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RESEARCH ARTICLE

DISTRIBUTION OF MATING TYPE ALLELES AND FERTILITY OF *MAGNAPORTHE ORYZAE* ISOLATES IN SOUTH INDIA.

[*]Khaled Fathy[1] and Prashanthi S. K.[2].

1. Genetics Department, Molecular Genetics Lab., Faculty of Agriculture, Zagazig University, 44519, Egypt.
2. Department of Biotechnology, IABT, University of Agricultural sciences, Dharward, Karnataka, 580005, India.

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Abstract

Ninety seven isolates of *Magnaporthe oryzae* collected from different locations of south India were evaluated to understand the mating type distribution and fertility of isolates by using MAT locus specific primers, forty six isolates showed mating MAT1-1, forty two showed mating type MAT1-2, seven isolates showed hermaphrodite and two unknown mating type. The observation that both mating-types were present in the states and districts of south India, suggests the possibility of sexual recombination in nature and this can affect diversity and dissemination. Clonal reproduction and sexual recombination may be the possible reasons for the population dynamics of *M. oryzae* in South India. Populations showing evidence for both types mating types may be assayed for fertility using a range of tester isolates from different regions. As locally obtained hermaphrodite isolates of both mating types from this study may be used as testers for a systematic survey of local field isolates in future.

Introduction:

Maqnaporthe oryzae, which causes rice blast, one of the top pathogens which has the ability to cause high level of losses in rice field. The life cycle of *Magnaporthe oryzae* involves both asexual and sexual reproduction. Asexual propagation can be initiated following standard laboratory procedures. Asexual spores (conidia) are easy to collect and can be used in rice or barley infection assays to evaluate virulence. Although the sexual cycle is rarely observed under field conditions, in the laboratory the sexual cycle can be induced using specific culture conditions, these are some of the characteristics that have made *Magnaporthe oryzae* an attractive system to perform genetic analysis and molecular biology (Valent *et al.*, 1986).

Main sources of variation in *Magnaporthe oryzae* populations, high capacity of asexual reproduction, Mutation and migration (Zeigler *et al.*, 1994). Parasexual recombination was found to be reason of variation in rice blast population even in putatively clonal populations (Zeigler *et al.*, 1997). Sexually fertile of *Magnaporthe oryzae* was known to be rare and found in few areas of Southeast Asia.

Magnaporthe oryzae is a heterotallic fungus, sexual reproduction occurs when mycelia of *Magnaporthe oryzae* of opposite mating contact each other. Sexual recombination controlled by a single locus (MAT1) that presents two mating types MAT1-1 and MAT1-2 which are required for sexual recombination (Kang *et al.*, 1994).

Corresponding Author: Khaled Fathy.
Address:- Genetics Department, Molecular Genetics Lab. Faculty of Agriculture, Zagazig University, 44519, Egypt.

The fertile interactions only possible between individuals of opposite mating type designated MAT1-1 (Male fertile) and MAT1-2 (Female fertile). However, the capacity of *M. grisea* isolates to produce perithecia (female fertility) is apparently controlled by genes at several loci, and these segregate independently of mating type and pathogenicity on different hosts (Kolmer and Ellingboe, 1988). In *Magnaporthe oryzae*, meiosis is immediately followed by a single mitotic division to generate four pairs of sister ascospores grouped into a single ascus. The ascospores are formed within the sexual structures (perithecia) produced by the female-fertile strain (or by both strains if both are female-fertile). Perithecia are flask shaped bodies that carry asci-bags containing ascospores, the products of meiosis- in abundance. Asci can be dissected to liberate the ascospores, which are arranged as un ordered octads. In either case the segregation patterns of genetic markers can be readily followed and the genetic basis of phenotypic traits determined (Valent *et al.,* 1991).

In fertility survey results were generally consistent in that female sterility was the norm, fertile isolates were typically male-fertile only, and mating type ratios were skewed (Notteghem and Silué, 1992; Yaegashi and Nishihara, 1976). This rarity of female fertility was consistent with the reported abundance of transposable elements in *Magnaporthe grisea* rice pathogens (Zeigler, 1998; Zeigler *et al.,* 1994). Translocations and non homologous crossover events can result in meiotic failure. Where the environment permits long-term persistence of well-adapted asexual lineages, mutations can accumulate at loci mediating fertility and result in a predominance of infertility in populations (Kistler and Miao,1992; Zeigler, 1998). In the late 1980s, hermaphroditic isolates were used as tester strains as well as in back-crossing programs to develop fertile rice pathogens of both mating types (Chao and Ellingboe, 1991).

Hermaphroditic isolates have been reported from southern China (Chengyun *et al*., 1992) and the Indian Himalayas (Kumar and Zeigler, 1995). However, a study from southern India reported no sexually fertile rice pathogens (Devulapalle and Suryanarayanan, 1995; Viji and Gnanamanickam, 1998).

Mekwatanakarn *et al.* (1999) studied mating type distribution and sexual fertility of *Magnaporthe grisea* isolates collected from different locations in Thailand. Three hundred forty-one single conidium isolates of *M. grisea* collected from five sites in north, northeast, and central Thailand were evaluated in vitro for sexual fertility and mating type. Ascospores were detected in isolates which isolated from the north-eastern and northern regions. Sixty seven per cent of *Magnaporthe grisea* isolates were infertile when crossed with the hermaphrodite strains. In bioassay mating type, fifty to seventy five per cent of isolates showed male fertility MAT1-1 and fifty to eighty five per cent of isolates showed MAT1-2 mating type from all locations in Thailand, hermaphroditic isolates were also detected. Back crossing of *Magnaporthe grisea* with weeping love grass (*Eragrostis curvularia*) was developed to show high level of fertility (Valent *et al.,* 1991).

Regulation of mating is controlled by a single mating type locus that contains genes that encode putative transcription factors which play role in transcription regulators of pheromone production and reception. Sexual mating compatibility in *Magnaporthe oryzae* is governed by the presence of two alleles of the mating type locus MAT1. The mating-type locus is found in the mating partner as an idiomorph, a non-homologues DNA sequence and gene set at the same chromosomal position. Ascogonia that are fertilized by male structures, which can be conidia, or spermatia (Metzenberg and Glass 1990; Coppin *et al.,* 1997). MAT locus could be linked to an avirulence gene, with no compatibility with the prevailing Rice cultivar, and this could represents a drawback for fitness, being eliminated along time (Notteghem and Silué, 1992).

Mycelium tufts were observed between crossed *Magnaporthe oryzae* cultures, new haplotypes were found, indicating the presence of recombination (Zeigler *et al.,* 1997). Crossing can occurs between fertile isolates, *Magnaporthe oryzae* isolates also found to be hermaphrodite (Zeigler, 1998).

When two *Magnaporthe oryzae* populations from Himalayas were analyzed, it was found that twenty two per cent of the isolates belonged to MAT1-1 and forty three per cent to MAT1-2, and hermaphrodite and male fertile isolates were found, suggesting a possible occurrence of sexual recombination in this Himalayan region. The structure of some *Magnaporthe oryzae* populations may be affected by sexual recombination which gives dynamic behave and diverse among populations (Kumar *et al.,* 1999).

There was a consensus that the majority of the *Magnaporthe oryzae* populations lost its viability of sexual reproduction. It was proposed that only one mating type could be found when new rice cultivar was planted in a

particular area (Zeigler, 1998). In Argentina, analysis of one hundred twenty five isolates collected between 2000 and 2003 showed that all of them belonged to mating type MAT1-1. Similar results were obtained in Korea, when the analysis of two hundred fifty four isolates demonstrated that all of them also belonged to MAT1-1 (Park *et al.*, 2008). Twenty nine per cent isolates belongs to MAT1-1 and seventy one per cent belongs to MAT 1-2 in Africa, but none of the isolates was hermaphrodite, avoiding crossing among them (Takan *et al.*, 2011).

DNA mobile genetic elements can cause chromosomic rearrangement and degeneration of sexual behavior without producing a drawback of fitness (Chuma *et al.*, 2011). One role of the sexual reproduction is to form resistance structures to enable the fungi to adjust against unfavourable conditions (www.intechopen.com, Klaus, Brazil).

Populations of *Magnaporthe oryzae* worldwide were characterized genetically to identify sexual reproduction (Saleh *et al.* 2012[a]). The two mating types in one Chinese population and almost all strains were female-fertile. Viable progenies of *Magnaporthe oryzae* were produced in vitro, sexual reproduction in *Magnaporthe oryzae* population was confirmed with the help of genotypic richness and linkage disequilibrium data. Computer simulations confirmed that the observed genetic characteristics were unlikely to have arisen in the absence of recombination. The authors concluded that *Magnaporthe oryzae*, population reproduces sexually in nature in Southeast Asia and evidenced the loss of sexual reproduction by a fungal plant pathogen outside its centre of origin.

Mating type distribution and lineage diversity was studied; sexual recombination might be the one reason for lineage diversity in *Magnaporthe oryzae* in fields of rice growing regions in North-East and Eastern states of India (Imam *et al.*, 2014).

Soma *et al.* (2014) studied the mating type alleles as a marker to measure population diversity in forty six isolates of *Magnaporthe oryzae* from various ecosystems of coastal Odisha, India. Mating types MAT1-1 and MAT1-2 was found in all the ecosystems in uplands and in irrigated fields. In irrigated ecosystem fields MAT1-1 and MAT1-2 could be found. The disease spreads was very fast in rice fields resulting in blast lesions looking as green islands was found in isolates showed MAT1-2.

Materials and methods:

Analysis of mating type alleles in *Magnaporthe oryzae* isolates:

In current study 97 *Magnaporthe Oryzae* isolates collected from different places in south India (Table 1) were studied for mating type analysis by *Mat* gene specific molecular markers. The pure culture of each isolate was grown in Rice straw Broth medium for approximately 8-15 days at room temperature (25-28^{o}C) to produce mycelia.

Mycelium mats of ninety seven blast isolates of *Magnaporthe oryzae* was used for genomic DNA isolation by adopting Cetyl Trimethyl Ammonium Bromide CTAB / NaCl method. Agarose gel electrophoresis was done to check the quality of DNA by running on 0.8% agarose (*Lonza*, USA) and DNA was quantified by using Nano drop spectrophotometer (ND-1000 V3.5.2, Nano Drop Technologies Inc., USA).

Amplification of *MAT* gene:

Mating type genes *MAT1-1* and *MAT1-2* were amplified according to Wang *et al.* (2004) using two pairs of specific primers. MAT1-1 Forward Primer (TCAGCTCGCCCAAATCAACAAT) and Reverse primer (ACTCAAGACCCGGCACGAACAT) yield a product of 809 bp and MAT1-2 Forward Primer (GAGTTGCCTGCCCGCTTCTG) and Reverse primer (GGCTTGGTCGTTGGGGATTGT) yield a product of 940 bp. Amplifications were performed in a final volume of 20 µl of reaction mixture: 10x Taq assay buffer, 2.5 mM dNTPs, 10 picoMole for forward and reverse primers, Taq DNA polymerase XT-5 PCR system (GeNei^{TM},Merck Biosciences, Bengalure) 3 units/µl, Template DNA 50 ng/µl and sterile distilled Millipore water. The reaction mixture was given a momentary spin for mixing of the reaction components except DNA template. Master mixture was distributed to all 0.2 ml PCR tubes and finally 1 µl of respective DNA template was added and short spin was given to mix template with all reaction components and then tubes were loaded in a thermal cycler. Thermal cycler conditions; 95°C Initial denaturation for 3 min, followed by 35 cycles of 95°C for 45 sec, The annealing temperature 70 ^{o}C for MAT1-1 and 71 ^{o}C for MAT1-2 for 1.45 min, 72°C for 2 min and a final extension step at 72°C for 7 min. PCR reaction was carried out using Master gradient 5331- Eppendorf version 2.30.31-09, Germany. Two per cent (6

g/ 300 ml) agarose was added to 1X TAE buffer (pH-8.0) for PCR separation, Ethidium bromide 12 µl was used as a staining agent. The gel was run at 8 V/cm for 1 to 1.5 hours and bands were visualized and documented in gel documentation system (Model Alpha Imager 1200, Alpha Innotech Corp, USA). Based on the results, 97 isolates were grouped into Male fertile, Female fertile, Hermaphrodite and Unknown based on presence or absence of MAT allele. Mating type frequencies were calculated.

Table 1:- Details of *Magnaporthe oryzae* isolates used for molecular study

Sl. No.	State	District	Place of collection	Host variety	Type of blast	Geographic coordinates	Code
1	Karnataka	Dharwad	A.R.S., Mugad	HR-12	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-105
2	Karnataka	Dharwad	A.R.S., Mugad	Intan Mugad	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-106
3	Karnataka	Dharwad	A.R.S., Mugad	Honasu	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-107
4	Karnataka	Dharwad	A.R.S., Mugad	Nyareminda	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-108
5	Karnataka	Dharwad	A.R.S., Mugad	Kiravanna	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-109
6	Karnataka	Dharwad	A.R.S., Mugad	Kari Esadi	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-110
7	Karnataka	Dharwad	A.R.S., Mugad	Pramod	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-111
8	Karnataka	Dharwad	A.R.S., Mugad	Jeerigesanna1	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-112
9	Karnataka	Dharwad	A.R.S., Mugad	Jaddu batta	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-113
10	Karnataka	Dharwad	A.R.S., Mugad	Intan	Neck blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-114
11	Karnataka	Dharwad	A.R.S., Mugad	Jirga	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-115
12	Karnataka	Dharwad	Mugad	Intan	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-16b
13	Karnataka	Dharwad	A.R.S., Mugad	Ashoka 200F	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-116
14	Karnataka	Dharwad	A.R.S., Mugad	Chippiga	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-200
15	Karnataka	Dharwad	A.R.S., Mugad	Gum Kadle	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-201
16	Karnataka	Dharwad	Mugad	Unknown	Neck blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-202
17	Karnataka	Dharwad	A.R.S., Mugad	Kempu Dodda	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-203
18	Karnataka	Dharwad	A.R.S., Mugad	Billi Hege	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-204
19	Karnataka	Dharwad	A.R.S., Mugad	Jondole Jeerge	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-205
20	Karnataka	Dharwad	A.R.S., Mugad	Bangar sanna	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-206
21	Karnataka	Dharwad	A.R.S., Mugad	Ambemohr-1	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-207
22	Karnataka	Dharwad	A.R.S., Mugad	Gum Kadle	Neck blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-208
23	Karnataka	Dharwad	A.R.S., Mugad	BPT5204	Neck blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-102
24	Karnataka	Dharwad	A.R.S., Mugad	Jeerige sanna-2	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-209
25	Karnataka	Dharwad	A.R.S., Mugad	BPT5204	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-210
26	Karnataka	Dharwad	A.R.S., Mugad	DHB1	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-99
27	Karnataka	Dharwad	A.R.S., Mugad	DHB4	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-100
28	Karnataka	Dharwad	Nigadi	Sali	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-211
29	Karnataka	Kodagu	Ponnampet	Asha	Leaf blast	12° 12′ N, 75° 54′ E; 851 m above sea level	Mo-si-048
30	Karnataka	Kodagu	Ponnampet	DHB-30	Leaf blast	12° 12′ N, 75° 54′ E; 851 m above sea level	Mo-si-117
31	Karnataka	Kodagu	Ponnampet	DHB-25	Leaf blast	12° 12′ N, 75° 54′ E; 851 m above sea level	Mo-si-118
32	Karnataka	Kodagu	Ponnampet	Unknown	Leaf blast	12° 12′ N, 75° 54′ E; 851 m above sea level	Mo-si-119
33	Karnataka	Kodagu	Ponnampet	HR-12	Leaf blast	12° 12′ N, 75° 54′ E; 851 m above sea level	Mo-si-120
34	Karnataka	Kodagu	Ponnampet	Mysore	Leaf blast	12° 12′ N, 75° 54′ E; 851 m above sea level	Mo-si-050
35	Karnataka	Mandya	Z.A.R.S., Mandya	NSN	Leaf blast	12° 31′ N, 76° 54′ E; 113 m above sea level	Mo-si-081
36	Karnataka	Mandya	Unknown	KMP	Neck blast	12° 31′ N, 76° 54′ E; 113 m above sea level	Mo-si-083
37	Karnataka	Mandya	Z.A.R.S., Mandya	Jaya	Leaf blast	12° 31′ N, 76° 54′ E; 113 m above sea level	Mo-si-079
38	Karnataka	Mandya	Unknown	Unknown	Neck blast	12° 31′ N, 76° 54′ E; 113 m above sea level	Mo-si-077
39	Karnataka	Mandya	Z.A.R.S., Mandya	Jaya	Neck blast	12° 31′ N, 76° 54′ E; 113 m above sea level	Mo-si-080
40	Karnataka	Mandya	Malavalli	IR64	Leaf blast	12° 22′ N, 77° 4′ E; 610 m above sea level	Mo-si-086
41	Karnataka	Mandya	Yamanahalli	MTU1001	Leaf blast	12° 35′ N, 77° 3′ E; 662 m above sea level	Mo-si-076a
42	Karnataka	Mandya	Maddur	MTU1001	Leaf blast	12° 35′ N, 77° 3′ E; 662 m above sea level	Mo-si-076b
43	Karnataka	Mandya	Unknown	HR-12	Leaf blast	12° 31′ N, 76° 54′ E; 113 m above sea level	Mo-si-082
44	Karnataka	Mandya	Unknown	AVT-IM T7	Neck blast	12° 31′ N, 76° 54′ E; 113 m above sea level	Mo-si-084
45	Karnataka	Mandya	Z.A.R.S., Mandya	BPT5204	Leaf blast	12° 31′ N, 76° 54′ E; 113 m above sea level	Mo-si-212
46	Karnataka	Raichur	Sirvar	Sona	Leaf blast	16° 10′ N, 77° 1′ E; 380 m above sea level	Mo-si-027
47	Karnataka	Raichur	Kavithal	Sona	Leaf blast	16° 10′ N, 76° 1′ E; 380 m above sea level	Mo-si-030
48	Karnataka	Raichur	Kalmala	Sona	Leaf blast	16° 10′ N, 77° 1′ E; 380 m above sea level	Mo-si-033
49	Karnataka	Raichur	Kelgaala Kamp	Sona	Leaf blast	16° 10′ N, 77° 1′ E; 380 m above sea level	Mo-si-032
50	Karnataka	Raichur	Yaraagera	Sona	Leaf blast	16° 10′ N, 77° 1′ E; 380 m above sea level	Mo-si-028
51	Karnataka	Raichur	Sindanoor	Sona	Leaf blast	16° 10′ N, 77° 1′ E; 380 m above sea level	Mo-si-029
52	Tamil Nadu	Erode	Bhavanisagar	ADT-29	Leaf blast	11° 28′ N, 77° 7′ E; 329 m above sea level	Mo-si-087
53	Tamil Nadu	Erode	Bhavanisagar	Deluxe	Leaf blast	11° 28′ N, 77° 7′ E; 329 m above sea level	Mo-si-88
54	Tamil Nadu	Coimbatore	Gudalore	NSN	Leaf blast	11° 30' N, 76° 30' E;1071 m above sea level	Mo-si-92
55	Tamil Nadu	Coimbatore	Gudalore	Bharti	Leaf blast	11° 30' N, 76° 30' E;1071 m above sea level	Mo-si-89
56	Tamil Nadu	Coimbatore	Near Gudloor	Gandhakasala	Leaf blast	11° 30' N, 76° 30' E;1071 m above sea level	Mo-si-93
57	Tamil Nadu	Coimbatore	Near Gudloor	Gandhakasala	Neck blast	11° 30' N, 76° 30' E;1071 m above sea level	Mo-si-94
58	Tamil Nadu	Coimbatore	Gudalore	Bharti	Panical blast	11° 30' N, 76° 30' E;1071 m above sea level	Mo-si-91
59	Tamil Nadu	Coimbatore	Gudalore	Bharti	Neck blast	11° 30' N, 76° 30' E;1071 m above sea level	Mo-si-90
60	Tamil Nadu	Coimbatore	Gudalore	ADT-29	Leaf blast	11° 30' N, 76° 30' E;1071 m above sea level	Mo-si-87
61	Karnataka	Udupi	Kavadigrama	IET	Leaf blast	13°19' N, 74°44'E; 215 m above sea level	Mo-si-55
62	Karnataka	Udupi	Brahavar	Purple Putta	Leaf blast	13°19' N, 74°44'E; 215 m above sea level	Mo-si-121
63	Karnataka	Udupi	Brahavar	GMS-23-121	Leaf blast	13°19' N, 74°44'E; 215 m above sea level	Mo-si-122
64	Karnataka	Udupi	Brahavar	Mo 4117	Leaf blast	13°19' N, 74°44'E; 215 m above sea level	Mo-si-123
65	Karnataka	Uttara Kannada	ARS Sirsi	Unknown	Leaf blast	14° 20′ N, 74° 26′ E; 48 m above sea level	Mo-si-036
66	Karnataka	Uttara Kannada	Haliyal	Jaya	Leaf blast	14° 20′ N, 74° 26′ E; 48 m above sea level	Mo-si-034
67	Karnataka	Uttara Kannada	Kumata	Rasi	Neck blast	14° 20′ N, 74° 26′ E; 48 m above sea level	Mo-si-060
68	Karnataka	Koppal	Unknown	IET	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-004

69	Karnataka	Koppal	Karatagi	BPT Rabi	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-003
70	Karnataka	Koppal	Unknown	G-B-30	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-007a
71	Karnataka	Koppal	Gangavathi	BPT5204	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-007
72	Karnataka	Koppal	Gangavathi	HR-12	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-005
73	Karnataka	Koppal	Gangavathi	BPT5204	Neck blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-006
74	Karnataka	Koppal	A.R.S., Gangavathi	DHB-19	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-124
75	Karnataka	Koppal	A.R.S., Gangavathi	DHA	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-125
76	Karnataka	Koppal	Sirguppa	Sona	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-214a
77	Karnataka	Koppal	Sirguppa	Sona	Neck blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-214b
78	Karnataka	Koppal	Unknown	G-B-30	Neck blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-si-215
79	Karnataka	Koppal	Unknown	A.B.Godi	Leaf blast	15° 25′ N, 76° 31′ E; 406 m above sea level	Mo-ni-216
80	Karnataka	Chikkamangalore	Mudegeri	Gamsali	Leaf blast	13° 32′ N, 75° 77′ E;1926 m above sea level	Mo-si-15a
81	Karnataka	Chikkamangalore	Mudegeri	Gamsali	Neck blast	13° 32′ N, 75° 77′ E;1926 m above sea level	Mo-si-15b
82	Karnataka	Chikkamangalore	Mudegeri	Gamsali	Seeds	13° 32′ N, 75° 77′ E;1926 m above sea level	Mo-si-15c
83	Karnataka	Chikkamangalore	Anegal	Chandibatta	Leaf blast	13° 32′ N, 75° 77′ E;1926 m above sea level	Mo-si-11
84	Karnataka	Chikkamangalore	Somvarpete	Holesalu chippiga	Leaf blast	13° 32′ N, 75° 77′ E;1926 m above sea level	Mo-si-13
85	Karnataka	Chikkamangalore	Unknown	Doddibatta	Leaf blast	13° 32′ N, 75° 77′ E;1926 m above sea level	Mo-si-12
86	Karnataka	Shimogga	Talaguppa	Intan	Leaf blast	13° 56′ N, 75° 34′ E; 569 m above sea level	Mo-si-23
87	Karnataka	Tumkur	Kunigal	Rasi	Leaf blast	13° 1′ N, 75° 34′ E; 773 m above sea level	Mo-si-78
88	Karnataka	Kalaburgi	Bheemrayanagudi	Sujatha	Leaf blast	16° 43′ N, 76° 47′ E; 441 m above sea level	Mo-si-53
89	Karnataka	Hassan	Sakleshpura	Intan	Leaf blast	12° 58′ N, 75° 46′ E; 949 m above sea level	Mo-si-98a
90	Kerala	Kasaragod	Mavangal	Athira	Leaf blast	10° 17′ N, 76° 32′ E; 80 m above sea level	Mo-si-64
91	Andhra Pradesh	Vizianagaram	Vizianagaram	MTU1010	Leaf blast	18° 7′ N, 83° 25′ E; 66 m above sea level	Mo-si-63
92	Uttar Pradesh	Meerut	Unknown	Basmati rice	Leaf blast	28° 59′ N, 77° 42′ E; 224 m above sea level	Mo-si-66
93	Kerala	Kasaragod	Narlan	Aravadha pille	Leaf blast	12° 30′ N, 75° 0′ E; 19 m above sea level	Mo-si-74
94	Karnataka	Ankola	Haladipura	Rasi	Leaf blast	14° 66′ N, 74° 3′ E; 16 m above sea level	Mo-si-60
95	Karnataka	Ankola	Apsarakonda	Jyoti	Leaf blast	14° 66′ N, 74° 3′ E; 16 m above sea level	Mo-si-61
96	Tamil Nadu	Unknown	Unknown	Gandhakasala	Leaf blast	11° 30' N, 76° 30' E;1071 m above sea level	Mo-si-93a
97	Karnataka	Dharwad	Mugad	BPT	Leaf blast	15° 27′ N, 74° 55′ E; 687 m above sea level	Mo-si-217

Determination of mating type and fertility status of unknown isolates:

The mating types of Mo-si-88 and Mo-si-15a (unknown mating types isolates) were determined by pairing each isolates three times with seven hermaphroditic testers Mo-si-007, Mo-si-215, Mo-si-15c, Mo-si-53, Mo-si-98a, Mo-si-076a and Mo-si-084.

Crosses were made by pairing in Petri dishes (TARSONS Petri dish with triple vent Radiation Sterile Manufactured by: Tarsons Products Pvt. Ltd.) containing Rice Straw Agar supplemented with streptomycin by pairing actively growing mycelia of *Magnaporthe oryzae* isolates and cultures were observed under phase contrast microscope **(Nikon Digital Sight ECLIPSE E600 - Olympus)** Nikon's NIS-Elements microscope imaging software used. All Observations, measurements and Microphotographs were taken and noted under this study. Sporulation process was observed in 4-5 microscopic fields. Sporulation rate was divided as Nil, Poor, Fair, Good and Excellent. The isolates were monitored regularly for the development of sexual structures for 1-3 months. Itoi *et al.*, 1983 described the way how we can identify the fertility status of unknown isolates (Fig.1).

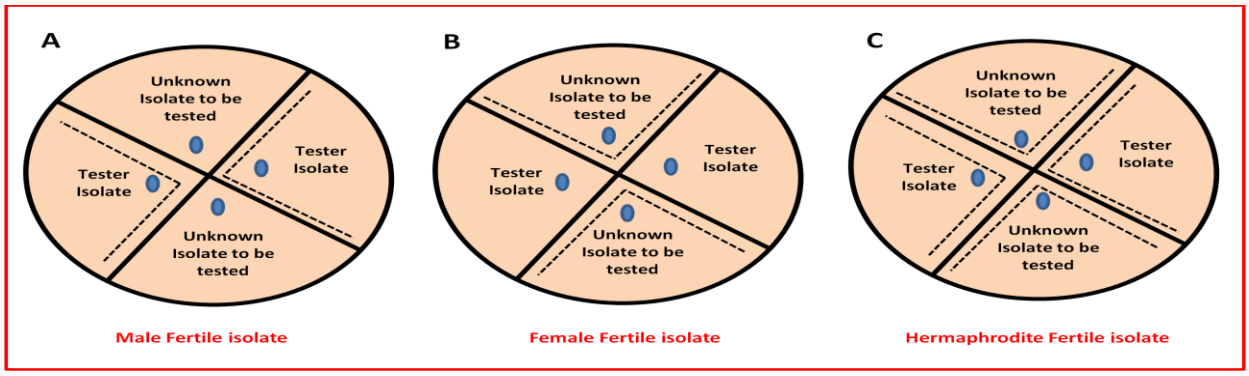

Fig. (1): Determination of mating type and fertility status of unknown isolates: A. Male fertile isolates induce perithecia on the tester isolate side. **B.** When perithecia formed on the side of unknown isolates, cultures considered as female fertile. **C.** Hermaphrodite isolates produce perithecia on both sides of the merger line (interface) between the unknown isolate and the tester isolate.

Results:

A total of ninety seven isolates of *Magnaporthe oryzae* collected from different locations of south India were evaluated to understand the mating type distribution and fertility of isolates by using MAT locus specific primers (Table 2). MAT1-1 (Male fertile) mating type generated the amplicon of 809 bp (Fig.2) where as MAT1-2 (Female fertile) yielded amplicon size of 940bp (Fig.3).

Table 2: Mating types distribution of *Magnaporthe oryzae* based on mating type alleles of MAT locus

Sr.No.	Isolate ID	Place of collection	Host variety	MAT1-1	MAT1-2	Male Fertile	Female Fertile	Hermaphrodite
1	*Mo-si-105*	A.R.S., Mugad	HR-12	+	-	✓		
2	*Mo-si-106*	A.R.S., Mugad	Intan L.B	+	-	✓		
3	*Mo-si-107*	A.R.S., Mugad	Honasu	-	+		✓	
4	*Mo-si-108*	A.R.S., Mugad	Nyareminda	+	-	✓		
5	*Mo-si-109*	A.R.S., Mugad	Kiravanna	+	-	✓		
6	*Mo-si-110*	A.R.S., Mugad	Kari Esadi	+	-	✓		
7	*Mo-si-111*	A.R.S., Mugad	Pramod	+	-	✓		
8	*Mo-si-112*	A.R.S., Mugad	Jeerigesanna	+	-	✓		
9	*Mo-si-113*	A.R.S., Mugad	Jaddu batta	+	-	✓		
10	*Mo-si-114*	A.R.S., Mugad	Intan N.B	+	-	✓		
11	*Mo-si-115*	A.R.S., Mugad	Jirga	+	-	✓		
12	*Mo-si-16b*	Mugad	Intan	+	-	✓		
13	*Mo-si-116*	A.R.S., Mugad	Ashoka 200F	+	-	✓		
14	*Mo-si-200*	A.R.S., Mugad	Chippiga	+	-	✓		
15	*Mo-si-201*	A.R.S., Mugad	Gum Kadle	+	-	✓		
16	*Mo-si-202*	Mugad	Unknown N.B	+	-	✓		
17	*Mo-si-203*	A.R.S., Mugad	Kempu Dodda	+	-	✓		
18	*Mo-si-204*	A.R.S., Mugad	Billi Hege	+	-	✓		
19	*Mo-si-205*	A.R.S., Mugad	Jondole Jeerge	+	-	✓		
20	*Mo-si-206*	A.R.S., Mugad	Bangar sanna	-	+		✓	
21	*Mo-si-207*	A.R.S., Mugad	Ambemohr-1	-	+		✓	
22	*Mo-si-208*	A.R.S., Mugad	Gum KadleN.B	+	-	✓		
23	*Mo-si-102*	A.R.S., Mugad	BPT5204	+	-	✓		
24	*Mo-si-209*	A.R.S., Mugad	Jeerige sanna-2	-	+		✓	
25	*Mo-si-210*	A.R.S., Mugad	BPT5204	+	-	✓		
26	*Mo-si-99*	A.R.S., Mugad	DHB1	-	+		✓	
27	*Mo-si-100*	A.R.S., Mugad	DHB4	-	+		✓	
28	*Mo-si-211*	Unknown2012	Unknown	-	+		✓	
29	*Mo-si-048*	Ponnampet	Asha	+	-	✓		
30	*Mo-si-117*	Ponnampet	DHB-30	-	+		✓	
31	*Mo-si-118*	Ponnampet	DHB-25	-	+		✓	
32	*Mo-si-119*	Ponnampet	Unknown	-	+		✓	
33	*Mo-si-120*	Ponnampet	HR-12	-	+		✓	
34	*Mo-si-050*	Ponnampet	Mysore	+	-	✓		
35	*Mo-si-081*	Z.A.R.S., Mandya	NSN	+	-	✓		
36	*Mo-si-083*	Unknown	KMP N.B	-	+		✓	
37	*Mo-si-079*	Z.A.R.S., Mandya	Jaya	-	+		✓	
38	*Mo-si-077*	Unknown	Unknown NB	-	+		✓	
39	*Mo-si-080*	Z.A.R.S., Mandya	Jaya N.B	-	+		✓	
40	*Mo-si-086*	Malavalli	IR64	-	+		✓	
41	*Mo-si-076a*	Yamanahalli	MTU1001	+	+			✓
42	*Mo-si-076b*	Maddur	MTU1001	-	+		✓	
43	*Mo-si-082*	Unknown	HR-12	-	+		✓	
44	*Mo-si-084*	Unknown	AVI-IM T7N.B	+	+			✓
45	*Mo-si-212*	Z.A.R.S., Mandya	BPT5204	-	+		✓	
46	*Mo-si-027*	Sirvar	Sona	+	-	✓		
47	*Mo-si-030*	Kavithal	Sona	-	+		✓	
48	*Mo-si-033*	Kalmala	Sona	+	-	✓		
49	*Mo-si-032*	Kelgaala Kamp	Sona	+	-	✓		
50	*Mo-si-028*	Yaraagera	Sona	+	-		✓	
51	*Mo-si-029*	Sindanoor	Sona	-	+		✓	
52	*Mo-si-087*	Bhavanisagar	ADT-29	-	+		✓	
53	*Mo-si-88*	Bhavanisagar	Deluxe	-	-			Unknown
54	*Mo-si-92*	Gudalore	NSN	+	-	✓		
55	*Mo-si-89*	Gudalore	Bharti	+	-	✓		
56	*Mo-si-93*	Near Gudloor	Gandhakasala	+	-	✓		
57	*Mo-si-94*	Near Gudloor	GandhakasalaN.B	+	-	✓		
58	*Mo-si-91*	Gudalore	Bharti seeds	-	+		✓	
59	*Mo-si-90*	Gudalore	Bharti N.B	-	+		✓	
60	*Mo-si-87*	Gudalore	ADT-29	+	-	✓		
61	*Mo-si-55*	Kavadigrama	IET	-	+		✓	
62	*Mo-si-121*	Brahavar	Purple Putta	-	+		✓	
63	*Mo-si-122*	Brahavar	GMS-23-121	+	-	✓		
64	*Mo-si-123*	Brahavar	Mo 4117	+	-	✓		
65	*Mo-si-036*	ARS Sirsi	Unknown	-	+		✓	
66	*Mo-si-034*	Haliyal	Jaya	+	-	✓		
67	*Mo-si-060*	Kumata	Rasi N.B	+	-	✓		
68	*Mo-si-004*	Unknown	IET	+	-	✓		
69	*Mo-si-003*	Karatagi	BPT Rabi	-	+		✓	
70	*Mo-si-007a*	Unknown	GB-30	+	-	✓		
71	*Mo-si-007*	Gangavathi	BPT5204	+	+			✓
72	*Mo-si-005*	Gangavathi	HR-12	-	+		✓	
73	*Mo-si-006*	Gangavathi	BPT5204 N.B	-	+		✓	
74	*Mo-si-124*	A.R.S., Gangavathi	DHB-19	-	+		✓	

75	***Mo-si-125***	A.R.S., Gangavathi	DHA	-	+		✓	
76	***Mo-si-214a***	Sirguppa	Sona L.B	-	+		✓	
77	***Mo-si-214b***	Sirguppa	Sona N.B	-	+		✓	
78	***Mo-si-215***	Unknown	GB-30 N.B	+	+			✓
79	***Mo-ni-216***	Unknown	A.B.Godi	+	-	✓		
80	***Mo-si-15a***	Mudegeri	Gamsali L.B	-	-			Unknown
81	***Mo-si-15b***	Mudegeri	Gamsali N.B	-	+		✓	
82	***Mo-si-15c***	Mudegeri	Gamsali seeds	+	+			✓
83	***Mo-si-11***	Unknown	Chandibatta	+	-	✓		
84	***Mo-si-13***	Somvarpete	Holesalu chippiga	+	-	✓		
85	***Mo-si-12***	Unknown	Doddibatta	+	-	✓		
86	***Mo-si-23***	Talaguppa	Intan	-	+		✓	
87	***Mo-si-78***	Kunigal	Rasi	-	+		✓	
88	***Mo-si-53***	Bheemrayanagudi	Sujatha	+	+			✓
89	***Mo-si-98a***	Sakleshpura	Intan	+	+			✓
90	***Mo-si-64***	Mavangal	Athira	-	+		✓	
91	***Mo-si-63***	Vizianagaram	MTU1010	-	+		✓	
92	***Mo-si-66***	Unknown	Basmati rice	-	+		✓	
93	***Mo-si-74***	Narlan	Aravadha pille	+	-	✓		
94	***Mo-si-60***	Haladipura	Rasi	+	-	✓		
95	***Mo-si-61***	Apsarakonda	Jyoti	-	+		✓	
96	***Mo-si-93a***	Unknown	Gandhakasala	-	+		✓	
97	***Mo-si-217***	Mugad	BPT	+	-	✓		

Among 97 isolates 46 isolates were MAT1-1 mating type accounting to 47.42 per cent frequency. 42 isolates were positive for MAT1-2 mating type with 43.29 per cent frequency. About seven isolates were hermaphrodite type with 7.21 per cent frequency and two isolates showed unknown mating type, which showed negative amplification for both MAT1-1 and MAT1-2 gene specific primers (Fig.4).

The highest frequencies of isolates that could be typed for MAT1-1 were detected in Dharward around 22 isolates with 75.86 per cent frequency (Fig.5), while MAT1-2 type recorded 24.13 per cent frequency in 7 isolates. MAT1-2 (Female fertile) was predominant in Kodagu isolates with 66.66 per cent of samples were female fertile where as MAT1-1 accounted 33.33 per cent frequency. No hermaphrodite and unknown mating types were observed in these two locations.

MAT1-1, MAT1-2 and hermaphrodite isolates were traced in Mandya cultures collection with 72.72 per cent frequency for MAT1-2, 9 per cent for MAT1-1 type and two isolates Mo-si-076a Yamanahalli (MTU1001L.B) and Mo-si-084 M.A.R.S. Mandya (Unknown, N.B) showed hermaphrodite isolates with 18.18 per cent frequency.

Both the mating types were observed in isolates collected from Raichur district which represents irrigated rice ecology and Uttara kannada district which is rainfed ecology encompassing MAT1-1 mating type with 60 per cent frequency and MAT1-2 mating type with 40%. Isolates collected from Koppal district contained MAT1-1, MAT1-2 and hermaphrodite mating types with 25, 58.33 and 16.66 per cent frequency respectively. Two isolates from Gangavathi and unknown location sample showed hermaphrodite mating type Similarly Chikkamangalore isolates showed MAT1-1, MAT1-2, hermaphrodite and unknown mating types with 50, 16.66, 16.66 and 16.66 per cent frequency respectively. In this location two of the isolates showed no amplification for either of the two mating types.

Kerela isolates consisting of only two cultures which showed MAT1-1 and MAT1-2 type1:1 ratio. Tamil Nadu rice blast isolates results showed 40 per cent of samples presented as MAT1-2, 50 per cent frequency recorded for MAT1-1 and 10 per cent unknown mating types. No hermaphrodite type was observed in Tamil Nadu isolates.

Different locations isolates in south India from Shimogga, Tumkur, Kalaburgi, Hassan, Vizianagaram and Meerut, showed 66.66 per cent frequency for mating type MAT1-2, and 33.33 per cent for hermaphrodite mating type. Hermaphrodite isolates were noticed, Bhhemrayana Gudi (Sujatha variety) Mo-si-53 isolate and Sakleshpura (Intan Variety) Mo-si-98(a) isolate.

Cultures were checked for sporulation and all of them were highly sporulated cultures (Fig.6) which used in crosses experiment. Production of Perithecia were not seeing when the cross between isolates of the opposite was done.

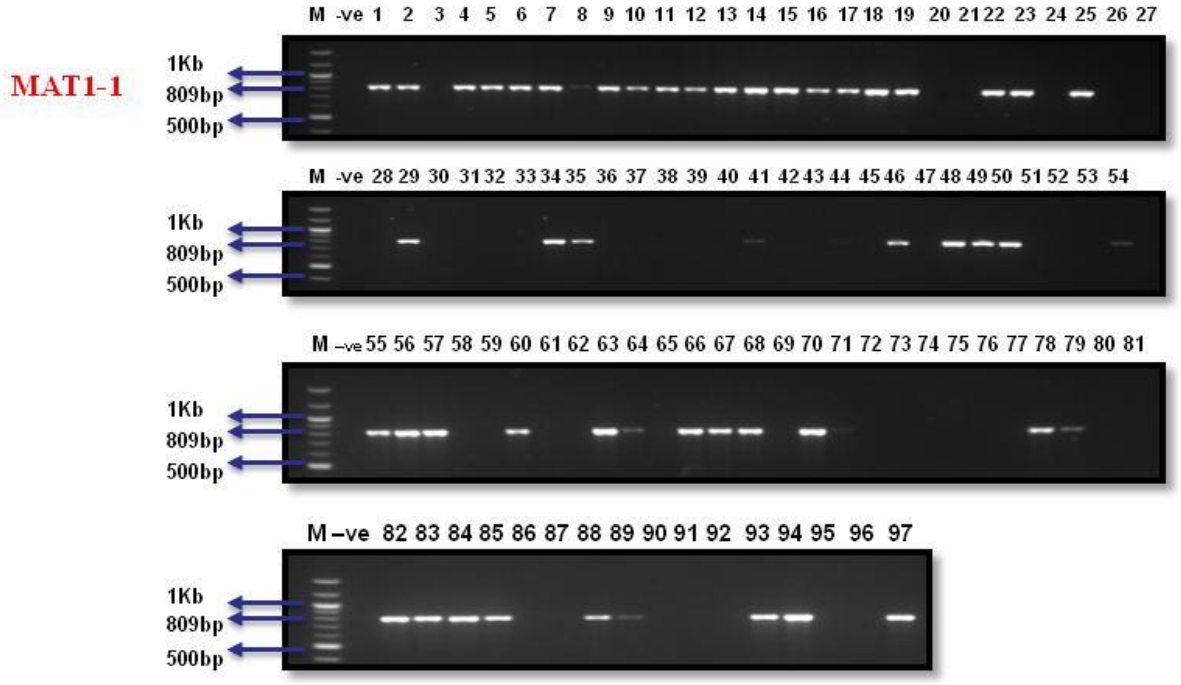

Fig. (2): Amplification of MAT1-1 mating types of *M. oryzae* by gene specific primers

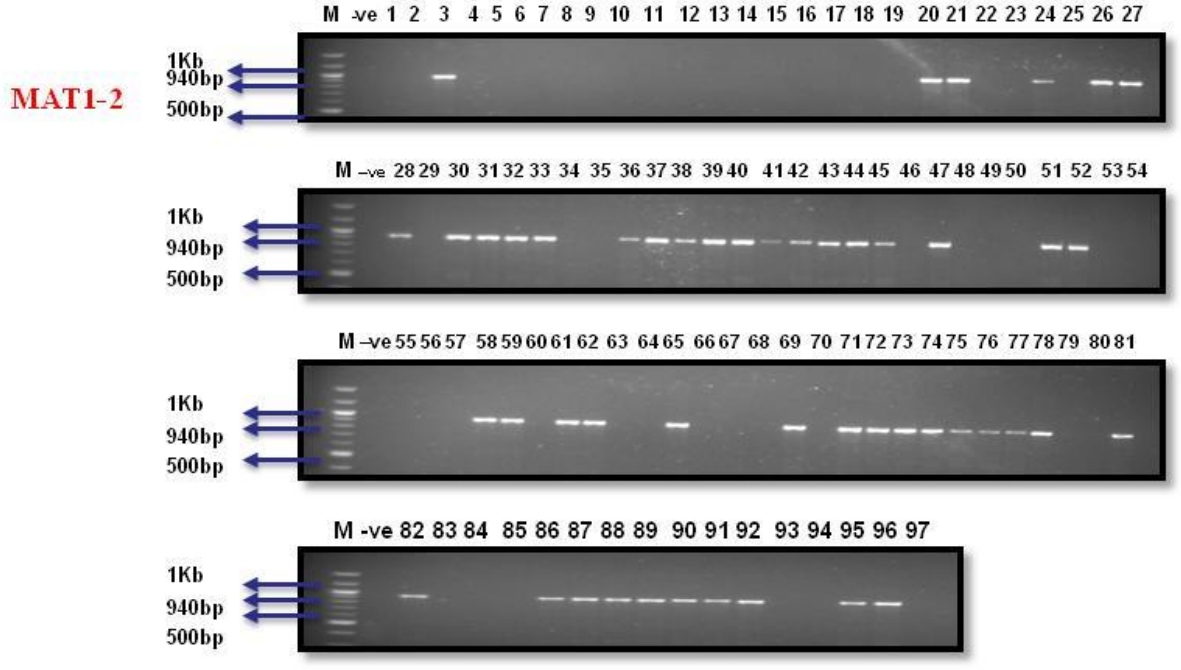

Fig. (3): Amplification of MAT1-2 mating types of *M. oryzae* by gene specific primers

(M- 100bp DNA ladder - -Ve Negative Control - 1-97 *Magnaporthe Oryzae* isolates)

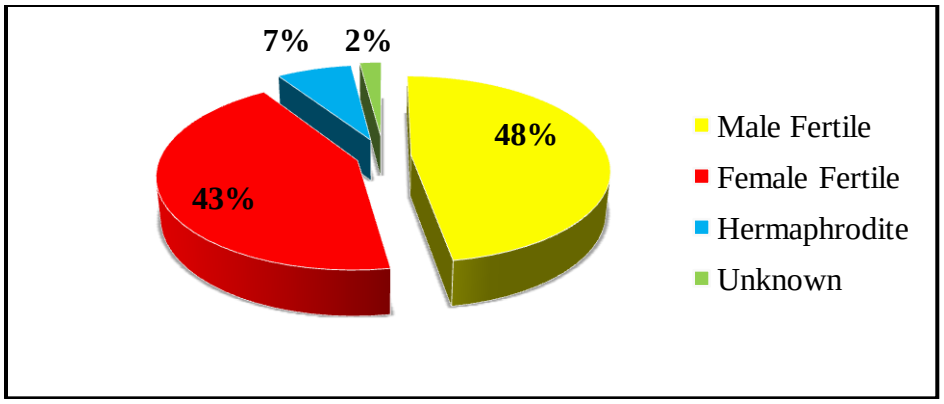

Fig. (4):- Mating types total frequencies in *Magnaporthe oryzae* isolates

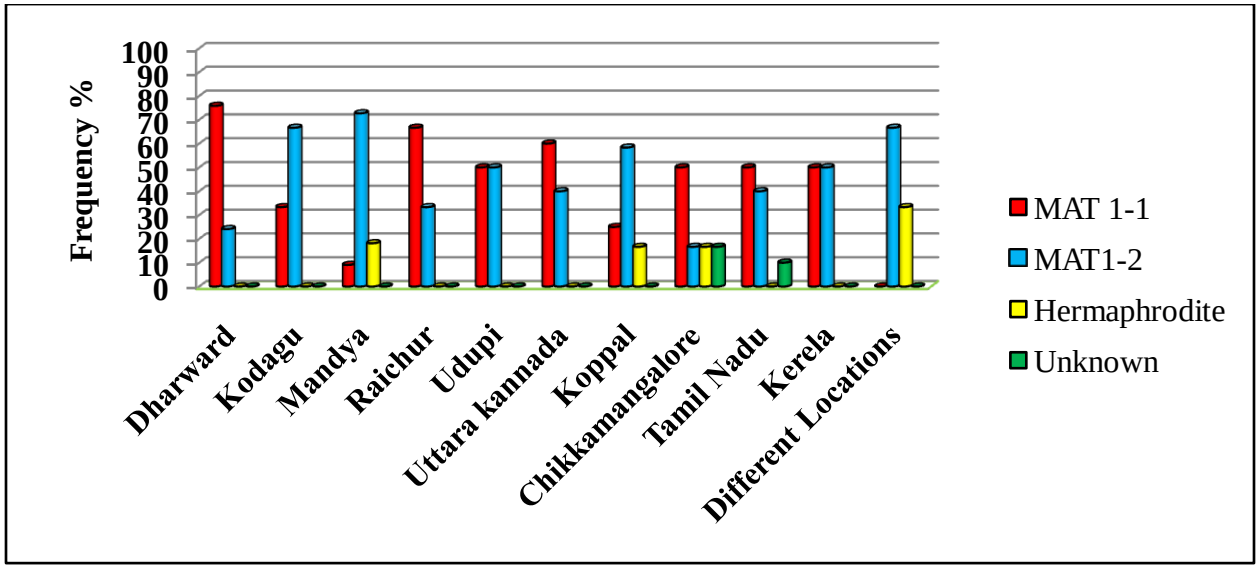

Fig.(5):- District /state wise distribution of mating types in *Magnaporthe oryzae* isolates

Discussion:

Magnaporthe is a hermaphroditic, heterothallic ascomycete (anamorph: *Pyricularia grisea*) pathogenic to a large number of gramineae species. *Magnaporthe oryzae* is known for its high capacity of asexual spore production and wide variation in the field. Both sexual and asexual stages are seen in the life cycles of ascomycetous fungi. In many fungi, including *M. oryzae* sexual cycle is controlled by mating type genes (Consola *et al.*, 2005; Leslie and Klein, 1996 and Zeng *et al.*, 2009). *M. oryzae* can only mate when two fertile opposite mating- types are present. Sexual propagation in *M. oryzae* is controlled by MAT (mating-type) locus, which is represented by two idiomorphs known as MAT1-1 (Male fertile) and MAT1-2 (Female fertile).

Until recently sexually fertile rice isolates were thought to be very rare and variations were attributed to parasexual recombination, mutations and clonal lineages.

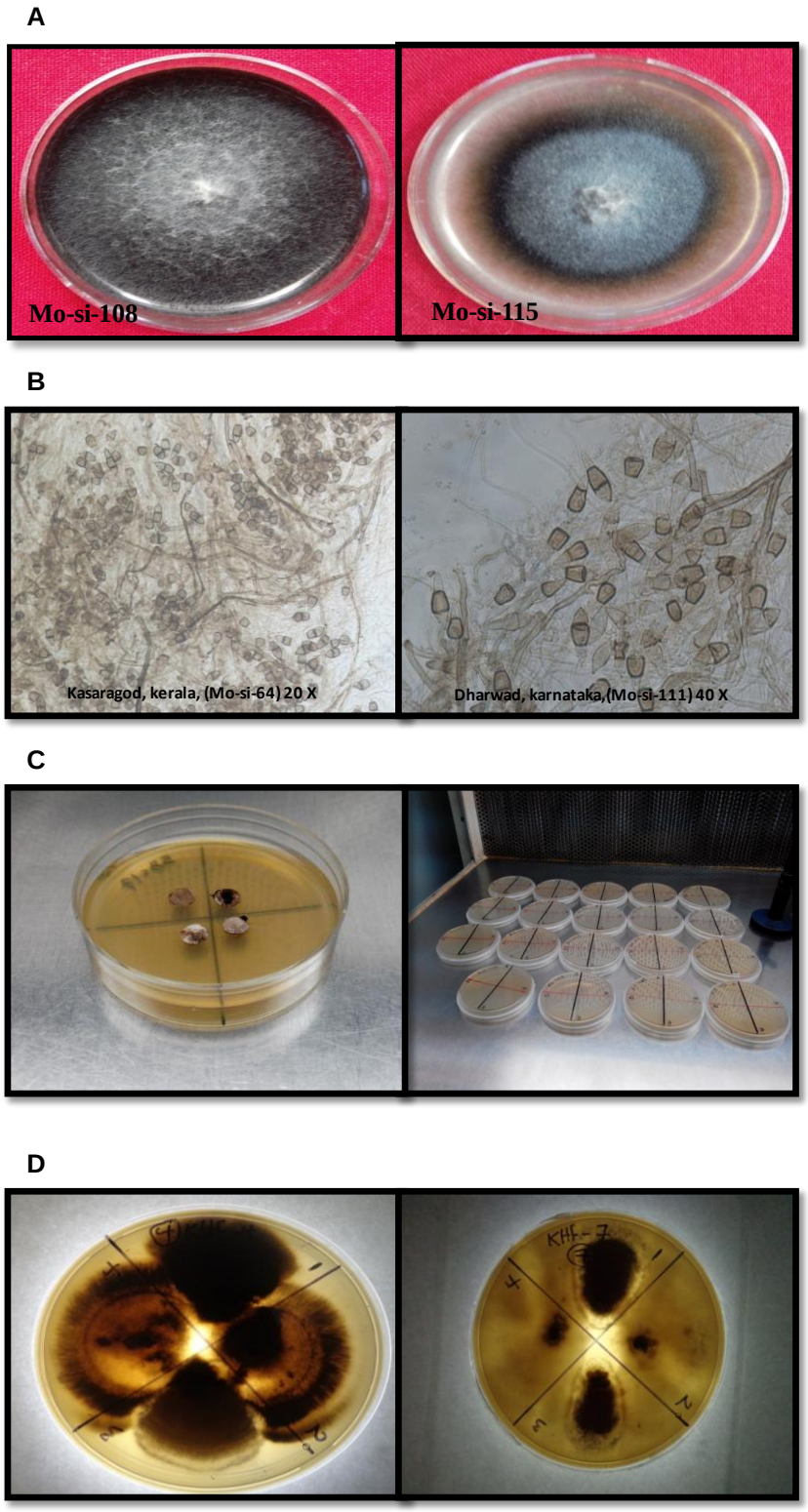

Fig. (6): Cultures, Sporulation and crosses between *Magnaporthe Oryzae* Isolates

A. Two cultures of *Magnaporthe oryzae* isolates on Rice Straw Agar. **B.** Pyriform conidia and highly sporulated *Magnaporthe oryzae*. **C.** *Magnaporthe oryzae* isolates pairing; Testers with unknown isolates using filter paper disk. **D.** Full growth of crossed cultures for mating type determination and fertility status of unknown isolates.

Sexual reproduction can reshuffle genetic material via recombination, thereby bringing together new alleles, which may influence pathogenicity to various rice cultivars and could influence the efficacy of fungicides. Capacity of *M. grisea* isolates to produce perithecia (female fertility) is apparently controlled by genes at several loci, and these segregate independently of mating type and of pathogenicity on different hosts (Mekwatanakarn *et al.,* 1999). In fertility surveys of rice pathogens results were generally consistent in that female sterility was the norm, fertile isolates were typically male-fertile only, and mating type ratios were skewed (Notteghem and Silué, 1992; Yaegashi and Nishihara, 1976). This rarity of female fertility was consistent with the reported abundance of transposable elements in *M. grisea* rice pathogens (Zeigler, 1998; Zeigler *et al.,* 1994). In the late 1980s, hermaphroditic (male and female-fertile) rice isolates were found and subsequently used as tester strains against field isolates as well as in back-crossing programs to develop fertile rice isolates of both mating types (Mekwatanakarn *et al.,* 1999). A study from Southern India reported no sexually fertile rice pathogens (Devulapalle and Suryanarayanan, 1995; Viji and Gnanamanickam, 1998).

However, in our present study among 97 isolates seven isolates showed the amplification for both MAT alleles indicating these isolates are male and female-fertile. Isolates Mo-si-076a, Mo-si-084, Mo-si-007, Mo-si-215, Mo-si-15c, Mo-si-53, Mo-si-98a, are hermaphroditic and hence these can be used as tester strains to identify and clone new avirulence genes in several field isolates. Both male 75.86 per cent and female fertile 24.13 per cent isolates were recovered from a single field of A.R.S Mugad. Similarly in other surveyed locations both mating alleles were recorded. Samples collected from Kodagu, Mandya and Koppal location was dominated by female fertile isolates 58.33 per cent to 72.72 per cent.

Conventional approach to determine mating type in the pathogen population depends upon the appearance of mature perithecia in a cross between known tester and an unknown strain on culture media which is time consuming and requires high technical expertise. PCR amplification methods using MAT gene specific primers are a rapid method to explore the mating type population of *M. oryzae* (Priyadarisini *et al.,* 1999; Notteghem and Silué, 1992: Hayashi *et al.,* 1997, Zheng *et al.,* 2008; Dong *et al.,* 2005).

Comparison of PCR method and the standard strain GUY11 / KA3 mating type assays showed good consensus 95.1 per cent (Bao-Hua *et al.,* 2004). In the present study same set of primers were used to amplify MAT alleles governing fertility in *M. oryzae*. The size of PCR product produced by MAT1-1 and MAT1-2 in this study was similar to the bands observed by earlier workers (Bau-Hua *et al.,* 2004; Soma *et al.,* 2014).

Analysis of mating type can provide an estimation of genetic diversity among *M. oryzae* populations from rice. Genetic diversity and mating-type studies suggested that *M. oryzae* exists as a recombinant population. However, detection of sexual form in natural field conditions and the pathogenicity of its progenies on rice still remain elusive despite the existence of firm populations (Saleh *et al.,* 2012[b]). The presence of both mating-type as detected by PCR based molecular markers are basically useful to identify the mating type locus in the field isolates of *M. oryzae* but not effective to identify the fertile *M. oryzae* strains because fertility is also influenced by genes other than the mating-type genes (Dayakar *et al.,* 2000).

Using this assay, we determined the mating type of 97 isolates from different locations in south India, 46 isolates showed mating type allele MAT1-1 (Male fertile), 42 showed mating type MAT1-2 (Female fertile), 7 isolates showed hermaphrodite and 2 unknown mating type. The observation that both mating-types were present approximately in equal proportion in the population sampled from South India suggesting the possibility of sexual recombination in nature which can affect diversity and dissemination. Two isolates Mo-si-15a from Mudegeri (Gamsali variety, Leaf blast) and Mo-si-88 from Bhavanisagar (Deluxe variety L.B) yielded no amplification consistently under standard conditions by using both MAT primers.

Several previous studies discussed about mating types in *Magnaporthe oryzae*, from various ecosystems of coastal Odisha, India. MAT1-1 mating type was dominating in all the ecosystems and MAT1-2 was found to be present in uplands as well as in irrigated fields. Both mating types could be found in the same field in irrigated ecosystem (Soma *et al*., 2014).

In the present study MAT1-2 and MAT1-1 were found to be present in both in irrigated and upland fields in Raichur and Uttara Kannada respectively. Where ever MAT1-1 and MAT1-2 are there we could expect

variation in the *M. oryzae* population due to recombination. In addition presence of hermaphrodite *M. oryzae* isolates in a location clues the occurrence of wide diversity in its population. Distribution of both the mating type in the same field among field isolates of *M. oryzae* was recorded by earlier workers and suggested the possibility of occurrence of sexual recombination in nature (Kolmer *et al.,* 1988; Kumar and Zeigler 1995).

Kumar *et al.* (1999) suggested possible occurrence of sexual recombination in *M. oryzae* population in Himalayan region. Imam *et al.* (2014) reported the presence of both mating types in North-East and Eastern India, of 63 *M. oryzae* isolates analysed, 16 of the isolates were of the mating type MAT1-1 while 35 were of the mating type MAT1-2 and eleven isolates did not produce a PCR product with either of these two mating types.

If a single mating-type predominates in a particular rice growing region, *M. oryzae* may not be sexually reproducing in that region and this may be important in the population dynamics of this pathogen. In past studies, MAT1-1 has been identified as the dominant mating-type associated with rice (Dayakar *et al.,* 2000). The presence of only MAT1-1 in the pathogens is not uncommon as it was also detected in the *M. oryzae* population in Japan and other regions (Notteghem and Silué, 1992). In this study, it is interesting to note that, in few locations Viz., Mandya, Chikkamangalore, Koppal districts both MAT1-1 and MAT1-2 mating types and hermaphrodite isolates were present. This information draws our attention to study in detail about host-pathogen population in these locations. Hence, in these locations variations due to sexual recombination may be expected in nature. Clonal reproduction and sexual recombination may be the possible reasons for the population dynamics of *M. oryzae* in South India. Populations showing evidence for both types mating types may be assayed for fertility using a range of tester isolates from different regions. As locally obtained hermaphrodite isolates of both mating types from this study may be used as testers for a systematic survey of local field isolates in future.

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