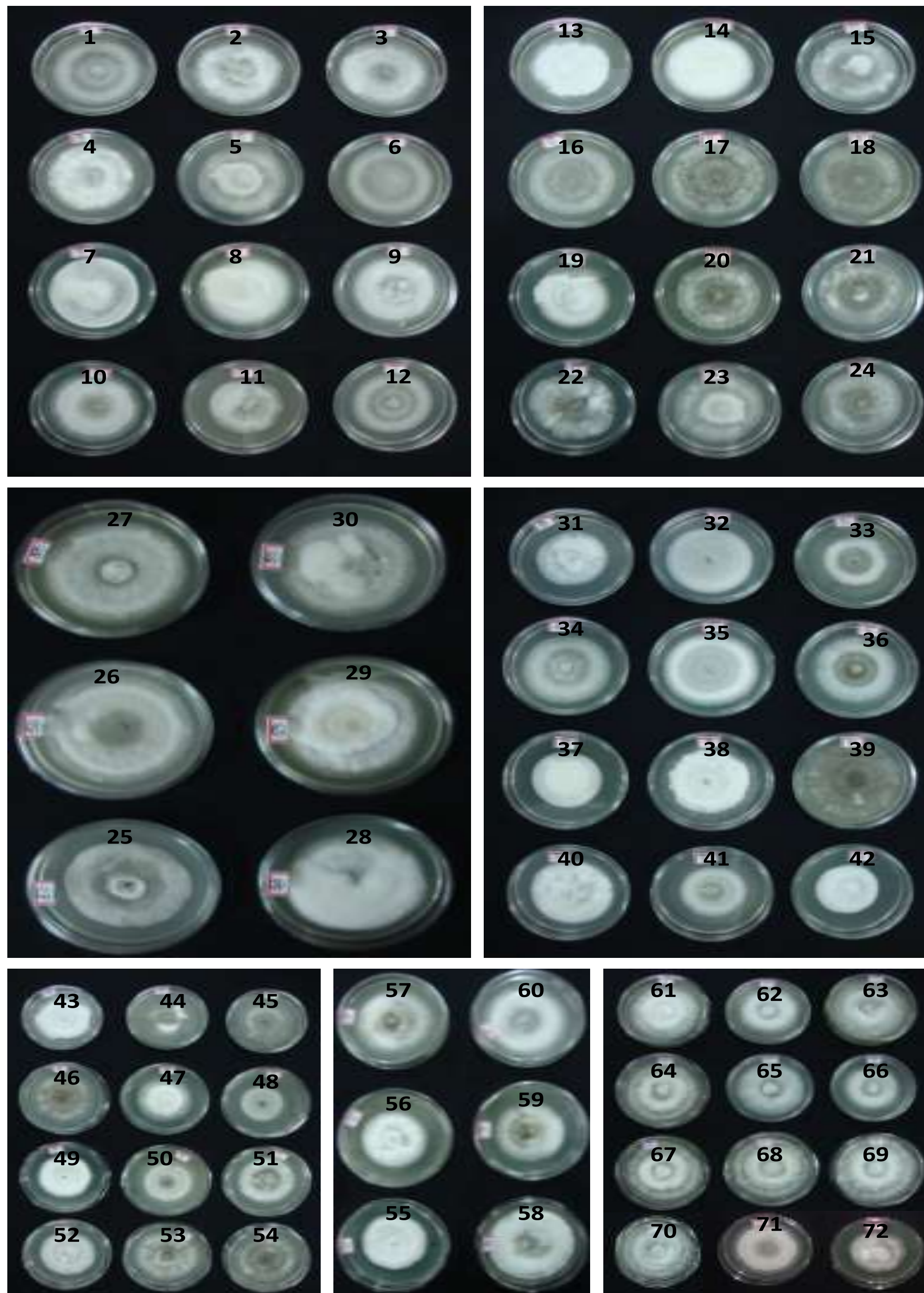

Figure 1. Sixteen-day old cultures of *Magnaporthe oryzae* isolates grown on oat meal agar collected from different geographical locations of Karnataka

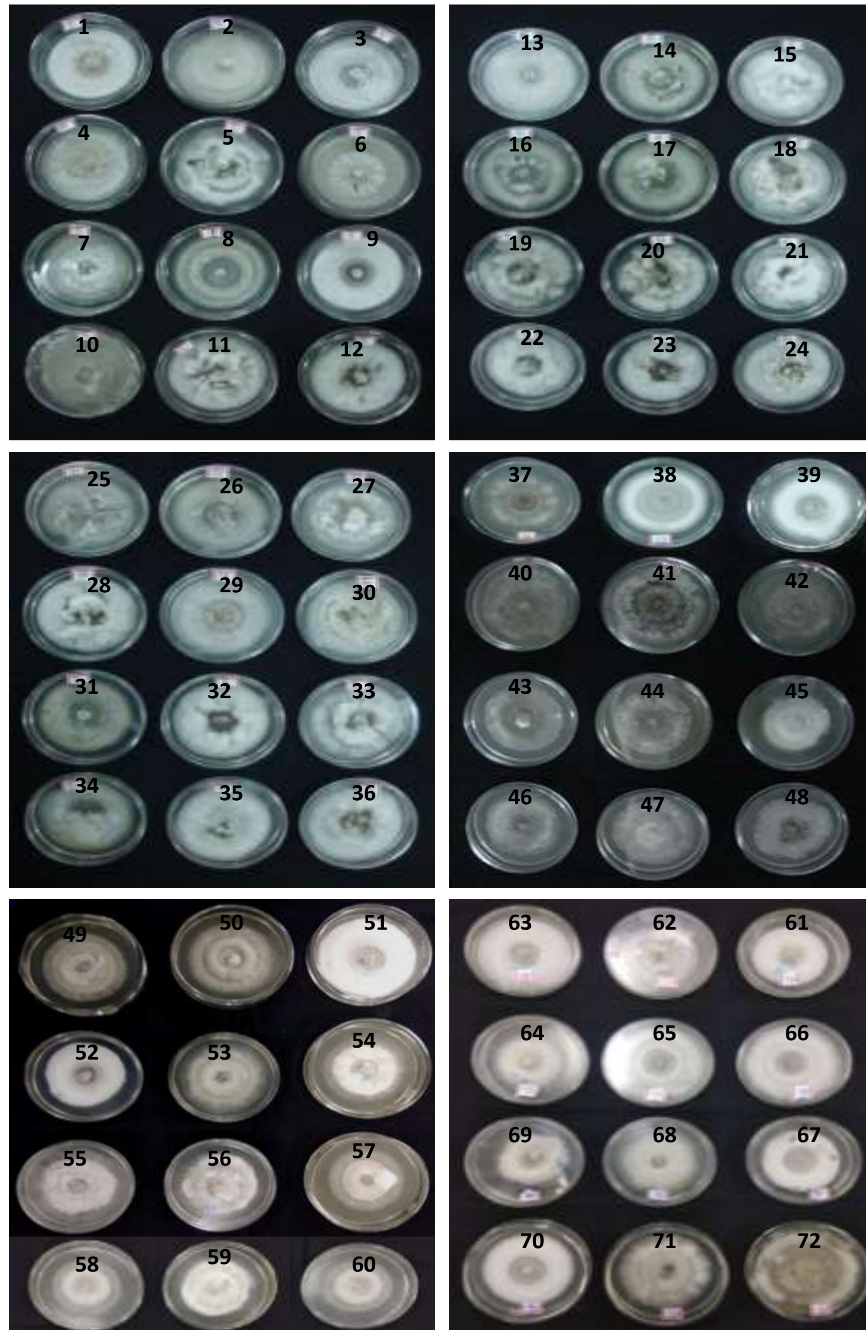

Figure 2. Sixteen-day old cultures of *Magnaporthe oryzae* isolates grown on potato dextrose agar collected from different geographical locations of Karnataka

Table 1. Cultural characteristics of 72 isolates of *Magnaporthe oryzae* on oat meal agar collected from different geographical locations of Karnataka, India

Isolate	Growth pattern	Sector formation	Colour of the medium	Colour of the mycelium	Texture/Surface	Sporulation
CKDS01	Compact	No	Brownish black	Grayish white	Rough surface	Uniform
CKHM02	Cottony	Yes	Bluish black	Grayish white	Smooth surface	Sector
CKHR03	Cottony	Yes	Black	Grayish white	Smooth surface	No
CKHR04	Cottony	No	Brown	Grayish white	Smooth surface	No
CKKL05	Cottony	Yes	Black	Grayish white	Rough surface	No
CKML06	Subdued	No	Black	Grayish white	Smooth surface	No
CKST07	Cottony	No	Black	Grayish white	Smooth surface	Uniform
CKST08	Cottony	No	Brown	Grayish white	Smooth surface	No
CKTR09	Cottony	No	Black	Grayish white	Smooth surface	Uniform
CYAG10	Compact	No	Bluish black	Grayish white	Smooth surface	Uniform
CYJM11	Cottony	Yes	Black	Grayish white	Smooth surface	Uniform
CYKT12	Compact	No	Bluish black	Grayish white	Rough surface	No
CYKS13	Cottony	Yes	Brown	Grayish white	Smooth surface	No
CYKK14	Cottony	No	Black	Grayish white	Smooth surface	No
CYLR15	Cottony	No	Black	Grayish white	Rough surface	No
KMBT16	Cottony	Yes	Black	Grayish white	Rough surface	No
KMHK17	Compact	No	Black	Grayish white	Smooth surface	Uniform
KMHD18	Submerged	No	Black	Grayish white	Rough surface	No
KVAM19	Cottony	Yes	Brown	Grayish white	Smooth surface	No
KVBL20	Subdued	No	Black	Grayish white	Rough surface	No

Table 1. (Con.)

Isolate	Growth pattern	Sector formation	Colour of the medium	Colour of the mycelium	Texture/Surface	Sporulation
KVBG21	Submerged	No	Bluish black	Grayish white	Smooth surface	No
KVBL22	Subdued	Yes	Black	Grayish white	Rough surface	No
KVHG23	Cottony	Yes	Black	Grayish white	Rough surface	No
KVKM24	Subdued	No	Bluish black	Grayish white	Smooth surface	No
KVKR25	Subdued	Yes	Bluish black	Grayish white	Smooth surface	Uniform
KVPN26	Compact	No	Bluish black	Grayish white	Smooth surface	No
KSAB27	Compact	No	Brown	Grayish white	Smooth surface	No
KSHB28	Cottony	Yes	Brown	Grayish white	Smooth surface	No
KSKK29	Cottony	Yes	Brown	Grayish white	Smooth surface	Corners
KSSR30	Submerged	Yes	Bluish black	Grayish white	Rough surface	No
MKKN31	Cottony	Yes	Black	Grayish white	Smooth surface	No
MKMD32	Compact	No	Bluish black	Grayish white	Smooth surface	No
MMMG33	Subdued	Yes	Black	Grayish green	Smooth surface	Sector
MMNG34	Compact	No	Brown	Grayish white	Smooth surface	Uniform
MMAD35	Cottony	No	Bluish black	Grayish white	Smooth surface	No
MMKO36	Compact	No	Black	Grayish green	Rough surface	No
MMGV37	Subdued	No	Brown	Grayish white	Smooth surface	No
MMSV38	Cottony	No	Brown	Grayish white	Smooth surface	No
MMVF39	Subdued	Yes	Brown	Grayish green	Rough surface	No
MMVF40	Cottony	Yes	Black	Grayish white	Smooth surface	center
MPAK41	Subdued	No	Black	Grayish white	Rough surface	Uniform

Table 1. (Con.)

Isolate	Growth pattern	Sector formation	Colour of the medium	Colour of the mycelium	Texture/Surface	Sporulation
MPDY42	Subdued	No	Brown	Grayish white	Rough surface	Uniform
MPPD43	Cottony	No	Brownish black	Grayish white	Smooth surface	No
MSCH44	Submerged	No	Bluish black	Grayish white	Smooth surface	No
MSKM45	Submerged	No	Black	Grayish white	Rough surface	Center
MHHM46	Submerged	No	Bluish black	Grayish white	Rough surface	Sector
MHSR47	Cottony	No	Black	Grayish white	Smooth surface	No
MHHN48	Subdued	No	Blackish green	Grayish white	Smooth surface	Uniform
MKBD49	Cottony	No	Black	White aerial	Smooth surface	No
MKHB50	Cottony	Yes	Blackish green	Grayish white	Smooth surface	No
MKML51	Cottony	Yes	Black	Grayish green	Rough surface	Center
MMKL52	Cottony	No	Brown	Grayish white	Rough surface	No
MMNG53	Subdued	Yes	Black	Grayish white	Rough surface	No
MMSD54	Submerged	No	Black	Grayish white	Rough surface	Uniform
MNHJ55	Cottony	Yes	Brown	Grayish white	Rough surface	No
MNHU56	Cottony	No	Bluish black	Grayish white	Smooth surface	No
MNHM57	Compact	Yes	Bluish black	Grayish white	Smooth surface	Center
MNRM58	Cottony	Yes	Brownish green	Grayish white	Rough surface	No
MTHR59	Compact	Yes	Bluish black	Grayish green	Smooth surface	No
MTSS60	Compact	No	Black	Grayish white	Smooth surface	Uniform
BBEM061	Cottony	No	Bluish black	Grayish white	Rough surface	No
BHDS62	Compact	No	Black	Grayish white	Smooth surface	No

Table 1. (Con.)

Isolate	Growth pattern	Sector formation	Colour of the medium	Colour of the mycelium	Texture/Surface	Sporulation
BHBG63	Cottony	Yes	Black	Grayish white	Smooth surface	Uniform
DDNS64	Cottony	Yes	Black	Grayish white	Rough surface	Center
DDMU65	Cottony	No	Bluish black	Grayish white	Smooth surface	No
HHCC66	Compact	No	Black	Grayish white	Rough surface	Uniform
HHHM67	Cottony	Yes	Brown	Grayish white	Smooth surface	No
KGGV68	Cottony	Yes	Black	Grayish green	Smooth surface	No
RRKD69	Cottony	Yes	Black	Grayish white	Smooth surface	No
SBGN70	Subdued	Yes	Black	Grayish white	Smooth surface	No
SBBM71	Compact	No	Black	Grayish white	Smooth surface	Uniform
SSHB72	Compact	Yes	Black	Grayish white	Smooth surface	Sector

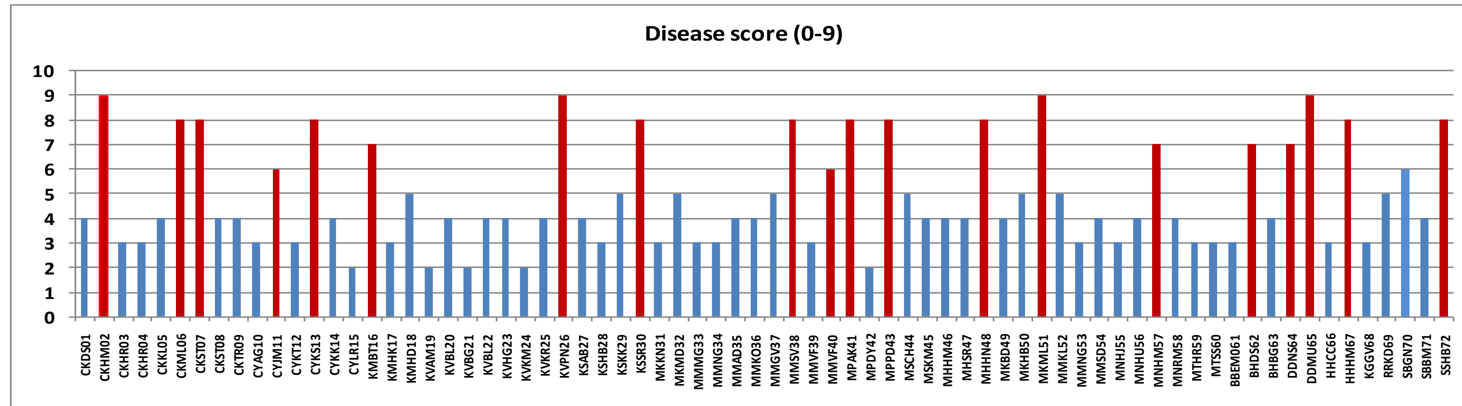

Figure 3. Virulence status of 72 rice blast isolates collected from different agro-climatic zones of Karnataka on susceptible cultivar HR12. (X- axis: Isolates; Y-axis: Numerical value indicates severity of the disease based on 0-9 scale, IRRI 1996)

The growth measurement parameters among 72 isolates of *M. oryzae* on two media and between two media showed the P-value less than 0.05 (P ≤ 0.05) showing that overall, there was a significant growth difference among isolates grown on a single medium as well as between these two growth media. The growth rates of all the isolates on two different media OMA and PDA were compared and grouped individually and obtained homogeneous subsets of 24 and 22 groups respectively when Post Hoc Test was applied. The test also showed the P-value less than 0.05 (P ≤ 0.05) and there was a significant growth difference among isolates grown on individual medium.

Pathogenicity test

Disease reactions of all the 72 isolates are given below (Figure 3). The isolates showing disease reaction ranging from 6 to 9 are indicated in red vertical bars (twenty isolates) and these are selected for isozyme analysis.

Isozyme analysis

SDS-PAGE protein profile of different isolates of *M. oryzae* showed common protein bands ranging from 30 KDa to 150 KDa in all *M. oryzae* isolates selected for isozyme study.

In catalase isozyme analysis 21 scorable bands were observed (Figure 4) with Rm value ranging from 0.37-0.44. There were six polymorphic bands found on the gel. Isolate KMBT16 showed the double band with the Rm of 0.37 and 0.40. In rest of the isolates, protein profile showed single band.

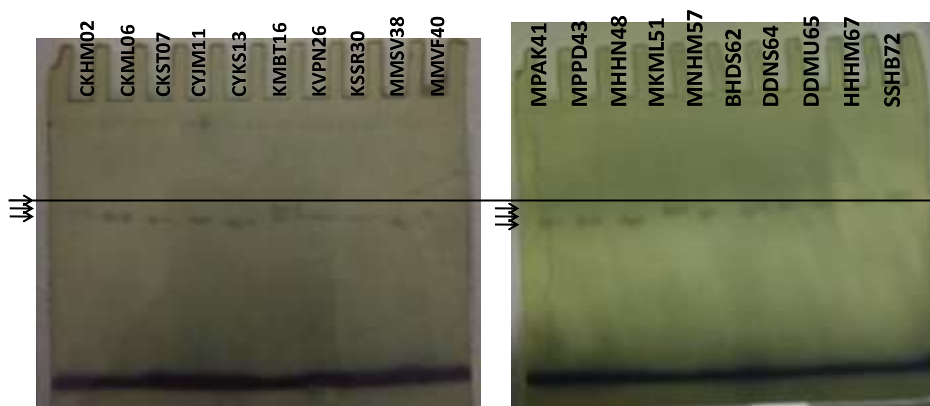

Figure 4. Catalase isozyme profile of 20 *Magnaporthe oryzae* isolates

Esterase analyses results showed a total of 65 scorable bands (Figure 5) with relative mobility (Rm) values ranging from 0.15-0.46 and eight distinct polymorphic bands. Isolate CYKS13 and MKML51 showed the presence of only two bands each with the absence of third band at the Rm site 0.18 and 0.40.

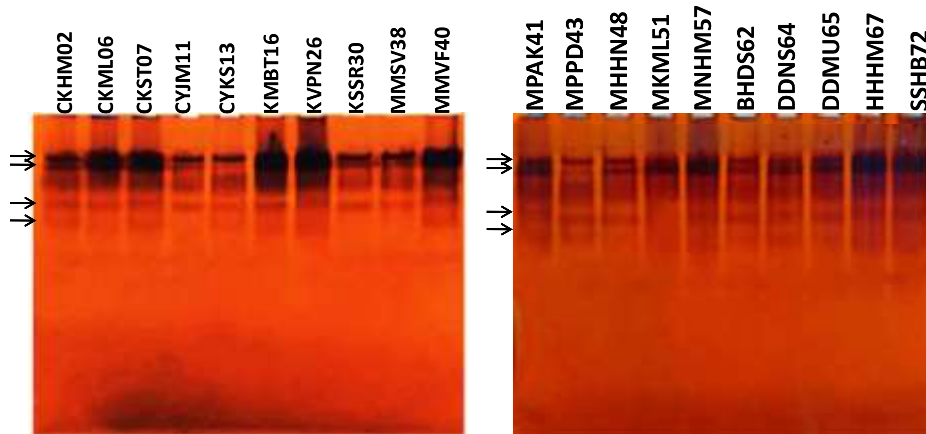

Figure 5. Esterase isozyme profile of 20 *Magnaporthe oryzae* isolates

In protease isozyme analysis 69 scorable bands were detected (Figure 6) with Rm value ranging from 0.10-0.42 with four polymorphic bands. Isolates MKML51, HHHM67, CKHM02 and CYKS13 did not show the band at Rm 0.35. Similarly, isolates MPAK41, MPPD43 and MNHM57 missed the common band at Rm 0.42 compared to rest of the isolates.

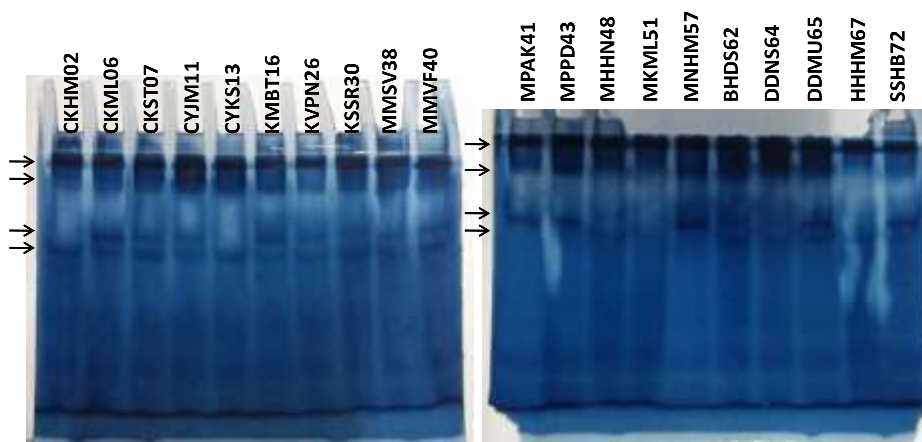

Figure 6. Protease isozyme profile of 20 *Magnaporthe oryzae* isolates

Cluster analysis carried out using simple matching coefficient and UPGMA resulted in the grouping of highly virulent 20 *M. oryzae* isolates. This was based on isozyme profile of three enzymes which showed two major clusters I and II with one outlier for each group CYKS13 and MKML51. The coefficient of similarity among these isolates ranged from 0.26 to 1. Cluster I is formed from the isolates from south Karnataka. Cluster II is formed from the other regions namely central and north Karnataka. In cluster II isolates BHDS62, DDNS64, DDMU65 and SSHB72 formed a separate subgroup (Figure 7).

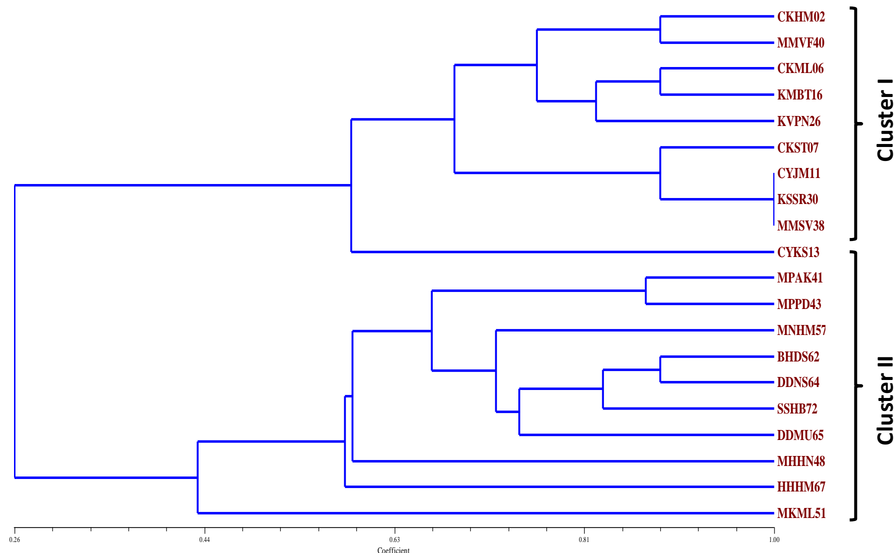

Figure 7. Dendrogram derived from isozyme banding patterns of 20 isolates of *Magnaporthe oryzae* obtained from different districts of Karnataka, India based on simple matching similarity coefficient (UPGMA) using NTSYs software

Discussion

The study on differential responses of *M. oryzae* isolates obtained from different host plants with relation to their growth characteristics on different cultural media was the preliminary step to understand the morphological variations. Many researchers reported the growth characteristics response of this pathogen in different media (Khadka *et al*., 2012; Vanaraj *et al*., 2013; Panda *et al*., 2017; Yashaswini *et al*., 2017). In our investigation, cultural morphology of 72 *M. oryzae* isolates on OMA and PDA varied significantly. An array of growth pattern, sector formation, colour of media and mycelia, texture and sporulation were observed among the 72 isolates cultured from different geographical locations of Karnataka. Teli *et al*. (2016) reported that

PDA and OMA were found to be the best media for radial growth of *M. grisea* isolated from Ponnampet. Previous report also showed that growth of *P. oryzae* was optimum on PDA by Vanaraj *et al*. (2013). Similar result was observed when the pathogen was grown on these two media by Gaitonde *et al*. (2016). In our study, *M. oryzae* growth rate was not significantly different in OMA and PDA medium. Among these two media, the *M. oryzae* on OMA showed a compact growth up to 22% with maximum grayish white mycelium (85%) compared to PDA where compact growth found was up to 21% and colour of the grayish mycelium was seen in 64% of the isolates. Interestingly, 50% of cottony growth and 43% sector formation was observed in colonies grown on both media. Overall colony diameters of different isolates varied from 50 mm to 86 mm. A single isolate showed two different cultural morphologies on different media. This could be due to the difference in the composition of nutrients, nature of the pathogen to utilize these nutrients, moisture available in two different media. This morphological variation pattern may be influenced by the environmental condition from which the diseased samples were collected and also genetic variation existing among the isolates. This type of study would help to understand the extent of morphological diversity among the *M. oryzae* isolates with a clue on the existence of variations at biochemical, physiological and genetic levels.

Biochemical parameters *viz.,* isozyme analyses of 20 isolates of *M. oryzae* showed a significant level of variations among the isolates with the coefficient of similarity ranging from 0.26 to 1. A total of 17 polymorphic bands were observed with an average of 5.66 bands for each marker. In UPGMA cluster analysis, two distinct clusters were formed in which isolates obtained from southern districts formed separate cluster that is cluster I and few of the isolates collected from northern region of Karnataka (BHDS62, DDNS64, DDMU65 and SSHB72) formed separate subgroup in the cluster II. This suggested that there is a correlation among the geographical boundaries and isolate clusters obtained based on UPGMA analysis.

Information on isozyme profiles of *M. oryzae* is lacking regarding the pathogen isolates of Karnataka as compared to other fungi like *Fusarium oxysporum*, *Ustilago hordei* and *Phytophthora* species (Hellmann and Christ, 1991; Bhuvanendra *et al*., 2010; Sumana *et al*., 2014). In 2006, Meena used peroxidase and polyphenol oxidase for isozyme analyses to identify the variations among the isolates collected from northern Karnataka region. Other than this study none of the researchers reported the use of isozyme analysis of this fungus in Karnataka. Current study has thrown light on the extent of variation existing among the isolates of 20 highly virulent isolates collected from different agro climatic locations which has given us information on the

diversity of virulent isolates at biochemical level where these enzymes are probably used by pathogen isolates as stable chemical weapons during infection and invasion.

The electrophoretic banding pattern is dependent on number of alleles present at a given locus which in turn is dependent on the nuclear conditions and also quaternary structure of the enzymes (Micales *et al*., 1992; Richardson *et al*., 2012). Variations in banding patterns shown in the current study may be caused by the expression of multiple alleles. Further characterization of these enzymes in *M. oryzae* is required at molecular level to find out whether they are formed from isozymes or allozymes. Occurrence of these forms of enzymes in virulent strains may be an adaptation shown by these isolates to varying agroclimatic zones.

Isozyme markers are neutral as these are not exposed to the tough selection pressures of host and can overcome resistance in the rice varieties unless these parameters are also considered during breeding (Newton, 1987). Thus, the extent of morphological diversity and diversity in the enzyme profiles identified during the current investigation appear to be possible adaptation strategies of the pathogen for its survival and virulence in varied environmental conditions.

Acknowledgements

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