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GENETICS & PLANT BREEDING

Status of *Magnaporthe oryzae* Infection in Different Districts of Karnataka, India and Establishment of Monoconidial Cultures for Understanding Genetic Diversity

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ABSTRACT

Blast disease caused by *Magnaporthe oryzae* B.C. Couch is one of the major production constraints and main pathological threats to rice crop around the world. A total of 171 places were visited and samples were collected from 101 diseased plots from different geographical regions of Karnataka, India during 2012-2014. Different parameters like locations, variety name, age of the crop, type of infection and severity of the disease were recorded. Among the total infection types, leaf blast was found to dominate followed by neck, collar, node and panicle blast. Disease incidence of 64.44%, 68.75%, 60.71% and 55.55% was recorded from major rice growing districts of South Karnataka *viz*., Chamarajanagar, Kodagu, Mandya and Mysuru districts respectively. Varieties MTU-1001, Jyothi and Sona Masuri remained highly susceptible in these four districts. This was followed by Minilong, IR64, Jaya, KCP-1, Rajamudi, Rajbhoga and KRH-4 varieties from different districts of Karnataka. Newly developed blast resistant varieties namely Rasi, KRH 4, Raksha and Mugad Siri 1253 were also found to be susceptible in some of the regions of Karnataka indicating breakdown of resistance. Thus, the emerging pathogenic variants among fungal populations trigger serious and incessant threat to the newly released resistant varieties. Seventy two monoconidial *M. oryzae* isolates were obtained and pure cultures were established for understanding molecular diversity of the pathogen.

Highlights

- Disease severity of *Magnaporthe oryzae* infecting rice ranged from 25% to 68.75% in different districts of Karnataka
- 72 monoconidial isolates were established to understand genetic diversity of the pathogen

Keywords: *Magnaporthe oryzae*, monoconidial isolates, resistant varieties

Rice (*Oryza sativa* L.) is one of the most important cereal crops of family Poaceae. It is the staple food crop of 60% of world's population. Rice has the world's largest area of cultivation. During 2016-17, worldwide cultivated area of rice was around 158.8 million hectares with a total production of 499.2 million tons (FAO 2017). Asia is the largest producer of rice contributing around 91% of total world rice production. The remaining rice production is divided between Africa and Latin America.

More than 90% of the world's rice is grown and consumed in Asian countries. China and India alone are responsible for nearly half of the world's output of rice. Rice is an important staple food crop of India occupying 41.92 million hectares with a production of 163.3 million tons. In Karnataka, rice occupies an area of 14.51 million hectares with a production of 36.58 million tons and a productivity of 2,583 kg/hectare.

Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma and nematodes. Among the fungal diseases, blast is considered

as a major threat to rice production because of its worldwide distribution and its destructiveness. Blast disease pathogen is considered as the highest loss causing fungus in the world (Dean *et al.* 2012).

Blast disease of rice plant is caused by *Magnaporthe oryzae* B.C. Couch, (Anamorph: *Pyricularia oryzae* Cavara) (Couch *et al*. 2002). The disease can infect paddy at all growth stages and all aerial parts of plant. Yield loss due to blast disease can be up to 50% when the disease occurs in epidemic proportions. Infection occurs on leaves during vegetative phase and on panicles and neck during reproductive phase of the crop. Yield losses associated with neck blast are much higher than yield losses associated with leaf blast (Ghatak *et al.* 2013). This results in significant loss in the yield and grain quality. The pathogen is known for its high genetic plasticity. Continuous shift in its genetic makeup results in the resistant varieties succumbing to infection. Breeding for resistance against blast pathogen is a constant challenge because this fungus overcomes resistance within a short time after the release of a new cultivar. An understanding of the structure and dynamics of pathogen population is essential for prudent implementation of strategies for management of the disease. Molecular tools like maker studies are currently being used to study the population dynamics of the rice blast fungus and such molecular data are employed to breed for durable resistance to rice blast disease. Monoconidial isolates are mandatory to undertake such investigations because they provide homogenous genetic clones. Hence detailed description of establishment of such cultures is warranted from the available literature for the researchers. These monoconidial cultures can be used for genetic diversity and virulence studies of the pathogen. The virulence pattern of *M. oryzae* isolates from upland rice cultivars namely Primavera and BRS Bonança was analyzed in Brazil using monoconidial isolates for understanding the resistance level of these two cultivars to the blast disease (Araújo *et al.* 2005). Assessment of genetic diversity of finger millet blast isolates using molecular marker RAPD was performed using monoconidial isolates collected from different parts of Tamil Nadu (Anju and Rabindran 2016). 75 monoconidial isolates of *M. grisea* obtained from rice, crabgrass, foxtail millet, barnyard millet, and some unknown weeds from Iran were analyzed with complementation tests to determine vegetative compatibility and genetic relationship between these isolates and standard mating type tester isolates (Motallebi *et al.* 2009). Information regarding the above mentioned aspects is scanty in Karnataka. Hence the present study was initiated to understand the disease incidence in different geographical regions of Karnataka, India and to obtain monoconidial cultures which can be used for further studies in understanding its virulence spectrum, host pathogen interaction, biochemical variation and molecular aspects of this pathogen. The information generated during the current investigation will be helpful for the breeders to develop rice varieties with durable blast resistance. Thus the output of the present research work will indirectly contribute to the increase in rice productivity of our country.

MATERIALS AND METHODS

Collection of diseased samples from the field in different districts of Karnataka, India

The Rice blast disease samples were collected from the farmers' fields from different districts of Karnataka including different agro climatic zones namely Northern Dry Zone, Central Dry Zone, Southern Dry Zone, Southern Transition Zone, Northern Transition Zone and in some parts of Hilly Zone (Fig. 1). District wise total number of samples collected is given in (Fig. 3) (Table 1). Different parameters like variety name, age of the crop, type of infection and severity of the disease (Fig. 2) were recorded. Samples were taken in different brown envelop paper covers and labeled separately and taken to laboratory for isolation of blast fungus.

Isolation of the pathogen, culturing and maintenance of pure cultures

The blast fungus *M. oryzae* was isolated from blast infected plant parts like leaves, neck, collar, node, stem and panicle. Diseased samples were cut into small pieces around the infected area showing the blast lesion including the edge of the lesion (1- 2 cm). The pieces were subjected to surface sterilization with 1% sodium hypochlorite for 1:30 min followed by 3 washes with sterile distilled water. Then they were fixed in the upper half of Petri dish lined with moist filter paper and to the

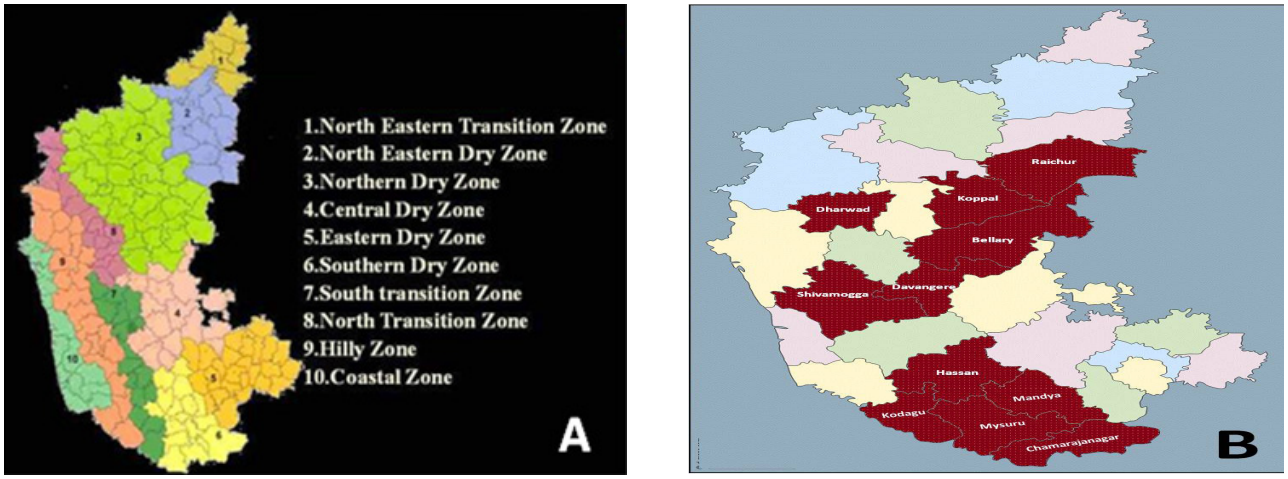

Fig. 1: (A) Agro climatic zones of Karnataka **(B)** Locations of samples collected from Karnataka, India (maroon shaded)

Fig. 2: A view of blast disease affected rice fields of different villages in Karnataka, India

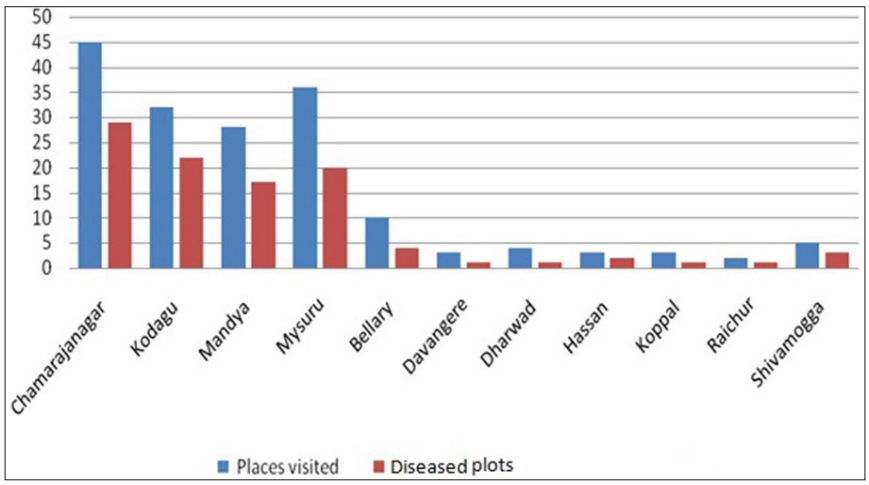

Fig. 3: Disease sample collection (X- axis: Districts of Karnataka visited, Y-axis: number of Places visited for sample collection)

Table 1: Locations of rice blast disease samples collected from different districts of Karnataka, India

Sl no.	District	Taluk	Villages	*Lat.* and *Long.**	Date of collection	Variety	Age of crop	Infection type	Isolate code**
1	Chamaraj-nagar	Kollegal	Dasanapura	12°10′55.0″N 77°05′49.7″E	21.11.2012	MTU 1010	4 Months	Neck, Leaf blast	CKDS01
			Hampapura	12°10′22.5″N 77°04′53.0″E	21.11.2012	Jaya	3 Months	Leaf blast	CKHM02
			Harale	12°10′49.9″N 77°06′57.7″E	18.10.2012	MTU 1001	2 Months 20 Days	Leaf blast	CKHR03
			Harale	12°10′48.6″N 77°06′59.8″E	18.10.2012	Gowri sanna	1 Month 20 Days	Leaf blast	CKHR04
			Kollegal	12°09′38.2″N 77°05′52.0″E	09.09.2013	Minilong	2 Months 15 Days	Leaf blast	CKKL05
			Mullur	12°09′33.7″N 77°03′57.7″E	09.09.2013	IR-64	3 Months 15 Days	Neck, Leaf blast	CKML06
			Settahalli	12°11′38.9″N 77°03′05.4″E	12.12.2012	Minilong	4 Months 25 Days	Leaf, Collar, Node, Neck, Panicle blast	CKST07
			Settahalli	12°11′25.1″N 77°03′23.9″E	12.12.2012	Jyothi	4 Months 15 Days	Leaf, Collar, Node, Neck, Panicle blast	CKST08
			Teramballi	12°08′14.1″N 77°03′26.5″E	16.09.2013	MTU 1001	3 Months	Leaf blast	CKTR09
		Yelandur	Agara	12°06′44.0″N 77°03′46.4″E	14.09.2013	KCP-1	2 Months 15 Days	Leaf blast	CYAG10
			Jodimellahalli	12°02′03.3″N 77°02′15.1″E	28.10.2013	Jyothi	4 Months	Neck, Leaf blast	CYJM11
			Katnavadi	12°06′44.4″N 77°02′40.8″E	22.10.2013	Gowri sanna	3 Months	Leaf blast	CYKT12
			Kestur	12°05′43.7″N 77°01′04.2″E	28.10.2013	MTU 1001	4 months 10 Days	Neck, Collar, Leaf blast	CYKS13
			Kinakahalli	12°06′57.5″N 77°03′23.0″E	27.09.2013	IR64	3 Months	Leaf blast	CYKK14
			Yelandur Rural	12°02′16.0″N 77°01′59.1″E	17.09.2013	KCP-1	2 months 20 Days	Leaf blast	CYLR15
2	Kodagu	Madikeri	Bethu	12°17′57.4″N 75°41′09.4″E	21.11.2012	Tunga	3 Months	Leaf blast	KMBT16
			Hakathoor	12°21′32.2″N 75°46′16.6″E	21.11.2012	Jeerige sanna	3 Months 15 Days	Neck, Leaf blast	KMHK17
			Hoddur	12°18′48.3″N 75°43′08.9″E	21.11.2012	Rajamudi	2 Months 15 Days	Leaf blast	KMHD18
		Virajpet	Ammatti	12°14′41.6″N 75°51′43.7″E	29.10.2013	Mangala	4 Months	Leaf, Collar, Node, Neck, Panicle blast	KVAM19
			Balele	12°09′57.7″N 76°02′02.8″E	24.09.2013	Rajbhoga	3 Months	Leaf blast	KVBL20

Lat. *and* ***Long.****: Latitude and Longitude,* ***Isolate Code****: The first, second, third and fourth letters of the isolates indicate the district, taluk and place of sample collection respectively. These letters are followed by collection numbers.*

			Bilugunda	12°12′33.1″N 75°50′33.1″E	09.12.2013	Sona Masuri	4 months 20 Days	Leaf, Collar, Node, Panicle blast	KVBG21
			Bilur	12°08′11.1″N 76°01′11.8″E	24.09.2013	Rajamudi	1 Month 20 Days	Leaf blast	KVBL22
			Halligattu	12°08′20.4″N 75°54′58.3″E	06.11.2013	MTU 1001	4 Months	Leaf, Collar, Node, Neck, Panicle blast	KVHG23
			Kalathmadu	12°11′36.8″N 75°53′09.4″E	19.09.2013	KHP-11	2 Months	Leaf blast	KVKM24
			Kirgur	12°08′33.6″N 75°58′10.3″E	19.09.2013	Athira	3 Months	Leaf blast	KVKR25
			Ponnampet	12°08′40.7″N 75°56′30.2″E	12.11.2013	BR 2655	4 Months 10 Days	Leaf, Collar, Node, Neck, Panicle blast	KVPN26
		Somwarpet	Abbimatta	12°37′41.0″N 75°48′25.4″E	02.12.2013	Jeerige sanna	4 Months 15 Days	Leaf blast	KSAB27
			Hebbale	12°31′32.4″N 75°58′51.4″E	02.12.2013	Jyothi	4 Months	Leaf blast	KSHB28
			Kalakandur	12°36′59.9″N 75°49′48.3″E	23.11.2013	Rajbhoga	3 Months	Leaf blast	KSKK29
			Sirangala	12°33′40.5″N 76°00′14.3″E	23.11.2013	Intan	4 Months	Leaf, Collar blast	KSSR30

Sl no.	District	Taluk	Villages	*Lat.* and *Long.**	Date of collection	Variety	Age of crop	Infection type	Isolate code**
3	Mandya	Krishnarajpete	Kurnena-halli	12°37′28.4″N 76°25′17.6″E	01.10.2013	Tunga	4 Months 15 Days	Leaf, Collar, Node blast	MKKN31
			Maduvina-kodi	12°35′03.4″N 76°26′23.2″E	01.10.2013	Mandya Vijaya	4 Months 25 Days	Leaf, Collar, Node, Neck, Panicle blast	MKMD32
		Maddur	Malagarana-halli	12°33′03.3″N 77°03′37.4″E	21.10.2013	Thanu	3 Months	Leaf blast	MMMG33
			Nagarakere	12°33′34.3″N 77°03′01.0″E	21.10.2013	KRH-4	2 Months 15 Days	Leaf blast	MMNG34
		Malavalli	Aladahalli	12°27′44.7″N 76°51′24.8″E	21.10.2013	Jaya	4 Months	Neck, Leaf blast	MMAD35
			Koregala	12°25′05.4″N 77°02′09.8″E	21.10.2013	MTU 1010	2 Months 15 Days	Leaf blast	MMKO36
		Mandya	Goravale	12°34′55.5″N 76°50′33.1″E	21.09.2013	KHP-10	4 Months	Leaf, Collar blast	MMGV37
			Shivalli	12°35′15.4″N 76°49′35.6″E	23.09.2013	Jaya	4 Months 20 Days	Leaf, Collar, Node, Neck, Panicle blast	MMSV38
			V.C. Farm	12°34′22.9″N 76°49′32.1″E	13.11.2013	IR64	4 Months 15 Days	Leaf, Collar, Node, Neck, Panicle blast	MMVF39
			V.C. Farm	12°34′24.1″N 76°49′43.4″E	13.11.2013	HR-12	4 Months 15 Days	Neck, Leaf blast	MMVF40
		Pandavapura	Aralakuppe-nala	12°25′58.7″N 76°37′31.8″E	20.09.2013	Rasi	3 Months	Leaf blast	MPAK41
			Devarayana-patna	12°33′21.9″N 76°39′04.3″E	20.09.2013	KRH 4	3 Months	Leaf blast	MPDY42

		Pandavapura	12°29′40.0″N 76°40′08.1″E	20.09.2013	Raksha	4 Months 15 Days	Leaf, Collar, Node, Neck, Panicle blast	MPPD43
		Srirangapatna	Chandagalu	12°32′24.2″N 76°49′57.1″E	24.10.2013	Sona Masuri	3 Months	Leaf blast	MSCH44
			Karimanti	12°24′53.9″N 76°39′22.3″E	24.10.2013	MTU 1001	4 Months	Collar, Leaf blast	MSKM45
4	Mysuru	Heggaa-dadevana kote	Hommar-agahalli	12°07′19.4″N 76°26′48.5″E	18.12.2012	Jyothi	4 Months 15 Days	Leaf, Collar, Node, Panicle blast	MHHM46
			Sagare	11°59′13.5″N 76°21′47.1″E	18.12.2012	Thanu	4 Months	Leaf blast	MHSR47
		Hunsur	Hunsur	12°18′08.0″N 76°16′40.1″E	07.11.2013	KRH-4	4 Months	Leaf blast	MHHN48
		Krishnara-janagar	Byadarahal-li Hantha	12°30′46.2″N 76°19′40.7″E	26.11.2013	MTU 1001	4 Months 20 Days	Leaf, Collar, Node, Panicle blast	MKBD49
			Hebbalu	12°27′59.1″N 76°21′09.2″E	26.10.2013	Jyothi	4 Months	Leaf, Collar, Node, Panicle blast	MKHB50
			Mirle	12°32′29.0″N 76°19′10.9″E	26.10.2013	MTU 1001	4 Months 10 Days	Leaf, Collar, Node, Panicle blast	MKML51
		Mysuru	Kalastha-vadi	12°22′44.6″N 76°40′24.0″E	22.10.2013	Thanu	3 Months	Neck, Leaf blast	MMKL52
			Nagana-halli	12°22′52.1″N 76°39′21.1″E	22.10.2013	Jyothi	1 month 15 Days	Leaf blast	MMNG53
			Siddalin-gapura	12° 22′ 10.9″ N76° 39′ 57.3″E	22.10.2013	MTU 1001	2 Months	Leaf blast	MMSD54
		Nanjangud	Hejjige	12°07′41.3″N 76°41′36.0″E	16.11.2013	Sona Masuri	4 Months	Neck, Collar, Leaf blast	MNHJ55
			Hullahalli	12°06′07.6″N 76°32′57.6″E	16.11.2013	Jyothi	4 Months 15 Days	Leaf, Collar, Node, Panicle blast	MNHU56
			Hullimavu	12°09′24.8″N 76°44′09.2″E	16.11.2013	Jyothi	4 Months 10 Days	Leaf, Collar, Node, Panicle blast	MNHM57
			Rampura	12°06′48.2″N 76°33′45.5″E	16.11.2013	JGL 1798	4 Months	Neck, Leaf blast	MNRM58
		Tirumakudal Narasipur	Hiriyuru	12°12′02.0″N 76°57′00.6″E	18.09.2013	Jyothi	2 months	Leaf blast	MTHR59
			Sosale	12°14′26.7″N 76°54′51.0″E	19.10.2013	KCP-1	4 Months	Neck, Leaf blast	MTSS60
5	Bellary	Bellary	Emmiganur	15°24′01.0″N 76°43′00.1″E	15.10.2014	Sona Ma-suri	4 Months 15 Days	Neck, Leaf blast	BBEM061
		Hospet	Devasamudra	15°20′42.6″N 76°38′38.2″E	15.10.2014	Sona Ma-suri	4 Months	Leaf blast	BHDS62
		Hagaribommana-halli	Belugoduhal	15°25′22.6″N 76°37′09.8″E	15.10.2014	Sona Ma-suri	4 Months	Leaf, Collar, Neck blast	BHBG63
6	Davangere	Davangere	Nagarasana-halli	14°19′15.1″N 75°50′39.7″E	25.09.2014	Mugad Siri 1253	3 Months 20 days	Neck, Leaf blast	DDNS64

7	Dharwad	Dharwad	Mugad	15°26′09.6″N 74°54′51.0″E	27.09.2014	Mugad Sugandha	4 Months 10 Days	Leaf, Collar, Node, Neck blast Panicle blast	DDMU65
8	Hassan	Holenarasipura	Chikkchagahalli	12°45′32.2″N 76°15′12.0″E	11.09.2013	MTU 1001	2 Months 15 Days	Leaf blast	HHCC66
			Hallimysore	12°38′52.3″N 76°15′33.2″E	19.09.2014	Rajamudi	3 Months	Leaf blast	HHHM67
9	Koppal	Gangavathi	Gangavathi	15°26′19.5″N 76°33′06.2″E	09.10.2014	Gangavathi Sona	3 Months 15 Days	Leaf,Collar, Neck blast	KGGV68
10	Raichur	Raichur	Kadlur	16°22′04.3″N 77°16′48.7″E	17.10.2014	Sona Masuri	3 Months 20 Days	Neck, Leaf blast	RRKD69
11	Shivamogga	Bhadravati	Guninarasipura	13°49′18.6″N 75°43′52.3″E	29.09.2014	KRH-4	4Months 10 Days	Leaf blast	SBGN70
			Balemaranahalli	13°47′44.7″N 75°41′27.0″E	29.09.2014	KHP-2	4 Months	Leaf blast	SBBM71
		Shivamogga	Holebenavalli	13°55′08.9″N 75°37′18.1″E	30.09.2014	Hemavathi	4 Months 20 Days	Leaf blast	SSHB72

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lower half 10ml of sterile water was transferred to create 100% relative humidity within the moist chamber (Fig. 4). Infected plant pieces were also placed on glass slides which were kept inside moist chamber. The set up was incubated at 28°C for 48hr to enhance sporulation. After incubation, these infected plant pieces were examined under stereo binocular microscope to confirm the typical elliptical or spindle shaped *M. oryzae* spores (Fig. 5).

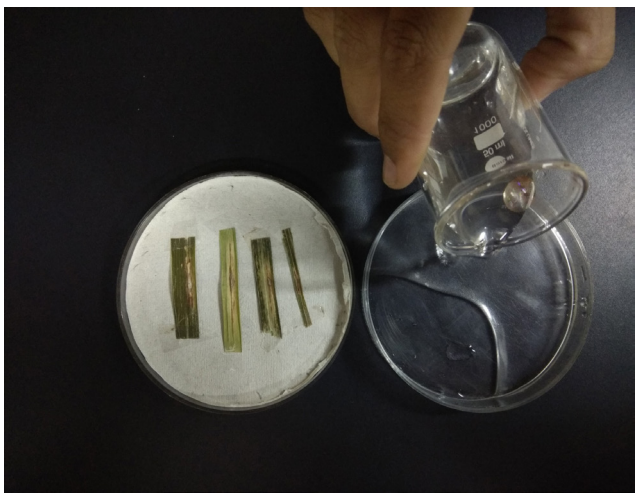

Fig. 4: Moist chamber for isolation of rice blast fungus *Magnaporthe oryzae*

After confirmation, the plant pieces were taken in Eppendorf tube with 1ml sterile water and inverted twice. Around 5 to 10µl of the conidial suspension was spread on water agar (2%) and incubated overnight at 25°C. A germinating conidium was picked up (single conidium) on to a fresh cornmeal rice straw agar plate with streptomycin sulfate (40mg/L) and incubated at 28°C (Muthumeenakshi *et al.* 2004) for 14 days.

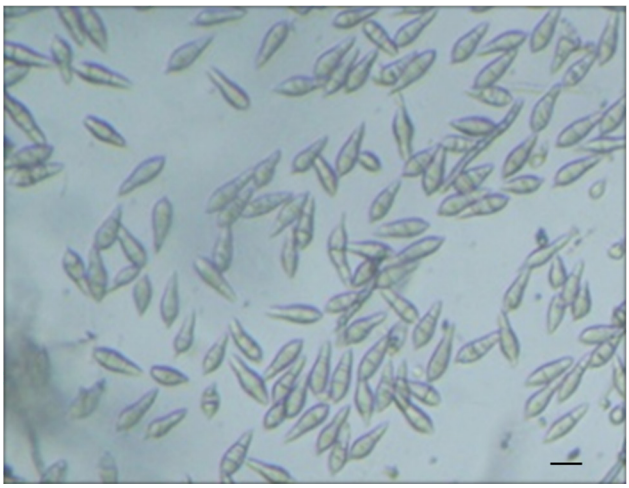

Fig. 5: A view of *Magnaporthe oryzae* conidia isolated from infected leaves of BR 2655 variety of rice collected in Ponnampet, Kodagu district of Karnataka, India (Scale bar 10 µm)

Preparation of media

(a) Water Agar (WA)

20gm of the Bacto Agar was suspended in 800ml of distilled water and mixed thoroughly and the

volume was made up to 1000ml by adding distilled water. This was sterilized at 121°C for 15min. 40mg of streptomycin sulfate was added just before plating.

(b) Corn Meal Rice Straw Agar (CMRSA)

Rice straw was cut into small pieces and boiled in 500ml distilled water for 20min and an extract was collected by filtering through a muslin cloth in a separate conical flask. 50gm of Corn powder, 15gm of agar and 5gm of sucrose were dissolved and mixed thoroughly in 400ml of distilled water in a separate conical flask. Finally these two solutions were mixed thoroughly and the volume was made up to 1000ml by adding distilled water. This was sterilized at 121°C for 20min. 40mg of streptomycin sulfate was added just before plating.

Long-term storage of *M. oryzae*

Whatman filter papers were cut in to small pieces about 15mm square. These filter papers were put in a Petri dish and sterilized. These sterile paper disks were placed on the surface of Oat Meal Agar (OMA) plate, then inoculated with a plug of 0.5cm diameter *M. oryzae* using transfer needle. Petri dishes were incubated at 28°C for 4-5 days i.e., until the filter paper was covered by the fungal mycelium. These colonized filter papers were kept inside sterilized Petri dishes which were dried by placing them in desiccators at room temperature for 4-5 days. These dried filter papers with fungal mycelium were cut in to small pieces of about 3-5mm square and were stored in microcentrifuge tubes which in turn were kept in storage boxes at -20°C (Fig. 6).

Fig. 6: Long term storage of *Magnaporthe oryzae* on filter papers

RESULTS AND DISCUSSION

Collection of diseased samples from different districts of Karnataka, India

A total of 171 places were visited during 2012-2014 (*kharif* season) in different districts of Karnataka, India. These showed 101 diseased plots. Disease incidence of 64.44%, 68.75%, 60.71% and 55.55% was recorded from Chamarajanagar, Kodagu, Mandya and Mysuru districts respectively from south Karnataka. Among total infection types, leaf blast was found to be predominant followed by neck blast, collar blast, node blast and panicle blast (Fig. 7).

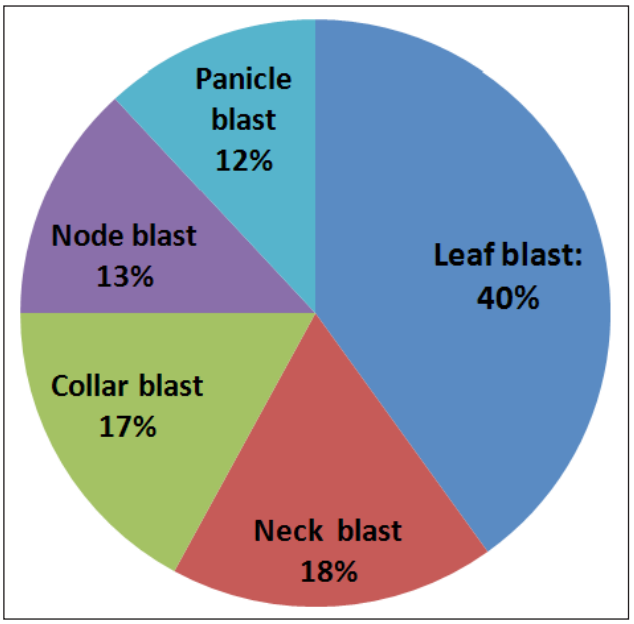

Fig. 7: Infection types of rice blast samples obtained during sample collection from different regions of Karnataka, India

This result suggests that the more aggressive isolates involved in epidemics were on leaves during the vegetative stage of the crop cycle and have a higher probability to infect necks, nodes, collars and panicles. This population shift may occur during disease transmission from leaves to panicle. 32 varieties of rice were recorded in 72 locations. MTU-1001, Jyothi and Sona Masuri varieties were found to be more susceptible to rice blast in these fields and remain as highly susceptible varieties in Southern Karnataka. In addition to this Minilong, IR64, Jaya, IR64, KCP-1, Rajamudi, Rajbhoga and KRH-4 varieties were also found to be susceptible. Newly developed resistant varieties namely Rasi, KRH 4, Raksha and Mugad Siri 1253 were also found to be susceptible in some of the regions of Karnataka (Fig. 8). Variety Sona Masuri in Bellary and Koppal was also found to be infected by blast in few plots.

Earlier researchers had carried out survey work

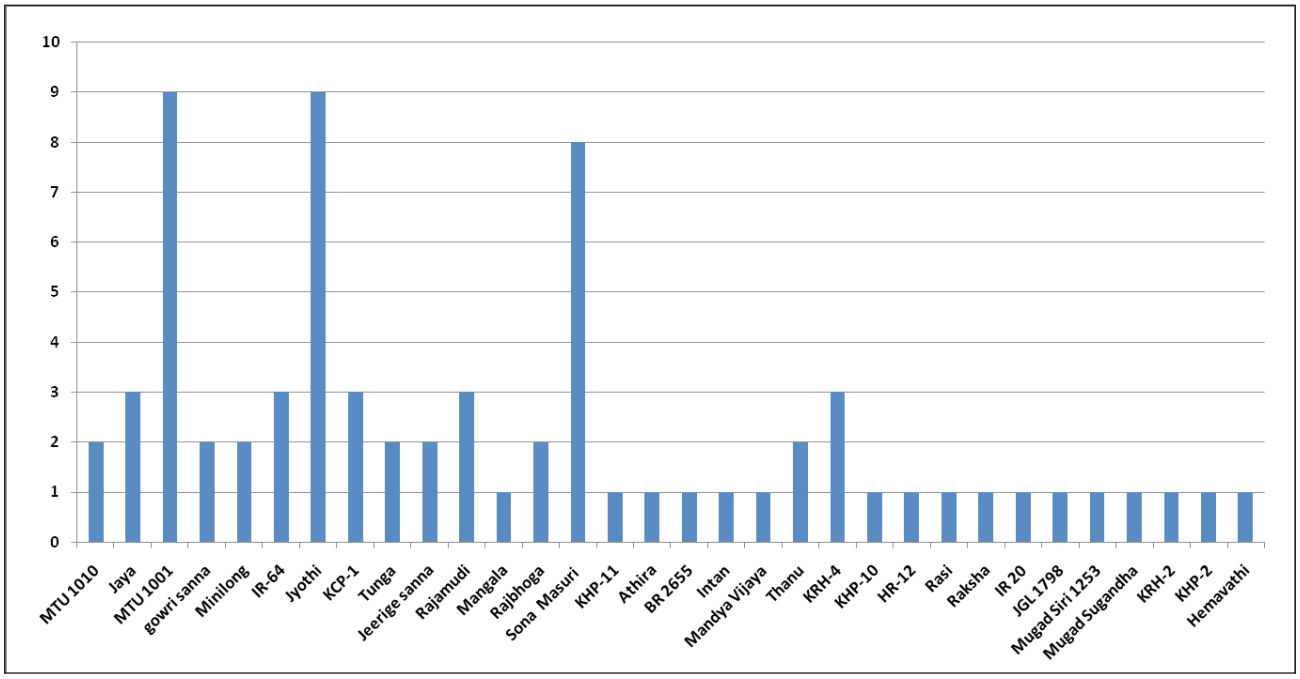

Fig. 8: Varieties of rice cultivated by farmers with number of locations visited during sample collection in different districts of Karnataka, India (X-axis: rice variety, Y-axis: number of locations of sample collection)

to understand the disease severity in different rice growing regions. Disease incidence, severity, cultivar susceptibility and yield loss across the ten agro ecological zones of Bangladesh were carried out to know the distribution and severity of the disease (Hossain *et al.* 2017). Similar type of work was carried out in Mandya district alone where the severity and incidence of leaf and neck blast was assessed and *P. oryzae* isolates were grouped based on host differentials (Kulmitra *et al.* 2017). During the present investigation we have collected large number of samples from different agro climatic zones which included 11 districts of Karnataka and this helped us to understand the infection range and number of susceptible varieties and breakdown of resistance in some of the newly released resistant cultivars.

Monoconidial isolation was carried out during present investigation as it is essential for understanding variation, mutation and segregation in fungi. This is identified by many researchers. Studies on genetic variability among 24 *M. grisea* isolates obtained from different parts of the plant namely leaf, neck and panicle was carried out through internal transcribed spacer (ITS) analysis to find out specificity and relationship among *M. grisea* isolates obtained from different districts of Tamil Nadu (Shanmugapackiam *et al.* 2015). In one of such studies, 35 monoconidial *M. oryzae* isolates were collected from rice variety MR219 in different rice growing regions of Malaysia to identify and provide a taxonomic position at the species level of blast pathogen using ITS, actin, β-tubulin and calmodulin gene, RAPD and ISSR as markers (Ashtiani *et al.* 2016). In Brazil, virulence pattern and genetic diversity in field populations of *P. grisea* from rice cultivar Metica-1 were studied using Pot2 inverted transposons through monoconidial isolates (Filippi *et al.* 2000). Similar study was also carried out using seventy two monoconidial isolates based on reactions type of leaf blast on wheat cultivars (Urashima *et al.* 2004). One of the studies on diversity of *P. oryzae* from different geographical regions of north India was carried out to understand morphological, pathological variations using monoconidial isolates. (Srivastava *et al.* 2014). Similarly in Colombia, Studies on genetic and virulence diversity of the rice blast fungus were carried out using MGR-DNA fingerprinting and differentially resistant rice cultivars through monoconidial isolates (Levy *et al.* 1993). Population dynamics of the rice blast fungus was examined by DNA fingerprinting of MGR586 in Japan to reveal the lineage of population structure using monoconidia isolates (Don *et al.* 1999).

Single spore isolation is the preliminary step to obtain pure form of particular isolate of a species of the pathogen. Further, this can be used for genetic diversity studies. This knowledge is helpful to breed resistant varieties.

CONCLUSION

The current study suggests that the incidence and prevalence of rice blast disease is a serious concern to these regions which directly affect the paddy production in coming years too. The study also revealed that *M. oryzae* is capable of breaking resistance in the newly released cultivars. Hence proper management of the disease is needed and it requires understanding of the pathogen distribution, diversity, identification of new sources of resistance, use of blast resistant cultivars, disease management strategies and good cultivation practices. Monoconidial isolation is the preliminary key step to understand the virulence spectrum and molecular aspects of the pathogen in developing durable resistant cultivars. Our study will help to breed resistant cultivars suitable to these regions.

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Compliance with Ethical Standards

This article does not contain any studies with human or animal subjects.

Conflict of interest

All authors declare that they have no conflict of interest.

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