“Epidemiology, virulence and molecular diversity in blast [*Magnaporthe grisea* (Hebert) Barr.] of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and resistance in the host to diverse pathotypes”

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DOCTOR OF PHILOSOPHY IN AGRICULTURE

(PLANT PATHOLOGY)

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CERTIFICATE

Mr T. YELLA GOUD has satisfactorily prosecuted the course of research and that the thesis entitled **"Epidemiology and virulence diversity in blast [*Magnaporthe grisea* (Hebert) Barr.] of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and resistance in the host to diverse pathotypes"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis nor its part thereof has not been previously submitted by him for a degree of any university.

Date: **Chairperson**

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This is to certify that the thesis entitled **"Epidemiology and virulence diversity in blast [*Magnaporthe grisea* (Hebert) Barr.] of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and resistance in the host to diverse pathotypes"** submitted in partial fulfillment of the requirements for the degree of 'Doctor of Philosophy in Agriculture' of the **Professor Jayashankar Telangana State Agricultural University**, Hyderabad is a record of the bonafide original research work carried out by Mr. T. YELLA GOUD under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I, **THANDU YELLA GOUD**, hereby declare that the thesis entitled **"EPIDEMIOLOGY, VIRULENCE AND MOLECUALR DIVERSITY IN BLAST [*Magnaporthe grisea* (Hebert) Barr.] OF PEARL MILLET [*Pennisetum glaucum* (L.) R. Br.] AND RESISTANCE IN THE HOST TO DIVERSE PATHOTYPES"** submitted to the **Professor Jayashankar Telangana State Agricultural University** for the degree of **Doctor of Philosophy in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

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ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
cm	:	Centimeter
CMIE	:	Centre for Monitoring Indian Economy
dia.	:	Diameter
DAS	:	Days after sowing
et al.	:	and others
Fig.	:	Figure
g	:	gram
GDP	:	grass domestic product
GR	:	guaranteed reagent
HCl	:	hydrochloric acid
i.e.,	:	That is
IDM	:	Integrated disease management
in vitro	:	in lab conditions
in vivo	:	on the live host
Kg ha^{-1}	:	Kilogram per hectare
M ha	:	million hectare
ml	:	millitre
mt	:	meter
NaOH	:	sodium hydroxide
^{o}C	:	Degree Celsius

PDA	:	Potato dextrose agar
PDI	:	Per cent Disease Index
pH	:	Hydrogen ion concentration
q ha^{-1}	:	quintal per hectare
S.Em	:	Standard Error Mean
viz.,	:	Namely
CRD	:	Completely Randomized Design
OMA	:	Oat-Meal Agar
ANOVA	:	Analysis of Variance
AMOVA	:	Analysis of Molecular Variance
DAI	:	Days After Inoculation
DI	:	Disease Incidence
etc.	:	etcetera
PMBVN	:	Pearl Millet Blast Virulence Nursery
LSD	:	Least Significant Difference
LWD	:	Leaf Wetness Duration
Iso	:	Isolate
No.	:	Number
SSR	:	Simple Sequence Repeat
URP	:	Universal Rice Primer
RCBD	:	Randomized Complete Block Design

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ABSTRACT

Blast has emerged as an important disease in the major pearl millet growing areas in India. The present investigation was undertaken to study cultural, pathogenic and molecular diversity in the *M. grisea* isolates infecting pearl millet, effect of temperature and leaf wetness duration on blast development and to screen pearl millet lines for resistance to blast disease. Culture characters of four monoconidial isolates of *M. grisea* MgPM 45, MgPM 53, MgPM 56 and MgPM 118 were tested on OMA and PDA medium at different pH ranging from pH 5.5 to pH 8.0 (pH 5.5, pH 6.0, pH 6.5, pH 7.0, pH 7.5 and pH 8.0) and different incubation temperatures 22^{o}C, 24^{o}C, 26^{o}C, 28^{o}C and 30^{o}C to select optimum conditions for growth and sporulation of the fungus. Results of this study indicated that pH of 6.5 and temperature of 28^{o}C is ideal for the growth and sporulation of *M. grisea* adapted to pearl millet. Based on these results, variability in the cultural and morphological characteristics of 65 isolates of *M. grisea* was studied on OMA medium at pH 6.5 by incubating at 28^{o}C. Culture morphology varied significantly among isolates. A range of colour variation in the medium was also observed from buff colour to black among field isolates with smooth or rough margin. The radial growth of the *M. grisea* isolates varied significantly; maximum radial growth of 4.25 cm was recorded for isolates MgPM 125 and MgPM 162 whereas minimum radial growth of 2.30 cm was recorded for the pearl millet isolate MgPM 148. Large variation was also observed for sporulation among field isolates. It was observed that isolates with grayish black and brownish black growth with sector formation produced more spores. In majority of the isolates, maximum sporulation was confined to sectored region. These 65 isolates were also tested for

pathogenic variation on a set of 10 host differentials (ICMB 93333, ICMB 95444, ICMB 97222-P1, ICMB 01333, ICMB 02444, ICMR 06444, 863B-P2, ICMR 06222 ICMR 11003 and IP 21187). The mean blast severity across the differentials was maximum for isolate MgPM 138 and minimum severity was observed for MgPM 132. Based on reaction type (avirulent/virulent), the 65 isolates were grouped into 28 different pathotypes. Pathotype G22 comprising isolates MgPM 121, MgPM 137, MgPM 138, MgPM 145, MgPM 148 from Rajasthan and MgPM 173 and MgPM 174 from Uttar Pradesh appeared as most virulent as it could infect all the 10 host differentials whereas pathotype G2 comprising MgPM 127, MgPM 129, MgPM 132, MgPM 149, MgPM 158, MgPM 159 and MgPM 39 was least virulent. These isolates could be grouped in five main clusters based on the results of molecular diversity study using URP markers. Among them cluster I (32) and III (31) included more than 95 per cent isolates whereas cluster II, IV and V contained 2-3 isolates. Studies on the effect of leaf wetness duration on disease development showed an overall increase in leaf blast severity, lesion length (mm), number of lesions per plant, lesion sporulation and leaf sporulation with the increase in leaf wetness duration (LWD). Based on the results it can be concluded, that both leaf wetness duration and temperature were essential for blast on pearl millet which becomes more severe at longer wetness durations beyond 48 hours during optimum day/night with a temperature ranging from 25±1/20±1^{o}C to 30±1/22±1^{o}C. For the identification of blast resistance, 160 designated B-lines of pearl millet were screened under greenhouse conditions against five pathotype-isolates *viz.*, MgPM 45, MgPM 53, MgPM 56, MgPM 118 and MgPM 119. Multiple-pathotype (3-5) resistance was found in 23 lines. Eight lines (81B, ICMB 88004, ICMB 92444, ICMB 97222-P1, ICMB 02111, ICMB 07111, ICMB 09333 and ICMB 09999) were found resistant to all the five pathotypes. Similarly for the identification of stable sources of adult plant resistance, 28 lines were evaluated in the disease nursery (PMBVN) at six locations, Aurangabad, Dhule, Durgapura, Gwalior, Jamnagar and Patancheru during 2013 and 2014. None of the entries in the blast nursery was resistant at all the test locations. However, ICMR 06444 was found resistant at three (Gwalior, Jamnagar and Patancheru) locations and showed moderate resistance at other three locations. ICMB 01333, ICMR 11009 and HHB 146 improved (a hybrid) were resistant at Gwalior and Jamnagar. Pearl millet lines identified in this study that are resistant at 2-3 locations can be selected for use in pearl millet breeding programs aiming to develop blast resistant hybrids.

Chapter - I

INTRODUCTION

Millets rank as the world's sixth most important food crops among cereals. There are major and minor millets, among the minor millets pearl millet is one. It has a great potential to grow on soils that are too sandy, light-textured, acidic, dry and too infertile for other cereals. Pearl millet is cultivated in over 30 countries of Asia, Africa and America where dry land system is possible. India and Africa are together occupying 90 percent area of total pearl millet production in the world (Yadav *et al.*, 2012).

The total area of pearl millet in India is 7.3 million hectare with a total production of 87 million tonnes In India approximately 90 percent of its production is contributed by five states *viz.,* Rajasthan, Gujarat, Uttar Pradesh, Haryana and Maharashtra. Rajasthan ranks first with an area of 3.98 M ha and annual production of 38.7 million tones (CMIE, 2014).

It is usually cultivated as a subsistence food, feed, fodder and fuel crop in parts of Africa and Asia. But more than 95 per cent is primarily grown for grain purpose only. It's grain is chiefly served as a food, because of high protein (27 to 32%), higher concentration of essential amino acids, twice the extract (fat) and higher gross energy than maize (Ejeta *et al.,* 1987; Davis *et al.*, 2003). The grain is also used as feed for poultry, swine and cat fish diets (Andrews and Kumar, 1992) and it is a good source of energy and nutrient for ruminant diet like dairy, beef cattle and goats (Dove and Myer, 1995). It produces highly palatable and nutritious forage.

Cultivation of pearl millet for grain and forage has been expanded into non-traditional areas in temperate and developed countries, where production constraints from biotic factors such as diseases, insect pests, parasitic and non-parasitic weeds assume greater importance. More than 50 fungal, bacterial and viral pathogens of pearl millet have been reported (Ramakrishnan, 1971; Ferraris, 1973). In India 60 per cent (Sharma *et al.*, 2012) or more of this crop is sown with genetically uniform single-cross hybrids that are particularly vulnerable to downy mildew (DM) disease caused by *Sclerospora graminicola* (Sacc.) J. Schrot. and Pyricularia leaf spot disease or blast disease of pearl millet caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*).

In India, the disease was first reported from Kanpur, Uttar Pradesh (Mehta *et al.*, 1953) and remained as a minor disease for a long time but has recently become a serious threat to pearl millet grain yield production and fodder production.

Management of diseases through resistant cultivars is the most economical and relevant way of controlling pearl millet blast mainly by resource poor and marginal farmers, who cannot afford to control blast disease by the application of chemical and pesticides. Chemical control of plant pathogens is most effective and yet the use of chemicals is not generally desired due to certain disadvantages *viz.*, ground water pollution, residues on food crops, effect on non-target organisms. Besides, their continuous use leads to the resurgence of resistant races of the pathogen under selection pressure. Although bio-control agents for blast have been successfully deployed to combat the disease in the laboratory, green house and field tests, the feasibility of such strategies on a commercial scale still remains to be tested.

Use of resistant cultivars is the best alternative to overcome yield losses caused by *M. grisea.* It has the ability to overcome resistance within two to three years after the release of resistant cultivars widely and thus made breeding for resistance a constant challenge (Sere *et al.,* 2007). There is a need to have clear understanding of the biology of the pathogen since major studies were carried out on rice blast pathosystem (Sere *et al.,* 2007). However, such studies are very limited with the pearl millet blast pathosystems (Sharma *et al.,* 2013).

The molecular genetic techniques using DNA fingerprinting by polymerase chain reaction (PCR) have been extensively employed to study the population dynamics of *M. grisea* and explore a promising new concept which utilizes such molecular data to breed for durable resistance. The use of molecular markers in population genetic studies has unraveled epidemiological information to levels of precision not possible previously. Unlike traditional markers, molecular markers are direct manifestations of genetic content and can therefore serve as reliable indices of genetic or pathotypic variations. They are not influenced by environmental factors and hence are highly reproducible. Besides, these are cost effective and less cumbersome.

Among the molecular markers URP markers are considered as useful, technically simple, cost effective and highly sensitive and informative marker system because no prior knowledge of the investigated species genomic sequence is required and these are highly polymorphic and provide genetic variation at intra and inter species level (Kang *et al.*,

2002). The advantageous of URP over SSR based genetic diversity was demonstrated by Jana *et al.* (2005) while working with genetic variability assays in *M. phaseolina.*

Weather variables, particularly relative humidity, leaf wetness duration and temperature play a major role in influencing infection and disease development in any host-pathogen systems. Blast disease has the potential to cause severe crop losses in pearl millet when environmental conditions are favorable for disease development. Therefore, information on relationship between weather variables and blast disease could be used to develop and improve techniques to screen for resistance. Improved knowledge of the effect of interaction of host cultivar with weather, pathogenic strain and the crop growth stages would be helpful in understanding and predicting the disease epidemics. These factors are more relevant with a polycyclic, airborne pathogen like *M. grisea*. In general, long periods of leaf wetness, high relative humidity (>90%) and high temperatures of 17 to 28°C favor the blast disease development (Lamey, 1970; Kim, 1994; Teng, 1994; Ou, 1985). Development of effective screening technique based on the basic knowledge of pathogen biology and epidemiology and identification of resistance in diverse germplasm accessions and breeding lines provides the basis for resistance utilization. With wide diversity in the pathogen population across geographical locations, multilocation evaluation of resistant lines, and greenhouse evaluation against diverse pathotypes help to identify the stable resistance sources. Such resistance sources can be used for breeding cultivars with stable and likely durable resistance.

Recognizing the importance of pearl millet and the constraint posed by the blast disease, the following objectives were formulated and research conducted.

1. To study the cultural, pathogenic and molecular diversity among pearl millet infecting populations of *M. grisea*.
2. To study effect of temperature and leaf wetness duration on disease development.
3. To identify resistance in pearl millet against diverse pathotypes of *M. grisea.*

CHAPTER II
REVIEW OF LITERATURE

A brief review on available literature pertaining to leaf spot or blast disease of pearl millet and various aspects related to the present study on cultural, pathogenic and molecular diversity of *Magnaporthe grisea*, their cross infectivity, effect of temperature and leaf wetness duration on disease development and screening for resistance have been presented in this chapter under the following headings and sub-headings.

2.1 THE DISEASE

Pearl millet (*Pennisetum glaucum* (L.) R. Br) is known by various names in different languages: pearl, bulrush, cattail, or spiked millet in English, bajra in Hindi, dukhn in Arabic, and *mil a chandelles* in French. Pearl millet originated in Africa and was subsequently introduced into India. Some researchers 'believe that millets were the first cultivated crops to be used for human food in prehistoric times. The crop is mainly grown for grain and stover in the hottest and driest areas of Africa and Asia where dry land crop production is possible. It is a staple grain for about 90 million people living in the semi-arid tropical regions of Africa and the Indian sub-continent. Pearl millet grain contains 27 to 32 per cent more protein, higher concentration of essential amino acids, twice the extract (fat) and higher gross energy than maize (Ejeta *et al*., 1987; Davis *et al*., 2003). Wu *et al.*, (2006) observed that the rate of fermentation of pearl millet was 30% greater than that of corn and distillers dried grains with solubles (DDGS) co-products were higher in protein and fat. In India, 70 per cent or more of the 9 M ha of this crop is sown to genetically uniform single-cross hybrids that are particularly vulnerable to downy mildew (DM) and leaf spot disease.

Pyricularia leaf spot, also called as blast disease of pearl millet caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*), has become a serious threat to pearl millet grain and fodder production in addition to downy mildew disease caused by *Sclerospora graminicola* (Sacc.) J. Schrot.. Pearl millet blast was first reported in India from Kanpur, Uttar Pradesh (Mehta *et al.,* 1953).

2.2 CAUSAL ORGANISM

The genus *Pyricularia grisea* (Cooke) Sacc. was established by Saccardo (1880) which was originally described from crabgrass (*Digiteria sanguinalis* L.). The name "Pyricularia" refers to the pyriform shape of the conidia. Subsequently Cavara (1892) described *P. oryzae* Cav. from rice (*Oryza sativa* L.) a species with similar morphology to *P. grisea.* These two species are similar in morphology.

The fungus *Pyricularia grisea* (Cooke.) Sacc. (formerly *Pyricularia oryzae* Cavara.) anamorph of *Magnaporthe grisea* (Hebert) Barr. is a heterothallic, filamentous fungus pathogenic to almost 50 plant species in 137 members of Poaceae (Ou, 1980; Choi *et al.,* 2013; Murakami *et al.,* 2000; Inukai *et al.,* 2006) including *Eleusine*. Initially, there was difference of opinion with regard to the nomenclature of the pathogen. Morphologically it is very close to *Pyricularia oryzae* (Ramakrishnan, 1948). The perfect stage of *Pyricularia grisea* was earlier named as *Ceratosphaeria grisea* (Hebert, 1971). Later Yaegashi and Nishihara (1976) suggested the genus *Magnaporthe*. Yaegashi and Udagawa (1978) finally proposed *M. grisea* as the perfect stage of *P. grisea* (Cke.) Sacc. instead of *Ceratosphaeria grisea*. Rossman *et al.* (1990) argued that *P. oryzae* should be synonymized with *P. grisea* and grouped these two anamorphs under the teleomorph *M. grisea* (Hebert) Barr.

The fungus *M. grisea* produces three-celled, pyriform macro conidia in the imperfect stage and four celled, spindle-shaped ascospores in the perfect stage (Barr, 1977; Hebert, 1971; Kato *et al.,* 1976). In 1994 Kato *et al.* (1994) found micro conidia in *M. oryzae* cultures on artificial media. Morphologically distinct from macro conidia, they were characterized by unique features such as a single cell with no septum, small size (average 0.7l m wide and 0.6l m long), lunate and hyaline. Kato *et al.* (1994) demonstrated that the small conidia were a third type of spores produced by *Magnaporthe oryzae* during its life cycle and named them micro conidia.

Mycelium in cultures is aerial or submerged, hyaline or olivaceous, 1.5 – 6.0 μm in width, septate branched, conidiophores one to many, fasciculate, simple or rarely branched, 2 – 4 septate, not or slightly constricted at septa; at first monosporic, then pleurogenous on sympodium, olivaceous to fuliginous, base swollen, dark coloured and becoming lighter colour towards the apex. Conidia variable in size and shape, terminal, pyriform to obclavate, base rounded, apex narrowed: 2 – septate, rarely 1 - 3 septate, not or slightly

constricted at septa, almost hyaline to pale olive, 14 – 40 x 6 – 13 µm in size, usually 19 – 23 x 7 – 9 µm, with small basal appendage. Basal appendage 1.6 – 2.4 µm (2 µm) in width; basal cell 4.8 – 10 µm (6.3 µm), middle cell 4.8 – 12.8 µm (7.8 µm), apical cell 4.8 – 10.7 µm (7.4 µm). Conidia germinate from apical or basal cell and less frequently from middle cell. Germinating hyphae hyaline, not or constricted at septa, branched, 3 – 5 µm in width (Shirai, 1896; Sawada, 1917; Nishikado, 1926). Under laboratory conditions the pathogen produces fertile perithecia (Viji and Gnanamanickam, 1998). However perithecium has not been observed for a pearl millet.

Taxonomy of *Magnaporthe grisea* (Hebert) is a member of

Kingdom	-	Mycota
Division	-	Eumycota
Sub division	-	Ascomycotina
Class	-	Pyrenomycetes
Order	-	Magnaporthales
Family	-	Magnaporthaceae
Genus	-	Magnaporthe
Species	-	grisea

2.3 SYMPTOMS

The disease appears as grayish, water-soaked lesions on foliage that enlarge and become necrotic, resulting in extensive chlorosis and premature drying of young leaves. Depending on the resistance level of the host cultivar, the lesion size varies from small, roundish, elliptical, diamond shaped to elongated, measuring 1-2 mm to 20 mm. Lesions are often surrounded by a chlorotic halo, which turns necrotic, giving the appearance of concentric rings. The lesions are usually confined to interveinal spaces on the foliage. Lesions grow and coalesce to cover large surface areas and cause necrosis of tissues. In case of a susceptible cultivar, the entire foliage gives a burnt appearance. Severely infected plants produce no grain or few shriveled grains in blasted florets. Leaf blast on pearl millet has been found to be negatively correlated with green-plot yield, dry matter yield and digestive dry matter (Wilson and Gates, 1993) thus affecting the productivity and quality of the crop (Thakur *et al.,* 2011).

2.4 YIELD LOSSES

Pyricularia leaf spot, also known as blast disease, is particularly important in pearl millet cultivars. The pathogen *M. grisea* is a worldwide disease capable of devastating pearl millet resulting in significant yield reduction. The disease affects the crop at all growth stages from seedlings (causing lesions and premature drying of young leaves) to affecting the panicle causing blast of florets. The pathogen is highly destructive and economically important and causing chronic yield losses of grain (Timper *et al*., 2002) and forage (Wilson and Gates, 1993). It is an important disease in the southern United States and more recently it has emerged as a serious disease of dual purpose (grain and fodder) pearl millet hybrids in India (Lukose *et al*., 2007; Anonymous, 2009). In India, the disease was first reported from Kanpur, Uttar Pradesh (Mehta *et al*., 1953) and remained as a minor disease for a long time, but is gaining importance owing to damage caused by it. Severe outbreaks of Pyricularia blast have occasionally been reported in northern India (Williams and Andrews, 1983).

2.5 *Magnaporthe grisea* SPECIES COMPLEX

The genus *Pyricularia* is an important blast pathogen causing devastating blast disease in members of graminaceous family including rice, wheat, millets and many other hosts of grass species. Recently Choi *et al.* (2013) spotted that the members of *Magnaporthe grisea* species complex cause blast disease on a wide range of graminaceous hosts, including cultivated rice and other grass species. Recently, based on phylogenetic analyses and mating tests, isolates from crabgrass were separated from the species complex and named *M. grisea*. To date, 137 members of Poaceae hosting this fungus have been described in fungal data base (http://nt.ars-grin.gov/fungaldatabases/, updated on Apr. 6, 2012). Since individual isolates have a limited host range, they were regarded as the *Magnaporthe grisea* species complex (Mg complex).

Dagdas *et al.* (2012) have studied how the fungus channels its pressure to form a narrow infection peg that breaches the rice leaf surface and first time observed the specialized group of proteins called septins in plant infection. In wheat and rice fields of Brazil Kohli *et al.* (2011) observed the secondary hosts of Pyricularia on grass weeds like *Cenchurus echinatus, Eleusine indica, Digiteria sanguinalis, Brachiaria plantaginea, Echinocloa crusgalli, Pennisetum setorum, Hyparrhe niarufa* and *Rhynchely trumroserum* but their role in epidemiology of wheat blast is not clearly understood.

A molecular study by Hirata *et al*. (2007) revealed cryptic species have been described on grasses *P. didyma* M. B. Ellis, *P. dubiosa* (Speg.) Viegas, *P. leersiae*

(Sawada) S. Ito, *P. panici-paludosi* (Sawada) S. Ito, *P. penniseti* Prasada & Goyal, *P. setariae* Y. Nasik and *P. zizzniicola* Hashioka. Some other species are reported specially on monocotyledonous plants including Cannaceae, Commelinaceae, Cyperaceae, Musaceae and Zingiberaceae, but some species are saprobes on leaf litter of dicotyledonous plants. Earlier in 1971 Siwasin and Giatgong named several invalid species of *Pyricularia* on grasses.

Bussaban *et al.* (2005) indicated that recent molecular and genetic analyses of *Pyricularia* species isolated from different hosts are genetically distinct. According to Uddin (*et al*. 2003), *M. grisea* is pathogenic to more than 50 gramineous hosts including small grains, forage and turf grass including rice, wheat, pearl millet, finger millet, foxtail millet and grasses. The fungus is highly variable, but highly specialized in their host range. The disease in broad leaf hosts such as species of Cteneenthe, Marantha and Stromanthe has been referred to as pyricularia leaf spot.

According to Fuentes *et al.* (2003) and Ou (1980) *P. grisea* is noted for expressing a large number of virulent forms or pathotypes. In the field, *P. grisea* reproduce asexually, but it's sexual stage (teleomorph *M. grisea*) was demonstrated in the laboratory by Barr (1977), Hebert (1971) and Valet *et al.* (1986). The majority of field isolates of *P. grisea* are infertile. Couch and Kohn (2002) confirmed that *M. oryzae* is a new species distinct from *M. grisea*. Gene trees were inferred for *Magnaporthe* species using portions of three genes: actin, beta-tubulin, and calmodulin. These gene trees were found to be concordant and distinguished two distinct clades within *M. grisea*. One clade is associated with the grass genus *Digitaria* and other clade is associated with *Oryza sativa* and other cultivated grasses, therefore nomenclatural tied to *M. grisea* and is described as a new species, *M. oryzae*. But these are morphologically similar in their characters, *M. oryzae* is distinguished from *M. grisea* by several base substitutions in each of three loci as well as results from laboratory matings *M. oryzae* and *M. grisea* are not interfertile. It has been specified that *M. oryzae* is the scientifically correct name for isolates associated with rice blast and gray leaf spot, continued use of *M. grisea* for such isolates would require formal nomenclatural conservation.

2.6 MORPHOLOGICAL AND CULTURAL CHARACTERISTICS

Little is known of the biology of *M. grisea* on pearl millet in spite of the crop's nutritional importance. Hence, this study was initiated to study and understand the media

requirements for the growth and development of the pathogen, which could serve as an input in disease management to minimize yield loss. This study was also undertaken to study the effects of growth factors on mycelial growth of *M. grisea* isolates.

Padmanabhan *et al.* (1970) isolated *P. grisea* from samples of diseased leaves, necks and nodes of the infected rice plant on oat meal agar (OMA) with traces of biotin and thiamine (B and T). Cultures were purified by dilution method, and single spore isolates were grown and multiplied on OMA + B and T at 25^{o}C.

Xia *et al.* (1993) collected the panicles with the symptoms of neck blast, washed once with sterile distilled water, and placed on moist filter paper in Petri dishes at room temperature to induce sporulation. Conidia from the lesion surface were spread onto 3% water agar with a sterile loop and incubated overnight. Single germinating conidium was isolated and transferred to potato dextrose agar.

Nishikado (1927) obtained good growth of *Pyricularia oryzae* isolated from rice on decoction of their host material. Ramakrishnan (1948) observed linear growth of the colonies of the *Pyricularia* isolated from rice on standard medium agar, Oat meal agar, French bean agar and decoction agar made out of the leaf material of rice. He also determined the weight of mycelial mat produced by the isolates in the standard medium, Richards's medium, Browns medium and decoctions of leaf material of rice. The isolates produced good growth on the decoctions of their host material. Later, Sun *et al.* (1989) studied the effects of 17 media on 41 isolates of *P. oryzae.* They found that, corn meal and rice straw agar media were most conducive for sporulation.

Awoderu *et al.* (1991) investigated that linear growth of *P. oryzae* was greatest on Potato dextrose agar, while conidial production was greatest on one per cent soluble starch yeast extract agar. Arunkumar and Singh (1995) studied *Pyricularia grisea* (*M. grisea*) from rice on different solid culture media. They found that, maximum colony diameter of rice isolate occurred on malt extract agar and Leonin agar.

Viji *et al*. (2000) could distinguish *Pyricularia* isolates from different hosts based on cultural and conidial variation. Sonah *et al.* (2009) studied the cultural and morphological variability of *M. grisea* isolates collected from rice and non-rice hosts revealed that isolates that showed fast vegetative growth as grey-green or grey-white produced more number of spores than those with slower vegetative growth (submerged or subdued growth patterns). Isolates derived from non-rice hosts also showed abnormal spore morphology which were longer, cylindrical and obpyriform.

Du Xinfa *et al.* (1995) stated that, *Pyricularia* isolates from hosts including rice and common weeds in paddy fields sporulated abundantly on sterilized barley or sorghum grains. Awoderu (1990) observed the effect of different pH levels on the radial growth and sporulation of *P. oryzae* on different media. They found that optimum pH range for growth of *P. oryzae* was 5.5 - 10.5. Arunkumar and Singh (1995) noticed that the best growth of the fungus *P. grisea* was observed at pH 6.5.

Hossain *et al.* (2004) investigated the effect of eight different temperatures (5, 10, 15, 20, 25, 30, 35 and 40^{o}C) and 10 pH levels (3.5, 4.5, 5.0, 5.5, 6.0, 7.0, 7.5 and 8.0) in Richard's medium. They observed that the maximum growth of the fungus was observed at pH 6.5 and the least growth at pH 3.5. Good growth of the fungus was observed between the pH range 6.0 to 7.0 and in temperature studies more growth of the fungus occurred at 30^{o}C.

Srivastava *et al.* (2009) conducted an experiment to develop and evaluate cultural characteristics of *P. grisea* isolates from *Eleusine coracana* (finger millet) on media derived from rice and finger millet *viz.* rice leaf agar, ragi leaf agar and ragi flour agar. Growth characteristics such as vegetative growth, sporulation and production of perfect stage were recoded and compared with those obtained on oat meal agar. Results revealed that ragi flour media recorded better growth and sporulation of *P. grisea* at 28^{o}C and pH 7.5 than other three media. Abundant perithecia were produced on ragi flour media at 20 – 22^{o}C when compared to other media.

Nishikado (1927) reported that the optimum temperature for the mycelial growth of *P. grisea* to be 25 to 30^{o}C while minimum temperature for the growth of the species is 8 – 9^{o}C and thermal death point is 51-52^{o}C. The minimum, optimum and maximum temperature for growth and conidial production of *P. grisea* were 10, 25 and 37^{o}C, respectively (Awoderu *et al.,* 1991).

Arun kumar and Singh (1995) studied the differential response of *P. grisea* isolates from rice, finger millet and pearl millet to temperature. They reported that, all the isolates exhibited maximum growth at 30^{o}C. The growth of *P. grisea* was optimum at 28^{o}C, moderate at 23^{o}C and minimum at 15^{o}C and growth was inhibited at a temperature of > 37^{o}C (Okeke *et al.,* 1992).

Mijan Hossain (2000) observed that among the non-synthetic media, potato dextrose agar supported maximum radial growth (85.00 mm), next was host extract + 2 per cent sucrose agar medium (80.33 mm) followed by oat meal agar (75.00 mm).

Ramakrishnan (1948) observed a positive correlation in the sporulating ability and aerial growth of *P. grisea*. Mutations of the SMO+ genetic locus were reported to cause a number of gross deviations from the normal process of conidiogenesis, resulting in conidia which exhibited a wide variety of unusual morphologies (Hamer *et al.,* 1989). Arase *et al.* (1994) reported that two mutant isolates of *P. oryzae* formed abnormal, longer, cylindrical spores with more septa than those of normal, obpyriform spores of wild isolates.

Mijan Hossain (2000) observed mycelium in cultures was first hyaline in colour, then changed to olivaceous, 1 – 5.2 µm in width, septate and branched. The size of conidia were 15 – 22 µm x 4 – 7 µm (Average, 17.4 µm x 5.2µm).

Aoki (1955) measured 16 isolates in potato dextrose agar culture and showed that, the average length of the isolate ranged from 21.2 to 28.4 µm, and the average width from 7.3 to 9.0 µm. Ono and Nakazato (1958) observed that, the size of conidia of *P. grisea* varied with the culture media also.

Nishikado (1917) described the size and morphology of *P. grisea* spores which measured 16 – 33 x 5 – 9 µm. Usually, the spores are 22 – 27 x 7 – 8 µm with a small basal appendage measuring the dimensions of basal appendage were 1.2 – 1.8 (1.6) µm in width, basal cell 4.8 – 11.5 (7.8 µm), middle cell 1.8 – 11.5 (6.6 µm), apical cell 6 – 14 (7) µm in length. The *Pyricularia* forms on other plants do not differ distinctly from the rice fungus in size of conidia. Sawada (1917) and Nishikado (1926) described the conidia of the *Pyricularia* on crab grass as somewhat slender.

2.7 PATHOGENIC VARIABILITY OF *Magnaporthe grisea* USING A SET OF PUTATIVE HOST DIFFERENTIALS

Information on the pathogen population structure, such as the type of variants present in a location, the amount and distribution of variation assist plant breeders in resistance breeding and deployment of resistant cultivars. Therefore, precise delineation of pathogenic variability in the target production area is a prerequisite for identifying pearl millet genotypes with a stable resistance to the variable pathogen populations. It is important from an ecological, epidemiological and breeding perspective to know how genetic diversity is maintained and how new, well adapted complex races arise in the pathogen population. Three characters are significant from pathogenicity point of view. First group, isolates may cause several types of diseases and symptoms, in second isolates may vary from avirulent to aggressively virulent state, and in third the host range among

isolates may vary from limited to extremely wide. For the pearl millet blast, there is limited information available (Sharma *et al.,* 2013) on development of a tentative set of differentials for assessing the racial differentiation.

To know the virulence pattern of pearl millet blast pathogen *M. grisea* Sharma *et al.* (2013) pathotyped 25 isolates collected from four major pearl millet growing states in India i.e., Rajasthan, Haryana, Maharashtra and Uttar Pradesh on ten pearl millet genotypes *viz.*, ICMB 02444, ICMB 02777, ICMB 06444, ICMB 93333, ICMB 96666, ICMB 97222, ICMB 99444, 863B, ICMR 06222 and ICMB 95444 under green house conditions. Differential reactions to the test isolates were recorded on ICMB 02444, ICMB 93333, ICMB 97222, 863B and ICMR 06222.

Similarly for the virulence pattern of the finger millet blast pathogen, Kumar *et al.* (2007) pathotyped 12 isolates using finger millet genotypes IE 1012, IE 2912, IE 2885, Indaf-5, Indaf-9 and GPU 28 as a new set of differentials, identified genotype IE 1012 as a differential host and Indaf-5 and Indaf-9 as susceptible controls in the differential set. For a better understanding of the pathogen diversity it is important to have the right number of differentials, including local commercial cultivars and other sources of resistance. For example, in case of rice blast, there are several site- specific differential sets and an International differential set have been developed (Atkins *et al.,* 1967; Ling and Ou, 1969; Ou, 1972; Bonman *et al.*, 1987), and these are being effectively used to discern the races/biotypes in the rice blast pathogen. Extensive work has been done with rice blast and detailed pathogenic variation has been reported from single-spores originating from single lesions and monoconidal subcultures (Ou and Ayad, 1968; Ou *et al.*, 1970).

Chen *et al.* (2001) tested pathogenicity reactions of 792 *M. grisea* isolates of rice using 13 host differentials consisting of six *indica* and seven *japonica* near isogenic lines (NILs) and identified that 48 pathotypes with the *indica* NILs, 82 pathotypes with the *japonica* NILs and a total of 344 pathotypes with both *indica* and *japonica* NILs. It is concluded that large differences in distribution of the pathotypes among the different rice growing areas. Sharma *et al.* (2002) pathotyped 119 isolates of *M. grisea* from north-western Himalayan region. Isolates were grouped into 52 pathotypes on the basis of disease reaction on international differential rice lines.

Takan *et al.* (2011) studied the compatibility of thirty-one isolates representing diverse sampling location and host range revealed that all isolates were compatible to the tested eight finger millet varieties and showed differences only in aggressiveness and over

all differences between isolates and varieties were highly significant for lesion number and leaf area affected.

Srivastav *et al.* (2013) examined the host specificity of blast fungus (*M. grisea*) on four finger millet (viz., K7, GE5230, GPU26, PR202) and one local landrace of foxtail millet using F1 progenies of a cross between two highly fertile and host specific pathogenic cultures isolated from a collection of field samples at central Himalayan region of Uttarakhand state in India. Parental isolate VII739 was virulent on finger millet and avirulent on foxtail millet cultivars, but VII769 showed virulent reaction on foxtail millet and avirulent on finger millet cultivars. Data revealed that pathogenicity developed from the cross between finger millet isolates and foxtail millet isolate was conditioned by one (on K7 and PR202) and two (on GE5230) genes. The segregating ratio for 1:1:1:1 and 3:1:3:1 in combined analysis between K7 and PR202 and between PR202 and GE5230, respectively suggested that genes present in the cultivars were different and independent, contrarily 2:0:1:1 ratio between cultivars K7 and GE5230 demonstrated that one gene in GE5230 was identical to the one in K7. Avirulent genes for pathogen on K7, PR202 and GE5230 were designated respectively as AVR1, AVR2 and (AVR1, AVR3) and their corresponding resistance genes in cultivars as R1, R2 and (R1, R3). Avirulent reaction on GPU26 and foxtail millet cultivar explained that complex host species specificity of *M. grisea* existed in nature.

Karthikeyan and Gnanamanickam (2008) collected a total of 128 isolates of *Magnaporthe grisea* strains infecting *Setaria italica* (foxtail millet) from different states of South India *viz.*, Tamil Nadu, Karnataka, Andhra Pradesh and Kerala to screen the 22 accessions of foxtail millet. The disease severity was scored 10 days after inoculation using the standard evaluation system (SES) developed by the International Rice Testing Program (1988), IRRI, Philippines on a 0 to 9 scale. Disease scores of 0 to 3 indicate the presence of incompatible (resistant) type lesions and scores of 4 to 9 indicate the presence of compatible (susceptible) type lesions. This result was found that 9 out of the 22 *Setaria* accessions were highly susceptible to *Setaria* strains of the blast fungus and seven cultivars/accessions were resistant to blast pathogen. Various virulence reactions were scored according to Standard Evaluation System.

Yong Feng *et al.* (2004) identified the races of *M. grisea* of Jiangsu Province, and studied its population structure, the virulence of the dominant race, and the interaction of virulence among *Magnaporthe grisea* with resistance genes in rice cultivars to

comprehensively control rice blast by using 342 mono-conidial isolates obtained from rice blast specimens collected from five typical areas in Jiangsu Province during 2000-2002. The isolates could be classified into seven groups, thirty races when assessed with seven Chinese differential rice cultivars. The race ZG1 was predominant one of *M. grisea* with a frequency of 65 per cent in 2000, 56.90 per cent in 2001 and 60.38 per cent in 2002; the races ZB and ZC groups were also important in Jiangsu Province. By inoculating 130 ZG1 race isolates on thirteen Japanese cultivars with known resistance genes, forty-two pathotypes were found. Among them, 30.77 per cent of ZG1 race isolates had virulence to rice cultivars Shin 2 (Pita-ks, Pi-sh), K1 (Pi-ta), Pi 4 (Pi-ta2, Pi-sh), being the predominant pathotype, indicating the resistances of cultivars Shin 2 (Pita-ks, Pi-sh), K1 (Pi-ta), Pi 4 (Pi-ta2, Pi-sh) had lost the resistance in Jiangsu Province. Rice cultivar K3 (Pi-kh) was highly resistant to *M. grisea* with 100% resistance frequency and resistant to the two single isolates, 2003-184 (ZC5) and 2003-14-1 (ZG1), however, it was infected by a mixture of the two isolates. It was suggested that the change in virulence stemmed from the interaction of different pathotype isolates was one of the reasons that made variety lose its resistance.

The virulence pattern of the isolates of *P. grisea* from commercial fields of the upland rice (*Oryza sativa*) cultivars 'Primavera' and 'BRS Bonança' was analyzed by Araujo *et al*. (2005). A hundred and seventy monoconidial isolates of the pathogen virulent to 'Primavera' and 139 to 'BRS Bonança' collected from eight fields, during two years (2001-2003) were tested, under greenhouse conditions, on six newly released rice cultivars. Differences in virulence pattern were observed in pathogenic populations of 'Primavera' and 'BRS Bonança'. Isolates with virulence to improved cultivars were common in samples from farmers' fields in the absence of infection. The virulence frequency of *P. grisea* isolates collected from 'Primavera'' to cultivars 'BRS Vencedora', 'BRS Colosso', 'BRS Liderança', 'BRS Soberana', 'BRS Curinga' and 'BRS Talento', was high in descending order. On the other hand, in the fungus population of 'BRS Bonança' virulence frequency was high in 'BRS Talento', followed by 'BRS Curinga', 'BRS Vencedora', 'BRS Liderança', 'BRS Colosso' and 'BRS Soberana'. While virulence to 'BRS Talento' was rare among isolates from 'Primavera', it was most frequent in isolates of 'BRS Bonança'. The six improved rice cultivars permitted agriculturally important virulence in the pathogen population which can be utilized in selecting breeding lines for specific resistance, in rice blast improvement program.

Genetic and phenotypic structure of *M. oryzae* populations of two upland rice cultivars was determined by Silva *et al.* (2009). Monoconidial isolates were obtained from rice blast affected fields, four from cv. BRS Bonança and four from cv. Primavera, in Goias State (2001- 2003). The pathotypes IB-41 and IB-9 were predominant in both leaf and panicle isolates of BRS Bonança and IF-1 in Primavera. A great majority of pathotypes were common to both leaf and panicle subpopulations of Bonança (42.8 %) and Primavera (66.6 %). The ANOVA of virulence data showed high variability within population of each cultivar. There was no significant difference in virulence pattern of isolates from leaves and panicles, independent of collection site and cultivar. The molecular characterization of isolates was done employing the rep-PCR analysis with two primer sequences from Pot2. The genetic analysis of 538 isolates showed a high genotypic diversity in both leaf and panicle pathogen populations with 103 haplotypes in Bonança and 49 in Primavera. The migration of pathotypes from leaves to panicles in each field was 70.8 and 36.6 per cent for Primavera and BRS Bonança, respectively. The results indicated that the diversity of *M. oryzae* population was influenced by cultivar of origin. A great amount of population diversity was encountered within the same field.

Nagaraja *et al.* (2010) evaluated core set of 520 finger millet accessions for blast resistance under prevailing weather conditions in field revealed that the incidence of neck and finger blast decreased significantly with increased temperature from 23.9 to 27.0°C and reduced rainfall from 303 to 83.4 mm during flowering period, however, the RH remained almost constant (88.34 to 88.90 %).

2.8 MOLECULAR DIVERSITY

Magnaporthe grisea is the most destructive fungal pathogen of rice and a model organism for studying plant-pathogen interaction. Molecular markers and genetic maps are useful tools for genetic studies. The most commonly used marker systems are random amplified polymorphic DNA (RAPD) (Guthrie *et al.,* 1992), restriction fragment length polymorphism (RFLP) (Soller and Beckmann, 1983), amplified fragment length polymorphism (AFLP) (Vos *et al*. 1995), Universal rice primers (URP) (Kang *et al.,* 2001) microsatellites such as simple sequence repeats (SSRs) (Brondani *et al.,* 2000; Kim *et al.,* 2000; Kaye *et al.,* 2003; Suzuki *et al.,* 2009), inter simple sequence repeats (ISSRs) (Zietkiewicz *et al*., 1994) and minisatellite markers (Li *et al.,* 2007).

Zheng *et al.* (2008) investigated 446 simple sequence repeat (SSR) loci and developed 313 SSR markers, which showed polymorphism among nine isolates from rice. The number of alleles of each marker ranged 2-9 with an average of 3.3. The polymorphic information content (PIC) of each marker ranged 0.20 – 0.89 with an average of 0.53. A genetic map was constructed by using 176 SSR markers covering a total length of 1247 cM, equivalent to physical length of about 35.0 Mb or 93 per cent of the genome, with an average distance of 7.1 cM between adjacent markers.

Molecular markers have also been used widely to characterize fungal plant pathogen populations, in particular for the assessment of genetic diversity, phylogenetic relationships and for characterization of pathotypes. The level of polymorphism revealed by a marker system is dependent on the number of markers used, the degree of genome coverage and the type of DNA sequence variation being assayed (Powell *et al*., 1996). One of the most powerful assays is amplified fragment length polymorphism (AFLP) analysis (Vos *et al*., 1995). But conversion of AFLPs to locus-specific markers can be problematic because it is difficult to capture the source of the polymorphism in the PCR amplicon. In contrast, URP-PCR based markers can be easily converted as the source of polymorphism can be easily deduced with the help of dendrograms, which provide a detailed inference about the phylogenetic relatedness and variations.

Analysis of the diversity of the plant pathogens has been revolutionized by molecular techniques and particularly PCR based techniques have helped to understand the taxonomy and population structure. According to Burdon and Silk (1997), plant pathogenic fungi most commonly rely on mutation and recombination as the main source of genetically based variations. The process of gene flow between the populations within species supplements as spread of propagules from one epidemiological area to another (McDermott and McDonald, 1993).

2.8.1 Molecular diversity study using universal rice primer (URP) markers

Magnaporthe grisea is clearly emerging as one of the most important model systems representing filamentous fungal pathogens of plants. The fungus *M. grisea* has capacity to quickly overcome the resistance within a short time after the release of a new cultivar has made breeding for resistance a constant challenge. In order to prevent breakdown of resistance genes, knowledge of genetic structure and dynamics of pathogen

population is essential for the prudent implementation of disease management strategies (Lavanya and Gnanamanickam, 2000).

Aggarwal *et al.* (2008) studied the molecular characterization of 15 different strains or isolates of *Chaetomium globosum* and one isolate of each *C. reflexum* and *C. perlucidium* collected from various locations of India with 12 universal rice primers by using polymerase chain reaction. Nine universal rice primers out of 12 gave polymorphic fingerprint patterns from DNA of *Chaetomium* spp. ranging from 250 to 3000 bp for each isolate. Phylogenetic analysis of entire fingerprint profile using the unweighted pair-group method with arithmetic averages (UPGMA) of a combined data showed formation of two main clusters with only 65.3 per cent similarity.

Inter specific genetic relationship among *Bipolaris* genera of 14 different isolates of *Bipolaris sorokiniana* and other four species of *Bipolaris* (*B. maydis*, *B. oryzae*, *B. tetramera* and *B. spicifera*) collected from different geographical locations of India, examined by Universal Rice Primers were evaluated by Banerjee *et al.* (2014) using 12 URP markers. Beyond 12 universal rice primers only seven primers were effectively produced multiple bands ranging from 75 to 5000 bp in all isolates of *Bipolaris*. Maximum number of bands (30) were obtained when amplified with URP 1F.

Kang *et al.* (2001) designed the 40 primers consisting of 20 oligonucleotides from the repetitive sequences of Korean weed rice DNA by using high stringent PCR conditions, 20 primers out of 40 produced characteristic fingerprints from diverse genomes including seven animals, 14 plants and six microorganisms as well as rice and named as universal rice primers (URP).

Experiments conducted by Kang *et al.* (2001) suggested that URP PCR is a valuable tool for characterization and grouping at inter and intra specific levels of various fungal, bacterial and plant species associated with medical, agricultural, industrial and environmental fields. It also observed the production of distinct PCR profiles in the isolates collected from different geographical regions, which permitted differentiation among them at inter species level and furthermore, ubiquitous strain types were observed.

Assessment of genetic variability among 60 monosporic isolates of *B. sorokiniana* from Brazil and other countries was carried out by Mann *et al.* (2014) by using 12 URP primers as described by Kang *et al.* (2002). PCR amplification generated 232 different DNA fragments ranging from 100 to 2018 bp. The primers URP 4R, URP-2R and URP-1R generated greater number of amplified fragments (36, 30 and 25, respectively) from the

single spore isolates and also among the isolates using these primers. The URP-PCR primers provided important information about the genetic profiles of the monosporic cultures, which showed to be conserved in fungi, enabling detection of intra specific variability among the monoconidial isolates and among the monosporic cultures that originated from the same polysporic strain. The isolates from Brazil were more efficiently amplified with URP primers compared to other countries.

Zhong and Steffenson (2001) found that there was no correlation between genetic similarity and geographic origin of *Cochhliobolus sativus* isolates based on virulence and AFLP markers studies. Whereas Kang *et al.* (2008) demonstrated that URP primers could be used to detect inter and intra specific polymorphism among 25 isolates of six *Alternaria* spp. and obtained results that allowed the isolates to be grouped according to their geographical regions.

Kiranbabu *et al.* (2013) studied the genetic diversity and population structure of 72 *M. grisea* isolates collected from finger millet (56), foxtail millet (6), pearl millet (7) and rice (3) from major crop growing areas in India using 24 SSR markers. None of the SSR markers detected polymorphism in the *M. grisea* isolates from pearl millet. Seventeen SSR markers were polymorphic in the 65 non pearl millet isolates and detected 105 alleles, of which one was rare, 83 common, 9 frequent and 12 most frequent. A model based population structure analysis of the genomic data identified two distinct populations with varying levels of ancestral admixtures among the 65 *M. grisea* isolates. Analysis of molecular variance (AMOVA) indicated that 52 per cent of the total variation among the isolates used in the study was due to differences between the pathogen populations adapted to different hosts, 42 per cent was due to differences in the isolates from the same host and the remaining 6 per cent due to heterozygosity with in isolates. High genetic variability present in *M. grisea* isolates calls for continuous monitoring of *M. grisea* populations anticipating blast resistance breakdown in finger millet cultivars grown in India.

Huff *et al.,* (1994) used random amplified polymorphic markers to survey genetic variability among 35 *M. poae* isolates. Amplification patterns for 23 of the 35 isolates were phenotypically unique and readily distinguishable from an out group of six additional ectotrophic, dematiaceous fungi. These results indicate the utility of RAPDs as an accurate and reproducible means of identifying individuals within the species. Analysis of molecular variance demonstrated that isolates of *M. poae* were significantly different (P=0.001) among 12 sample locations.

2.9 EPIDEMIOLOGY

The influence of temperature and leaf wetness duration on the development plant disease has been well defined for a number of pathosystems but to our knowledge, this is the first study to demonstrate the interaction of temperature and leaf wetness duration on the development of blast of pearl millet. Specific temperatures and durations of free moisture are required for infection and subsequent disease development by many plant pathogenic fungi *M. grisea* on rice (Kim, 1994; Ou, 1985; Teng, 1994; Greer and Webster, 2001), on rye grass (Uddin *et al.,* 2003; Bain *et al.,* 1972; Carver *et al.,* 1972) and on finger millet (Kiranbabu, 2011), *Colletotrichum coccodes* on tomato (Byrne *et al.,* 1998), *Rhizoctonia solani* on perennial rye grass (Gross *et al.,* 1998), *A. brassicae* on oil seed rape (Hong *et al.,* 1996), *Botrytis cinerea* on geranium (Sirjusingh and Sutton, 1996)

The weather plays an important role in the variability of disease development, undoubtedly when there are no fluctuations in the relative humidity and temperature there appears to be no modifications in disease (Asai *et al*., 1967). Suzuki (1975) stated that the climate has a strong influence on the appearance of blast epidemics. In 1979, Marin Sanchez reported *P. oryzae* infections affected all plant organs and caused serious yield losses when the climate conditions are favourable to the disease. According to Makowski *et al.* (2011) wetness of the host surface is a critical environmental factor for the development of foliar fungal diseases.

Castejon munoz (2008) conducted a field trial to investigate how temperature and relative humidity affect the air borne concentration of *P. oryzae* spores and the development of rice blast. A relative humidity of 95 per cent and an average of 26-27^{o}C were optimum for infection and substantially favoured spore release. The first symptoms of infection were detected on the leaves when the crop was at the mid-tillering stage, the number of lesions increased as the plant developed. After the onset of heading the number of leaf lesions decreased while the number of node and panicle base lesions increased. A temperature increase of 1°C in early August (mid-tillering stage) led to an increase in the mean intensity of disease. The peak spore concentration in August could be used to forecast panicle blast. Assessing the air borne concentration of *P. oryzae* could help in understanding the population dynamics of this pathogen.

Kato and Kozaka (1974) conducted experiment on effect of temperature on lesion enlargement and sporulation of *P. oryzae* in rice leaves by exposing to alternating day and

night temperatures. Inoculated plants were incubated after initiation of lesion development in separate growth cabinets with day (12 h) and night (12 h) temperature of 20 and 16^{o}C, 25 and 16^{o}C, 32 and 25^{o}C, 32 and 20^{o}C respectively and observed that blast lesions on rice leaves expanded rapidly for eight days and approached a maximum length of 35 mm during the next 12 days at temperatures 32/25^{o}C, 32/20^{o}C and 25/16^{o}C day/night and lesion expansion is slower at temperature 20/16^{o}C day/night to a length of 25 mm in 20 days. But highest potential of sporulation recorded at median temperature regime of 25/16^{o}C day/night treatment.

According to Lamey (1970) long periods of leaf wetness, high relative humidity and temperatures of 17 to 28^{o}C were favorable to rice blast development. Kim, (1994), Teng, (1994), Ou, (1985) stated that sporulation of *P. grisea* is favoured by relative humidity $\geq$ 89%, optimal temperatures of 25 to 28^{o}C and minimum of 4 h of leaf wetness is required. Leaf wetness of 7 to 14 h is essential for infection of rice by *P. grisea* (Barksdale and Asai, 1965; Khan and Libby, 1958; Teng, 1994).

Ou, 1987 reported that reproduction is asexual by mitotically produced spores (conidia) from lesions on above ground plant parts. A single lesion can produce 2000-6000 conidia/day for up to 14 days, with multiple cycle of infection and reproduction during on growing season. Some conidia may disperse beyond usual 1m range. Although asexual reproduction occurs *in vitro*, the morphological structures, perithecia are not observed in nature (Hebert, 1971; Silue and Notteghem, 1990; Zeiger, 1998).

Shirasawa *et al.* (2012) investigated the effect of red light on lesion formation in comparison with white and natural light conditions. For this rice seedlings at 4 leaf stage were inoculated with *M. oryzae* and kept in the dark for 24 h and then continuously lit with white and red fluorescent lamps. Control plants were placed under natural light without white and red fluorescent lamps. They observed that under red light, the formation of blast lesions with white, collapsed tissues was significantly inhibited and the formation of necrotic lesions was increased as compared to white and natural light treatment.

Shafaullah *et al.* (2011) studied the effect of epidemiological factors- temperature, relative humidity and rainfall on the incidence of paddy blast caused by *P. oryzae*, during the growing season 2008. The blast disease incidence and temperature was negatively correlated i.e. -0.88, -0.80, -0.95 and -0.84, respectively, this indicated that the disease incidence increases with the decrease in temperature. However, humidity and rainfall were positively correlated with paddy blast disease incidence.

In general, long periods of leaf wetness, high relative humidity and temperatures of 17 to 28^{o}C favours rice blast development (Webster and Gunnell, 1992). Important processes in the disease cycle include sporulation, germination and infection. Sporulation of *P. grisea* is favoured by relative humidity ≥ 89%, optimal temperatures of 25 to 28^{o}C and a minimum of 4 h of leaf wetness (Kim, 1994; Ou, 1985; Teng, 1994). Under optimal conditions, conidiophores and the first conidia were produced 4 to 6 h after dew formation and the conidia were released shortly thereafter (Kim, 1994; Teng, 1994). Optimal conditions for *P. grisea* conidial germination were 92 to 96% relative humidity and temperature of 25 to 28^{o}C (Kim, 1994; Ou, 1985). In water conidial germination may occurs within 3 h (Kato, 1974). Leaf wetness of 7 to 14 h is essential for infection of rice by *P. grisea* (Barksdale and Asai, 1965; Kahn and Libby, 1958; Kato, 1974; Teng, 1994, Yoshino 1974).

According to Greer and Webster (2001) in fields where moderate rice blast pressure was present, long leaf wetness periods of 14 to 18 h and warmer mean temperatures 18 to 22^{o}C during the leaf wetness period resulted in higher *P. grisea* conidia production.

Turechek and Stevenson (1998) evaluated the effects of partial host resistance, temperature, leaf wetness duration and leaf age on infection and lesion development of pecan scab caused by *Cladosporium caryigenum*. Trees of cultivars Wichita (susceptible) and Sumner (resistant) were inoculated with conidia of *C. caryigenum* and placed in mist chambers set at 15, 25 and 35°C. The trees were removed from the chambers after 3, 6, 12, 24, 36, or 48 h of leaf wetness and placed in a greenhouse to allow disease development. After 8 to 16 days, disease began to develop on both 'Wichita' and 'Sumner'. Logistic regression analysis showed that the probability of a leaf becoming infected was greatest for 'Wichita', it decreased with increasing leaf age and temperature and increased with increasing leaf wetness. Results showed that infection frequency, lesion size, and conidia production decreased proportionately with increasing leaf age. The magnitude of this effect was greatest on 'Sumner'. Conidia production was positively correlated with lesion size, and both were positively correlated with infection frequency on both cultivars.

According to Vander plank's equivalence theorem (1963), quantitative information concerning the effect of environment on specific disease components should also be useful in estimating the quantitative impacts of plant genotypes with partial resistance (i.e., components of resistance) on the rate of disease progress in the field (Aquino *et al.,* 1995; Pedersen and Morrall, 1994; Ricker *et al.*, 1985; Webb *et al.,* 1996). Applying this theorem

at disease components level, a 50 per cent reduction in infection efficiency, whether it is due to a less favorable environment, partial resistance or a less aggressive pathogen isolate, would all have equivalent epidemiological effects on the rate of disease progress in the field (Vander plank, 1963).

Uddin *et al.* (2003) evaluated the effects of temperature and leaf wetness duration on the development of gray leaf spot of perennial rye grass turf in controlled environment chambers. Six week old Legacy II rye grass plants were inoculated with an aqueous conidial suspension of *P. grisea* (approximately 8 x 10^4 conidia per ml of water) and subjected to four different temperatures (20, 24, 28 and 32^{o}C) and 12 h leaf wetness durations (3 to 36 h at 3 h intervals). Three days after inoculation, gray leaf spot developed on all plants at all temperatures and leaf wetness durations. Disease incidence and severity were assessed 7 days after inoculation. There were significant effects (α = 0.0001) of temperature and leaf wetness duration on disease incidence and severity and there were significant interactions (α = 0.0001) between them. Among the four temperatures tested 28^{o}C was most favorable to gray leaf spot development. Disease incidence and severity increased with increased leaf wetness duration at all temperatures. A shorter duration of leaf wetness was required for disease development under warmer temperatures.

A study on gray leaf spot of forage Italian rye grass in the early 1980s conducted by Moss and Trevathan (1987) indicated 26^{o}C to be the most favourable temperature for gray leaf spot development and disease severity reportedly increased with increasing leaf wetness duration up to 24 h. However, in tall fescue turf, a slightly higher temperature (28^{o}C) was the most favourable to gray leaf spot development and an increase in disease incidence was associated with an increase in temperature (Uddin *et al.*, 1998).

Wetness duration and temperature are generally the main microclimatic parameters determining the development of fungal plant diseases. Thus, models to predict anthracnose and other foliar diseases in different host systems have been based on climatic parameters (Danneberger *et al.*,1984; Madden *et al.*,1993; Peres *et al.*, 2002; Timmer and Zitko, 1993; Timmer and Zitko, 1996), relationships between temperature and leaf wetness duration derived from experiments under controlled conditions (Carisse *et al.*, 2000; Diéguez-Uribeondo *et al.*, 2003; Montesinos *et al.*, 1995; Shaw *et al.*, 1990; Uddin *et al.*, 2002) and laboratory studies on conidial germination (Fitzell *et al.*, 1984). Some of these models have been modified for particular climates and have allowed a significant improvement in the timing of fungicide applications (Peres *et al.*, 2002; Timmer and Zitko, 1996).

As per the literature, most of the research on sporulation and conidial release from blast lesions on rice have been conducted during the leaf blast stage (Kato, 1974) and this was probably due to the importance of primary inoculum potential of leaf blast lesions to neck blast development. The pathogen from rice grows luxuriantly on oatmeal, potato dextrose, ragi-meal agar medium at pH of 6.9 and temperature 30°C (Sirkant Kulkarni and Govindu, 1976). Perezsendin *et al.* (1982) recorded 30°C as the optimum temperature for sporulation of *M. grisea* from rice. Sporulation of *M. oryzae* and disease progress was favored by high relative humidity (>89%), optimal temperature (25-28°C), and a minimum of 4 h of leaf wetness (Teng, 1994).

Moss and Trevathan (1987) found that blast infection of 3 week old plants of susceptible ryegrass cultivar 'Gulf' increased exponentially with increasing inoculum densities up to 8×10^5 conidia ml^{-1} and optimum temperature for infection was predicted to be 26°C, few lesions occurred at 35°C and none observed at 5°C. A continuous leaf wetness of at least 24 h was required for maximum infection and may be the critical factor in epidemic disease development.

According to the Bisht *et al*. (1984) the climatic conditions that prevailed from 15th July were more favourable for blast development with average minimum and maximum atmospheric temperature of around 20 and 30°C respectively and relative humidity of >80%. The investigations on effect of temperature and relative humidity on finger millet blast incidence made by Chaudary and Vishwadhara (1988), Gowda and Gowda (1995) and Kumar *et al.* (2005) revealed that a temperature range of 18 to 24°C was more congenial for the development of neck and finger blast in ragi, than at other temperature ranges.

Thakur *et al.* (2009) developed the greenhouse and field screening technique for pearl millet blast by artificial inoculation. The field screening technique involved the use of a highly susceptible line as an infector row grown after every four test rows, artificial spray inoculation of 30 day old plants using *P. grisea* spore suspension (1×10^5 spores ml^{-1}) and maintaining high humidity (>90% RH) through perfo-irrigation for 2 weeks following inoculation. The greenhouse screening technique involved spray inoculation of 15 day old potted seedlings with *P. grisea* spore suspension and maintaining moderate temperature (25±1°C) and high humidity through a misting system for 10 days after inoculation.

2.10 HOST PLANT RESISTANCE

Pearl millet is a low value crop and is generally grown as rainfed crop and most often on marginal soils. Development of varieties with genetic resistance is the best means of combating the disease problem and is more relevant in pearl millet, which is predominantly grown by resource-poor and marginal farmers who cannot afford controlling diseases using chemicals. The success of such programme depends on the identification of stable resistant sources and its subsequent utilization in breeding. As the germplasm is the basic raw material, one has to bank upon a broad genetic base now and in the future (Nagaraja *et al.*, 2007). As a result, the search continues for sources of high levels of host-plant resistance (HPR). However, large scale evaluation of germplasm collections against various biotic or abiotic stresses is resource and time consuming. To overcome the need for a large scale evaluation of the entire germplasm collection of a species, Frankel and Brown (1984) proposed the concept of a core collection (10% of the entire collection) representing over 70 per cent of the genetic variation available in the entire collection.

Leaf blast susceptible varieties of rice have shown the resistance to neck blast and *vice versa* (Ono and Suzuki, 1960). Balal *et al.* (1977), Bhardwaj and Singh (1983) showed the positive correlation between leaf and neck blast infection. However, Koh *et al.* (1987) found some cultivars resistant at seedling stage appeared leaf susceptible to neck infection. Bonman *et al.* (1989) reported that two lines out of 27 were susceptible to leaf blast but resistant to neck blast and concluded that leaf and neck blast were not linked. Similar findings were obtained by Padmanabhan (1965) and Puri *et al.* (2009) concluded that resistance to neck blast might be expressed in some lines of rice independently to leaf blast. Ou (1985), Ou and Nuque (1963) reported rice lines resistant to leaf blast at seedling stage, are completely resistant to neck blast and susceptible at the seedling stage are susceptible to neck blast.

Vingnanakulasingam (1991) and Puri *et al.* (2009) screened the rice lines for neck blast resistant under greenhouse conditions by injecting spore suspension of 10^5 spores ml^{-1} with syringe at photosynthetic leaf sheath base of the individual tillers at Booting stage (beginning with panicle initiation, growth stage 4 of IRRI growth scale of a 0 – 9) (IRRI, 2002). Bonman (1992) showed the correlation between leaf and neck blast incidence in most of lines of rice, Barkhe 3017, Masuli × MT4P # 137, Masuli × MT4 P # 168 and Masuli × MTP # 86 except IR 25604, which was susceptible to leaf blast but resistant to neck blast and concluded that genetic makeup and environmental parameter were the prominent factors for differential interaction.

Jia *et al.* (2003) developed novel spot method for evaluation of blast resistance in rice and indicated no deleterious effects of Tween 20 to rice blast development and Tween-20 (0.02% vol/vol) was necessary for promoting adherence of spore suspensions to the detached leaves. Puri *et al.* (2009) assessed 182 rice lines for leaf and neck blast resistance, among them 77 were resistant, 43 were moderately resistant, 39 were moderately susceptible and 23 were susceptible to leaf blast while among the selected 31 lines evaluated for neck blast, each 4 lines were resistant and moderately resistant, 16 were moderately susceptible and 7 were susceptible. Leaf and neck infection was significant and positively correlated.

Ravi Kumar *et al.* (1990) evaluated 316 accessions of finger millet over four seasons under natural epiphytotic conditions at UAS, Bangalore. However, none were completely free from finger blast. Six genotypes GE 75, 669, 866, 1309, 1319, and 1407 showed resistance to both neck and finger blast and these were identified as source of stable resistance for resistance breeding programmes. Out of 25 finger millet cultivars tested in two fields, none of the cultivars were resistant to leaf blast bur HPB IE11-1 had small sized lesions. When scored for neck and finger blast IE 1012 was completely immune to infection and cultivars HPBIE11-1, indaf 15, MR 1, MR 2 and MR 3 had less than 5% infection (Somashekhara *et al.*, 1991).

Evaluation of 21 genotypes of ragi under natural epiphytotic conditions for three consequent years to know the stability of resistance to neck and finger blast showed that the genotypes VL 145, VL 149, PR1158-9, GPU 16 and RHRN82-Y84 exhibited stable resistance while HR8-19-1 and PR 202 exhibited moderate resistance and stability (Jain *et al.*, 1994).

Fakrudin *et al.* (2000) evaluated 15 selected accessions of finger under field conditions IE 2897, 2912, 2885 and 1012 were resistant while others were intermediate to highly susceptible for leaf blast whereas, IE 1012, 2885, GPU 28 and GPU26 were completely resistant to neck blast. Over the 2 years of evaluation under natural epiphytotic conditions, finger millet genotypes, GPU 26, GPU 28, AKE 1033, VL 149 and MR 2 were found to be moderately resistant to neck and finger blast (Rajanna *et al.*, 2000).

Mantur and Madhukeshwara (2001) and Mantur *et al.* (2001) screened 66 genotypes of finger millet over two seasons in field conditions under natural epiphytotic conditions revealed that neck blast incidence in susceptible check was >50 per cent

whereas, genotypes 2400, 4913, 4914, 4915, 4929, 4966, 5102, 5126, 5148 were completely free from blast while as many as 36 genotypes showed <2 per cent incidence.

Sunil (2002) screened 100 finger millet germplasm lines resistance to blast over two seasons, of these GE 632, 637, 659, 665, 669, 674, 676, 682, 696, 704, 705, 710, 728, 730 were found to possess partial resistance in the form of slow blasting. Karmakar *et al.* (2002) identified finger millet blast tolerant cultivars, GPU 28, MR 20, VR 550, MR 19, GPU 34 and VR 687 during two years of evaluation under field conditions and also reported that sowing on 2nd July allowed the crop to escape the disease and obtained higher grain yield in comparison to 18th July sowing, where disease severity increased and reduced grain yields by 21.5 per cent probably due to high inoculum pressure which coincides with favorable weather conditions.

Ravishankar *et al.* (2004) studied the effect of different maturity groups on blast disease under field conditions at UAS, Bangalore and found that long duration (MR-33) and medium-duration (KMR-9 and KMR-3) cultivars were more tolerant than the early-maturing cultivars (KMR-7 and KMR-4). Sixty-five varieties collected from farmers and 30 germplasm lines were evaluated by Takan *et al.* (2004) for blast resistance under natural infection at Alupe, Kenya revealed that ICRISAT germplasm lines KNE 620, 629, 688, 814 and 1149, and farmer variety accessions 14, 29, 32 and 44 were identified with low blast severity levels and good agronomic performance.

CHAPTER III
MATERIAL AND METHODS

This chapter describes all the materials used and methods adopted in the present investigation. All the techniques used are detailed under respective headings and their original references quoted. The present investigation was carried out at Cereals Pathology and Center for Excellence in Genomics (CEG), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The field experiment - Pearl millet blast Virulence Nursery (PMBVN) was conducted at six locations at Aurangabad, Dhule, Jamnagar, Gwalior and Durgapura in India during the rainy (*Kharif*) season 2013 and 2014.

3.1 COLLECTION, PURIFICATION AND TESTING PATHOGENICITY OF *Magnaporthe grisea* ISOLATES

3.1.1 Glassware

Borosil glassware was used throughout the present investigation. The glassware was first washed with a detergent followed by thorough cleaning with tap water before placing them in cleaning solution for 24 hours and finally rinsed with distilled water for 3-4 times and air dried.

3.1.2 Chemicals

Chemicals of Analytical Reagent (AR) and Guaranteed Reagent (GR) grades of standard make were used. The pH of the media was adjusted using either 0.1 N HCl or 0.1 N NaOH.

3.1.3 Sterilization

Glassware used in the present investigation were sterilized in hot air oven at 160°C for 90 minutes. Surface of Laminar Air Flow chamber (LAF) was sterilized by wiping with cotton swab dipped in alcohol. Inoculation loop, cork borer and scalpel were sterilized by dipping in alcohol and flame sterilization. Culture media and distilled water were sterilized in autoclave at 15 lb for 20 minutes.

3.1.4 Laboratory Techniques

The general laboratory techniques described by Dhingra and Sinclair (1995), Nene and Thapliyal (1993) and Aneja (2007) were followed for preparation of media,

sterilization, isolation and maintenance of fungal cultures with slight modifications wherever necessary.

The pH of the medium was adjusted to 6.5 with 0.1 N NaOH or 0.1 N HCl as the case may be with the pH meter. The medium was dispensed to test tubes and conical flasks at 8.0 ml and 100 ml each, respectively. The medium was sterilized in an autoclave at 15 lb for 20 min.

3.1.5 Equipments

Optical microscope (10x and 40x magnifications) was used for observing the *M. grisea* cultures. Hot air oven and autoclave were used for sterilization of glassware and media, respectively. Incubators were used for incubating test materials at different temperatures. The cultures were stored in a refrigerator. Chemicals were weighed on a single pan electronic balance with a sensitivity of 0.001 g. Other tools used in the present investigation for various purposes included camel hair brush, inoculation needle, pots, etc.

3.1.6 Culture media used

For isolation, culturing and maintenance of *M. grisea* isolates in the laboratory Potato Dextrose Agar (PDA) (potato: 200 g, dextrose: 20 g, agar: 20 g, distilled water: 1000 ml) and Oat meal agar (rolled oats: 20 g, agar: 15 g, distilled water: 1000 ml) were used.

Peeled potato pieces or rolled oats were boiled for respective media in 500 ml of distilled water in a 1000 ml beaker. The extract was filtered through a double layered muslin cloth. In a separate 1000 ml beaker, 500 ml of distilled water was taken to which 20 g of agar was added and boiled till it got dissolved. Both the solutions were mixed in another 1000 ml beaker into which 20 g of dextrose was added. The final volume of the medium was made up to 1000 ml by addition of sterile distilled water.

The pH of the medium was adjusted with 1 N NaOH or 1 N HCl as the case may be with the pH meter. The medium was dispensed to test tubes and conical flasks at 8.0 ml and 100 ml each, respectively. The medium was sterilized in an autoclave at 15 lb for 20 min.

3.1.7 Plating of medium

The sterilized medium in conical flasks was melted and poured in Petri plates (9 cm dia.) @ 20 ml per plate aseptically in the laminar air flow chamber and allowed to solidify. The plates containing the medium were used for culturing and maintenance of *M. grisea* isolates.

3.1.8 Collection of blast disease samples and isolates

Blast-infected leaf samples of pearl millet were collected from different locations in India during *Kharif* season 2010 and 2012. Sampling sites also included "hot spots" where blast occurs in severe form.

The isolates were designated with three part code such as MgPM 21, MgFM 57, MgC 3, MgD 2 and MgR 1 and so on. The first part of the letters represented the pathogen name (Mg: *Magnaporthe grisea*) and the next alphabet letter represented the host name (e.g. PM: Pearl millet, FM: Foxtail millet, C: Cenchrus, D: Dicanthium and R: Rice) followed by numerical number indicated the serial number of isolate.

3.2 ISOLATION OF MONO-CONIDIAL ISOLATES OF *Magnaporthe grisea*

Blast-infected leaf tissues were cut into small bits. These bits were washed in distilled sterilized water twice, surface sterilized in 0.1 per cent sodium hypochlorite for 2 min, rinsed three times in sterilized water, dried with sterilized filter paper and placed onto OMA medium in Petri dishes. Following incubation for 7 days at 28±1°C, a dilute spore suspension was prepared in sterilized distilled water and plated onto 4 per cent water agar in Petri plates. After 10–12 h incubation at 28±1°C, single germinating conidia were marked with help of a dummy objective lens under a microscope and transferred to fresh Petri plates containing OMA medium, one conidium per plate. The Petri plates were incubated at 28±1°C for 7 days and the identity of the fungal cultures developing from the single spores was established based on spore morphology (Ou, 1985). Cultures were maintained on OMA for further study.

3.3 PATHOGENICITY TEST

3.3.1 Plant material

Susceptible pearl millet line ICMB 95444 was used for testing the pathogenicity of each isolate. Seedlings of the susceptible cultivar were grown in 15 cm diameter plastic pots filled with sterilized soil-sand-FYM (farmyard manure) mix (2:1:1) and placed in a green house bay maintained at 30°C with two replications. Seedlings were thinned at one-leaf stage to keep 10 plants per pot.

3.3.2 Inoculum preparation and inoculation

Mycelial discs of 5 mm of each isolate were cut from 7 day old culture of *M. grisea* grown on OMA medium at 28±1ºC. Mass multiplication of spores for inoculation was achieved by growing each isolate (3 discs/plate) on OMA medium at 28±1ºC for 8 to 10 days. The plates were flooded with 10 ml of distilled water and the fungal growth containing mycelium and conidia was gently removed by scrapping with a sterile plastic inoculation loop. Harvested spores were filtered through a double-layer muslin cloth, the resultant concentration was adjusted to 1×10^5 conidia ml^{-1} and Tween 20 (0.02% vol/vol) (polyoxylethylene sorbitan monolaurate) (Jia *et al*., 2003) was added to the suspension just before inoculation. Twelve day old pot grown seedlings were artificially inoculated by spraying the inoculum on the foliage using a hand operated atomizer (Plate 3.1a). Seedlings sprayed with water were maintained as control. All the inoculated seedlings were covered with polythene bags and incubated at 25°C for 48 h to prevent cross contamination. After 48 h bags were removed and inoculated seedlings were exposed to more than 90 per cent relative humidity (RH) under misting for 6 days in green house (Plate 3.1b).

3.3.3 Data recording

Data on leaf blast severity of each isolate was recorded eight days after inoculations using a 1-9 progressive scale on individual plant basis (Sharma *et al.,* 2013) (Table 3.1 and Plate 3.2). To complete Koch's postulates, re-isolations of the each isolate from the artificially inoculated leaves were made following the protocol previously described.

3.4 CULTURAL, MORPHOLOGICAL, PATHOGENIC AND MOLECULAR DIVERSITY AMONG THE *Magnaporthe grisea* ISOLATES

3.4.1 Effect of different pH levels and different temperatures on growth and sporulation of *Magnaporthe grisea* isolates

Two different media potato dextrose agar (PDA) and oat meal agar (OMA) were tested at six different pH levels and five different incubation temperatures (22^oC, 24^oC, 26^oC, 28^oC and 30^oC) in the present investigation based on earlier studies on rice blast (Hossain *et al.,* 2004; Awoderu, 1990; Arunkumar and Singh, 1995) to select the best medium suitable for the growth and sporulation of the pathogen.

Plate 3.2. Progressive scale (1-9) for recording leaf blast severity in pearl millet seedlings infected with *Magnaporthe grisea.*

Table 3.1. Disease rating scale (1-9) of foliar blast of pearl millet.

Rating Scale	Symptoms and lesions	Disease reaction
1	No lesion to small brown specks of pin head size	Resistant
2	Large brown specks	
3	Small, roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter with a brown margin	
4	Typical blast lesions, elliptical, 1-2cm long, usually confined to the area between main veins, covering < 2% of the leaf area	Susceptible
5	Typical blast lesions covering < 10% of the leaf area	
6	Typical blast lesions covering 10-25% of the leaf area	
7	Typical blast lesions covering 26-50% of the leaf area	
8	Typical blast lesions covering 51-75% of the leaf area and many leaves dead	
9	> 75 % leaf area covered with lesions or all leaves dead	

To test the optimum pH for the growth of *M. grisea*, culture characters of four monoconidial isolates of *M. grisea* MgPM 45, MgPM 53, MgPM 56 and MgPM 118

selected based on previous studies conducted at ICRISAT (Sharma *et al.,* 2013) were tested on OMA and PDA medium at different pH ranges from pH 5.5 to pH 8.0 (pH 5.5, pH 6.0, pH 6.5, pH 7.0, pH 7.5 and pH 8.0) and incubated at different temperatures 22^{o}C, 24^{o}C, 26^{o}C, 28^{o}C and 30^{o}C for 10 days and then radial growth (cm) and sporulation of these isolates was recorded to select optimum pH and temperature.

3.4.2 Morphological and cultural characteristics of *Magnaporthe grisea* isolates

The variability in cultural characteristics of 65 isolates of *M. grisea* collected from different states was carried out on OMA medium at pH 6.5 by incubating at 28^{o}C (selected based on pH and temperature studies). The morphological characteristics such as colour of the fungus, type of growth of the fungus (cotton, subdued, tufted, submerged, sectored or non-sectored growth), smooth or rough surface and compressed or raised margins were recorded for the *M. grisea* isolates from pearl millet at 10 days after incubation.

3.4.2.1 Sporulation measurement: The sporulation capacity of each isolate on media was assessed by microscopic observations. After 10 days of incubation at 28±1°C, each Petri plate of *M. grisea* isolate were flooded with 5 ml of sterile distilled water and gently scraped with sterile inoculation loop to produce spore suspension and the conidia were counted by using a haemocytometer.

Rate of sporulation (Rating)	**No. of spores / microscopic field**
Excellent (++++)	Above 40
Good (+++)	21-40
Fair (++)	11-20
Poor (+)	1-10
Nil (-)	0

3.5 PATHOGENIC VARIABILITY OF *Magnaporthe grisea* ISOLATES USING A SET OF PUTATIVE HOST DIFFERENTIALS

3.5.1 Evaluation of different pearl millet lines for pathogenic variability of *Magnaporthe grisea*

Pathogenic variability was studied among 65 isolates of *M. grisea* collected from major pearl millet growing areas in India based on their reaction on a set of host differentials**.** Differential hosts are sets of plant cultivars used to distinguish pathotypes (races) by their qualitative differences in their reactions (susceptible and resistant) to different isolates of the pathogen. Ten pearl millet genotypes (ICMB 93333, ICMB 95444, ICMB 97222-P1, ICMB 01333, ICMB 02444, ICMR 06444, 863B-P2, ICMR 06222 ICMR 11003 and IP 21187) that had shown differential reactions in the pearl millet blast variability nursery (PMBVN) evaluated at several locations in India as well as in the greenhouse screen were selected as host differentials (Table 3.2).

Planting, multiplication of the pathogen, inoculum preparation, inoculum concentration, inoculations and incubation conditions were followed as mentioned in pathogenicity studies (Chapter 3.3)**.**

The experiment was conducted in a completely randomized design (CRD) with three replicates; 1pot/replicate with 10 seedlings. Blast severity was recorded 8 days after inoculation using a 1-9 progressive scale (Sharma *et al.*, 2013). Based on the reaction type (avirulent reaction = score $\leq$ 3.0 (no lesion to small necrotic spots) on a differential line, and virulent reaction = score $\geq$ 4.0 (typical blast lesions) on 1-9 scale), isolates were grouped in different pathogenic groups/pathotypes. The experiment was repeated to confirm the reaction (virulent/avirulent) of isolates on host differentials.

3.5.2 Genetic diversity in *Magnaporthe grisea* using SSR markers

Genetic diversity among the 65 isolates of *M. grisea* from pearl millet collected from different locations was studied using the SSR (simple sequence repeat) markers and URPs (Universal Rice Primers). Five *M. grisea* isolates two from rice and each one from *Dicanthium, Cenchrus* and foxtail millet were included for comparison.

3.5.2.1 Genomic DNA isolation: Total DNA was extracted from the single spore cultures of *M. grisea* isolates from pearl millet, foxtail millet, rice, *Dicanthium* and *Cenchrus* according to Viji *et al*. (2000) with minor modifications. *M. grisea* isolates were grown

Table 3.2. List of Pearl millet differential lines/genotypes along with their pedigree

S. No.	Genotype	Pedigree
1	**ICMB 93333**	(843B x ICMPS 900-9-3-8-2)-21-8-4
2	**ICMB 95444**	(81-1164 DB/85-1856 LR-16-B x 843DMR1)-14-6-3
3	**ICMB 97222-P1**	[(ICMB 88006 x ICMB 88005) x (ICMB 89111 x ICMB 88004)]-28-2-B
4	**ICMB 01333**	HHVBC S1-64-3-2-3-2-1
5	**ICMB 02444**	(BSECBPT/91-38 x SPF3/S91-529)-2-1-B-2
6	**ICMR 06444**	[((MC 94 S1-34-1-B × HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-37-1-1-1-2-B 2.0 2.1 0
7	**863BP$_2$**	Togo-13-4-1
8	**ICMR 06222**	SDMV 90031-S1-3-3-2-1-3-2-2-1-B
9	**ICMR 11003**	(((J 2340-P4-P2 x (J 2340-P4 x P310-17-P2)-P2)-P6 x J 2340-P4-P2)-P3 x J2340-P4-P2)-P10-P18-P391-Bulk
10	**IP 21187**	IP – 8695-1

separately in 100 ml of potato dextrose broth for seven days at 28 ± 1ºC. The mycelia were harvested by filtration through double layers of sterilized Whatmann No.3 filter paper and dried. About 200 mg of the mycelial mat was ground to fine powder using liquid nitrogen. Powdered mycelia were vortexed in pre-heated 2 per cent CTAB buffer (N-cetyl-N,N,N trimethyl ammonium bromide (1%), 0.1 M Tris Hcl (pH: 8.0), 1.4 M NaCl, 0.5 M EDTA (pH: 8.0), polyvinyl pyrolidone (PVP) (1%) and 2-mercaptoethanol (1%) and incubated at 65°C in water bath with occasional shaking. After one hour incubation, the tubes were allowed to

cool down to room temperature. Then, equal volume of chloroform: isoamylalcohol (24:1) was added to the each tube and mixed well by inverting the tube for 4-5 times and centrifuged at 10000 rpm for 10 min. The upper aqueous, supernatant (approximately 400 µl) containing DNA was transferred to a new 1.5 ml micro centrifuge tube and added with equal volume of chloroform and isoamylalcohol (24:1), mixed well by inverting the tube for 4-5 times and centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to sterile tube carefully and equal volume of ice cold isopropanol was added and the tubes were subsequently incubated in a freezer (-20^{o}C) for 30 min to precipitate DNA. Following incubation, the tubes were centrifuged at 10,000 rpm for 10 min. After centrifugation, supernatant was carefully decanted from each tube and pellet was retained. The pellet was washed with 70 per cent ethanol by centrifuging the DNA at 3000 rpm for 3 min and dried in vacuum for 10 min. In order to remove the co-isolated RNA, 200 µl of low salt TE buffer ($T_1E_{0.1}$) and 3 µl of RNase (stock 10 mg/µl) were added to each tube containing dry pellet and incubated at 37^{o}C for one hour.

After incubation, equal volume of phenol-chloroform-isoamylalcohol mixture (25:24:1) was added to each tube, carefully mixed and centrifuged at 10,000 rpm for 5 min. The aqueous layer was transferred to fresh tubes and chloroform-isoamylalcohol (24:1) mixture was added to each tube, carefully mixed and centrifuged at 10,000 rpm for 10 min. The aqueous layer was transferred to fresh tubes. To the tubes containing aqueous layer, 20 µl (approximately 1/10th volume) of 3M sodium acetate (pH 5.2) and 300 µl (2 volume) of ice cold ethanol (kept at -20ºC) were added and the tubes were subsequently placed in a freezer (-20ºC) for 30 min. Following incubation, the tubes were centrifuged at 10000 rpm for 10 min. After centrifugation, supernatant was carefully decanted from each tube and pellet was retained. Pellets were obtained by carefully decanting the supernatant from each tube and then pellet was washed with 70 per cent ethanol. Completely dried pellets were re-suspended in 100 µl of $T_{10}E_1$ buffer and incubated overnight at room temperature to allow them to dissolve completely. Dissolved DNA samples were stored at -20°C for further use.

3.5.2.2 DNA quality and quantity check: Qualitative analysis of DNA was performed by agarose gel electrophoresis as described below. For this procedure reagents TBE buffer (10X and 1X TBE buffer), ethidium bromide (10 mg/ml), agarose, orange loading dye (0.5 M EDTA (pH 8.0): 10 ml; 5 M NaCl: 1 ml; glycerol: 50 ml; distilled water: 39 ml; orange dye powder (Orange G, Gurr Certistain®) were used.

Procedure: Agarose (0.8 g) was added to 100 ml of 1X TBE buffer and heated using microwave oven until the agarose was completely dissolved. After cooling the solution to about 60°C, 5 µl of ethidium bromide solution was added and the resulting mixture was poured into the gel-casting tray for solidification. Before the gel solidified, an acrylic comb of desired well number was placed on the agarose solution to form wells for loading the samples. Each well was loaded with 5 µl of sample aliquot having 3 µl distilled water, 1 µl orange dye and 1 µl of DNA sample. The DNA samples in known concentration (lambda DNA of 50 ng/µl, 100 ng/µl and 200 ng/µl) were also loaded on to the gel to estimate the DNA concentration of the experimental samples. The gel was run at 80 V for 30 min. After completing the electrophoresis run, DNA on the gel was visualized under UV light and photographed (SynGene Inc, Cambridge, UK). If the DNA was observed as a clear and intact band, the quality was considered good, whereas a smear of DNA indicating poor quality was discarded and reisolated. Relative concentration of DNA present in the samples approximately derived by visual comparison with lambda DNA.

3.5.3 Genetic diversity

The genetic diversity of *M. grisea* isolates was studied by using two types of molecular markers simple sequence repeats (SSR) and universal rice primers (URPs) in the present investigation.

3.5.3.1 SSR genotyping: A set of 48 SSR markers were selected based on literature (Suzuki *et. al*., 2009; Brondani *et. al*., 2000) for studying the genetic diversity of *M. grisea* isolates. These primers were synthesized by Eurofins (Eurofins Genomics India Pvt. Ltd. Bangalore, India). The forward primers of these markers were synthesized by adding M13 to forward primer sequence (5'CACGACGTTGTA AAACGAC3') at the 5'end of each primer.

Genomic DNA of all the isolates were diluted to 10 ng μl^{-1} and used as template for amplification of SSR loci. The PCR reactions were performed in 5 µl volume consisting of 2 µl of 10 ng DNA template, 1 µl of 2mM dNTPs, 0.4 µl of 50 mM $MgCl_2$, 0.7 µl of primer containing 1:5:1 ratio of 100 pmole/µl M13 tailed forward primer, 100 pmole/µl reverse primer and 100 pmole/µl of M13-Forward primer labeled with either Fam or Vic or Ned or Pet (Applied Biosystems), 1.0 µl of 10X PCR buffer and 0.04 U of *Taq* DNA polymerase (SibEnzymes Ltd, Russia). The reaction mixture was vortexed and briefly centrifuged. PCR amplification was performed in a ABI thermal cycler (GeneAmp, PCR

system 9700, PE Applied Biosystems) with the following temperature profiles: 94^{o}C for 5 min of initial denaturation cycle, followed by 35 cycles of denaturation at 94^{o}C for 30 seconds, with constant annealing temperature (45^{o}C) for 30 sec and extension at 72^{o}C for 30 sec, followed by a final extension at 72^{o}C for 20 min. The PCR products were tested for amplification on 1.5 per cent agarose.

3.5.4 Universal Rice Primer (URP) analysis

A total of 12 oligonucleotide URP primers (20 mer), originally derived from repetitive sequences of weedy rice (Kang *et al.,* 2002) were used for this study. All primers were custom synthesized by Eurofins (Eurofins Genomics India Pvt. Ltd. Bangalore, India). PCR amplification was performed in a Temperature Gradient Thermal Cycler (BIORAD BT100, USA). The reactions were performed in a 20 µL volume containing 50 ng of genomic DNA (1 µl), 0.5 U *Taq* DNA polymerase (0.1 µl), 0.2 mM of dNTPs (2 µl), 20 pmoL primer (2 µl), 10X reaction buffer (2 µl) and remaining volume was adjusted with sterile Milli-Q water. The PCR amplification was performed with the following thermal cycling conditions: initial denaturation for 1 min at 94^{o}C, followed by 35 cycles of denaturation at 94^{o}C for 1 minute, annealing at 58^{o}C for 1 minute, extension at 72^{o}C for 2 minutes and final extention at 72^{o}C for 7 min. Amplicons were analyzed by electrophoresis in 1.5 per cent agarose gels in TBE buffer and visualized by staining with ethidium bromide and recorded with Gene Genius gel documentation system (SynGene Inc, Cambridge, UK). The sizes of the PCR products were determined by comparison with standard 100 bp or 1 kb DNA ladder (Bangalore Genei Pvt. Ltd., Bangalore, India).

3.5.5 Data analysis

The amplified fragments of each isolate were scored as 1 (present) or 0 (absent). Comigrating bands were considered homologous characters. Faint bands and bands showing variable levels of intensity were not considered for scoring. The Dice dissimilarity index was calculated using Darwin 5 version 5.0.158 to compute pair wise Dice dissimilarity coefficient and dissimilarity matrix was used in cluster analysis to construct the dendrogram using un-weighted pair group method with an arithmetic average (UPGMA) algorithm. Bootstrap values with 1000 replications were calculated by using the same software.

Polymorphic information content (PIC) for each marker was calculated by using the formulae described by Ghislain *et al.* (1999) as $PIC=1-p^2-q^2$, where p = band frequency, q = no-band frequency.

3.5.5.1 Population structure analysis: The Bayesians model based structure analysis was performed by using the structure software version 2.3.4. (Pritchard *et al.,* 2010) to determine the genetically homogeneous/heterogeneous *M. grisea* populations from different geographical regions in India. For this three independent runs with 20,000 burn-in cycles and 20,000 Markov Chain Monte Carlo (MCMC) replications were performed by setting the number of populations (*K*) from 1 to 10 groups using a model allowing for admixture and correlated allele frequencies. Other parameters in MCMC were set as follows: prior mean= 0.01, prior SD= 0.05 and λ= 1.0. All runs were repeated five times. Since *K* = 1 to *K* = 10 were predicted as optimum number of clusters in simulation method (Evano *et al*., 2005) and in web based software "Structure Harvester version 0.6.894" (Earl and Holdt, 2011), we set the number of clusters *K* = 1-10 for the run. The *K* value was determined by Ln P(D) (log probability of data) in structure output based on the rate of change in Ln P(D) between successive *K*. In addition, the *K* values with the highest likelihood (Δ*K*) were determined from the same structure output (Evano *et al*., 2005).

Analysis of molecular variance (AMOVA) was also performed using the ARLEQUIN software for *M. grisea* population differentiation (Arlequin version 3.5.1.2) (Excoffier *et al*., 1992).

3.6 EFFECT OF TEMPERATURES AND LEAF WETNESS DURATION ON PEARL MILLET BLAST DISEASE DEVELOPMENT

3.6.1 Influence of leaf wetness duration (LWD) on disease development

Influence of leaf wetness duration (0, 6, 12, 24, 36, 48, 60 and 72 h) on the development of leaf blast was studied under greenhouse conditions with three replications. In this present investigation one susceptible line ICMB 95444 and one resistant line ICMR 06444 were used. Isolates of *M. grisea* isolates MgPM 45, MgPM 53, MgPM 56 and MgPM 118 were used in this study. The experiment was conducted in a completely randomized block design (CRD).

Planting, multiplication of the pathogen, inoculum preparation, inoculum concentration, inoculations and incubation conditions were followed as mentioned in pathogenicity studies (Chapter 3.3).

Immediately after inoculations, inoculated plants, except those exposed to 0 h of leaf wetness duration, were covered with pre-wetted polyethylene bags for different durations (6, 12, 24, 36, 48, 60 and 72 h) and then incubated at 25°C with 12 h photoperiod for three days. After completion of each wetness duration, the bags were removed and incubated at above mentioned conditions for five days. Observations on lesion size (mm), number of lesion per leaf and leaf blast severity using a 1–9 scale (Sharma *et al.*, 2013) were recorded eight DAI and then per cent disease index was calculated by using the formula.

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of the scores x 100}}{\text{Number of observations x highest number of rating scale}}$$

3.6.1.1 Measurement of lesion length and sporulation: The lesion number and length of the lesions was measured eight days after inoculations. To evaluate sporulation potential of the pathogen, the detached lesion bearing leaves were brought to the laboratory, lesions were cut from the leaf and incubated in moist chamber for 24 hours. Following incubation, lesions were scraped in 5 ml distilled sterile water to harvest conidia using sterile spatula. Collected spore suspension was placed in small test tubes and the conidia were immediately counted using a haemocytometer. The entire experiment was repeated twice to confirm the results.

3.6.2 Effect of temperatures on disease development

Effect of different temperatures 40±1/28±1^{o}C, 35±1/25±1^{o}C, 30±1/22±1^{o}C, 28±1/20±1^{o}C and 25±1/20±1^{o}C on disease development was studied under growth chambers by using one susceptible line ICMB 95444 and resistant line ICMR 06444 with three replications. The experiment was conducted in completely randomized design (CRD) with three replications (one pot per replication). In this experiment also *M. grisea* isolates (MgPM 45, MgPM 53, MgPM 56 and MgPM 118) were used.

Planting, multiplication of the pathogen, inoculum preparation, inoculum concentration and inoculations data recording were similar as described under section mentioned in pathogenicity studies (Chapter 3.3). After inoculations the seedlings were covered with polythene bags for 48 h to maintain suitable leaf wetness (based on effect of

leaf wetness duration on pearl millet blast disease development 4.3.1) duration, also to avoid cross contamination and incubated at different temperatures (40±1/28±1^{o}C, 35±1/25±1^{o}C, 30±1/22±1^{o}C, 28±1/20±1^{o}C, and 25±1/20±1^{o}C) for eight days, but after 48 h bags were removed and inoculated seedlings were exposed to more than 90 per cent relative humidity (RH) at respective temperatures.

Observations such as lesion length (mm), number of lesions per leaf, lesion sporulation and disease severity were recorded DAI and per cent disease index (PDI) were calculated similarly mentioned in influence of leaf wetness duration on disease development (Chapter 3.6.1).

3.7 IDENTIFICATION OF RESISTANCE IN PEARL MILLET AGAINST DIVERSE PATHOTYPES OF *Magnaporthe grisea*

3.7.1 Evaluation of B-lines of pearl millet for blast resistance under green house conditions

3.7.1.1 Plant material: One hundred sixty designated B-lines (designated till 2009 at ICRISAT's hybrid parent breeding program) of pearl millet were screened for blast resistance under greenhouse conditions along with a resistant designated restorer parent (ICMR 06444) and a susceptible designated seed parent (ICMB 95444) check. The screening was carried out against five pathotype-isolates (MgPM 45 collected from Telangana, MgPM 53 and MgPM 56 from Rajasthan and MgPM 118 and MgPM 119 from Haryana) of *M. grisea* selected from the pathogenic variability study (Sharma *et al.*, 2013). Fifteen seeds of each line were planted in 10 cm diameter pots filled with sterilized soil-sand-FYM mix (2:1:1 by volume) and seedlings were thinned to 10 per pot after germination and placed in a greenhouse bay maintained at 30±1^{o}C.

3.7.1.2 Inoculum preparation and inoculations: Inoculum of each of the mono-conidial pathotype-isolate of *M. grisea* was multiplied on oatmeal agar plates by incubating the inoculated plates at 25^{o}C with 12 h darkness for 7-10 days. Spores were harvested by flooding the plates with sterilized distilled water and scraping the growth by a spatula. The spore suspension was adjusted to desired concentration (1×10^{5} spore mL^{-1}) with the help of a haemocytometer and a drop of surfactant (Tween 20) was added to ensure the uniform dispersal of spores.

Twelve-day old seedlings maintained in the pots were spray-inoculated with spore suspension of each isolate separately, covered with polythene bags and incubated at 25 °C

for 48 h to prevent cross contamination. After 48 h bags were removed and inoculated seedlings were exposed to >90% relative humidity (RH) under misting for 6 days in a greenhouse. The experiment was conducted in a completely randomized design (CRD) with three replicates (one pot per replicate) and 10 seedlings were maintained per replicate.

3.7.1.3 Disease scoring: Leaf blast severity on each line was recorded 6 days after inoculation using 1-9 progressive rating scale (Sharma *et al.*, 2013). Pearl millet lines exhibiting score 1.0–3.0 were considered resistant, with 3.1-5.0 score as moderately resistant, 5.1-7.0 score as susceptible while those showing >7.0 score were considered highly susceptible.

3.7.1.4 Data analysis: Analyses of variance (ANOVA) for blast scores was done using GENSTAT statistical package version 14.0 (Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK) to determine significant differences among pathotypes, host genotypes and their interactions (Kiranbabu, 2011).

3.7.2 Identification of sources of blast resistance in pearl millet blast virulence nursery (PMBVN) 2013-14

The pearl millet blast virulence nursery (PMBVN) consisted of 28 pearl millet lines as test entries including appropriate susceptible (ICMB 95444) and resistance (ICMR 06444) checks were evaluated for blast resistance under natural epiphytotic conditions during *Kharif* 2013 and 2014 at Aurangabad, Dhule, Jamnagar, Gwalior, Durgapura and under artificial inoculation at ICRISAT, Patancheru during 2013 and 14 *Kharif* season (Table 3.3 and Figure 3.1). The nursery was conducted in a randomized complete block (RCB) design with 2 replications and each entry was grown in two rows, 2 m long in each replication according to plot numbers *i.e.* 1001 to 1028 in replication 1 and 2001 to 2028 in replication 2. In order to increase disease pressure, the varieties (ICMB 95444 and ICMB 89111) were planted in border rows. Row-to-row spacing was maintained as 60 cm at all location. Thinning was done 2 weeks after seedling emergence by maintaining 20 plants per row with 10 cm spacing between plants per row.

The agronomic practices were followed as per the local practices. Multiplication of the pathogen, inoculum preparation, inoculum concentration (Plate 3.3a and Plate 3.3b) was followed as mentioned in pathogenicity studies (Chapter 3.3)**.** Perfo-irrigation was provided twice a day for 30 min each, on rain free days from inoculation to soft dough

stage to maintain high humidity and leaf wetness to promote infection and disease development at Patancheru location.

3.7.1.4 Data recording: The data on disease severity was recorded at all the locations at hard dough stage of crop growth based on 1–9 scale reported by Sharma *et al.,* (2013).

3.7.1.5 Statstical analysis: Analysis of variance (ANOVAs) for data on blast disease severity of pearl millet was done by using the GENSTAT statistical package (version 14; Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK). The Bartlett's test of homogeneity was conducted, which indicated that the error variances were homogeneous. The data of both the years (2013 and 2014) was pooled and ANOVA performed using a mixed model (considering years and replications as random and genotypes as fixed). The significance of main effects, year and genotypes were tested against residual mean squares.

3.8 STATISTICAL ANALYSIS

3.8.1 Cultural diversity among the *Magnaporthe grisea* isolates

The effect of incubation temperature and pH level of media on radial growth of the *M. grisea* isolates was determined by analysis of variance (ANOVA, Regression or REML) performed by using GENSTAT statistical package (version 14.0., Rothamsted Experiment Station, Herpenden Herts AL52JQ, UK) to determine significant differences among the isolates, pH levels, temperature and their interaction. The ANOVA model was performed with the radial growth as the variate and the temperature, isolates and pH levels were considered as the factors.

3.8.2 Pathogenic variability of *Magnaporthe grisea* isolates using a set of putative host differentials

The experiments to detect pathogenic variability among the isolates of *M. grisea* were conducted in completely randomized block design under greenhouse conditions. The data on leaf blast severity studies were subjected to Analyses of variance using GENSTAT statistical package (version 14.0., Rothamsted Experiment Station, Herpenden Herts AL52JQ, UK) to determine significant differences among the isolates, accessions and their interaction (Kiranbabu, 2011). The dendrogram was constructed based on disease reaction of the differentials considering the resistance/avirulent reaction (score $\leq$ 3.0 on 0-9 scale) as 0 and susceptible/virulent reaction (score $\geq$ 4.0 on 0-9 scale) as 1. The data were then analysed using NTSYSpc version 2.2 using similarity coefficient and a dendrogram was constructed by unweighted pair group method of arithmetic averages (UPGMA) using the

SAHN (Sequential Agglomerative Hierarical Nested) cluster analysis module for grouping of the isolates in to different pathotypes.

3.8.3 Epidemiology

A completely randomized design was used for all the epidemiology experiments. An analysis of variance (ANOVA) was performed to determine statistical significance of the main factors and their interactions using GENSTAT statistical package (version 14.0., Rothamsted Experiment Station, Herpenden Herts AL52JQ, UK). Significant differences among the treatments at P <0.01, separation of means was conducted using the least significant difference (LSD) at P<0.01 (LS MEANS DUNCAN). Regression procedures were performed using WINBOOT statistical package to determine the relationship between the environmental factors and isolates and disease index (dependent variable y), sporulation, lesion size and number of lesions (independent variable x).

Chapter IV

RESULTS AND DISCUSSION

The results of investigations carried out to determine the morphological, cultural, pathogenic and genetic variation among the isolates of *Magnaporthe grisea*, effect of leaf wetness duration and temperature on disease development and screening of pearl millet lines for their resistance against blast disease are presented in this chapter. Further, the results are discussed critically in the light of the published research work on the subject.

4.1 COLLECTION AND ISOLATION OF *Magnaporthe grisea* ISOLATES FROM INDIA

4.1.1 Collection, isolation and purification of *Magnaporthe grisea* isolates

The samples of the blast disease from different hosts (pearl millet, Cenchrus and Dicanthium) were collected from different states of India. The blast affected leaves were identified in the field based on key symptoms of large elliptical lesions, with large, grey necrotic centers and brown to grey margins (Plate 4.1). A total of 102 blast samples from pearl millet were collected from major pearl millet growing states of India *viz.*, Rajasthan, Uttar Pradesh, Maharashtra, Gujarat, Haryana, New Delhi and Telangana and one isolate each from *Cenchrus* and *Dicanthium* were collected from Rajasthan, India during *Kharif* 2010 and 2012, two rice blast disease samples were obtained from Department of Plant Pathology, College of Agriculture, Rajendranagar and one foxtail millet blast disease sample was obtained from Cereals Pathology, ICRISAT, Patancheru. The pathogen was identified as *Magnaporthe grisea* (Hebert) Barr. (anamorph = *Pyricularia grisea* Sacc.) based on microscopic observations of conidia. A total of 65 monoconidial isolates of *M. grisea* from pearl millet were established from the samples collected from different locations in India (Table 4.1). Of the 65 *M. grisea* isolates 30 from Rajasthan, 13 from Uttar Pradesh, nine from Gujarat, five from Haryana, four from Maharashtra, three from New Delhi and one from Telangana were selected. Five *M. grisea* isolates from non-pearl millet hosts (two from rice and one each from *Dicanthium*, *Cenchrus* and foxtail millet) were included in the genetic variability studies for comparison (Table 4.2).

Table 4.1. Sources of *Magnaporthe grisea* isolates collected from different states of India.

S. No	Identity	Host of origin	Year	Cultivar	Site of Collection (Location/District/State)
1	MgPM 21	*Pennisetum glaucum*	2010	Unknown hybrid	Jalna/Jalna/Maharashtra
2	MgPM 32	*P. glaucum*	2010	AHT-IIB	Nagpur/Nagpur/Maharashtra
3	MgPM 39	*P. glaucum*	2010	ICMB 95222	Hissar/Hissar/Haryana
4	MgPM 40	*P. glaucum*	2010	Unknown hybrid	Bawal/Bawal/Haryana
5	MgPM 49	*P. glaucum*	2010	Supremo	Mahendergarh/Mahendergarh/Haryana
6	MgPM 45	*P. glaucum*	2010	ICMB 95444	Patancheru/Medak/Telangana
7	MgPM 53	*P. glaucum*	2010	Pioneer 86M64	Kherapa/Jodhpur/Rajasthan
8	MgPM 56	*P. glaucum*	2010	Pioneer 86M52	Gotan/Nagaur/Rajasthan
9	MgPM 118	*P. glaucum*	2010	Unknown hybrid	Rewari/Rewari/Haryana
10	MgPM 119	*P. glaucum*	2010	Unknown hybrid	Bhojawas/Bhojawas/Haryana
11	MgPM 121	*P. glaucum*	2012	PB 106	Bhomio ki Dhani/Nagaur/Rajasthan
12	MgPM 122	*P. glaucum*	2012	GHB 539	Rol Chandavata,/Nagaur/Rajasthan
13	MgPM 124	*P. glaucum*	2012	Dhania 7692	Chopron ki Dhani/Nagaur/Rajasthan
14	MgPM 125	*P. glaucum*	2012	Unknown	Sathin/Jodhpur/Rajasthan
15	MgPM 126	*P. glaucum*	2012	RHB 177	Kasti/Jodhpur/Rajasthan
16	MgPM 127	*P. glaucum*	2012	Unknown	Dambra/Jodhpur/Rajasthan
17	MgPM 129	*P. glaucum*	2012	HHB 67-2	Netda/Jodhpur/Rajasthan
18	MgPM 131	*P. glaucum*	2012	9444	Bhrai/Jodhpur/Rajasthan
19	MgPM 132	*P. glaucum*	2012	HOPE 3	Bhrai/Jodhpur/Rajasthan
20	MgPM 133	*P. glaucum*	2012	ICMR 01333	ARS Mandore/Jodhpur/Rajasthan
21	MgPM 134	*P. glaucum*	2012	RHB 177	Ujliya/Jodhpur/Rajasthan
22	MgPM 135	*P. glaucum*	2012	Proagro-9444	Motukabas/Jaipur/Rajasthan
23	MgPM 137	*P. glaucum*	2012	Unknown	Rampura Dabri/Jaipur/Rajasthan
24	MgPM 138	*P. glaucum*	2012	Unknown	Chomu/Jaipur/Rajasthan
25	MgPM 139	*P. glaucum*	2012	Mixture	Haduta/Jaipur/Rajasthan
26	MgPM 144	*P. glaucum*	2012	Local	Malikpur/Sikar/Rajasthan
27	MgPM 145	*P. glaucum*	2012	Unknown	Saphara/Sikar/Rajasthan
28	MgPM 147	*P. glaucum*	2012	Unknown	Mangad Khokavala/Jaipur/Rajasthan
29	MgPM 148	*P. glaucum*	2012	Pioneer-86M86	MangadKhokavala/Jaipur/Rajasthan
30	MgPM 149	*P. glaucum*	2012	Yoddha	Bassi/Jaipur/Rajasthan
31	MgPM 150	*P. glaucum*	2012	Pioneer-86M86	Goribasko/Jaipur/Rajasthan

32	MgPM 151	*P. glaucum*	2012	Pioneer-86M86	Jatwada/Jaipur/Rajasthan
33	MgPM 152	*P. glaucum*	2012	Pioneer-86M86	Dausa/Dausa/Rajasthan
34	MgPM 153	*P. glaucum*	2012	Pioneer-86M86	Bhandarej/Dausa/Rajasthan
35	MgPM 154	*P. glaucum*	2012	Unknown	Dubbi/Dausa/Rajasthan
36	MgPM 156	*P. glaucum*	2012	Proagro-9450	Antarheda/Dausa/Rajasthan
37	MgPM 158	*P. glaucum*	2012	Pioneer-86M86	Kamalpura/Bharatpur/Rajasthan
38	MgPM 159	*P. glaucum*	2012	Pioneer-86M86	Mauloni/Bharatpur/Rajasthan
39	MgPM 162	*P. glaucum*	2012	Unknown	Mahuwar/Agra/Uttar Pradesh
40	MgPM 165	*P. glaucum*	2012	Kaveri-Fauji	Choumuha/Aligarh/Uttar Pradesh
41	MgPM 167	*P. glaucum*	2012	JK-778	Baijala/Aligarh/Uttar Pradesh
42	MgPM 169	*P. glaucum*	2012	Kaveri Super Boss	Jaugama/Bulandshahr/Uttar Pradesh
43	MgPM 171	*P. glaucum*	2012	Hybrid	Gunnaur/Sambhal/Uttar Pradesh
44	MgPM 172	*P. glaucum*	2012	Kaveri Super Boss	Gunnaur/Sambhal/Uttar Pradesh
45	MgPM 173	*P. glaucum*	2012	Kaveri Super Boss	Junawai/Sambhal/Uttar Pradesh
46	MgPM 174	*P. glaucum*	2012	Local	Duwari/Sambhal/Uttar Pradesh
47	MgPM 175	*P. glaucum*	2012	Unknown	Jarifnagar/Badaun/Uttar Pradesh
48	MgPM 178	*P. glaucum*	2012	Pioneer-86M86	Shahbajpur/Badaun/Uttar Pradesh
49	MgPM 179	*P. glaucum*	2012	Kaveri Super Boss	Shahpur/Kasganj/Uttar Pradesh
50	MgPM 181	*P. glaucum*	2012	Pioneer 86M66	Aagsauli/Hathras/Uttar Pradesh
51	MgPM 182	*P. glaucum*	2012	Pioneer 86M86	Habipur/Hathras/Uttar Pradesh
52	MgPM 186	*P. glaucum*	2012	Unknown	Phulambri/Aurangabad/Maharashtra
53	MgPM 188	*P. glaucum*	2012	Unknown	Dhule/Dhule/Maharashtra
54	MgPM 190	*P. glaucum*	2012	Local	Hariyala/Kheda/Gujarat
55	MgPM 191	*P. glaucum*	2012	Unknown	Prakashnagar/Kheda/Gujarat
56	MgPM 193	*P. glaucum*	2012	Unknown	Shekhapura/Kheda/Gujarat
57	MgPM 194	*P. glaucum*	2012	Local	Malawada/Kheda/Gujarat
58	MgPM 196	*P. glaucum*	2012	Local	Bhandhani/Anand/Gujarat
59	MgPM 197	*P. glaucum*	2012	Unknown	Haripura/Anand/Gujarat
60	MgPM 200	*P. glaucum*	2012	Unknown	Savali/Kheda/Gujarat
61	MgPM 204	*P. glaucum*	2012	Unknown	Palundra/Gandhinagar/Gujarat
62	MgPM 206	*P. glaucum*	2012	Unknown	Aluva/Gandhinagar/Gujarat
63	MgPM 208	*P. glaucum*	2012	Unknown	IARI/New Delhi/New Delhi
64	MgPM 209	*P. glaucum*	2012	Unknown	IARI/New Delhi/New Delhi
65	MgPM 210	*P. glaucum*	2012	Unknown	IARI/New Delhi/New Delhi

Note: ICRISAT: International Crops Research Institute for the Semi-Arid Tropics; IARI: Indian Agriculture Research Institute; ARS: Agriculture Research Station.

Table 4.2. Isolates of *Magnaporthe grisea* of different hosts collected from different states.

S. No.	Crop	State	No. of Collected samples	Total No. of isolates
1	Pearl Millet	Rajasthan	30	65
		Uttar Pradesh	13	
		Gujarat	9	
		Haryana	5	
		Maharashtra	4	
		New Delhi	3	
		Telangana	1	
2	Cenchrus	Rajasthan	6	1
3	Dicanthium	Rajasthan	5	1
4	Rice	Telangana	1	2
		Andhra Pradesh	1	
5	Foxtail Millet	Telangana	1	1
	Total			**70**

4.1.2 Pathogenicity Test

Koch's postulates were performed for the 102 *M. grisea* isolates from pearl millet by artificial inoculations. A pearl millet line ICMB 95444 known for its susceptible reaction based on previous field and green house studies, was used to prove Koch's

postulates (Table 4.3). All the isolates were pathogenic to ICMB 95444. Out of 102 pearl millet isolates, 65 isolates were established and continued further studies.

4.2 Study of Cultural, Morphological, Pathogenic and Molecular diversity among the *Magnaporthe grisea* isolates

4.2.1 Effect of different pH levels and different temperatures on growth and sporulation of *Magnaporthe grisea*

This work was aimed to identify suitable medium for the growth, sporulation and other cultural characters of the *M. grisea* monoconidial isolates collected from pearl millet. Lilly and Barnett (1951) reported that temperature and pH of the medium has profound effect upon the rate and the amount of growth and many other life processes. Hence, two different media potato dextrose agar (PDA) and oat meal agar (OMA) at six different pH levels (5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) and incubation temperatures (22^{o}C, 24^{o}C, 26^{o}C, 28^{o}C and 30^{o}C) were tested by in the present investigation on earlier studies (Hossain *et al.*, 2004; Awoderu, 1990; Arun Kumar and Singh, 1995) to select the best medium suitable for the growth and sporulation of the pathogen.

The analysis of variance (ANOVA) of radial growth of *M. grisea* isolates showed significant variation at different temperatures and different pH levels ($p< 0.001$) on both OMA and PDA (Table 4.4), indicating that different pH levels of culture media and different incubation temperatures influences the mycelium growth of *M. grisea* isolates.

Both the media favoured the growth of all the isolates of *M. grisea* at all the tested temperatures and pH levels (Table 4.5). The growth of the pathogen was more on OMA over the PDA medium at all the pH levels and incubation temperatures. There was increase in mean radial growth of isolates across different temperatures from pH 5.5 to pH 7.0 and thereafter there was decrease in the growth with the increase in temperature. Maximum mean radial growth (3.33 cm) of the four isolates was observed at pH 7.0 on OMA. Whereas on PDA, maximum mean radial growth was observed at pH 6.5. This clearly shows that pH 6.5 to pH 7.0 is ideal for the growth of *M. grisea.* With respect to incubation temperature, there was increase in radial growth with the increase in temperature from

Table 4.3. Pathogenicity of *Magnaporthe grisea* isolates collected from states.

S. No	Isolate	District	State	Disease severity (1-9 scale)
1	MgPM 03	Aurangabad	Maharashtra	6.5
2	MgPM 07	Ahmednagar	Maharashtra	8.0
3	MgPM 09	Aurangabad	Maharashtra	8.0
4	MgPM 21	Jalna	Maharashtra	3.5
5	MgPM 23	Aurangabad	Maharashtra	7.5
6	MgPM 25	Dhule	Maharashtra	8.5
7	MgPM 26	Jalgaon	Maharashtra	5.5
8	MgPM 27	Jalgaon	Maharashtra	5.5
9	MgPM 31	Dhule	Maharashtra	8.0
10	MgPM 32	Nagpur	Maharashtra	7.5
11	MgPM 37	Aurangabad	Maharashtra	8.0
12	MgPM 39	Hissar	Haryana	7.5
13	MgPM 40	Bawal	Haryana	8.0
14	MgPM 41	Jaipur	Rajasthan	7.5
15	MgPM 43	Aligarh	Uttar Pradesh	6.5
16	MgPM 45	Patancheru	Telangana	7.5
17	MgPM 49	Mahendergarh	Haryana	7.5
18	MgPM 50	Sundrah	Haryana	8.0
19	MgPM 52	Koka	Haryana	8.0
20	MgPM 53	Kherpa	Rajasthan	9.0
21	MgPM 55	Mulana	Rajasthan	8.0
22	MgPM 56	Gotan	Rajasthan	7.5
23	MgPM 57	Rudhia	Rajasthan	8.0
24	MgPM 118	Rewari	Haryana	8.0
25	MgPM 119	Bhojawas	Haryana	9.0
26	MgPM 121	Nagaur	Rajasthan	9.0
27	MgPM 122	Nagaur	Rajasthan	8.5
28	MgPM 124	Nagaur	Rajasthan	8.0
29	MgPM 125	Jodhpur	Rajasthan	9.0
30	MgPM 126	Jodhpur	Rajasthan	9.0
31	MgPM 127	Jodhpur	Rajasthan	7.5
32	MgPM 129	Jodhpur	Rajasthan	6.5
33	MgPM 131	Jodhpur	Rajasthan	6.5
34	MgPM 132	Jodhpur	Rajasthan	5.5
35	MgPM 133	Jodhpur	Rajasthan	8.5
36	MgPM 134	Jodhpur	Rajasthan	6.5
37	MgPM 135	Jaipur	Rajasthan	7.5

38	MgPM 136	Jaipur	Rajasthan	6.0
39	MgPM 137	Jaipur	Rajasthan	5.0
40	MgPM 138	Jaipur	Rajasthan	8.5
41	MgPM 139	Jaipur	Rajasthan	8.5
42	MgPM 140	Jaipur	Rajasthan	8.0
43	MgPM 141	Jaipur	Rajasthan	7.5
44	MgPM 142	Sikar	Rajasthan	8.0
45	MgPM 143	Sikar	Rajasthan	7.5
46	MgPM 144	Sikar	Rajasthan	7.5
47	MgPM 145	Sikar	Rajasthan	8.0
48	MgPM 146	Sikar	Rajasthan	5.5
49	MgPM 147	Jaipur	Rajasthan	7.5
50	MgPM 148	Jaipur	Rajasthan	5.5
51	MgPM 149	Jaipur	Rajasthan	6.5
52	MgPM 150	Jaipur	Rajasthan	6.5
53	MgPM 151	Jaipur	Rajasthan	5.5
54	MgPM 152	Dausa	Rajasthan	8.5
55	MgPM 153	Dausa	Rajasthan	4.0
56	MgPM 154	Dausa	Rajasthan	5.5
57	MgPM 155	Dausa	Rajasthan	8.0
58	MgPM 156	Dausa	Rajasthan	7.5
59	MgPM 157	Dausa	Rajasthan	7.5
60	MgPM 158	Bharatpur	Rajasthan	6.0
61	MgPM 159	Bharatpur	Rajasthan	5.5
62	MgPM 160	Bharatpur	Rajasthan	4.0
63	MgPM 161	Agra	Uttar Pradesh	3.5
64	MgPM 162	Agra	Uttar Pradesh	7.5
65	MgPM 164	Aligarh	Uttar Pradesh	6.5
66	MgPM 165	Aligarh	Uttar Pradesh	7.5
67	MgPM 166	Aligarh	Uttar Pradesh	3.0
68	MgPM 167	Aligarh	Uttar Pradesh	7.0
69	MgPM 168	Aligarh	Uttar Pradesh	6.5
70	MgPM 169	Bulandshahr	Uttar Pradesh	6.5
71	MgPM 170	Bulandshahr	Uttar Pradesh	7.5
72	MgPM 171	Sambhal	Uttar Pradesh	4.0
73	MgPM 172	Sambhal	Uttar Pradesh	7.5
74	MgPM 173	Sambhal	Uttar Pradesh	7.5
75	MgPM 174	Sambhal	Uttar Pradesh	6.5
76	MgPM 175	Badaun	Uttar Pradesh	7.0

77	MgPM 176	Badaun	Uttar Pradesh	4.5
78	MgPM 177	Badaun	Uttar Pradesh	8.5
79	MgPM 178	Badaun	Uttar Pradesh	9.0
80	MgPM 179	Kasganj	Uttar Pradesh	3.5
81	MgPM 180	Kasganj	Uttar Pradesh	7.5
82	MgPM 181	Hathras	Uttar Pradesh	8.0
83	MgPM 182	Hathras	Uttar Pradesh	9.0
84	MgPM 183	Mathura	Uttar Pradesh	8.5
85	MgPM 185	Aurangabad	Maharashtra	8.0
86	MgPM 186	Aurangabad	Maharashtra	8.0
87	MgPM 187	Jalgaon	Maharashtra	8.5
88	MgPM 188	Dhule	Maharashtra	7.0
89	MgPM 190	Kheda	Gujarat	4.0
90	MgPM 191	Kheda	Gujarat	6.5
91	MgPM 192	Kheda	Gujarat	4.5
92	MgPM 193	Kheda	Gujarat	5.0
93	MgPM 194	Kheda	Gujarat	8.0
94	MgPM 195	Anand	Gujarat	5.5
95	MgPM 196	Anand	Gujarat	8.5
96	MgPM 197	Anand	Gujarat	3.5
97	MgPM 200	Kheda	Gujarat	8.0
98	MgPM 204	Gandhinagar	Gujarat	7.0
99	MgPM 206	Gandhinagar	Gujarat	6.5
100	MgPM 208	New Delhi	New Delhi	3.5
101	MgPM 209	New Delhi	New Delhi	6.5
102	MgPM 210	New Delhi	New Delhi	8.0

22^{o}C to 28^{o}C, whereas with the further increase in temperature at 30^{o}C, the radial growth was decreased. The maximum growth was observed on both the media at 28^{o}C (Plate 4.2a and Plate 4.2b). It is concluded from this experiment that about neutral pH of the medium (pH 6.5 to 7.0) and 28^{o}C incubation temperature are optimum to support the growth of this fungus.

Similar results were obtained for sporulation of four isolates on OMA and PDA at different pH and temperatures. In general, profuse sporulation was observed on OMA,

whereas it was sparse on PDA. As observed for radial growth, maximum sporulation was observed at pH 6.5 and temperature 28^{o}C to 30^{o}C. This further indicated that pH of about 6.5 and temperature of about 28^{o}C is ideal for the growth and sporulation of *M. grisea* adapted to pearl millet (Table 4.6).

Irrespective of differences in composition and pH levels of culture media, good and uniform mycelial growth was observed on both the media. However considerable variation was registered in respect of growth type, colour of vegetative growth and surface appearance of the isolates of *M. grisea* on two different media (Table 4.7 and Table 4.8). OMA produced grayish brown, good and uniform mycelial growth with sector formation. Whereas on PDA medium, dull brown with gray white fluffy margins were observed. However, there was no difference for cultural character of a particular isolates at different pH and temperature profiles. Srivastava (2009) and Onofeghara *et al.* (1973) also observed similar variation in colour of the colonies of *P. oryzae* while culturing the isolate in different media. Our results suggests that different factors affect the growth and sporulation of the fungus *M. grisea* and are in agreement with the results of Srivastava *et al.* (2009), who reported that response of different media were not uniform because the requirements are not the same for growth, sporulation and perithecia development of *M. grisea* species.

As observed in the present study, (Abe, 1930; Yoshii, 1936; Henery and Anderson, 1948; Shumakova and Petrova, 1961) 28^{o}C was found optimum for the growth of the *M. grisea* fungus. Kulakarni (1973) and Awoderu (1990) reported that among the solid media, Oat meal agar (OMA) and among the liquid media both ragi meal and oat meal broth were good for growth and sporulation of the *M. grisea* isolates. Srivastava (2009) also reported oat meal agar (OMA) medium is widely used for *in vitro* culture as well as sporulation of *P. grisea*. Similar types of results were reported by Hossain *et al.* (2004) and Arun Kumar and Singh (1995) who obtained best growth of the fungus at pH 6.5. Awoderu (1990) and Hossain *et al*. (2004) found that good growth of the fungus *P. oryzae* of 5.5 to 10.5 at pH 6.0 to 7.0. Hence, based on these results, cultural and morphological characters of all the 65 *M. grisea* isolates collected from pearl millet was studied on OMA medium at pH 6.5 by incubating at 28^{o}C.

4.3 Morphological and cultural characteristics of *Magnaporthe grisea* isolates

Morphological and colony characteristics of the fungus are the important basic factors for identification of a fungus and its variability. The variability in cultural and morphological characteristics of sixty five isolates of *M. grisea* was carried out on OMA medium at pH 6.5 by incubating at 28^{o}C. The pH of medium and temperature were selected based on the results of pH and temperature studies (above mentioned). The morphological characteristics such as colour of the fungus, type of growth of the fungus (cottony, subdued, tufted, submerged, sectored or non-sectored growth), smooth or rough surface, compressed or raised margins were studied among the isolates of *M. grisea*. Culture morphology varied greatly among isolates. A range of colour variation in the medium was also observed among field isolates (Table 4.9).

4.3.1 Colony characters

The isolates showed significant differences ($P< 0.01$) in their growth rate and colour between the isolates. Almost all the 65 isolates exhibited submerged to subdued growth with sector or concentric growth on the OMA except five isolates MgPM 126, MgPM 145, MgPM 148 and MgPM 153 collected from Rajasthan and MgPM 173 isolate collected from Uttar Pradesh that did not show sectors or concentric rings on the OMA. Of the 65 *M. grisea* isolates, only two isolates MgPM 131 and MgPM 132 exhibited similar morphology of submerged growth with sector formation and contained smooth margins with smooth surface.

The observations pertaining to colony colour were made 10 days after incubation on OMA medium and results are presented in Table 4.9. The colony colour varied from buff colour to black and had smooth or rough margin. Most of the isolates had grayish brown or black colour mycelial growth. MgPM 40 and MgPM 56 had buff coloured mycelium, whereas five isolates showed brownish vegetative growth. In most of the cases the colour of the media turned brownish black following growth of the *M. grisea* isolates and some cases it was found brown (11), black (9) and buff colour (2) coloured. For most of the isolates (61) texture of mycelial growth was rough while some isolates had smooth surface with either raised or smooth margins.

Present investigation indicated significant variation in the cultural characters of the *M. grisea* isolates collected from pearl millet. Studies on morphological variation in the *M. grisea* isolates infecting pearl millet are almost negligible. Most of observations have been made on the *M. grisea* isolates infecting rice (Ou, 1985; Srivastava, 2009) and finger millet

(Getachew *et al.,* 2013; Kiran babu, 2011). Our results are supported by the findings of Barnett and Hunter (1960), Getachew *et al.,* (2013), Ou (1985), who found the variation in the mycelium of the *P. grisea* isolates.

4.3.2 Radial growth and sporulation

The data on radial growth of the mycelium was recorded 10 days after incubation (DAI) and presented in Table 4.9. The radial growth of the *M. grisea* isolates significantly varied from 2.30 cm to 4.25 cm. Among all the isolates, maximum radial growth of 4.25 cm was recorded for isolates MgPM 125 and MgPM 162, followed by isolates MgPM 40, MgPM 169 and MgPM 208, which recorded a radial growth of 4.23 cm, whereas minimum radial growth was recorded for the pearl millet isolate MgPM 148. Our results are in agreement with Kumar and Singh (1995) and Meena (2005), who reported the variability in aerial mycelial growth of *M. grisea* isolates from different hosts.

Large variation was observed for sporulation ability of the field isolates. The level of sporulation was compared with the growth patterns of the pathogen. It was observed that isolates with grayish black and brownish black growth with sector formation produced more spores. The isolates with cottony and submerged growth were poor spore producers with some exceptions (MgPM 154). The lower surface of the colonies was usually brown or black. Colony texture or surface of all the isolates was rough to smooth with trace to abundant sporulation. In majority of the isolates, maximum sporulation was confined to sectored region. Excellent sporulation was observed in 14 isolates *viz.*, MgPM 121, MgPM 138, MgPM 144, MgPM 145, MgPM 169, MgPM 174, MgPM 179, MgPM 181, MgPM 182, MgPM 186, MgPM 188, MgPM 200, MgPM 204 and MgPM 210 from Table 4.9. The present investigation indicated a close correlation between the sporulation ability with colour and sector formation. Correlation between sporulating ability and aerial growth was also observed by Ramakrishnan (1948). The present observations on sporulation are similar to the earlier reports (Sonah *et al*., 2009).

4.4 Pathogenic diversity among pearl millet infecting populations of *Magnaporthe grisea*

The pearl millet blast pathogenic diversity study consisting of 10 host differentials from different parentage were evaluated for pathogenicity of sixty five isolates of *M. grisea* in green house. In this experiment an attempt was made to distinguish pathotypes of *M. grisea* isolates collected from major pearl millet growing states of India by their qualitative

differences in their reactions (susceptible and resistant) on pearl millet differential lines. The experiment was repeated to confirm the reaction (virulent/avirulent) of isolates on host differentials.

The test isolates induced clear blast symptoms on the susceptible line ICMB 95444. The ANOVA revealed highly significant ($P< 0.001$) differences among isolates, host genotypes and their interaction for blast severity (Table 4.10). The mean blast severity across the differentials was maximum for isolate MgPM 138 collected from Jaipur, Rajasthan (score 7.4 on 1-9 scale) followed by MgPM 137 (score 7.1) also from Jaipur, Rajasthan. Minimum severity was observed for MgPM 132 (score 2.4) collected from Jodhpur, Rajasthan across the differentials. Mean blast scores across isolates was minimum (score 3.0) on IP 21187 followed by ICMR 11003 (3.2 score). All the isolates were virulent on susceptible genotype ICMB 95444 and least virulent on pearl millet line IP 21187, followed by genotype ICMR 11003, while moderate disease reaction was observed for all the isolates on the remaining seven host differential lines and also highly variable reaction was observed within and across the isolate- genotype combinations (Table 4.11).

Isolates induced differential reaction on all the genotypes except susceptible check ICMB 95444. On the basis of the reaction type (avirulent/virulent), the 65 isolates were grouped into 28 different pathotypes (Figure 4.1). Most pathotypes (18) were represented by only one isolate, while 11 pathotypes comprised two to ten isolates.

Of these groups, less virulence was exhibited by pathotype G2 which had seven less virulent isolates *viz.*, MgPM 127, MgPM 129, MgPM 132, MgPM 149, MgPM 158 and MgPM 159 from Rajasthan and one isolate MgPM 39 from Haryana. These isolates were virulent only on ICMB 95444. The next less virulent group was pathotype G1 which was virulent on two genotypes, ICMB 95444 and ICMB 01333. G1 included nine *M. grisea* isolates, among them one each from Rajasthan (MgPM 139), Uttar Pradesh (MgPM 169), Haryana (MgPM 188), New Delhi (MgPM 210), two from Gujarat (MgPM 193, MgPM 196) and three from Maharashtra (MgPM 21, MgPM 32 and MgPM 49). Pathotype G21 appeared as most virulent among all as it could infect all the 10 host differentials. It was represented by seven isolates *viz.*, MgPM 121, MgPM 137, MgPM 138, MgPM 145, MgPM 148 from Rajasthan and MgPM 173 and MgPM 174 from Uttar Pradesh. This was followed by group G24 having seven isolates, four (MgPM 167, MgPM 172, MgPM 179 and MgPM 182) from Uttar Pradesh, two isolates MgPM 191, MgPM 204 from Gujarat and one isolate MgPM 147 from Rajasthan and were virulent on nine of the ten differential

lines. Pathotypes G22 (MgPM 154 from Rajasthan) and G20 (MgPM 40 from Haryana and MgPM 134 from Rajasthan) were also virulent on nine genotypes. The virulence of these three pathotypes was differentiated by their avirulent reaction on ICMB 93333 (G20), ICMB 01333 (G23) and IP 21187 (G25).

Occurrence of 28 pathotypes among 65 isolates supports the differences in the virulence of the pathogen populations and the presence of different resistance gene candidates (Reddy *et al*., 2010). Besides, composition of landraces and cultivars of pearl millet grown in this geographic region can also play a crucial role in structuring the pathogen populations. Similar findings were reported by Sharma *et al*. (2002) who found that 119 *M. grisea* isolates from north-western Himalayan region were grouped into 52 pathotypes on the basis of disease reaction on international differential rice lines. Similar observations have been reported by Chen *et al*. (2001) and Le *et al*. (2010) in rice blast.

Based on mean blast severity, score 3.0 or less than 3 on a 1–9 scale considered as resistant and the lines displaying score more than 3 were considered as susceptible. All the isolates showed susceptible reaction on susceptible check ICMB 95444, whereas resistant reaction was observed by 41 isolates on ICMR 06444. None of the differential lines were found resistant to all the 65 *M. grisea* isolates tested. However, clear differential reactions were obtained on the remaining differential lines (Table 4.11).

In this study high level of blast resistance was exhibited by accession IP 21187 when tested against maximum (23) pathotypes, followed by ICMR 11003 that was found resistant to 20 pathotypes out of 28 identified in this study. ICMR 06444 and ICMB 93333were found resistant to 17 pathotypes. Elite parental lines ICMR 06222 and ICMB 97222-P1 included in the differential set were found resistant to 14 and 15 pathotypes, respectively. It would be prudent to collect diverse sources of multiple pathotype resistance (≥10) and deploy them in pearl millet hybrid parent lines to prevent disease out breaks (Sharma *et al.,* 2013).

Knowledge about the pathotype composition of the pathogen population is crucial for the development of strategies for manipulating the disease resistance genes for crop protection. Pathogenic variation in the 25 isolates of *M. grisea* adapted to pearl millet has been reported by Sharma *et al.* (2013) and five pathotypes were identified. However, we tested more isolates from almost all the pearl millet growing regions in India to understand the virulence structure of *M. grisea* in India. In addition, the differential set used in the present study was different than that used by Sharma *et al.* (2013). The results of this study

have practical utility in the selection of geographically and pathologically diverse isolates for use in the identification and utilization of blast resistance in pearl millet.

4.5 Molecular diversity of *Magnaporthe grisea* isolates collected from different hosts

4.5.1 Genetic diversity of *M. grisea* isolates using SSR markers

For characterize genetic diversity in *M. grisea* isolates, a total of 28 SSR markers were used. However, only two SSR markers (MGM 01 and MGM 158) exhibited amplification with more than 54 (70%) *M. grisea* isolates collected from pearl millet, though not revealed any polymorphism among *M. grisea* populations of pearl millet. Similarly Kiranbabu *et al.*, (2013) also reported that none of the primer pairs detected polymorphism in pearl millet infecting *M. grisea* populations, out of 17 SSRs, but only three SSR markers (Pyrms 47-48, Pyrms 63-64 and Pyrms 67-68) were amplified with DNA of pearl millet isolates. Thus, the data from SSR marker was excluded and the *M. grisea* isolates from pearl millet were characterized using 12 universal rice primers (URP) that are repeat motifs obtained from Korean weedy rice and have been utilized in diverse genome like animals, plants and microbes (Kang *et. al.*, 2002).

4.5.2 Genetic diversity of *M. grisea* isolates using Universal Rice primers (URP)

Molecular diversity among 65 isolates of *M. grisea* from pearl millet was studied with 12 URP markers (Table 4.12 and Plate 4.3). Five isolates of *M. grisea* from other hosts (two from rice and one each from foxtail millet, *Cenchrus* and *Dicanthium*) were included for comparison. Three primers (URP-2R, URP-13R and URP-32F) could amplify template DNA of only 3-10 isolates; thus were not used for the further study. The remaining nine primers (URP-1F, 2F, 4R, 6R, 9F, 17R, 25F, 30F and 38F) produced 105 easily scorable amplified fragments. The number of scorable fragments produced per URP primer ranged from 8 to 15, and size of the fragments ranged from 0.2 to 4 Kb. Basic data set from URP analysis revealed the presence of 99 and 59 polymorphic loci, with an average gene diversity of 0.21± 0.10 and 0.23± 0.12 over loci in pearl millet and non-pearl millet isolates of *M. grisea*, respectively. The polymorphic information content (PIC) for each marker varied from 0.32 to 0.50 with an average of 0.43. Three primers URP 4R, URP 9F and URP 25F showed highest PIC value of 0.50 (Table 4.12).

4.5.3 Genetic diversity of *Magnaporthe grisea*

Cluster analysis with UPGMA clearly separated the isolates into five main clusters. In the dendrogram, most of the isolates were grouped in cluster I (32) and III (31) whereas cluster II, IV and V contained 2-3 isolates. All the four pearl millet *M. grisea* isolates (MgPM 21, MgPM 32, MgPM 186 and MgPM 188) collected from Maharashtra were grouped in cluster I along with 28 isolates collected from different states of India. Isolates MgPM 53 from Rajasthan and MgPM 173 from Uttar Pradesh were grouped in cluster II, and isolate MgPM 135 from Rajasthan was grouped along with two isolates MgPM 165 and MgPM 167 from Uttar Pradesh in cluster IV. Group III had 27 *M. grisea* isolates infecting pearl millet and two isolates from rice and one each from foxtail millet and *Dicanthium*. Large variation was also observed with in the clusters. The isolates from rice (MgR 1 and MgR 2) appeared separately in the cluster III. Similarly, isolate from *Dicanthium* was also quite diverse from the other isolates from pearl millet included in this group. *M. grisea* isolates MgPM 196 from pearl millet and MgC 3 from *Cenchrus* were grouped in cluster V (Figure 4.2).

4.5.4 Population structure of *Magnaporthe grisea*

Bayesian model analysis with 65 pearl millet and five isolates of *M. grisea* from other hosts revealed the presence of distinct population structure in *M. grisea*. Given that the log likelihood values increased progressively as *K* increased, the method of Evanno *et al*. (2005) was applied as a criterion to infer the most likely *K* value. The maximum delta *K* was detected at $K = 2$ with a second maximum at $K = 4$ (Figure 4.3a) as the Ln P(D) shows highest peak at $K = 2$ followed by at $K = 4$ (Figure 4.3b). Hence, bar plots were drawn using the values of $K = 2$ and $K = 4$ for grouping of populations. As shown in the Figure 4.4a, *M. grisea* isolates were grouped into two sub populations (clusters) where 32 of the pearl millet isolates and four non-pearl millet isolates (two isolates from rice and one each from foxtail millet and *Dicanthium*) were grouped in cluster I and remaining 33 isolates of pearl millet and one isolate from *Cenchrus* were included in cluster II with an average heterozygosity of 0.489 and 0.511, respectively. On the other hand, isolates grouped in cluster I of the bar plot at $K = 2$ were distributed into three clusters (I, III and IV) in the bar plot at $K = 4$, whereas cluster II (with 0.21 heterozygosity) had *M. grisea* isolates of pearl millet and *Cenchrus* as that of cluster II in Figure 4.4a (bar plot at $K = 2$) with the addition of two more pearl millet isolates (MgPM 165 and MgPM 196) (Figure 4.4b). In detail, cluster I (red colour) in Figure 4.4b comprised *M. grisea* isolates of

pearl millet (MgPM 53, MgPM 132, MgPM 133, MgPM 135, MgPM 137 and MgPM 149), *Dicanthium* (MgD 2) and foxtail millet (MgFM 57) with 0.0398 heterozygosity whereas cluster III had four pearl millet isolates MgPM 138, MgPM 139, MgPM 145 and MgPM 148 (blue colour) (with 0.1564 heterozygosity). Cluster IV (with 0.3418 heterozygosity) had 22 isolates including two isolates from rice (yellow colour) (Figure 4.4b).

4.5.5 Molecular variability of *Magnaporthe grisea*

AMOVA revealed significant genetic variation between and within the studied populations. About 16 per cent genetic variation was detected (F_{ST} = 0.1594) due to differences between populations of *M. grisea* adapted to pearl millet and non-pearl millet hosts and 84 per cent variation was due to difference in the isolates (Table 4.13a). Within pearl millet isolates, nine per cent (F_{ST} = 0.087) of the total variation was contributed by the difference in the populations from different states (Table 4.13b).

Blast management through host plant resistance is economical for resource poor farmers and is also considered as an ecofriendly disease management strategy. Breeding for pearl millet blast resistance has just gained importance in India through understanding the inheritance of blast resistance in pearl millet (Gupta *et al*., 2012) and pathogenic variability in *M. grisea* populations (Sharma *et al*., 2013). Genetic diversity in the pathogen populations plays a major role in disease dynamics and consequently, in the success of disease management strategies, including the development of resistant cultivars. Although a number of reports are available for the genetic diversity of *M. grisea* infecting rice (Xia *et al*., 1993; Shull and Hamer, 1994; Viji *et al*., 2000; Chadha and Gopalakrishna, 2005; Srivastava *et al*., 2009; Suzuki *et al*., 2009; Kumar *et al*., 2010; Madhavan *et al*., 2014), wheat (Kaye *et al*., 2003), finger millet (Takan *et al*., 2004; Takan *et al*., 2011; Tanaka *et al*., 2009; Singh and Kumar 2010; Kiranbabu *et al*., 2013), there is no published information on the genetic diversity of *M. grisea* populations adapted to pearl millet. Hence, we used URPs to understand the genetic diversity among mono-conidial isolates of *M. grisea* from pearl millet collected from major pearl millet growing areas of India where blast occurs in severe form. URPs have been previously used by various authors to determine the genetic diversity of fungal plant pathogens such as *Phellinus linteus* (Kang *et al*., 2002), *Fusarium* sp. (Prasad *et al*., 2005), *Macrophomina phaseolina* (Jana *et al*., 2005), *Alternaria* spp. (Kang *et al*., 2002), *Aspergillus* sp. (Goltabeh *et al*., 2007), *Tilletia indica* (Aggarwal *et al*., 2010), *Rhizoctonia solani* (Gonzàlez *et al*., 2012), *Ascochyta*

rabiei (Ali *et al*., 2013), *Bipolaris sorokiniana* (Banerjee *et al*., 2014; Mann *et al*., 2014) and bio-control agent *Chaetomium* sp. (Aggarwal *et al*., 2008).

Dendrogram constructed using binary data with bootstrap analysis showed presence of high degree of polymorphism in the isolates of *M. grisea* irrespective of their host origin and geographical location. It was also noted that only isolates from Maharashtra (MgPM 21, MgPM 32, MgPM 186 and MgPM 188) were grouped together while other isolates from different states of India were clustered irrespective of their geographic origin. Xia *et al*. (1993) while studying DNA polymorphism in *M. grisea* isolates from rice also reported that there is no relationship between genetic diversity and geographic distribution of isolates. A similar result was reported by Takan *et al*. (2011) for *M. grisea* isolates of finger millet.

The results of population structure analysis also revealed that isolates were grouped in different clusters according to their genetic relatedness irrespective of their host origin as well as geographical distribution. Micro-organisms undergo mutation, natural selection, genetic drift and gene flow to adapt themselves in local environmental conditions which leads to development of genetic variation among populations (Slatkin, 1987). Selection, random drift and migration then acts on this basic variation to shape the structure of individual populations (Burdon and Thrall, 2008). Recombination and selection are the major evolutionary forces for *M. oryzae* (Couch and Kohn, 2002). However, Kumar *et al*. (1999) found the presence of same lineages of *M. oryzae* in Himalayan hills and 1,000 km away in lowland plains in India and they concluded the reason as migration of *M. oryzae* between the populations. Though, genetically similar *Magnaporthe* populations are distributed in different geographical regions, analysis of molecular variance in the present investigation shows the presence of high degree of genetic variation (84%) in pearl millet infecting populations of *M. grisea* (Table 4.13b) collected from different geographical regions of India. Similar results were obtained in the DNA fingerprinting analysis of *M. oryzae* isolates collected from the same cultivar in different locations of Korea (Park *et al*., 2008). They suggested that the reason for presence of genetic variation within the subpopulations of *M. oryzae* collected from same cultivars in different locations is due to some degree of restricted gene flow within Korea not only between cultivars but between geographic areas (collection sites).

Magnaporthe grisea exhibits a great degree of pathogenic variability and adaptability in nature, and its ability to generate new pathogenic races adapted to deployed

resistance genes has rendered most resistant cultivars ineffective within a few years (Ahn, 1994). If the resistant cultivars are to be effective and durable, control methods should focus on the populations of pathogens rather than individuals which lead to avoid the breakdown of resistance (McDonald, 1997). Hence, knowledge on genetic diversity and populations structure of pearl millet-infecting *M. grisea* generated from this study will be helpful for the breeders while breeding for pearl millet resistant to blast.

4.6. Effect of temperature and leaf wetness duration on development of blast disease of pearl millet

The present study was conducted to understand the effect of temperature and leaf wetness duration on the development of *M. grisea* on pearl millet under controlled conditions. In this experiment different leaf wetness durations and different incubation temperatures were tested separately on both blast disease susceptible line ICMB 95444 and resistant line ICMR 06444. High disease pressure was observed only on ICMB 95444 in both leaf wetness duration and temperature experiments, while there was no infection on ICMR 06444. To our knowledge, this is the first study to demonstrate the interaction of temperature and leaf wetness duration on the development of blast of pearl millet.

4.6.1. Effect of leaf wetness duration on development of Pearl millet blast

The pearl millet blast severity and disease index was evaluated on susceptible ICMB 95444 and resistant line ICMR 06444 at different leaf wetness durations of 0, 6, 12, 24, 36, 48, 60 and 72 hours. Disease severity was recorded using 1-9 scale given by Sharma *et al.* (2013) then disease intensity (Per cent disease index (PDI)), lesion length, number of lesions per leaf and lesion sporulation was recorded and represented in table 4.14 to 4.18. The blast infection was found only on susceptible line ICMB 95444 but not on the resistant line ICMR 06444 at all the leaf wetness durations. Hence, the other observations such as lesion length, number of lesions per leaf, lesion sporulation and leaf sporulation were analysed only for ICMB 95444.

There was an overall trend of increase in leaf blast incidence, the lesion length (mm), number of lesions per plant, lesion sporulation and leaf sporulation increased with the increase in leaf wetness duration (LWD) while number of lesions decreased at 60 and 72 hours of leaf wetness durations.

Symptoms first appeared three to four days after inoculations on the ICMB 95444 in all the treatments except in control (0 hour leaf wetness duration) by isolates MgPM 45,

MgPM 53 and MgPM 118 which did not produce any infection, whereas isolate MgPM 56 from Nagaur district of Rajasthan successfully produced the symptoms of 44.81 per cent PDI. The per cent disease index varied from 11.11 to 100 per cent (Table 4.14 and Figure 4.5). The per cent disease index attained its maximum (100%) by 48 hour and above in highly susceptible line when inoculated with the four isolates. The isolate MgPM 56 recorded PDI 44.81 per cent at zero hour (0 h) leaf wetness duration and reached maximum of 100 per cent at 24 hour and above wetness durations with mean maximum PDI (89.58 %), whereas isolates MgPM 45, MgPM 53 and MgPM 118 recorded >40 per cent disease index at 6 hour leaf wetness duration to 100 per cent PDI at 36-48 hours leaf wetness duration. The mean disease index at 36, 48, 60 and 72 h of leaf wetness duration was non-significant. This indicated that 36 h of leaf wetness is optimum for the blast development.

The wetness durations tested were significant on the lesion length (mm). With increase in the wetness duration there was gradual increase in the lesion length at all the time intervals. Highest lesion length was recorded at 72 hour wetness duration (5.91 mm) followed by 60 h wetness duration (5.31 mm) and lowest lesion length was recorded at zero hour leaf wetness duration where only one isolate MgPM 56 produced the infection out of four isolates. Mean maximum lesion length across wetness durations was observed in isolate MgPM 56 (3.71 mm) compared to other isolates, the highest lesion length of 6.30 mm at 72 hour leaf wetness duration and lowest lesion length of 1.54 mm at 0 hour wetness duration were also recorded for this isolate table 4.15. For all the isolates, lesion length increased with increase in wetness durations (Figure 4.6).

The lesion density counted at all wetness durations was statistically significant table 4.16. Unlike other parameters/ variables lesion density was not increased in response to longer leaf wetness durations, initially the mean lesion number across isolates increased from 1.37 at 0 hour to 41.30 at 48 hour leaf wetness duration and then decreased to 37.25 and 33.86 at 60 hour and 72 hour leaf wetness duration respectively (Figure 4.7). It could be due to the fact that lesions formed in longer wetness durations (60 h and above) coalesced to large necrotic spots leading to decreased number of lesions/plant. It may also be supported by corresponding significant increase in lesion size in leaf wetness durations of 60 hour and 72 hour. However, there may not be any change in disease intensity. These results are in broad agreement with earlier studies which have shown that lesion number increased exponentially with increased wetness duration up to 24 h in gulf ryegrass and did not increase beyond 24 h (Moss and Trevathan, 1987).

Among different isolates lesion density (number of lesions per leaf) ranged from minimum 2.67 (for isolate MgPM 53 at 6 h) to maximum 45.70 by isolate MgPM 56 at 48 hour leaf wetness durations. Mean maximum number of lesions per leaf 24.91 were produced by isolate MgPM 56 and least number of lesions 20.26 produced by isolate MgPM 118 and it was on par with MgPM 45 and MgPM 53.

The effect of leaf wetness duration on sporulation, lesion sporulation (spores per lesion) and leaf sporulation (spores per leaf) of pearl millet line ICMB 95444 was studied and presented in table 4.17 and 4.18, respectively. Spores per lesion and spores per leaf increased with increase in wetness duration from 0 to 72 hour. For all the four isolates data on sporulation was significant ($P< 0.05$). Spores per lesion ranged from (73.33×10^4 spores for isolate MgPM 53 (at 6 h leaf wetness) to 567.50×10^4 spores for isolate MgPM 56 (72 h leaf wetness). Similarly spores per leaf also recorded minimum (195.56×10^4 spores) for isolate MgPM 53 and maximum ($21,489.33 \times 10^4$ spores) with isolate MgPM 56. In this study sporulation in infected lesion and leaf increased in response to increase in wetness duration (Figure 4.8 and 4.9). Mean minimum and maximum sporulation of lesion and leaf recorded 21.25×10^4 and 511.04×10^4 spores per lesion and 116.17×10^4 and 17322.21×10^4 spores per leaf were recorded at zero and 72 hour leaf wetness durations, respectively.

Pearson correlation and multiple regression modeling was performed to quantify the relationship between disease incidence with leaf wetness duration, lesion length, number of lesions per leaf, spores per lesion and spores per leaf individually across tested *M. grisea* isolates. Correlation results provided significant positive correlations among all traits tested (Table 4.19). Multiple regression and linear regression analysis were carried out to quantify the relationship between disease incidence and leaf wetness duration, lesion length, number of lesions per leaf, spores per lesion and spores per leaf individually across tested *M. grisea* isolates.

The regression (R^2) value obtained by multiple regression analysis was similar to adjusted R^2 value of 95 per cent ($R^2 = 0.95$). Linear model of regression curve indicates that prediction of disease incidence by using variables like leaf wetness duration, lesion length, number of lesions per leaf, spores per lesion and spores per leaf will be more accurate. This relationship was best described by a quadratic model,

$$Y = 8.9151 - 0.5365X_{LWD} + 5.5991X_{LL} + 2.7187X_{LD} + 0.29758X_{LS} - 0.00852X_{LFS}$$

where *Y* is the disease index or disease severity index, X_{LWD} is leaf wetness duration X_{LL} is lesion length, X_{LD} = lesion density (number of lesions per leaf), X_{LS} = spores per lesion and X_{LFS} is spores per leaf (Figure 4.5).

However, the regression model evaluated explained 96 per cent or more association between predicted probabilities and observed disease incidence. The model provided very high R^2 (0.95) and adjusted R^2 (0.95), both were similar with no significant deviation between observed and predicted blast disease incidence. To validate this model individual regression models were performed to reduce R^2 value.

Over all disease intensity progressed much more rapidly (> 92%) at 12 hours and more wetness durations compared with 0 and 6 hour and maximum (100%) PDI recorded at 48 hour and above wetness durations. Thus, for 48, 60 and 72 hour wetness duration periods, disease symptoms were first visible 3 days after inoculation and increased to final average severities of 4.55 to 5.91 lesion length, 33.86 to 41.30 lesions per leaf 8 days after inoculation. Among the *M. grisea* isolates tested, MgPM 56 from Nagaur district of Rajasthan was found more aggressive.

We found wetness duration a critical microclimatic parameters for blast development. Disease incidence at 0 hour wetness duration occurred only with MgPM 56 out of all tested isolates. It indicated that infection by conidia of MgPM 56 occurred even with no leaf wetness and when the RH was high. Periods of high RH may be important for infection when duration of wetness is short or absent (Jacome and Schuh, 1992). Conidial germination infection plays important role in the epidemiology of pearl millet blast during the dry season, when humidity is the limiting factor for infection. For other isolates of *M. grisea* disease developed when inoculations were followed by a period of wetness. This is in agreement with others Teng, (1994) showed that germination of conidia of *Magnaporthe* spp. is negligible in absence of free moisture. Similar observations were made by Wastie, (1972) and Moss and Trevathan (1987) in rice and by Uribeondo *et al.* (2011) in the case of *Colletotrichum* spp. These regression results show a high degree of fit of the model to the data (R^2 = 0.98). The model predicted optimum wetness duration of 48 h for formation of maximum number lesions per plant.

To our knowledge, this is the first systematic study to demonstrate the effect of leaf wetness duration on development of leaf blast in pearl millet. Leaf wetness of 7 to 14 h is essential for infection of rice by *P. grisea* (Barksdale and Asai, 1965; Kato, 1974; Yoshino, 1974; Kingsolver *et al*., 1984; Teng, 1994; Greer and Webster, 2001) and similar results

were obtained in the present study that 12 hour leaf wetness duration caused >91 per cent blast infection in pearl millet. The length of required wetness period for infection depends upon the temperature. If leaf wetness ends before infection is completed, the process is terminated (Teng, 1994). The effect of leaf wetness duration on leaf blast development in pearl millet was consistent with the findings of Green *et al*. (2004) who found 25°C temperature and 32 h and 48 h leaf wetness were optimum conditions for infection of *P. setariae* in green foxtail. Similar findings on leaf wetness duration and *Pyricularia* spp. have been reported in earlier studies in tall fescue by Uddin *et al*. (1998) and in perennial ryegrass turf by Uddin *et al*. (2002).

In the present study, sporulation increased with increase in leaf wetness durations. Effects of water availability on spore production have not been investigated as much as water effects on infection. Basically, sporulation generally requires longer wetness durations than does the infection (Huber and Gillespie, 1992). McCartney and Lacey (1990) reported that free moisture plays a positive role in the production and release of primary inoculum of *Pyrenopeziza brassicae* on oilseed rape.

In our experiments, leaf blast severity, lesion size, number of lesions, spores per lesion and spores per leaf increased with LWD. A severe outbreak of this disease seems to require 48 h and more of leaf wetness duration and a linear relationship was found between wetness and blast severity, although low level of disease appeared in 12 h wetness duration as well. Further, more critical experiments are needed to better understand the interaction of LWD and temperature for blast infection for development of disease prediction models.

4.6.2 Effect of temperature on pearl millet blast disease development

The present experiment was conducted to determine the relationship between temperature and infection of pearl millet by *M. grisea* under controlled environmental conditions. For this, pearl millet lines ICMB 95444 and ICMR 06444 were tested by four *M. grisea* isolates infecting pearl millet at different day/night temperatures (25±1/20±1^{o}C, 28±1/20±1^{o}C, 30±1/22±1^{o}C, 35±1/25±1^{o}C and 40±1/28±1^{o}C) with 48 hours of leaf wetness duration (based on the results of leaf wetness duration experiment). The disease symptoms were first visible three days after inoculation, with an additional 4 days of incubation the disease increased to final average severities. Consequently eight days after inoculation plants were evaluated for disease intensity by recording the disease severity on

1-9 scale, lesion length, lesion density (number of lesions), lesion sporulation and leaf sporulation.

Under these conditions, the isolates induced typical blast disease symptoms on ICMB 95444, but not on ICMR 06444. Hence, the data on ICMR 06444 was excluded from the analysis. Variation in disease incidence and in other variables, between and within treatments was observed. Overall, the per cent disease incidence, lesion length, number of lesions, spores per lesion and spores per leaf induced by the isolate MgPM 56 was higher than other isolates, whereas lesion length measured by other isolates was statistically similar with each isolates.

The disease index for all temperatures varied from 85.93 by MgPM 45 at 40±1/28±1^{o}C to 100 per cent by isolates MgPM 56 and MgPM 118 at 25±1/20±1^{o}C and 30±1/22±1^{o}C, respectively. Whereas maximum disease index obtained by isolates MgPM 45 (92.22%) and MgPM 53 (96.30%) was recorded at temperature 30±1/22±1^{o}C and minimum disease incidence was recorded at 40±1 and 28±1^{o}C temperature by all the four isolates (Table 4.20 and Figure 4.10).

The optimum temperature observed for disease development (>94% PDI) ranged from 25±1/20±1^{o}C to 35±1/25±1^{o}C. The mean maximum disease index (96.48%) occurred at 30±1/22±1^{o}C temperature and it was statistically similar with other temperatures 25±1/20±1^{o}C, 28±1/20±1^{o}C and 35±1/25±1^{o}C. The mean disease index increased from 25±1/20±1^{o}C to 30±1/22±1^{o}C temperature and declined with the further increase in the temperature (35±1/25±1^{o}C and 40±1/28±1^{o}C). Among the tested isolates the mean maximum disease index was recorded by MgPM 56 (96.15%) and it was on par with disease index induced by other isolates MgPM 53 (94.74%) and MgPM 118 (95.33%) while PDI for MgPM 45 (89.19%) was significantly lower than the other isolates.

The measured lesion length (Table 4.21 and Figure 4.11) in response to temperature ranged from 2.56 (at 40±1/28±1^{o}C) to 4.21 mm (at 28±1/20±1^{o}C) for isolate MgPM 56. The mean lesion length across isolates increased with the increase in temperature from 25±1/20±1^{o}C to 28±1/20±1^{o}C and declined with the further increase in the temperature. This clearly shows that the temperature 28±1/20±1^{o}C is optimum for the blast development. Similar trend was observed for the number of lesion per leaf. The observed number of lesions per infected leaf of pearl millet for all temperatures and tested isolates varied significantly (Table 4.22 and Figure 4.12). The mean lesion number across isolates increased with the increase in temperature from 25±1/20±1^{o}C to 30±1/22±1^{o}C and declined with the further

increase in the temperature. The lowest and highest lesion counts occurred with isolates MgPM 45 at 25±1/20±1^{o}C (22.60) and with isolate MgPM 56 at 30±1/22±1^{o}C (63.30) temperature, respectively. However, lowest lesion count (27.53) across isolates was recorded at 40±1/28±1^{o}C. Maximum lesions (46.70) were observed at 30±1/22±1^{o}C and this number was significantly higher than the rest that recorded at other temperature profiles.

It was observed that lesions formed at temperature 28±1/20±1^{o}C were elongated, coalesced and enlarge to necrotic spots leading to decreased number of lesions per leaf, whereas at 30±1/22±1^{o}C temperature were produced, and then lesion length and lesion number decreased irrespective of decrease or increase in temperatures beyond these two temperature profiles (Figure 4.10 and 4.11).

For sporulation studies (Table 4.23 and 4.24), maximum sporulation was recorded with isolate MgPM 56 i.e., 710 x 10^4 spores per lesion and 42,187.42 x 10^4 spores per leaf at 28±1/20±1^{o}C and 30±1/22±1^{o}C temperature, respectively. Minimum sporulation of 404.17 x 10^4 spores per lesion and 10,325.58 x 10^4 spores per leaf was observed with isolate MgPM 45 at 40±1/28±1^{o}C temperature. In general, mean sporulation counted at different temperatures was maximum at 28-30/20-22^{o}C. Maximum lesion sporulation (636.25 x 10^4 spores per lesion) and leaf sporulation (29001.60 x 10^4 spores per leaf) were observed at 28±1/20±1^{o}C and 30±1/22±1^{o}C, respectively, while mean minimum sporulation was observed at 40±1/28±1^{o}C temperature for both lesion (457.29 x 10^4 spores per lesion) and leaf sporulation (12,680.88 x 10^4 spores per leaf). The results of this study indicates that a day temperature of 28-30^{o}C and a night temperature of 20-22^{o}C are ideal for sporulation of *M. grisea* on pearl millet (Figure 4.13 and 4.14).

Pearson correlation and multiple regression modeling was performed to quantify the relationship temperature and disease incidence, lesion length, number of lesions per leaf, spores per lesion and spores per leaf individually across the tested *M. grisea* isolates.
Correlation results (Table 4.25) provided significant negative correlations between temperature (-0.40) and all the variables tested.

The regression (R^2) value obtained by multiple regression analysis was similar to adjusted R^2 value of 61 per cent (R^2 = 0.61). Linear model of regression curve indicates that prediction of disease incidence by using variables like leaf wet ness duration, lesion length, number of lesions per leaf, spores per lesion and spores per leaf will be more accurate. This relationship was best described by a quadratic model,

$Y = 92.45 - 0.20X_T - 1.99\ X_{LL} + 0.30\ X_{LD} + 0.01\ X_{LS} - 8.38\ X_{LFS}$

where *Y* is the disease index, X_T is temperature, X_{LL} is lesion length, X_{LD} = lesion density (number of lesions per leaf), X_{LS} = spores per lesion and X_{LFS} is spores per leaf (Figure 4.10).

To understand the effect of epidemiological parameters on pearl millet blast, disease intensity correlation statistics were calculated separately for both leaf wetness duration and temperature experiments. The correlation analysis indicated significant positive correlation in both experiments with respect to disease intensity on pearl millet line ICMB 95444, except the interaction between temperature and disease incidence which showed negative correlation in temperature studies. In both experiments, maximum correlation (89-95%) was observed among the leaf sporulation and lesion density. It can be concluded based on the results of both leaf wetness duration and temperature experiments that blast on pearl millet becomes more severe at longer wetness durations beyond 48 hours during optimum day/night temperature ranges from 25±1/20±1^{o}C, to 30±1/22±1^{o}C.

Temperature and wetness period are important factors for blast disease development of pearl millet. Temperatures of 25±1/20±1^{o}C, 28±1/20±1^{o}C, 30±1/22±1^{o}C and 35±1/25±1^{o}C favoured the disease development when accompanied with 48 hours of wetness duration. This indicates that the effect of temperature was more pronounced at the longer wetness duration periods than at the shorter periods as suggested by Lalancette *et al.* (1988) and Gross *et al.* (1998).

Disease was observed at all temperatures evaluated in this study (25±1/20±1^{o}C to 40±1/28±1^{o}C). Disease incidence decreased when temperatures increased from the optimum range to 40±1/28±1^{o}C. In the wetness studies, there was a general trend for disease incidence to increase with an increase in wetness period duration. This finding is not unexpected, since continues free moisture is required for the germination of spores and penetration of leaf tissues. The longer the duration of leaf wetness the greater the likelihood of infection and disease development (Sullivan *et al.*, 2002; Godfrey 1954).

According to Gross *et al.* (1998), the effects of temperature are more pronounced for longer wetness duration periods than shorter periods indicating that the leaf wetness is perhaps the critical environmental variable for infection, though temperature regulates the rapidity and level of disease development.

Comparison of the isolates at each level of temperature and leaf wetness indicated that the isolate MgPM 56 induced more disease intensity under all the wetness durations (0

to 72 hours) and different temperatures (25±1/20±1^{o}C, 40±1/28±1^{o}C) tested The differences observed between isolates suggest some degree of variability for the aggressiveness of the isolates.

In general, long periods of leaf wetness, high relative humidity and temperatures of 17 to 28^{o}C favor rice blast development (Webster and Gunnel, 1992). Of the two temperature regimes (22 and 29^{o}C) tested on gray leaf spot of perennial rye grass turf, 29^{o}C was reported to be more favourable to disease development (Landschoot and Hoyland, 1992). According to Uddin *et al.* (2003) the highest disease incidence and severity were observed at 28^{o}C for gray leaf spot development and these results support our findings.

4.7 Identification of sources of blast resistance in pearl millet diverse pathotypes of *Magnaporthe grisea*.

4.7.1 Evaluation of designated hybrid seed parents of pearl millet for blast resistance under green house conditions

Disease severity in the susceptible check ICMB 95444 was quite high (≥7.0) against all the five pathotypes which indicated a reliable disease screen. As expected, the resistant check ICMR 06444 included in the study was found resistant to all the five pathotypes. Significant differences for blast severity were observed for pathotypes, genotypes (B-lines) and their interaction which indicated different levels of virulence in the pathotypes and resistance in the host lines (Table 4.26). Twenty-three lines were found resistant (1.0-3.0 score) each to isolate MgPM 118 and MgPM 45. Similarly, 22 lines were resistant to MgPM 56 followed by 19 to MgPM 53 and 15 to MgPM 119 (Figure 4.15). Twenty-eight lines showed moderate resistance (3.1-5.0 score) to MgPM 45, 24 to MgPM 119, 10 to MgPM 118, seven to MgPM 53 and four to MgPM 56.

Most of the B-lines exhibited susceptible to highly susceptible reaction against five pathotypes used in this study. Based on mean disease score across pathotypes, 63 lines were found susceptible (score 5.1-7.0) and 70 lines were highly susceptible (score >7.0) to blast. Thus, these lines are not expected to develop blast resistant hybrids unless crossed with blast resistant pollinator. However, we could identify 23 lines having resistance to 3-5 pathotypes. Eight lines (81B, ICMB 88004, ICMB 92444, ICMB 97222-P1, ICMB 02111, ICMB 07111, ICMB 09333 and ICMB 09999) were found resistant to all the five pathotypes (Table 4.27). Nine lines had resistance against four pathotypes and six lines were found resistant to any three pathotypes. The diverse parentage of the blast resistant

lines suggests that the resistance in these lines has been derived from diverse blast resistant germplasm used in the hybrid-parent breeding program. However, a few lines shared common pedigree; e.g., ICMB 97222 and ICMB 02111, and ICMB 00111 and ICMB 01777 have same parents. In fact, resistance in ICMB 97222 and ICMB 02111 appears to be derived from another blast resistant (resistant to all pathotypes) line ICMB 88004 involved in the parentage of these lines as two (ICMB 88006 and ICMB 89111) of the remaining three lines used to develop ICMB 97222 and ICMB 02111 are highly susceptible to blast.

Many pathogenic races have been identified in *M. grisea* infecting rice and this variability has been cited as the principal cause for the frequent breakdown of resistance in rice varieties (Suh *et al*., 2009). Pathogenic variation in the pearl millet infecting populations of *M. grisea* has also been reported (Sharma *et al*., 2013). Evidence also exists for the intra-population variability in the pathogenicity of mono-conidial cultures of *P. oryzae* from single lesions (Bonman *et al*., 1987). The migration of pathotypes within the same field from one phenological stage to the other is quite common in *M. oryzae*-rice pathosystem (Chen *et al*., 1995; Silva *et al*., 2009). Under such conditions, it is essential to deploy multiple-pathotype resistance in the crop cultivars. We could identify eight B-lines having resistance to all the five pathotypes of *M. grisea* in India. Therefore, these lines can serve as potential source of multiple-pathotype resistance in the development of blast resistant pearl millet hybrids and hybrid parents.

Sources of blast resistance in pearl millet were identified in the 1980s and efforts have been made to incorporate resistance into improved cultivars and elite breeding lines by Hanna *et al*. (1988) and Wilson and Hanna, (1992). Though sources of blast resistance have also been identified against *M. grisea* populations in India (Sharma *et al.,* 2013), concerted efforts are required to use these resistance sources in the breeding programs to develop blast resistant hybrid parent lines for use in the development of hybrid cultivars. However, the lines screened and identified as blast resistant in the present study are designated B-lines that are ready for use in hybrid development; thus, these lines can be directly used in the hybrid development programs to manage blast of pearl millet through host plant resistance.

4.7.2 Identification of sources of blast resistance in pearl millet blast virulence nursery (PMBVN) 2013-14 at six different locations

Pearl millet lines (28) were evaluated for blast resistance in the disease nursery (PMBVN) at six locations, Aurangabad, Dhule, Durgapura, Gwalior, Jamnagar and Patancheru during *Kharif* 2013 and 2014. In general, blast severity was adequate (8 to 9 score on 1-9 scale) on the known susceptible line, ICMB 95444 across locations indicating a reliable disease screen. For each field experiment conducted during 2013 and 2014, data on blast severity were analyzed separately using ANOVA. The Bartlett's test of homogeneity was conducted, which indicated that the error variances were homogeneous. The data of both the years were pooled and ANOVA performed. Pooled analysis of blast severity indicated that there was a significant ($P< 0.001$) differences among pathogen populations in India (Table 4.28), and genotypes also showed significant ($P< 0.001$) differential reactions to discern the pathogenic variation in the pathogen populations and different levels of resistance in the genotypes.

None of the entries in the blast nursery was resistant at all the test locations. However, ICMR 06444 was found resistant at three (Gwalior, Jamnagar and Patancheru) locations and showed moderate resistance at other three locations (Table 4.29). This line recorded minimum blast severity (2.99 score) across locations. ICMB 01333, ICMR 11009 and HHB 146 improved (a hybrid) were resistant at Gwalior and Jamnagar. Ten lines showed resistance at any one location. Most of these accessions had moderate resistance at other locations. Remaining lines were mostly found susceptible at the test locations.

The variation in the pathogen population was evident from the mean severity levels at different locations. Some entries exhibited differential reaction to the pathogen populations across locations and thus, detected pathogenic variation in the pearl millet infecting populations of *M. grisea*. Considering the blast severity at hard dough stage, pathogen population at Dhule, Aurangabad and Durgapura appeared highly virulent as none of the entry was found resistant at these locations. However, maximum mean blast severity (score 5.57) was recorded at Dhule. High relative humidity (90%) and optimum temperature (mean temperature around 28°C) play an important role in blast development. Conducive environment for the blast development was observed at the test locations for the blast development.

Multi-location evaluation of pearl millet in the downy mildew virulence nursery has led to the identification of many sources stable resistance that have been used to develop downy mildew resistant pearl millet hybrids (Thakur *et al.,* 2007). Similarly, pearl millet lines identified in this study that are resistant at 2-3 locations can be potential source for the

pearl millet breeding programs aiming to develop blast resistant hybrids. In addition, there is a need for the multi-location evaluation of the pearl millet lines that have been identified as blast resistant in the greenhouse screens (Sharma *et al.*, 2013) for the identification of stable sources of blast resistance.

Table 4.5. Radial growth (cm) of pearl millet blast (*Magnaporthe grisea*) isolates MgPM 45, MgPM 53, MgPM 56 and MgPM 118 on PDA and OMA at different temperatures and pH levels under *in vitro* conditions

Temperature	Isolate	pH 5.5 (cm)		pH 6.0 (cm)		pH 6.5 (cm)		pH 7.0 (cm)		pH 7.5 (cm)		pH 8.0 (cm)		Mean (cm)	
		PDA	OMA	PDA	OMA	PDA	OMA	PDA	OMA	PDA	OMA	PDA	OMA	PDA	OMA
22°C	**MgPM 45**	1.20	1.43	1.50	1.67	1.70	2.07	1.97	1.87	1.73	1.63	1.70	2.23		
	MgPM 53	1.03	1.50	1.13	2.13	1.30	2.37	1.50	2.40	1.13	2.77	1.53	3.10	**1.90**	**2.01**
	MgPM 56	1.50	2.77	1.33	2.57	3.20	2.03	2.07	1.73	2.03	1.87	1.43	2.87		
	MgPM 118	1.90	1.30	1.33	2.47	2.57	2.77	2.83	2.33	2.23	2.50	1.63	2.43		
24°C	**MgPM 45**	1.27	1.50	1.53	1.70	2.13	2.50	1.90	3.63	1.80	3.00	1.73	4.00		
	MgPM 53	1.53	2.10	2.10	2.83	2.50	2.60	2.50	2.93	2.50	3.10	2.20	2.80	**2.44**	**2.60**
	MgPM 56	1.20	2.53	1.80	3.50	2.77	3.47	2.80	3.83	1.70	3.50	1.40	3.60		
	MgPM 118	1.40	1.90	1.70	1.97	3.70	2.53	3.50	3.83	2.60	3.23	2.73	2.30		
26°C	**MgPM 45**	1.40	2.27	1.77	3.90	3.50	2.90	2.17	3.10	1.73	4.00	1.67	3.23		
	MgPM 53	1.93	2.67	2.50	3.07	3.30	3.10	3.10	3.10	2.87	2.27	2.73	3.13	**2.69**	**2.83**
	MgPM 56	1.37	2.10	1.40	3.03	2.73	3.37	2.07	3.83	1.87	3.63	1.73	3.03		
	MgPM 118	1.83	2.40	2.73	3.77	2.97	4.13	3.17	3.90	1.73	3.67	1.87	3.23		
28°C	**MgPM 45**	1.50	2.23	1.80	3.27	1.80	3.43	2.60	3.67	2.03	3.80	1.80	3.43		
	MgPM 53	1.80	2.20	2.73	3.33	3.60	3.40	3.83	3.63	3.37	3.73	3.03	3.63	**2.93**	**3.12**
	MgPM 56	1.30	2.27	1.53	3.40	3.50	3.40	2.83	3.47	2.30	3.90	1.83	3.53		
	MgPM 118	1.30	2.33	3.00	3.63	3.60	4.17	3.80	3.93	3.63	3.80	3.47	3.77		
30°C	**MgPM 45**	1.40	2.90	1.70	3.47	3.37	3.07	1.97	4.02	1.77	3.03	1.70	3.43		
	MgPM 53	1.47	3.07	2.70	3.27	3.37	3.37	3.23	3.90	2.73	3.60	2.73	3.13	**2.75**	**2.92**
	MgPM 56	1.50	2.53	1.33	3.40	3.20	3.40	2.07	3.60	2.03	3.33	1.57	3.60		
	MgPM 118	1.60	2.77	1.67	3.57	3.57	3.57	3.43	3.95	1.83	3.50	1.57	3.43		
	Mean	**1.47**^{f}	**2.24**^{e}	**1.86**^{e}	**3.00**^{d}	**2.92**^{a}	**3.08**^{c}	**2.67**^{b}	**3.33**^{a}	**2.18**^{c}	**3.19**^{b}	**2.00**^{d}	**3.20**^{b}		

Table 4.4. Analysis of variance (ANOVA) of radial growth of pearl millet blast (*Magnaporthe grisea*) isolates MgPM 45, MgPM 53, MgPM 56 and MgPM 118 on PDA and OMA at different temperatures and pH levels under *in vitro* conditions

Source of variation	Degrees of freedom	PDA				OMA			
		Sum of squares	Mean sum of squares	Variance	LSD	Sum of squares	Mean sum of squares	Variance	LSD
Rep	2	0.088	0.04	0.98		0.03	0.02	0.42	
Isolate (Iso)	3	25.38	8.46	186.84**	0.06	2.63	0.87	23.56**	0.06
Temperature (Temp)	4	28.82	7.20	159.12**	0.07	71.12	17.78	476.71**	0.06
pH level (pH)	5	87.98	17.5	388.52**	0.08	46.20	9.24	247.78**	0.07
Iso*Temp	12	21.59	1.79	39.73**	0.14	12.33	1.02	27.56**	0.13
Iso*pH	15	11.15	0.74	16.42**	0.15	8.46	0.56	15.14**	0.14
Temp*pH	20	10.59	0.52	11.70**	0.17	14.43	0.72	19.35**	0.16
Iso*Temp*pH	60	18.63	0.31	6.86**	0.34	21.55	0.35	9.63**	0.31
Residual	238	10.77	0.04			8.87	0.03		
Total	359	215.02				185.66			

Note: ** Highly significance at p< 0.01.

Table 4.6. Sporulation of isolates of *Magnaporthe grisea* MgPM 45, MgPM 53, MgPM 56 and MgPM 118 on OMA and PDA at different temperature and pH levels under *in vitro* conditions

Temperature	Isolate /pH	pH 5.5		pH 6.0		pH 6.5		pH 7.0		pH 7.5		pH 8.0	
		OMA	PDA	OMA	PDA	OMA	PDA	OMA	PDA	OMA	PDA	OMA	PDA
22°C	MgPM 45	+	+	+	+	+	+	+	+	+	+	+	+
	MgPM 53	+	+	+	+	+	+	+	+	+	+	+	+
	MgPM 56	+	+	+	+	+	+	+	+	+	+	+	+
	MgPM 118	+	+	+	+	+	+	+	+	+	+	+	+
24°C	MgPM 45	+	+	++	+	++	+	++	+	++	+	++	+
	MgPM 53	+	+	++	+	++	+	++	+	++	+	++	+
	MgPM 56	+	+	++	+	++	+	++	+	++	+	++	+
	MgPM 118	+	+	++	+	++	+	++	+	++	+	++	+
26°C	MgPM 45	+	+	++	++	++	+	++	+	++	+	++	+
	MgPM 53	+	+	++	+	+++	+	+++	+	++	+	++	+
	MgPM 56	+	+	++	+	++	+	+++	+	++	+	++	+
	MgPM 118	+	+	++	+	+++	+	++	+	++	+	++	+
28°C	MgPM 45	+	+	+++	++	++++	+	+++	+	++	+	++	+
	MgPM 53	+	+	+++	+	++++	+	+++	+	+++	+	+++	+
	MgPM 56	++	+	+++	+	++++	++	+++	+	++	+	++	+
	MgPM 118	+	+	+++	+	++++	+	+++	+	++	+	++	+
30°C	MgPM 45	+	+	++	+	++++	+	+++	+	++	+	++	+
	MgPM 53	++	+	++	+	++++	+	+++	+	++	+	++	+
	MgPM 56	++	+	++	+	++++	++	++	+	++	+	++	+
	MgPM 118	++	+	++	+	++++	+	+++	+	++	+	++	+

(**Note:** +: Poor, ++: Fair, +++: Good, ++++: Excellent sporulation)

Table 4.7. Effect of different pH levels of OMA medium and temperatures on colony morphology of *Magnaporthe grisea* isolates MgPM 45, 53, 56 and 118 infecting pearl millet.

Temperature	pH level	Isolate No.	Cultural Morphology	Colour on the media	Colour of the vegetative growth	Texture /Surface appearance	Margins
22°C	**pH 5.5**	**MgPM 45**	Subdued + Small tufted growth + No sector formation	Black	Blackish gray	Rough	Smooth
		MgPM 53	Subdued + Submerged + Tuft + Sector formation	Brown	Grayish brownish	Smooth	Smooth
		MgPM 56	Subdued + Tuft + No sector formation	Black	Grayish black	Smooth	Smooth
		MgPM 118	Subdued + Submerged + Tuft + No sector formation	Black	Grayish black	Smooth	Smooth
	pH 6.0	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Smooth
		MgPM 53	Subdued + Submerged + Tuft + No Sector formation	Brown	Grayish brownish	Rough	Smooth
		MgPM 56	Subdued + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Smooth
	pH 6.5	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Brown	Blackish gray	Rough	Smooth
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 56	Subdued + Cotton growth + Sector formation	Black	Brownish black	Rough	Smooth

		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Smooth
	pH 7.0	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Smooth
		MgPM 53	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 56	Subdued + Tuft + No sector formation + Sector formation in radial rings	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Smooth
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Smooth
		MgPM 56	Subdued + Tuft + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Cotton growth + Sector formation in radial rings	Brown	Brownish black	Rough	Raised
	pH 8.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Smooth
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 56	Subdued + Tuft + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Smooth
24^{o}C	**pH 5.5**	**MgPM 45**	Subdued + Small tufted growth + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Grayish black	Smooth	Smooth
		MgPM	Subdued + Tuft + Sector formation	Black	Grayish	Smooth	Smooth

		56			black		
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Brown	Brownish black	Rough	Smooth
	pH 6.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Smooth
		MgPM 53	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish white	Smooth	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish black	Smooth	Smooth
		MgPM 118	Subdued + Tuft + Sector formation	Black	Brownish black	Rough	Raised
	pH 6.5	**MgPM 45**	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 53	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 56	Subdued + Small tufted sectors in concentric rings	Black	Brownish black	Rough	Smooth
		MgPM 118	Subdued + Tuft + Sector formation	Brown	Brownish black	Rough	Raised
	pH 7.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 53	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish white	Rough	Raised
		MgPM 56	Subdued + Tuft + Sector formation	Black	Grayish black	Rough	Raised
		MgPM 118	Subdued + Cotton growth + Sector formation in radial rings	Black	Grayish black	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish	Rough	Raised

		53			black		
		MgPM 56	Subdued + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Tuft + Sector formation	Black	Grayish black	Rough	Smooth
	pH 8.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 53	Subdued + Radial sector formation	Black	Grayish black	Rough	Smooth
		MgPM 56	Subdued + Tuft + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Raised
26^{o}C	**pH 5.5**	**MgPM 45**	Subdued +Tuft + Cottony growth + No sector formation	Brown	Brownish black	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Grayish black	Smooth	Smooth
		MgPM 56	Subdued + Tuft + No sector formation	Black	Grayish black	Smooth	Smooth
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Smooth
	pH 6.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Buff colour	Grayish white	Rough	Smooth
		MgPM 53	Subdued + Cotton growth + Sector formation	Black	Grayish black	Rough	Smooth
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 118	Subdued + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Raised
	pH	**MgPM**	Subdued + Tuft + Sector formation	Buff	Grayish	Rough	Raised

	6.5	**45**		colour	white		
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Smooth
		MgPM 56	Subdued + Cotton growth + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 118	Subdued + Tuft + Cotton growth + Sector formation	Brown	Brownish black	Rough	Raised
	pH 7.0	**MgPM 45**	Subdued + Tuft + Sector formation	Brown	Grayish brown	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Raised
		MgPM 56	Subdued + Tuft + Cotton growth + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Cotton growth + Sector formation	Brown	Brownish black	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + Cottony growth + Sector formation	Brown	Grayish brown	Rough	Raised
		MgPM 53	Subdued + Cotton growth + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 56	Subdued + Tuft + Cotton growth + Sector formation	Black	Grayish black	Rough	Raised
		MgPM 118	Subdued + Cotton growth + Sector formation	Brown	Brownish black	Rough	Raised
	pH 8.0	**MgPM 45**	Subdued + Cotton growth + Sector formation	Brown	Grayish brown	Rough	Raised
		MgPM 53	Subdued + Cotton growth + Sector formation	Black	Grayish black	Rough	Raised
		MgPM 56	Subdued + Tuft + Cotton growth + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Cotton growth + Sector formation	Brown	Brownish black	Rough	Raised

28^{o}C	**pH 5.5**	**MgPM 45**	Subdued + Tuft + Sector formation	Brown	Grayish brown	Rough	Smooth
		MgPM 53	Subdued + Tuft + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 56	Subdued + Tuft + No sector formation	Black	Grayish black	Smooth	Smooth
		MgPM 118	Subdued + Tuft + No sector formation	Black	Grayish black	Rough	Smooth
	pH 6.0	**MgPM 45**	Subdued + Scanty aerial mycelium + Tuft + Sector formation	Black	Grayish black	Rough	Raised
		MgPM 53	Subdued + Cotton growth + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium +Sector formation	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
	pH 6.5	**MgPM 45**	Subdued + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 53	Subdued + scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium +Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Raised
	pH 7.0	**MgPM 45**	Subdued + Cottony growth + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 53	Subdued + Cotton growth + Sector formation	Brown	Brownish black	Rough	Smooth
		MgPM 56	Subdued + Cotton growth + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM	Subdued + Cotton growth + Sector formation	Brown	Grayish	Rough	Raised

		118			black		
	pH 7.5	**MgPM 45**	Subdued + Tuft + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 56	Subdued + Tuft + Cotton growth + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Brown	Grayish brown	Rough	Raised
	pH 8.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Smooth
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium +Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Grayish brown	Rough	Raised
30^{o}C	**pH 5.5**	**MgPM 45**	Subdued + Scanty aerial mycelium+ Sector formation	Brown	Brownish black	Rough	Smooth
		MgPM 53	Subdued + Tuft + Sector formation	Black	Grayish black	Smooth	Smooth
		MgPM 56	Subdued + Tuft + No sector formation	Black	Brownish black	Rough	Smooth
		MgPM 118	Subdued + Scanty aerial mycelium + Sector formation	Brown	Grayish black	Rough	Smooth
	pH 6.0	**MgPM 45**	Subdued + Cotton growth + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation in radial rings	Black	Grayish black	Rough	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium +Sector formation	Black	Brownish black	Rough	Smooth

		MgPM 118	Subdued + Tuft + Cotton growth + Sector formation	Brown	Grayish black	Rough	Smooth
	pH 6.5	**MgPM 45**	Subdued + Tuft + cotton growth + Sector formation	Black	Grayish black	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 118	Subdued + Tuft + Cotton growth + Sector formation	Black	Grayish black	Rough	Smooth
	pH 7.0	**MgPM 45**	Subdued + Cotton growth + Sector formation	Black	grayish black	Rough	Smooth
		MgPM 53	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Smooth
		MgPM 56	Subdued + Scanty aerial mycelium + Sector formation	Black	Grayish black	Rough	Smooth
		MgPM 118	Subdued + Cotton growth + Sector formation	Brown	Grayish brown	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 53	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium +Sector formation	Black	Grayish black	Rough	Raised
		MgPM 118	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
	pH 8.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Brownish black	Rough	Raised
		MgPM 53	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
		MgPM	Subdued + Scanty aerial mycelium + Sector formation	Black	Brownish	Rough	Raised

		56			black		
		MgPM 118	Subdued + Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised

Table 4.8. Effect of different pH levels of PDA medium and temperatures on colony morphology of *Magnaporthe grisea* isolates MgPM 45, 53, 56 and 118 infecting pearl millet

Temperature	pH level	Isolate No.	Cultural Morphology	Colour on the media	Colour of the vegetative growth	Texture /Surface appearance	Margins
22°C	**pH 5.5**	**MgPM 45**	Submerged + No sector formation	Brown	Brown	Rough	Raised
		MgPM 56	Submerged + No sector formation	Brown	Brown	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued +Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
	pH 6.0	**MgPM 45**	Submerged + No sector formation	Brown	Brown	Rough	Raised
		MgPM 56	Submerged + No sector formation	Brown	Brown	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish black	Rough	Raised
	pH 6.5	**MgPM 45**	Submerged + No sector formation	Brown	Brown	Rough	Raised
		MgPM 56	Submerged +Tuft + No sector formation	Brown	Brown	Rough	Raised
		MgPM 53	Subdued + Cottony growth + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Brownish black	Rough	Raised
	pH	**MgPM 45**	Submerged + No sector formation	Brown	Brown	Rough	Raised

	7.0						
		MgPM 56	Submerged + No sector formation	Brown	Brown	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish black	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + No sector formation	Brown	Brown	Rough	Raised
		MgPM 56	Subdued + No sector formation	Brown	Brown	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued +Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
	pH 8.0	**MgPM 45**	Subdued + No sector formation	Brown	Brown	Rough	Raised
		MgPM 56	Subdued + No sector formation	Brown	Brown	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued +Scanty aerial mycelium + Sector formation	Brown	Brownish black	Rough	Raised
24^{o}C	**pH 5.5**	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
		MgPM 53	Subdued + Cottony growth +Scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
		MgPM 118	Subdued +Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
	pH	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish	Rough	Raised

	6.0				gray		
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish brown	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
		MgPM 118	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
	pH 6.5	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
		MgPM 118	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
	pH 7.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued +Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
		MgPM 118	Subdued + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + Tufty + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish brown	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony growth +Scanty aerial mycelium + No Sector formation	Black	Blackish white	Rough	Raised

	pH 8.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Cottony growth +Scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony growth +Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
26^{o}C	**pH 5.5**	**MgPM 45**	Subdued + Cottony + scanty aerial mycelium + No sector formation	Black	Blackish white	Rough	Raised
		MgPM 56	Subdued + Tuft + No sector formation	Brown	Brownish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued +Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
	pH 6.0	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Tuft + Sector formation	Brown	Brownish gray	Rough	Raised
		MgPM 53	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
		MgPM 118	Subdued + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
	pH 6.5	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish gray	Rough	Raised
		MgPM 53	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM	Subdued + Tuft + Cottony fluffy growth +Scanty aerial	Black	Blackish	Rough	Raised

		118	mycelium + Sector formation		gray		
	pH 7.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Brown	Brownish gray	Rough	Raised
		MgPM 53	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Brown	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Tuft + Cottony growth +Scanty aerial mycelium + No Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Cottony growth +Scanty aerial mycelium + No Sector formation	Black	Blackish gray	Rough	Raised
	pH 8.0	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
28^{o}C	**pH 5.5**	**MgPM 45**	Subdued + Cottony + scanty aerial mycelium + No sector formation	White	Blackish white	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised

		MgPM 118	Subdued +Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
	pH 6.0	**MgPM 45**	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish gray	Rough	Raised
		MgPM 53	Subdued + Tuft + Cottony growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
	pH 6.5	**MgPM 45**	Subdued + Tufted growth + Sector formation	Black	Blackish white	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Cottony growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
	pH 7.0	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + Cottony + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + Tuft + Scanty aerial mycelium + Cottony + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish	Rough	Raised

					gray		
		MgPM 118	Subdued + Cottony growth +Scanty aerial mycelium + No Sector formation	Black	Blackish gray	Rough	Raised
	pH 8.0	**MgPM 45**	Subdued + Tuft + Cottony growth + Scanty aerial mycelium	Blackish ash	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Cottony growth +Scanty aerial mycelium + No Sector formation	Black	Blackish gray	Rough	Raised
30^{o}C	**pH 5.5**	**MgPM 45**	Subdued + cottony+ Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued +Tuft + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
	pH 6.0	**MgPM 45**	Subdued + Tufted + Scanty aerial mycelium + No sctor formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish white	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Cottony fluffy growth + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
	pH 6.5	**MgPM 45**	Subdued + Tufted + Scanty aerial mycelium + Fluffy + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish gray	Rough	Raised

		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
	pH 7.0	**MgPM 45**	Subdued + Tufted + Scanty aerial mycelium + Fluffy + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + No sector formation	Brown	Brownish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish white	Rough	Raised
	pH 7.5	**MgPM 45**	Subdued + Tufted + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish white	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony growth +Scanty aerial mycelium + No Sector formation	Black	Blackish gray	Rough	Raised
	pH 8.0	**MgPM 45**	Subdued + cottony+ Tufted +Scanty aerial mycelium + No sector formation	Black	Blackish gray	Rough	Raised
		MgPM 56	Subdued + Scanty aerial mycelium + No sector formation	Brown	Brownish gray	Rough	Raised
		MgPM 53	Subdued + Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised
		MgPM 118	Subdued + Tuft + Cottony fluffy growth +Scanty aerial mycelium + Sector formation	Black	Blackish gray	Rough	Raised

Table 4.9. Cultural and morphological characteristics of sixty five *Magnaporthe grisea* isolates collected from pearl millet from different location on OMA medium

S.No	Isolate No.	Cultural Morphology	Colour on the media	Colour of the vegetative growth	Texture /Surface appearance	Margins	Radial growth (cm)	Sporulation
1	**MgPM 21**	Subdued + Tufted growth + Scanty aerial mycelium + Sector formation	Brownish black	Grayish brown	Rough surface	Raised margins	3.50	++
2	**MgPM 32**	Subdued + Small tufted growth forming sectors + Radial sector formation	Brownish black	Grayish brown	Rough surface	Raised margins	3.60	++
3	**MgPM 39**	Subdued + Submerged + Scanty aerial mycelium + Sector formation	Brownish black	Grayish black	Rough surface	Smooth margins	3.30	++
4	**MgPM 40**	Subdued + Small tufted growth forming sectors + Radial sector formation	Brownish black	Buff	Rough surface	Smooth margins	4.23	++
5	**MgPM 49**	Subdued + Tufted growth + Radial sector formation	Brownish black	Grayish brown	Rough surface	Smooth margins	3.60	++
6	**MgPM 45**	Subdued + Tufted growth + Radial sector formation	Brownish black	Grayish black	Rough surface	Smooth margins	3.30	++
7	**MgPM 53**	Subdued + Tufted growth + Scanty aerial mycelium + Radial sector formation	Brownish black	Grayish black	Rough surface	Smooth margins	3.50	++
8	**MgPM 56**	Subdued + Small tufted growth forming sectors + Radial sector formation	Brownish black	Buff	Rough surface	Smooth margins	3.90	++
9	**MgPM 118**	Subdued + submerged + Scanty aerial mycelium + Sector formation +Tufted growth	Brownish black	Grayish black	Rough surface	Smooth margins	3.90	++
10	**MgPM 119**	Subdued + tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish black	Rough surface	Smooth margins	3.50	++
11	**MgPM 121**	Subdued + small tufted growth forming sectors	Buff colour	Grayish white	Smooth surface	Smooth margins	2.70	+
12	**MgPM 122**	Cottony + tuft + sector formation	Buff colour	Brown	Rough Surface	Smooth margins	3.40	+++
13	**MgPM 124**	Subdued + Small tufted sectors in	Brown	Grayish	Rough	Smooth	3.30	++

		concentric rings		brown	Surface	margins		
14	**MgPM 125**	Subdued + Small tufted radiated sectors	Black	Brownish black	Rough surface	Smooth margins	4.25	+++
15	**MgPM 126**	Submerged scanty aerial mycelium + No sector formation	Brown	Grayish brown	Rough surface	Smooth margins	2.90	+
16	**MgPM 127**	Tuft + Subdued + sector formation	Brown	Grayish white	Rough surface	Smooth margins	3.00	++
17	**MgPM 129**	Subdued + Sector formation	Brown	Grayish white	Rough surface	Smooth margins	3.10	++
18	**MgPM 131**	Submerged + Sector formation	Black	Grayish black	Smooth surface	Smooth margins	2.50	++
19	**MgPM 132**	Submerged + Sector formation	Black	Grayish black	Smooth surface	Smooth margins	3.10	++
20	**MgPM 133**	Subdued + Sector formation + Cotton growth + tufted growth	Brown	Grayish brown	Rough surface	Raised margins	3.50	++
21	**MgPM 134**	Subdued + Tuft + Cotton growth	Brown	Grayish brown	Rough surface	Smooth margins	2.80	+++
22	**MgPM 135**	Subdued + Small tufted sectors in concentric rings	Brown	Brownish black	Rough surface	Raised margins	3.10	+
23	**MgPM 137**	Cottony + Radiating sector formation + Subdued	Brownish black	Grayish black	Rough surface	Raised margins	3.80	++
24	**MgPM 138**	Cottony + Radiating sector formation + Subdued	Brownish black	Grayish black	Rough surface	Raised margins	2.80	+++
25	**MgPM 139**	Submerged + Sectors in concentric rings	Brownish black	Grayish brown	Smooth surface	Smooth margins	2.90	++
26	**MgPM 144**	Cottony + Sector formation+ Subdued	Brownish black	Grayish brown	Rough surface	Smooth margins	3.30	++++
27	**MgPM 145**	Subdued + tuft + Scanty aerial mycelium + No sector formation	Brownish black	Grayish brown	Rough surface	Smooth margins	2.30	++++
28	**MgPM 147**	Subdued + Sector formation + Tufted growth	Brownish black	Grayish black	Rough surface	Smooth margins	2.80	+++

29	**MgPM 148**	Subdued + tuft + Scanty aerial mycelium + No sector formation	Black colour	Grayish green	Rough surface	Raised margins	2.20	++
30	**MgPM 149**	Subdued + tuft + Sector formation	Brownish black	Grayish black	Rough surface	Smooth margins	2.70	++
31	**MgPM 150**	Subdued + Sector formation + Tufted growth	Buff colour	Brown	Rough surface	Raised margins	2.80	+++
32	**MgPM 151**	Subdued + submerged + Radial sector formation	Black colour	Grayish black	Rough surface	Smooth margins	2.80	++
33	**MgPM 152**	Subdued + Sector formation + Tuft + Scanty aerial mycelium	Brown	Grayish brown	Rough surface	Smooth margins	3.10	++
34	**MgPM 153**	Subdued + tuft + Scanty aerial mycelium + No sector formation	Black colour	Grayish black	Rough surface	Raised margins	2.50	++
35	**MgPM 154**	Submerged + Sector formation	Brownish black	Grayish black	Smooth surface	Smooth margins	3.70	+++
36	**MgPM 156**	Subdued + Sectors in concentric rings + Tuft	Buff colour	Brown	Rough surface	Smooth margins	2.90	++
37	**MgPM 158**	Subdued + Tuft + Cotton growth + Scanty aerial mycelium + Sector formation	Brown colour	Grayish black	Rough surface	Raised margins	2.90	++
38	**MgPM 159**	Subdued + Tuft + Scanty aerial mycelium + sector formation	Brownish black	Grayish black	Rough surface	Raised margins	3.10	+
39	**MgPM 162**	Subdued + Tuft + Cottony growth + Sector formation	Brownish black	Grayish brown	Rough surface	Raised margins	4.25	+++
40	**MgPM 165**	Subdued + Tuft + Scanty aerial mycelium + Radial sector formation	Buff colour	Brown	Rough surface	Raised margins	3.20	+++
41	**MgPM 167**	Subdued + Tuft + Cottony growth + Radial sector formation	Brownish black	Grayish black	Rough surface	Raised margins	3.60	++
42	**MgPM 169**	Subdued + Tuft + Cottony growth + Scanty aerial mycelium	Brownish black	Grayish black	Rough surface	Raised margins	4.23	++++
43	**MgPM 171**	Subdued + tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish black	Rough surface	Raised margins	3.70	+

44	**MgPM 172**	Subdued + tuft + Scanty aerial mycelium + Sector formation	Brownish black	Brownish black	Rough surface	Raised margins	3.10	+
45	**MgPM 173**	Subdued + Scanty aerial mycelium + No sector formation	Black	Grayish black	Rough surface	Smooth margins	2.50	+++
46	**MgPM 174**	Subdued + Tuft + scanty aerial growth + Sector formation	Black	Grayish black	Rough surface	Raised margins	2.30	++++
47	**MgPM 175**	Subdued + Scanty aerial mycelium + Sector formation	Black colour	Brownish black	Rough surface	Raised margins	3.20	++
48	**MgPM 178**	Subdued + Submerged + Sector formation + Scanty aerial mycelium	Brownish black	Grayish black	Rough surface	Smooth margins	3.50	++
49	**MgPM 179**	Subdued + Cottony growth + Tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish green	Rough surface	Smooth margins	2.70	++++
50	**MgPM 181**	Subdued + Scanty aerial mycelium + Tuft + Radial sector formation	Brown	Brown	Rough surface	Smooth margins	3.40	++++
51	**MgPM 182**	Subdued + Tufted growth + Scanty aerial mycelium	Brownish black	Grayish black	Rough surface	Smooth margins	3.20	++++
52	**MgPM 186**	Subdued + Tuft + Radial Sector formation	Brownish black	Grayish black	Rough surface	Raised margins	3.90	++++
53	**MgPM 188**	Subdued + Tuft + Scanty aerial mycelium + Sector formation in concentric rings	Brownish black	Grayish green	Rough surface	Smooth margins	3.20	++++
54	**MgPM 190**	Subdued + Cottony growth + Tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish brown	Rough surface	Raised margins	4.20	+++
55	**MgPM 191**	Subdued + Cottony growth + Tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish black	Rough surface	Smooth margins	3.00	+++
56	**MgPM 193**	Subdued + Tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish green	Rough surface	Smooth margins	2.70	++
57	**MgPM 194**	Subdued + Submerged + Scanty aerial mycelium	Brownish black	Grayish black	Rough surface	Smooth margins	2.60	++
58	**MgPM 196**	Subdued + Cottony growth + Tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish black	Rough surface	Raised margins	3.90	++
59	**MgPm 197**	Subdued + Radial Sector formation	Brown	Brown	Rough	Raised	3.70	+

					surface	margins		
60	**MgPM 200**	Subdued + Cottony growth + Tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish brown	Rough surface	Raised margins	3.90	++++
61	**MgPM 204**	Subdued + small tufted growth forming sectors	Brownish black	Grayish brown	Rough surface	Smooth margins	3.90	++++
62	**MgPM 206**	Subdued + Submerged + Small tufted growth forming sectors	Brownish black	Grayish black	Rough surface	Smooth margins	3.60	++
63	**MgPM 208**	Subdued + Cottony growth + Tuft + Scanty aerial mycelium + Sector formation	Brownish black	Grayish black	Rough surface	Raised margins	4.20	++
64	**MgPM 209**	Subdued + Scanty aerial mycelium + tuft + Sector formation	Brownish black	Grayish brown	Rough surface	Smooth margins	3.30	+
65	**MgPM 210**	Subdued + Tufted growth + Scanty aerial mycelium	Brownish black	Grayish black	Rough surface	Raised margins	3.90	++++
	LSD (at 5%)						**0.31**	

(**Note:** +: Poor, ++: Fair, +++: Good, ++++: Excellent sporulation).

Table 4.10. Analysis of variance (ANOVA) for leaf blast severity of sixty five isolates of *Magnaporthe grisea* on ten pearl millet host differential lines

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	Variance (F value)
Rep	2	0.77	0.38	1.74
Isolate	64	2188.51	34.19	153.13**
Genotype	9	3235.37	359.48	1609.77**
Isolate*Genotype	576	1840.05	3.19	14.31**
Residual	1298	289.86	0.22	
Total	1949	7554.58		

**Highly Significant (P< 0.001)

Table 4.11. Disease severity (1-9 scale) of blast on ten different pearl millet lines tested against sixty five isolates of *Magnaporthe grisea* collected from India under green house conditions

S. No	Isolate/Cultivar	ICMB 93333	ICMB 95444	ICMB 97222-P1	ICMB 01333	ICMB 02444	ICMR 06444	863 B P2	ICMR 06222	ICMR 11003	IP 21187	Total
1	**MgPM 21**	3.0	9.0	3.0	6.0	3.0	3.0	3.0	3.0	3.0	3.0	**3.9**
2	**MgPM 32**	3.0	8.0	3.0	5.0	3.0	3.0	3.0	3.0	3.0	3.0	**3.7**
3	**MgPM 39**	3.0	9.0	3.0	3.0	3.0	3.0	3.0	3.0	2.3	2.0	**3.4**
4	**MgPM 40**	2.6	7.2	3.4	3.6	4.6	3.8	3.4	3.4	2.9	2.9	**3.8**
5	**MgPM 45**	2.9	8.1	2.1	5.3	3.6	2.8	2.7	2.7	2.0	2.5	**3.5**
6	**MgPM 49**	3.0	8.0	3.0	5.0	3.0	3.0	3.0	3.0	2.0	2.0	**3.5**
7	**MgPM 53**	1.7	8.6	2.3	5.5	4.6	2.8	2.3	2.3	1.8	1.5	**3.3**
8	**MgPM 56**	1.6	8.1	2.1	3.9	4.9	2.3	4.8	3.4	1.6	1.4	**3.4**
9	**MgPM 118**	3.7	8.6	2.7	6.2	5.4	2.7	5.3	3.9	1.7	1.4	**4.2**
10	**MgPM 119**	2.8	8.7	1.8	5.6	3.0	1.8	6.3	2.2	1.8	1.8	**3.6**
11	**MgPM 121**	7.0	8.0	5.0	5.0	6.0	5.0	7.0	7.0	6.0	6.0	**6.2**
12	**MgPM 122**	2.8	7.7	4.3	5.6	5.1	3.0	4.5	4.9	2.8	2.6	**4.3**
13	**MgPM 124**	3.5	7.0	3.3	4.5	3.5	2.0	3.5	3.0	2.0	1.5	**3.4**
14	**MgPM 125**	2.5	8.5	3.2	4.0	4.5	3.0	2.5	2.5	2.5	2.5	**3.6**
15	**MgPM 126**	1.5	7.8	2.0	3.8	3.0	2.5	2.8	3.5	2.5	3.0	**3.3**
16	**MgPM 127**	3.0	9.0	3.0	2.0	3.0	3.0	2.0	2.0	2.0	3.0	**3.2**
17	**MgPM 129**	3.0	6.3	2.0	2.7	2.0	3.0	2.0	3.0	2.0	2.0	**2.8**
18	**MgPM 131**	6.0	8.0	5.0	5.0	7.0	3.0	7.0	7.0	7.0	2.0	**5.7**
19	**MgPM 132**	1.0	8.0	2.0	1.0	3.0	3.0	1.0	3.0	1.0	1.0	**2.4**
20	**MgPM 133**	3.0	8.0	3.0	5.0	6.0	3.0	5.0	3.0	2.0	2.0	**4.0**
21	**MgPM 134**	2.8	8.5	4.3	5.2	6.1	3.3	5.1	3.3	4.5	3.7	**4.7**

22	**MgPM 135**	2.8	8.4	3.1	4.7	3.0	3.3	2.0	2.1	2.4	2.1	**3.4**
23	**MgPM 137**	7.0	8.0	7.0	5.0	8.3	6.0	7.0	7.0	8.7	7.3	**7.1**
24	**MgPM 138**	7.0	8.0	7.3	6.0	8.0	8.0	8.0	8.0	7.0	7.0	**7.4**
25	**MgPM 139**	1.7	8.0	2.0	5.3	2.0	2.7	2.3	3.0	3.0	3.0	**3.3**
26	**MgPM 144**	3.0	7.6	4.2	4.7	4.3	3.0	4.3	3.0	3.0	2.3	**3.9**
27	**MgPM 145**	8.0	8.0	7.0	5.0	8.0	6.0	6.0	6.0	6.0	7.0	**6.7**
28	**MgPM 147**	5.6	6.7	4.4	4.5	5.4	3.9	6.7	6.7	4.3	2.6	**5.1**
29	**MgPM 148**	6.5	7.0	5.0	3.5	6.0	6.0	6.0	4.5	5.0	5.5	**5.5**
30	**MgPM 149**	2.0	9.0	3.0	3.0	3.0	2.0	3.0	2.0	2.0	3.0	**3.2**
31	**MgPM 150**	7.0	7.9	3.0	6.3	7.3	3.0	6.8	6.8	5.1	3.4	**5.7**
32	**MgPM 151**	2.3	6.8	2.8	3.5	4.3	2.5	5.3	4.9	2.5	2.5	**3.7**
33	**MgPM 152**	2.7	5.4	4.0	5.1	5.5	3.7	4.4	4.0	4.0	2.7	**4.1**
34	**MgPM 153**	4.3	7.1	2.1	4.1	4.1	1.6	6.1	4.8	1.7	2.0	**3.8**
35	**MgPM 154**	6.2	7.0	5.3	3.0	5.3	4.7	4.7	4.8	4.5	4.8	**5.0**
36	**MgPM 156**	2.7	8.3	2.6	4.8	3.2	3.1	4.2	2.7	2.9	3.0	**3.7**
37	**MgPM 158**	3.0	9.0	3.0	3.0	2.7	3.0	3.0	3.0	3.0	2.0	**3.5**
38	**MgPM 159**	2.0	8.0	3.0	3.0	3.0	3.0	2.0	3.0	2.0	3.0	**3.2**
39	**MgPM 162**	2.8	6.7	3.7	5.1	4.8	2.9	4.4	3.3	3.0	2.4	**3.9**
40	**MgPM 165**	3.0	7.7	3.0	5.0	7.0	3.0	6.0	6.3	3.0	3.0	**4.7**
41	**MgPM 167**	6.7	8.4	4.4	5.3	6.3	4.3	6.9	7.1	5.0	2.4	**5.7**
42	**MgPM 169**	3.0	8.0	2.0	4.7	2.0	3.0	2.7	2.0	2.0	3.0	**3.2**
43	**MgPM 171**	5.6	6.4	2.9	5.2	5.5	2.7	6.3	5.4	4.4	2.6	**4.7**
44	**MgPM 172**	3.9	7.7	4.0	3.9	6.3	3.9	5.9	6.8	3.4	2.6	**4.8**
45	**MgPM 173**	7.1	7.8	5.8	5.0	5.2	5.3	7.0	6.0	5.1	6.4	**6.1**
46	**MgPM 174**	7.1	7.8	6.4	5.5	6.9	6.0	6.6	6.4	5.6	6.3	**6.5**

47	**MgPM 175**	4.9	8.0	3.0	4.5	4.3	5.4	4.9	3.0	3.0	2.4	**4.3**
48	**MgPM 178**	4.2	7.1	2.0	4.6	5.6	2.1	6.6	6.9	2.0	2.6	**4.4**
49	**MgPM 179**	6.3	7.5	3.9	4.6	5.3	4.0	6.8	6.9	4.6	2.5	**5.2**
50	**MgPM 181**	3.0	8.3	3.3	5.0	2.5	2.5	2.5	2.2	3.0	2.0	**3.4**
51	**MgPM 182**	5.5	6.8	3.7	5.0	5.5	3.9	6.7	6.2	4.7	2.5	**5.1**
52	**MgPM 186**	6.2	8.1	4.4	5.4	5.3	4.7	5.0	5.1	2.7	2.7	**5.0**
53	**MgPM 188**	3.0	8.0	3.0	5.0	3.0	2.0	3.0	3.0	2.0	3.0	**3.5**
54	**MgPM 190**	2.5	7.2	5.0	5.0	6.3	4.2	4.8	4.5	4.5	4.7	**4.9**
55	**MgPM 191**	4.7	8.0	4.2	5.5	6.0	4.4	4.7	4.7	4.2	2.6	**4.9**
56	**MgPM 193**	3.0	9.0	3.0	5.0	3.0	3.0	3.0	3.0	3.0	2.0	**3.7**
57	**MgPM 194**	2.3	8.8	3.0	3.5	5.8	2.7	2.0	2.0	2.0	3.3	**3.6**
58	**MgPM 196**	3.0	9.0	3.0	5.0	3.0	3.0	3.0	3.0	2.0	3.0	**3.7**
59	**MgPM 197**	1.5	5.0	2.5	5.0	3.5	3.0	2.0	3.0	2.5	2.5	**3.1**
60	**MgPM 200**	3.0	8.0	2.6	5.9	4.7	3.4	3.4	2.1	2.6	2.6	**3.8**
61	**MgPM 204**	6.8	8.0	5.8	5.2	6.5	5.3	6.8	7.1	5.5	2.1	**5.9**
62	**MgPM 206**	4.2	5.5	3.2	3.6	4.5	2.0	4.4	3.2	1.5	2.0	**3.4**
63	**MgPM 208**	2.5	4.0	2.0	3.0	4.5	3.5	4.0	2.5	2.7	2.0	**3.1**
64	**MgPM 209**	2.6	7.9	4.1	4.0	5.7	1.6	5.1	4.6	3.7	2.6	**4.2**
65	**MgPM 210**	2.0	9.0	2.3	5.0	3.0	3.0	3.0	3.0	2.0	2.0	**3.4**
	Mean	**3.8**	**7.8**	**3.5**	**4.6**	**4.7**	**3.4**	**4.4**	**4.1**	**3.3**	**3.0**	

LSD at 5%**:** Isolate = 0.23, Genotype = 0.09, Isolate x Genotype = 0.75

Table 4.12. Genetic diversity among isolates of *Magnaporthe grisea* collected from different hosts by Universal Rice Primers (URP).

Primer	Sequences	Percentage of Amplification	Percentage of polymorphism	Range of fragment length (Kb)	PIC
URP 1F	ATCCAAGGTCCGAGACAACC	50	100	0.3-3	0.41
URP 2F	GTGTGCGATCAGTTGCTGGG	52.86	100	0.25-1.5	0.43
URP 4R	AGGACTCGATAACAGGCTCC	71.43	92.85	0.2-2.5	0.50
URP 6R	GGCAAGCTGGTGGGAGGTAC	95.71	91.66	0.25-2.0	0.33
URP 9F	ATGTGTGCGATCAGTTGCTG	75.71	92.85	0.2-4	0.50
URP 17R	AATGTGGGCAAGCTGGTGGT	88.57	33.33	0.25-2	0.45
URP 25F	GATGTGTTCTTGGAGCCTGT	77.14	81.81	0.2-2	0.50
URP 30F	GGACAAGAAGAGGATGTGGA	35.71	100	0.2-1.5	0.32
URP 38F	AAGAGGCATTCTACCACCAC	64.29	100	0.4-2	0.48

Table 4.13a. Analysis of molecular variance (AMOVA) for pearl millet and non-pearl millet infecting populations of *Magnaporthe grisea*

Source of variation	**Variance components**	**Percentage of variation**
Among pearl millet and non-pearl millet isolates	2.05	15.94
Within pearl millet and non-pearl millet isolates	10.83	84.06
Total	12.88	

Table 4.13b. Analysis of molecular variance (AMOVA) for pearl millet infecting populations of *Magnaporthe grisea*

Source of variation	**Variance components**	**Percentage of variation**
Among populations (states)	0.96	8.73
Within populations	10.06	91.27
Total	11.02	

Table 4.15. Effect of different leaf wetness durations on pearl millet (ICMB 95444) blast lesion length (mm) development

S.No	Isolate	Leaf wetness duration (LWD)/ Lesion length (mm)								
		0 h	**6 h**	**12 h**	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**	**Mean**
1	**MgPM 45**	0.00	2.55	2.70	3.17	3.67	4.83	5.63	5.67	**3.53^{b}**
2	**MgPM 53**	0.00	2.40	2.67	3.00	3.27	4.53	5.03	6.10	**3.38^{c}**
3	**MgPM 56**	1.54	2.43	2.80	3.20	3.50	4.53	5.40	6.30	**3.71^{a}**
4	**MgPM 118**	0.00	2.20	2.43	2.60	3.33	4.30	5.17	5.57	**3.20^{d}**
	Mean	**0.39^{h}**	**2.40^{g}**	**2.65^{f}**	**2.99^{e}**	**3.44^{d}**	**4.55^{c}**	**5.31^{b}**	**5.91^{a}**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	0.39	0.19	2.85		
Iso	3	3.45	1.15	16.63	<.0001	0.156
Lwd	7	264.68	37.81	546.75	<.0001	0.221
Iso*Lwd	21	5.44	0.25	3.75	<.0001	0.441
Residual	62	4.28	0.06			
Total	95	278.26				

Table 4.16. Effect of different leaf wetness durations on pearl millet (ICMB 95444) blast lesion density

S.No	Isolate	Leaf wetness duration (LWD)/ No. of lesions per leaf								
		0 h	**6 h**	**12 h**	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**	**Mean**
1	**MgPM 45**	0.00	4.33	10.13	15.00	24.10	42.37	38.33	32.70	**20.87^{c}**
2	**MgPM 53**	0.00	2.67	13.03	24.87	30.80	39.70	35.70	34.37	**22.64^{bc}**
3	**MgPM 56**	5.47	5.83	13.27	22.50	27.70	45.70	40.93	37.87	**24.91^{a}**
4	**MgPM 118**	0.00	4.62	11.33	20.40	23.80	37.43	34.03	30.50	**20.26 ^{c}**
	Mean	**1.37^{h}**	**4.36^{g}**	**11.94^{f}**	**20.69^{e}**	**26.60^{d}**	**41.30^{a}**	**37.25^{b}**	**33.86^{c}**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	0.45	0.22	0.07		
Iso	3	312.91	104.30	32.25	<.0001	1.02
Lwd	7	19275.28	2753.61	851.31	<.0001	1.44
Iso*Lwd	21	331.09	15.76	15.76	<.0001	2.89
Residual	62	200.54	3.23454			
Total	95	20120.30				

Table 4.17. Effect of different leaf wetness durations on lesion sporulation of blast of pearl millet (ICMB 95444)

S.No	Isolate	Leaf wetness duration (LWD)/ Spores per lesion (x 10^4)								
		0 h	**6 h**	**12 h**	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**	**Mean**
1	**MgPM 45**	0.00	74.17	126.67	214.17	265.00	340.00	390.00	425.00	**229.38^{c}**
2	**MgPM 53**	0.00	73.33	171.67	250.00	324.17	375.83	403.33	510.00	**263.54^{b}**
3	**MgPM 56**	85.00	150.83	207.50	251.67	322.50	382.50	440.00	567.50	**300.94^{ab}**
4	**MgPM 118**	0.00	102.50	199.17	232.50	314.17	370.00	435.00	541.67	**274.38^{b}**
	Mean	**21.25^{h}**	**100.21^{g}**	**176.25^{f}**	**237.08^{e}**	**306.46^{d}**	**367.08^{c}**	**417.08^{b}**	**511.04^{a}**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	4914.58	2457.29	2.19		
Iso	3	63209.57	21069.85	18.81	<.0001	19.65
Lwd	7	2291976.49	327425.21	292.35	<.0001	27.79
Iso*Lwd	21	29784.70	1418.31	1.27	0.2332	55.59
Residual	62	69439.58	1119.99			
Total	95	2459324.93				

Table 4.18. Effect of different leaf wetness durations on leaf blast sporulation of pearl millet (ICMB 95444)

S.No	Isolate	Leaf wetness duration (LWD)/ Spores per leaf (x 10^4)								
		0 h	**6 h**	**12 h**	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**	**Mean**
1	**MgPM 45**	0.00	321.39	1283.56	3212.50	6386.50	14404.67	14950.00	13897.50	**6807.01^{d}**
2	**MgPM 53**	0.00	195.56	2237.39	6216.67	9984.33	14920.58	14399.00	17527.00	**8185.07^{b}**
3	**MgPM 56**	464.67	879.86	2752.83	5662.50	8933.25	17480.25	18010.67	21489.33	**9459.17^{a}**
4	**MgPM 118**	0.00	473.45	2257.22	4743.00	7477.17	13850.33	14804.50	16375.00	**7497.58^{c}**
	Mean	**116.17^{f}**	**467.56^{f}**	**2132.75^{ef}**	**4958.67^{d}**	**8195.31^{c}**	**15163.96^{b}**	**15541.04^{b}**	**17322.21^{ab}**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	1442408	721204	0.67		
Iso	3	90593230	30197743	28.10	<.0001	594.79
Lwd	7	4281097077	611585297	569.03	<.0001	841.16
Iso*Lwd	21	87362809	4160134	3.87	<.0001	1682.32
Residual	62	66637286	1074795			
Total	95	4527132810				

Table 4.14. Effect of different leaf wetness durations on per cent disease index on pearl millet (ICMB 95444) blast

S.No	Isolate	Leaf wetness duration (LWD)/ Per cent disease incidence (PDI)								
		0 h	6 h	12 h	24 h	36 h	48 h	60 h	72 h	Mean
1	**MgPM 45**	11.11	40.00	90.37	98.15	100.00	100.00	100.00	100.00	**79.95^{c}**
2	**MgPM 53**	11.11	45.93	93.33	99.63	100.00	100.00	100.00	100.00	**81.25^{b}**
3	**MgPM 56**	44.81	78.15	93.70	100.00	100.00	100.00	100.00	100.00	**89.58^{a}**
4	**MgPM 118**	11.11	41.11	89.26	96.30	99.63	100.00	100.00	100.00	**79.68 ^{c}**
	Mean	**19.54^{e}**	**51.30^{d}**	**91.67^{c}**	**98.52^{b}**	**99.91^{a}**	**100.00^{a}**	**100.00^{a}**	**100.00^{a}**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	5.42	2.71	1.08		
Iso	3	1587.39	529.13	210.44	<.001	0.91
Lwd	7	78003.44	11143.35	4431.89	<.001	1.29
Iso*Lwd	21	3981.12	189.57	75.40	<.001	2.58
Residual	62	155.89	2.51			
Total	95	83733.28				

Table 4.19. Correlation matrix exhibiting correlation between per cent disease index (PDI) and other variables under different leaf wetness durations (LWD)

Variables	Leaf wetness duration	Lesion length	No. of lesions per leaf	Spores per lesion	Spores per leaf	PDI
Leaf wetness duration	1.00					
Lesion length	0.94	1.00				
No. of lesions per leaf (Lesion density)	0.90	0.88	1.00			
Spores per lesion	0.97	0.95	0.91	1.00		
Spores per leaf	0.96	0.91	0.95	0.95	1.00	
PDI	0.69	0.80	0.77	0.79	0.64	1.00

Table 4.21. Effect of different day/night temperatures on development of lesion length (mm) of blast disease on pearl millet (ICMB 95444)

S.No	Isolate	Temperature (day/night)/lesion length (mm)					
		25±1/ 20±1°C	**28±1/ 20±1°C**	**30±1/ 22±1°C**	**35±1/ 25±1°C**	**40±1/ 28±1°C**	**Mean**
1	**MgPM 45**	3.27	4.03	3.57	3.13	2.83	**3.37[a]**
2	**MgPM 53**	3.23	4.03	3.60	3.20	2.80	**3.37[a]**
3	**MgPM 56**	3.42	4.21	4.10	3.00	2.56	**3.46[a]**
4	**MgPM 118**	3.40	3.70	3.57	3.10	2.77	**3.31[a]**
	Mean	**3.33[c]**	**3.99[a]**	**3.71[b]**	**3.11[d]**	**2.74[e]**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	0.04	0.02	0.50		
Isolate	3	0.17	0.05	1.35	0.271	0.15
Temperature	4	11.67	2.91	69.64	<.001	0.16
Isolate*Temperature	12	1.14	0.09	2.27	0.027	0.33
Residual	38	1.59	0.04			
Total	59	14.61				

Table 4.22. Effect of different day/night temperatures on production of blast lesions on pearl millet (ICMB 95444)

S.No	Isolate	Temperature (day&night)/No. of lesions per leaf					
		25±1 & 20±1^{o}C	**28±1 & 20±1^{o}C**	**30±1 & 22±1^{o}C**	**35±1 & 25±1^{o}C**	**40±1 & 28±1^{o}C**	**Mean**
1	**MgPM 45**	22.60	25.03	28.67	26.70	25.53	**25.71^{d}**
2	**MgPM 53**	30.13	35.47	36.53	33.17	25.17	**32.09^{c}**
3	**MgPM 56**	40.40	49.07	63.30	41.77	31.90	**45.29^{a}**
4	**MgPM 118**	35.67	41.57	58.30	34.93	27.53	**39.60^{b}**
	Mean	**32.20^{c}**	**37.78^{b}**	**46.70^{a}**	**34.14^{c}**	**27.53^{d}**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	14.37	7.19	0.53		
Isolate	3	3159.19	1053.06	77.90	<.001	2.71
Temperature	4	2302.84	575.71	42.59	<.001	3.03
Isolate*Temperature	12	1421.56	118.46	8.76	<.001	6.07
Residual	38	513.66	13.52			
Total	59	7411.62				

Table 4.23. Effect of different day/night temperatures on sporulation of blast lesion on pearl millet (ICMB 95444)

S.No	Isolate	Temperature (day & night)/Spores per lesion (x 10^4 spores)					
		25±1 & 20±1°C	**28±1 & 20±1°C**	**30±1 & 22±1°C**	**35±1 & 25±1°C**	**40±1 & 28±1°C**	**Mean**
1	**MgPM 45**	480.00	550.83	530.00	464.17	404.17	**485.83^{d}**
2	**MgPM 53**	544.17	619.17	577.50	480.00	420.83	**528.33^{b}**
3	**MgPM 56**	625.00	710.00	667.50	567.50	521.67	**618.33^{a}**
4	**MgPM 118**	588.33	665.00	644.17	535.83	482.50	**583.17^{a}**
	Mean	**559.38^{b}**	**636.25^{a}**	**604.79^{a}**	**511.88^{c}**	**457.29^{d}**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	1303	651	0.26		
Isolate	3	154424	51475	20.17	<.001	37.34
Temperature	4	246009	61502	24.10	<.001	41.75
Isolate*Temperature	12	5305	442	0.17	0.999	83.50
Residual	38	96989	2552			
Total	59	504030				

Table 4.24. Effect of different day/night temperatures on sporulation of blast infected leaf of pearl millet (ICMB 95444)

S.No	Isolate	Temperature (day & night)/Spores per leaf (x 10^4 spores)					
		25±1 & 20±1°C	**28±1 & 20±1°C**	**30±1 & 22±1°C**	**35±1 & 25±1°C**	**40±1 & 28±1°C**	**Mean**
1	**MgPM 45**	10943.50	13668.58	15172.75	12499.58	10325.58	**12522.00^{d}**
2	**MgPM 53**	16423.42	22026.50	21108.00	16034.67	10585.33	**17235.58^{c}**
3	**MgPM 56**	25289.08	34724.00	42187.42	23636.00	16569.75	**28481.25^{a}**
4	**MgPM 118**	21066.67	27633.75	37538.25	18735.67	13242.83	**23643.43^{b}**
	Mean	**18430.67^{c}**	**24513.21^{b}**	**29001.60^{a}**	**17726.48^{c}**	**12680.88^{d}**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	997396.63	498698.32			
Isolate	3	2218244393.13	739414797.80	95.21	<.001	2060.02
Temperature	4	1937904683.10	484476170.80	62.38	<.001	2303.17
Isolate*Temperature	12	612700571.50	51058380.96	6.57	<.001	4606.35
Residual	38	295120117.20	7766318.87			
Total	59	5064967162.0				

Table 4.20. Effect of different day/night temperatures on per cent disease index (PDI) of blast of pearl millet (ICMB 95444)

S.No	Isolate	Temperature (day & night)/Per cent disease index (PDI)					
		25±1 & 20±1°C	**28±1 & 20±1°C**	**30±1 & 22±1°C**	**35±1 & 25±1°C**	**40±1 & 28±1°C**	**Mean**
1	**MgPM 45**	90.37	90.00	92.22	87.41	85.93	**89.19[b]**
2	**MgPM 53**	93.70	91.85	96.30	99.26	92.59	**94.74[a]**
3	**MgPM 56**	100.00	97.04	97.41	95.19	91.11	**96.15[a]**
4	**MgPM 118**	95.56	95.19	100.00	97.78	88.15	**95.33[a]**
	Mean	**94.91[a]**	**93.52[a]**	**96.48[a]**	**94.91[a]**	**89.44[b]**	

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	LSD
Rep	2	24.73	12.37	0.94		
Isolate	3	450.53	150.18	11.37	<.001	2.68
Temperature	4	344.16	86.04	6.51	<.001	3.04
Isolate*Temperature	12	210.58	17.55	1.33	0.243	6.00
Residual	38	502.02	13.21			
Total	59	1532.02				

Table 4.25. Correlation matrix showing the correlation between per cent disease index (PDI) with other variables under different temperature conditions

Variables	Temperature	Lesion length	No. of lesions per leaf	Spores per lesion	Spores per leaf	PDI
Temperature	1.00					
Lesion length	-0.71	1.00				
No. of lesions per leaf	-0.29	0.47	1.00			
Spores per lesion	-0.63	0.76	0.72	1.00		
Spores per leaf	-0.38	0.62	0.89	0.89	1.00	
PDI	-0.40	0.38	0.75	0.61	0.67	1.00

Table 4.26. Analysis of variance (ANOVA) of 160 designated B lines against five different pathotypes of *Magnaporthe grisea* infecting pearl millet

Source of variation	Degrees of freedom	Sum of square	Mean sum of square	Variance	F probability
Rep	2	0.15	0.07	0.69	
Pathotype (P)	4	389.21	97.30	884.24	<.001
Breeding lines (B)	159	7262.07	45.67	415.05	<.001
P*B	636	1218.78	1.92	17.41	<.001
Residual	1598	175.84	0.11		
Total	2399	9046.07			

Table 4.27. Disease severity (on 1-9 scale) in selected B-lines showing resistance to 3-5 pathotypes of *Magnaporthe grisea.*

Entry	Pedigree	Blast severity (1-9 scale)[a]						Patho types[b]
		MgPM 45	MgPM 53	MgPM 56	MgPM 118	MgPM 119	Mean	
81B	Induced downy mildew resistant selection from Tift 23D2B	2.0	1.0	1.0	1.0	1.0	1.2	5
ICMB 88004	Togo-11-5-2 selection	3.0	2.0	2.0	2.0	2.0	2.2	5
ICMB 92444	(843B x ICMPS 1500-7-4-1-6)-23-1-B-1-4	3.0	2.0	2.0	1.0	2.0	2.0	5
IICMB 97222-P1	[(ICMB 88006 x ICMB 88005) x (ICMB 89111 x ICMB 88004)]-28-2-B	3.0	2.0	2.0	1.0	3.0	2.2	5
ICMB 02111	[(ICMB 88006 x ICMB 88005) x (ICMB 89111 x ICMB 88004)]-99-B	3.0	3.0	3.0	3.0	2.0	2.8	5
ICMB 07111	(ICMB 96111 x 4038-4-2-B)-2-1-5-4	2.0	1.0	1.0	1.0	2.0	1.4	5
ICMB 09333	[(SRC II C3 S1-103-1-1 x HHVBC)-20 x (81B x ICMP 451)-5-4-2-3]-5-2-1-B-B-3-B	2.0	1.0	1.0	1.0	1.0	1.2	5
ICMB 09999	(81B x 4025-3-2-B)-8-1-B	2.0	1.0	1.0	1.0	2.0	1.4	5
ICMB 92666	[ICMPES 34 x (843B x ICMPES 34)]-155-4-2	2.0	3.0	3.0	1.0	4.0	2.6	4
ICMB 92777	[843B x (ICMPS 500-4-4-3 x ICMPS 1800-3-1-2-C3-4)]-7-1-3	3.0	3.0	3.0	3.0	4.0	3.2	4
ICMB 93222	(26B x 834B)-11-2-B-B	3.0	4.0	3.0	3.0	3.0	3.2	4
ICMB 93333	(843B x ICMPS 900-9-3-8-2)-21-8-4	2.9	2.0	1.8	3.6	2.7	2.6	4
ICMB 00111	(BSECBPT/91-40 x SPF3/S91-3)-1-2-2-4	2.0	3.0	1.0	1.0	4.0	2.2	4
ICMB 01777	(BSECBPT/91-38 x SPF3/S91-529)-10-1-6	2.0	3.0	3.0	3.0	4.0	3.0	4
ICMB 04222	(843B x EEBC S1-407)-12-3-B	5.0	2.0	3.0	2.0	3.0	3.0	4
ICMB 05777	(D2BLN/95-98 x EEBC C1-1)-7-B-B	4.0	3.0	3.0	2.0	2.0	2.8	4
ICMB 06444	EEBC S1-407-1-B-B-B-B-1	2.8	2.8	2.5	3.1	1.7	2.6	4
ICMB 00222	{[(81B x SRL 53-1) x 843B]-3-5-2 x (843B x 834B)-25-B-B-1}-84-6-B-B	3.0	5.0	3.0	3.0	4.0	3.6	3
ICMB 00999	(ICMB 89111 x 863B)-65-8-B-B	2.0	2.0	4.0	2.0	4.0	2.8	3
ICMB 03333	9035/S92-B-3	3.0	5.0	3.0	3.0	6.0	4.0	3
ICMB 03888	[(ICMB 88006 x ICMB 88005) x (ICMB 89111 x ICMB 88005)]-1-1-3-B-9	4.0	2.0	5.0	2.0	2.0	3.0	3
ICMB 03999	(ICMB 89111 x IP 9402-2-1-1-2)-31-1-B-B	2.0	4.0	3.0	2.0	4.0	3.0	3
ICMB 04111	(81B x 4017-5-4-B)-12-3-1-3	4.0	3.0	2.0	2.0	4.0	3.0	3

[a] Mean of 3 replicates; LSD (P<0.01): Isolate = 0.04; Genotype = 0.23, Isolate × Genotype = 0.53

[b] Resistant to number of pathotypes

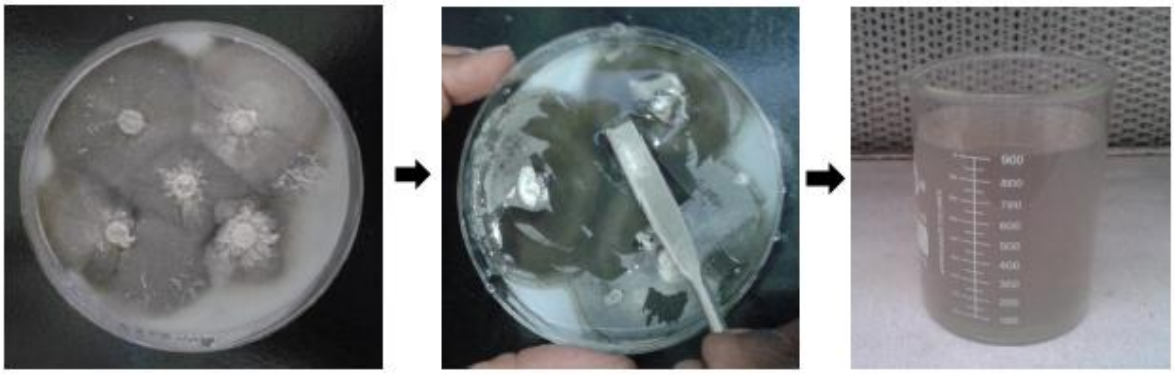
4.28. Analysis of variance (ANOVA) of twenty eight pearl millet genotypes against blast disease during *Kharif* 2013 - 2014 at six locations

Source	DF	Sum of Squares	Mean Square	F Value	P > F	LSD
Year (Y)	1	2.73	2.73	3.30	0.211	0.30
Replication (Year)	2	1.65	0.82	1.75		
Genotype (G)	27	1390.17	51.48	108.97	<.001	0.39
Location (L)	5	219.59	43.91	92.93	<.001	0.18
G*L	135	382.78	2.83	6.00	<.001	0.95
G*Y	27	52.0878	1.92	4.08	<.001	0.56
L*Y	5	154.82	30.96	65.52	<.001	0.28
G*L*Y	134	225.83	1.68	3.57	<.001	1.35
Residual	322	151.49	0.47			
Total	668	2581.18				

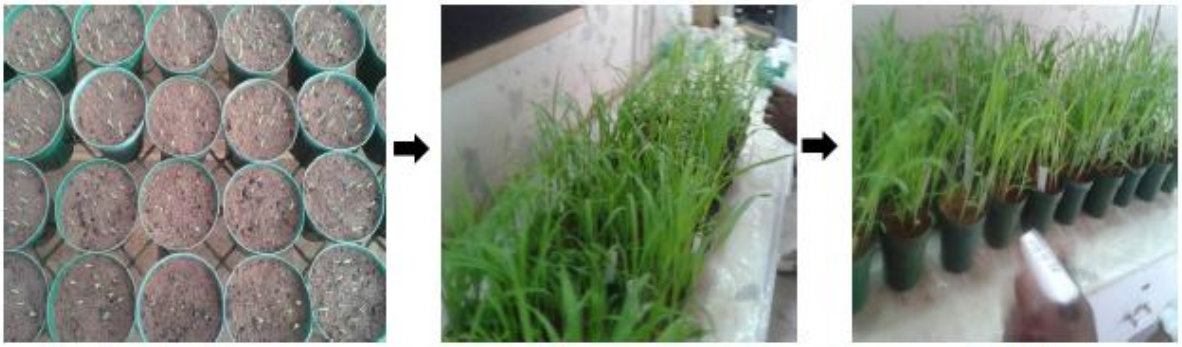
4.29. Disease severity of twenty eight pearl millet accessions against blast disease during *Kharif* 2013 -2014 at six locations

S No.	Genotype	Disease severity (1-9 Scale)						Mean
		Aurangabad	Dhule	Durgapura	Gwalior	Jamnagar	Patancheru	
1	IP 7846	4.50	4.25	4.50	3.25	2.98	3.50	**3.83^{lmn}**
2	IP 11036	6.25	4.50	4.75	4.00	3.10	3.50	**4.35^{hijk}**
3	IP 15256	5.38	5.25	4.75	4.50	3.18	5.00	**4.68^{ghi}**
4	IP 21187	5.25	4.75	4.00	3.75	2.68	3.50	**3.99^{klmn}**
5	ICMB 93333	6.25	5.50	5.00	5.00	3.58	4.50	**4.97^{fg}**
6	ICMB 92666	6.00	7.00	6.75	6.50	5.18	5.50	**6.15^{d}**
7	ICMB 92777	4.25	5.00	5.75	3.75	4.00	4.50	**4.54^{ghij}**
8	ICMR 06222	5.00	5.25	5.25	4.00	2.50	3.75	**4.29^{ijk}**
9	ICMR 06444	4.75	3.75	4.00	1.25	1.20	3.00	**2.99^{p}**
10	ICMB 01333	5.38	5.00	3.75	2.75	1.50	6.25	**4.10^{jkl}**
11	ICMB 97222	3.25	5.75	5.25	4.75	2.80	4.25	**4.34^{hijk}**
12	ICMB 02444	5.38	7.00	5.25	7.25	4.85	4.50	**5.70^{e}**
13	ICMB 89111	8.00	8.75	7.00	8.25	7.13	8.25	**7.90^{b}**
14	ICMB 95444	8.88	9.00	8.25	8.75	8.05	9.00	**8.65^{a}**
15	ICMB 93222	4.63	5.29	5.00	5.75	3.78	5.00	**4.91^{fg}**
16	ICMB 00111	6.25	5.25	5.25	4.25	2.75	4.75	**4.75^{gh}**
17	ICMB 02111	3.75	5.25	4.00	3.25	2.70	5.25	**4.03^{klm}**
18	ICMB 07111	4.50	4.75	4.00	3.75	2.60	5.00	**4.10^{jkl}**
19	ICMB 09999	3.88	3.50	4.75	4.00	1.43	4.25	**3.63^{mno}**
20	863B-P2	4.00	7.75	5.25	6.50	5.98	6.00	**5.91^{de}**
21	HHB 146 Improved	3.63	3.25	4.75	3.00	1.48	3.50	**3.27^{op}**
22	ICMR 01004	4.50	5.50	5.00	4.25	2.78	4.00	**4.34^{hijk}**
23	ICMR 11009	4.00	3.25	4.00	2.25	1.60	4.25	**3.23^{op}**
24	ICMR 11019	3.75	3.25	4.50	4.53	1.90	3.50	**3.57^{no}**
25	ICMB 95222	7.00	8.25	6.75	7.75	7.70	8.25	**7.62^{b}**
26	ICMB 95222-672	3.13	6.25	5.75	6.50	3.13	6.50	**5.21^{f}**
27	ICMB 95222-760	4.75	6.75	5.75	6.75	6.23	6.75	**6.16^{d}**
28	IP 22303	5.88	7.00	6.00	8.00	7.88	7.50	**7.04^{c}**
	Mean	**5.08^{bc}**	**5.57^{a}**	**5.18^{b}**	**4.94^{c}**	**3.74^{d}**	**5.13^{bc}**	

Figure 3.1. Map showing the six locations of Pearl millet blast virulence nursery (PMBVN) conducted during 2013 and 2014.

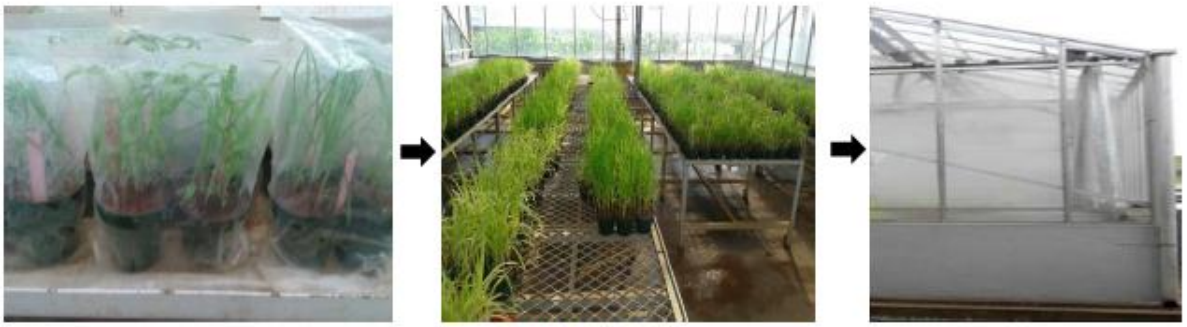


Pure culture | Conidia harvesting | Inoculum

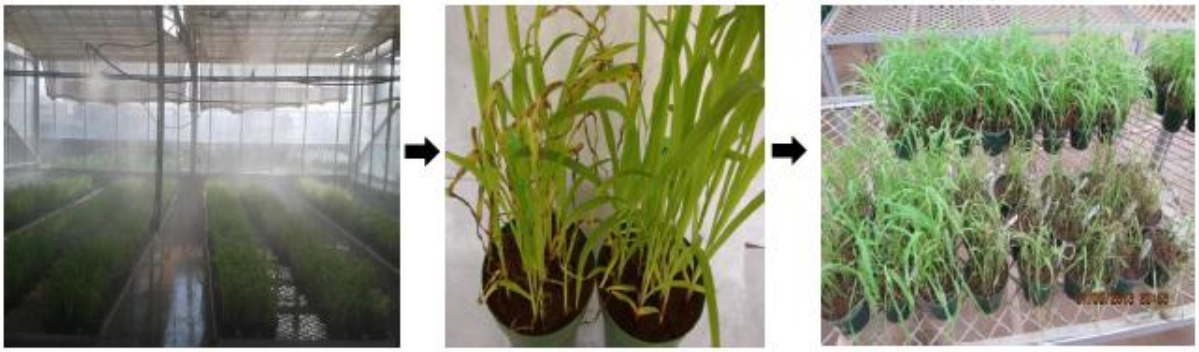


Germinated seedlings | Seedlings at 3rd leaf stage (or) 12 day old seedlings | Inoculation

Plate 3.1a. Glass house screening technique for pearl millet blast disease.



Incubation

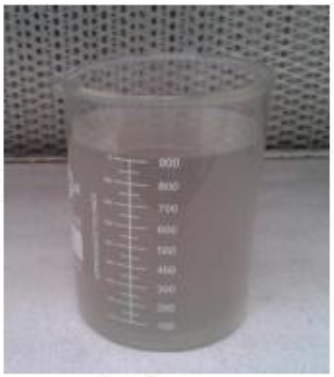


Under misting condition | Disease assessment

Plate 3.1b. Glass house screening technique for pearl millet blast disease.

Pure culture

Conidia harvesting



Inoculum

Inoculations in PMBVN

Perfo irrigation

Plate 3.3a. Inoculations in Pearl millet blast virulence nursery.

Blast infected PMBVN Field

Pearl millet cultivar exhibiting typical leaf blast symptoms

Blast disease symptoms produced on highly susceptible and moderately resistant cultivar in PMBVN at Patancheru

Plate 3.3b. Pearl millet cultivar exhibiting blast symptoms in Pearl millet blast Virulence Nursery.

Plate 4.1 Pearl millet plant infected with blast disease under field conditions.

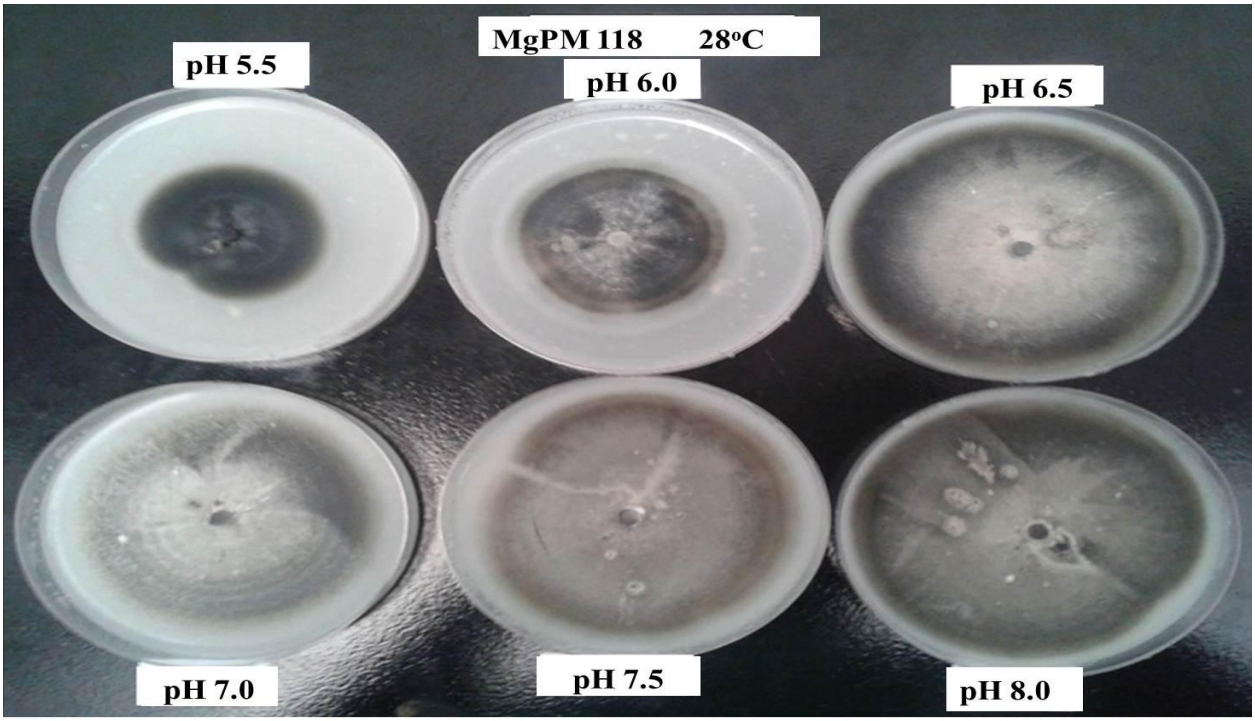


Plate 4.2a. Effect of different pH levels of OMA media on growth of *M. grisea* isolate MgPM 118 incubated at 28°C

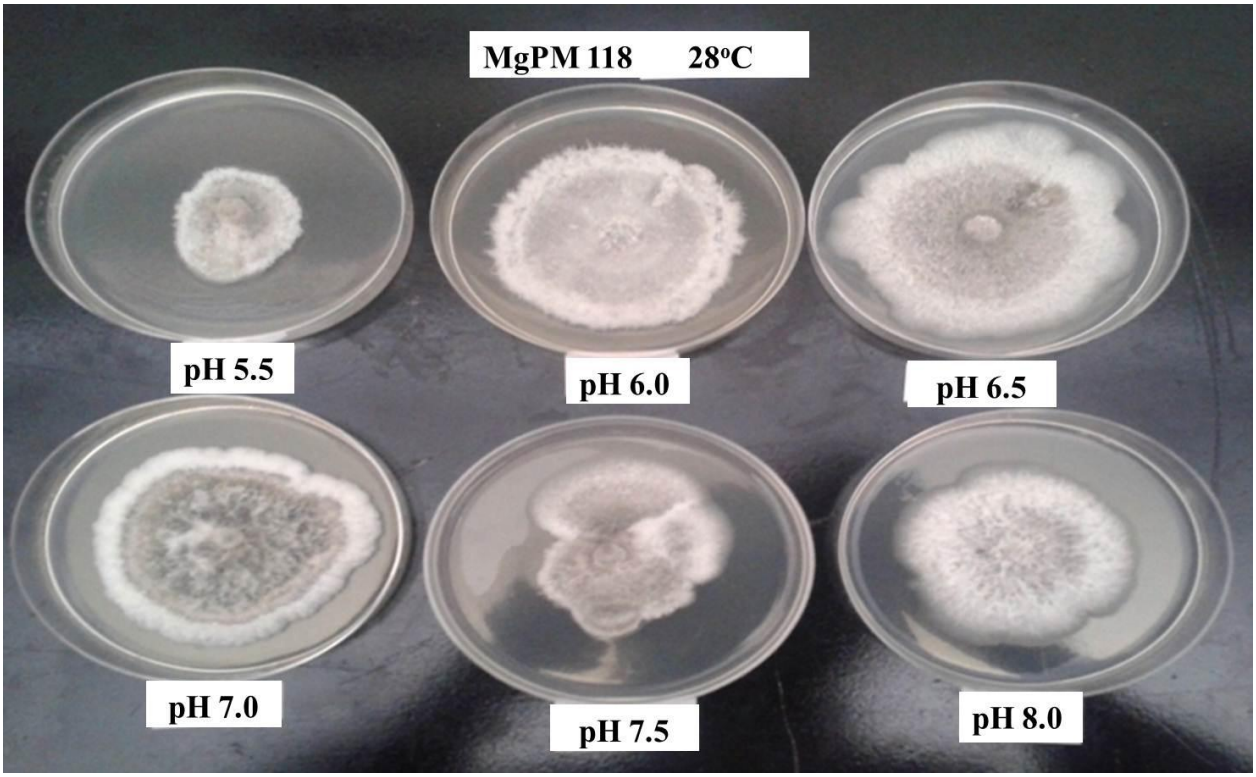


Plate 4.2b. Effect of different pH levels of PDA media on growth of *M. grisea* isolate MgPM 118 incubated at 28°C

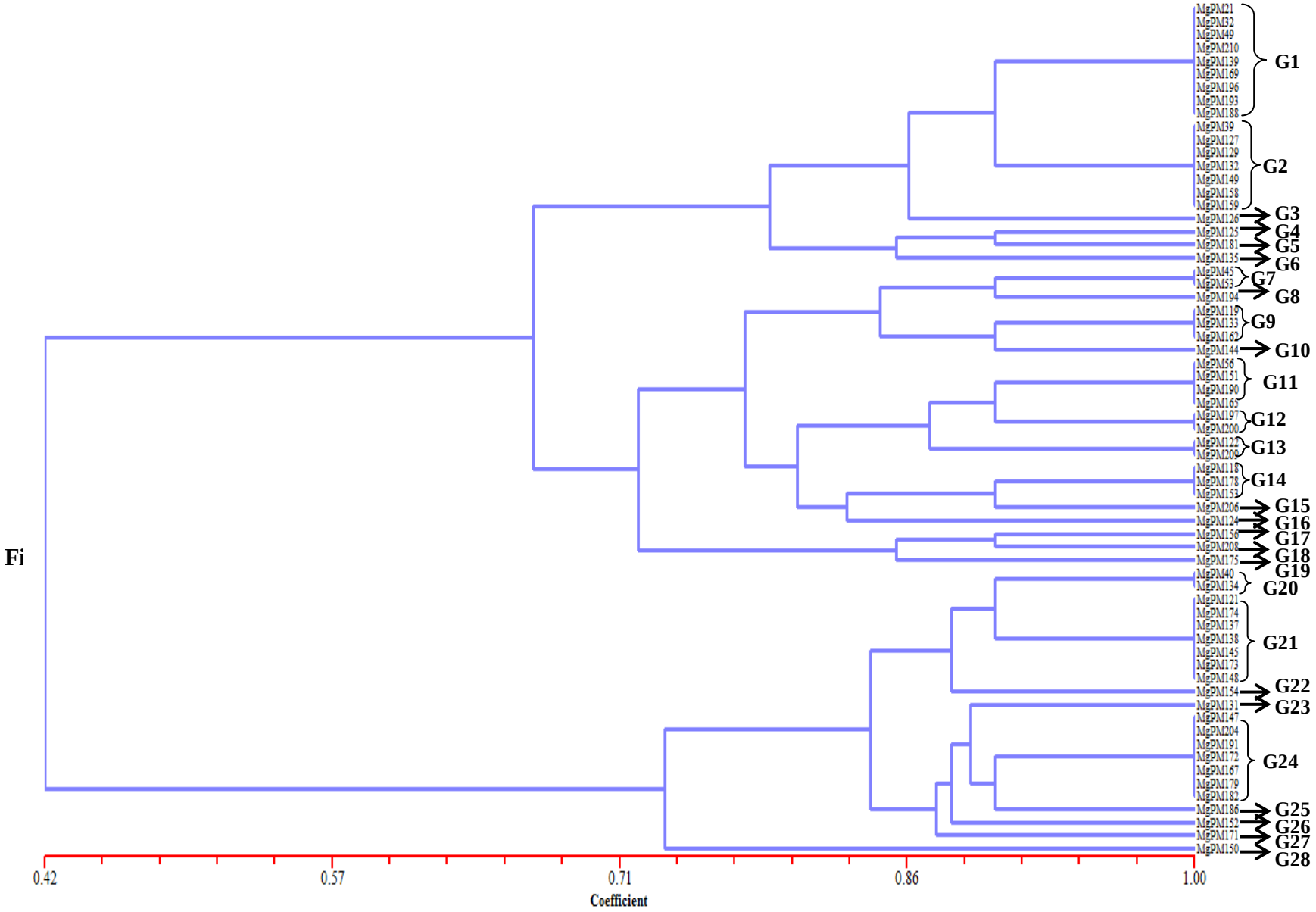


Figure 4.1. Grouping of the isolates based on the virulence reaction on different genotypes of pearl millet.

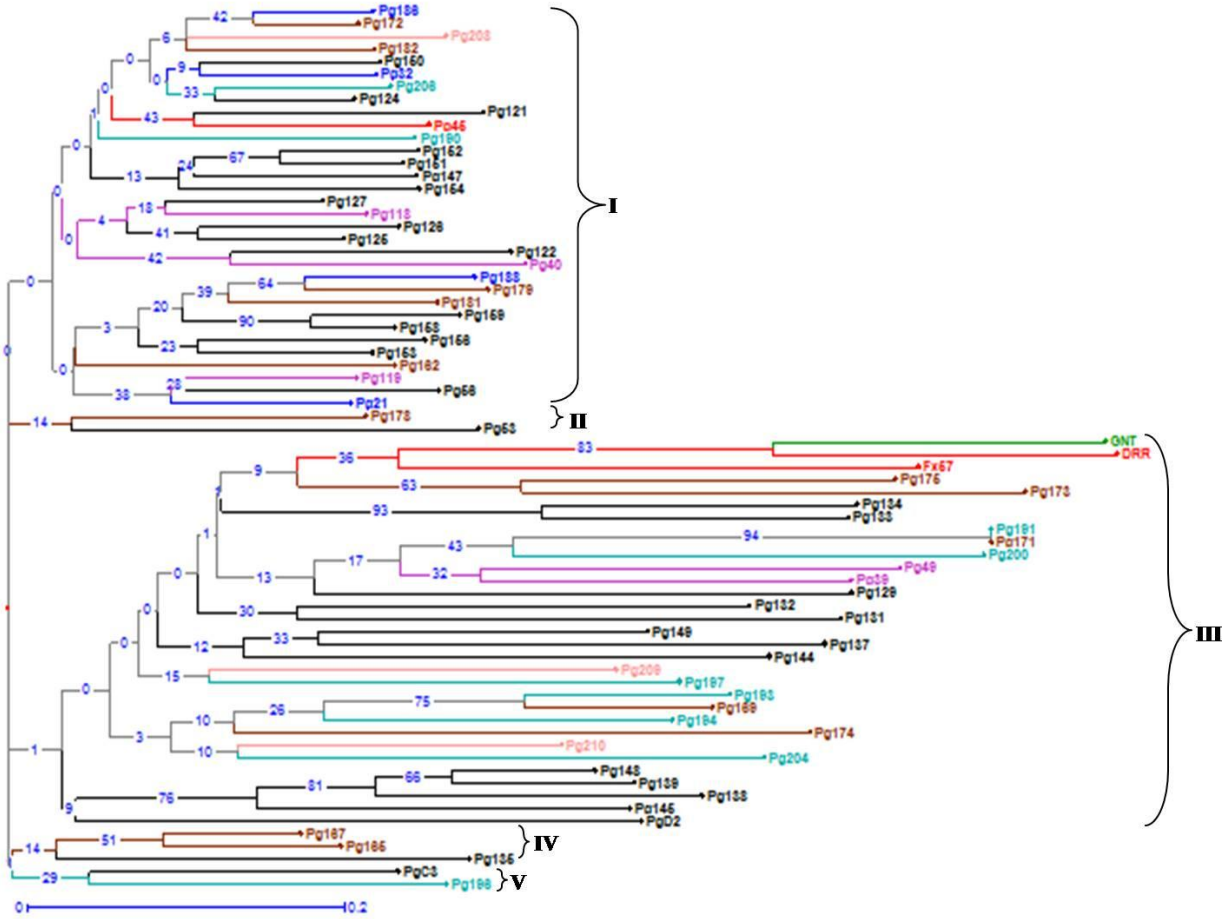
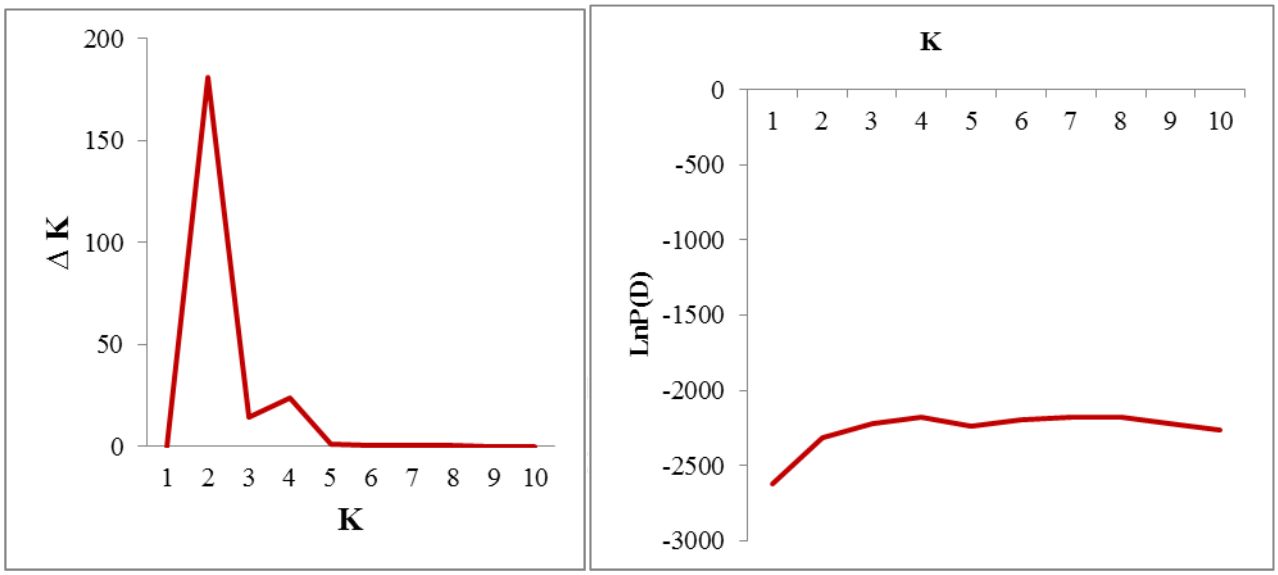
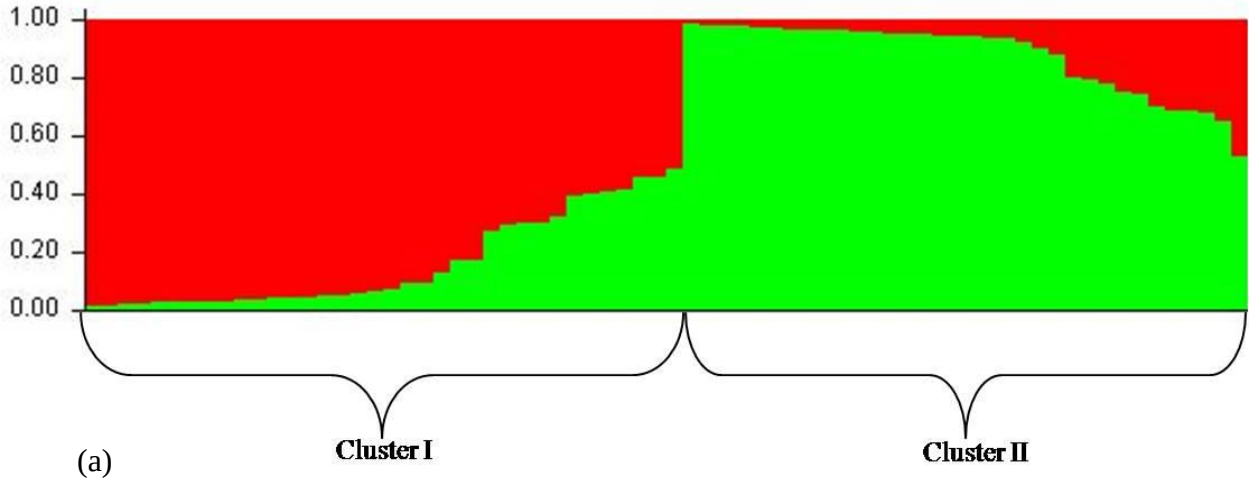


Figure. 4.2. Unweighted pair group method arithmetic average dendrogram constructed from URP PCR data indicating the relationship among the isolates of *M. grisea* from different hosts.

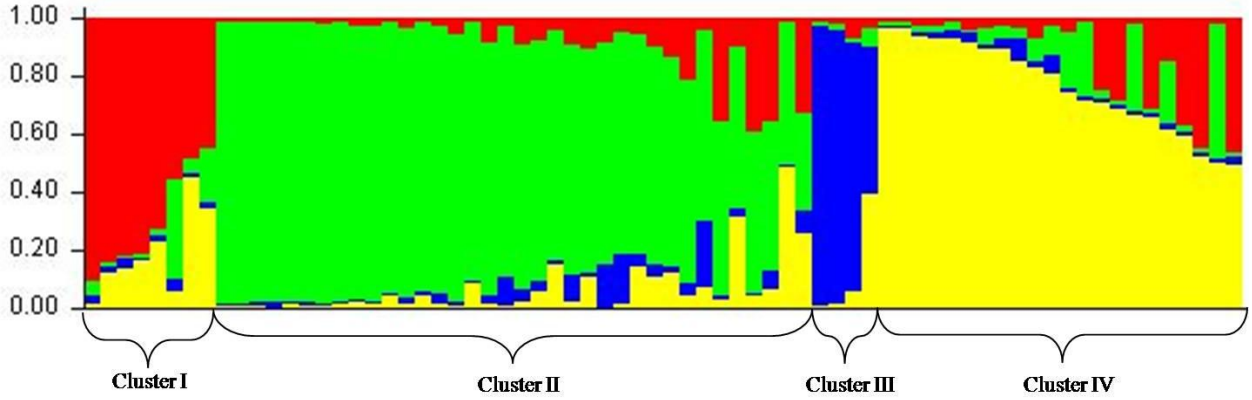


(a) (b)

Figure 4.3. Likelihood distribution of subpopulations of the *Magnaporthe grisea* isolates from different hosts based on the average of Δ*K* over ten runs for each *K* value (a), Log likelihood of data (n=70), L (*K*), as a function of *K* (b).



(a)



(b)

Figure 4.4. Model-based ancestry for each of the 70 isolates of *M. grisea* based on the nine universal rice primers using STRUCTURE version 2.3.4 at K=2 (a) and K=4 (b).

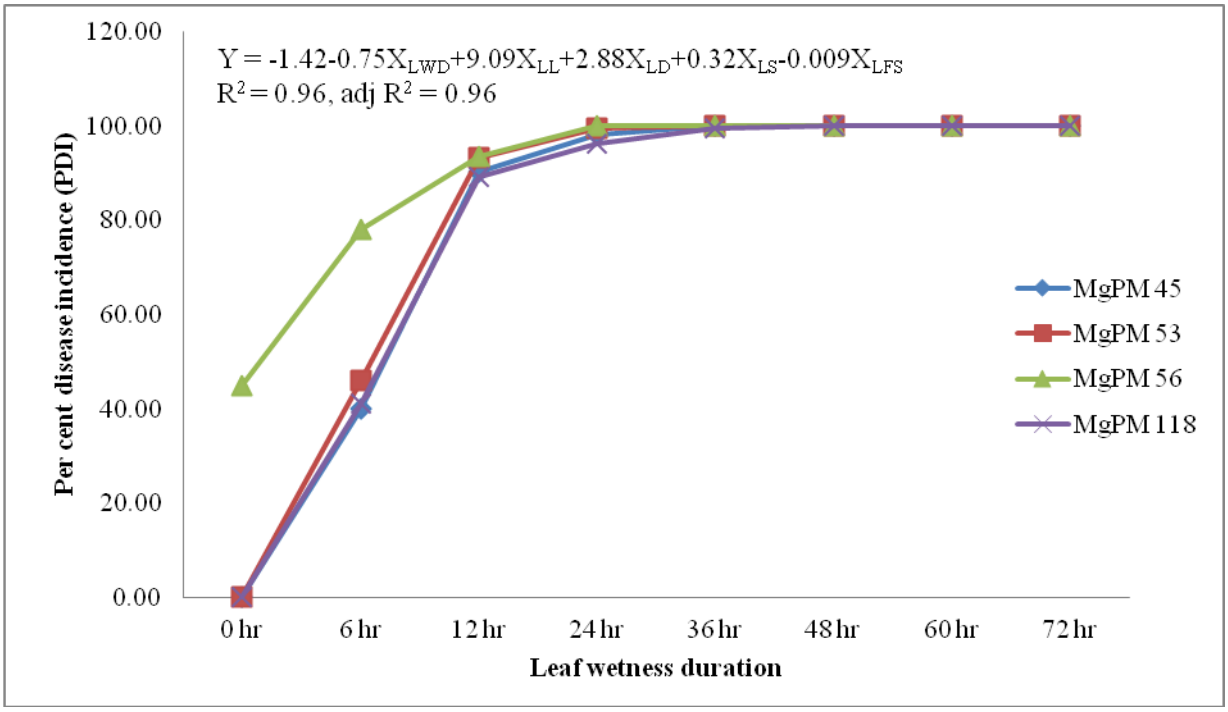


Figure 4.5. Effect of different leaf wetness durations on per cent disease index (PDI) of blast of pearl millet.

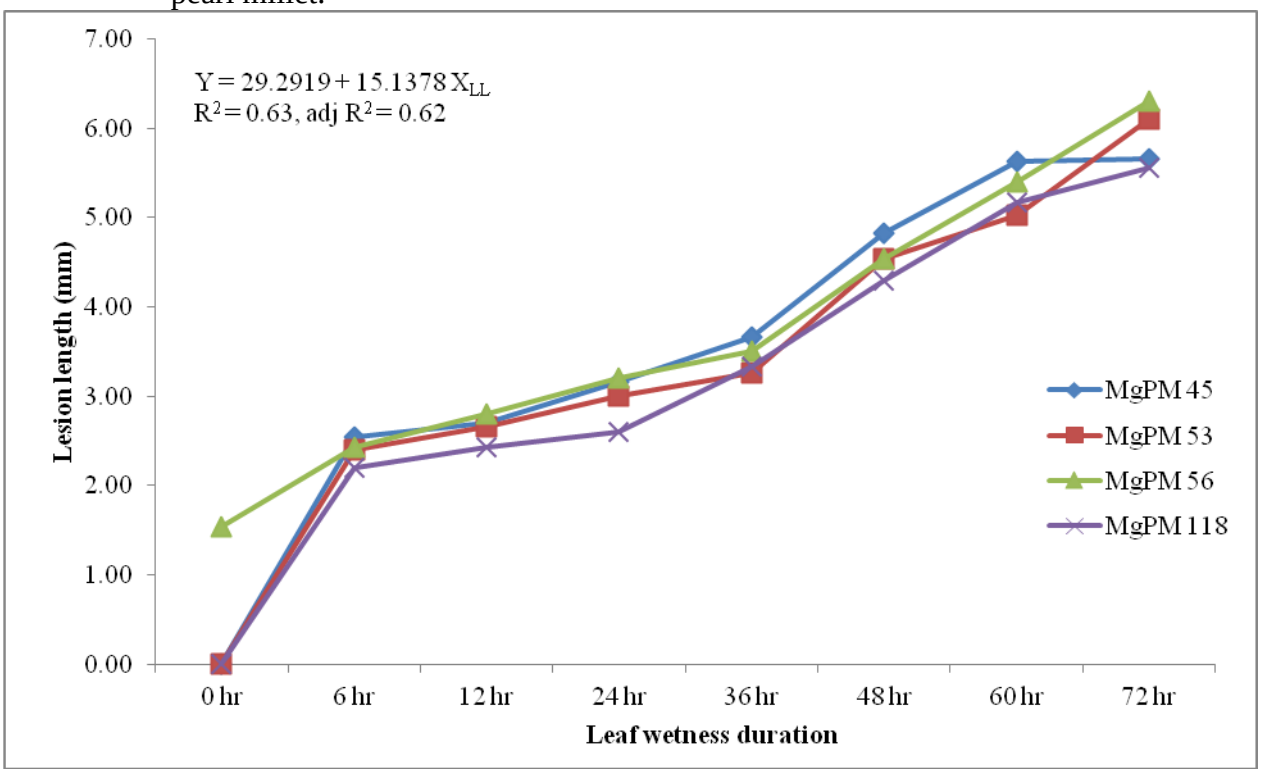


Figure 4.6. Effect of different leaf wetness durations on blast of pearl millet lesion length development.

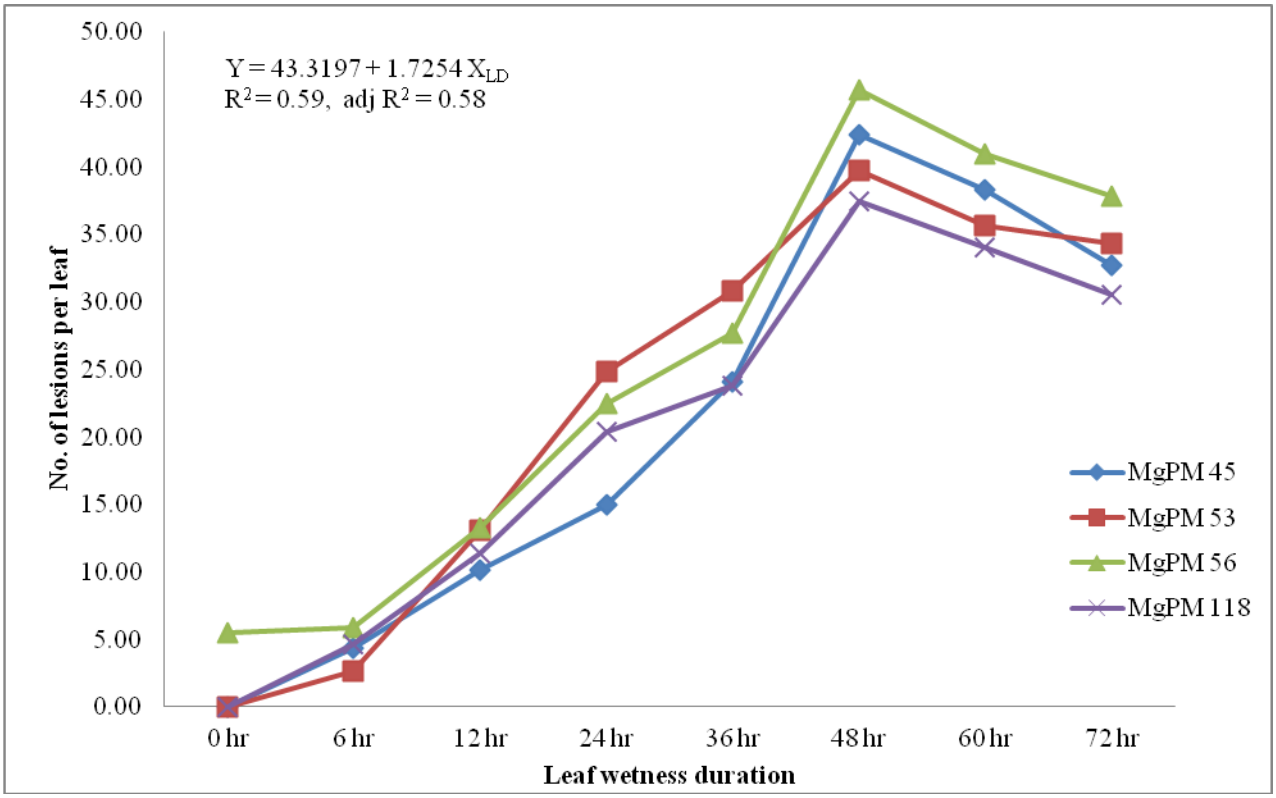


Figure 4.7. Effect of different leaf wetness durations on lesion density of blast of pearl millet.

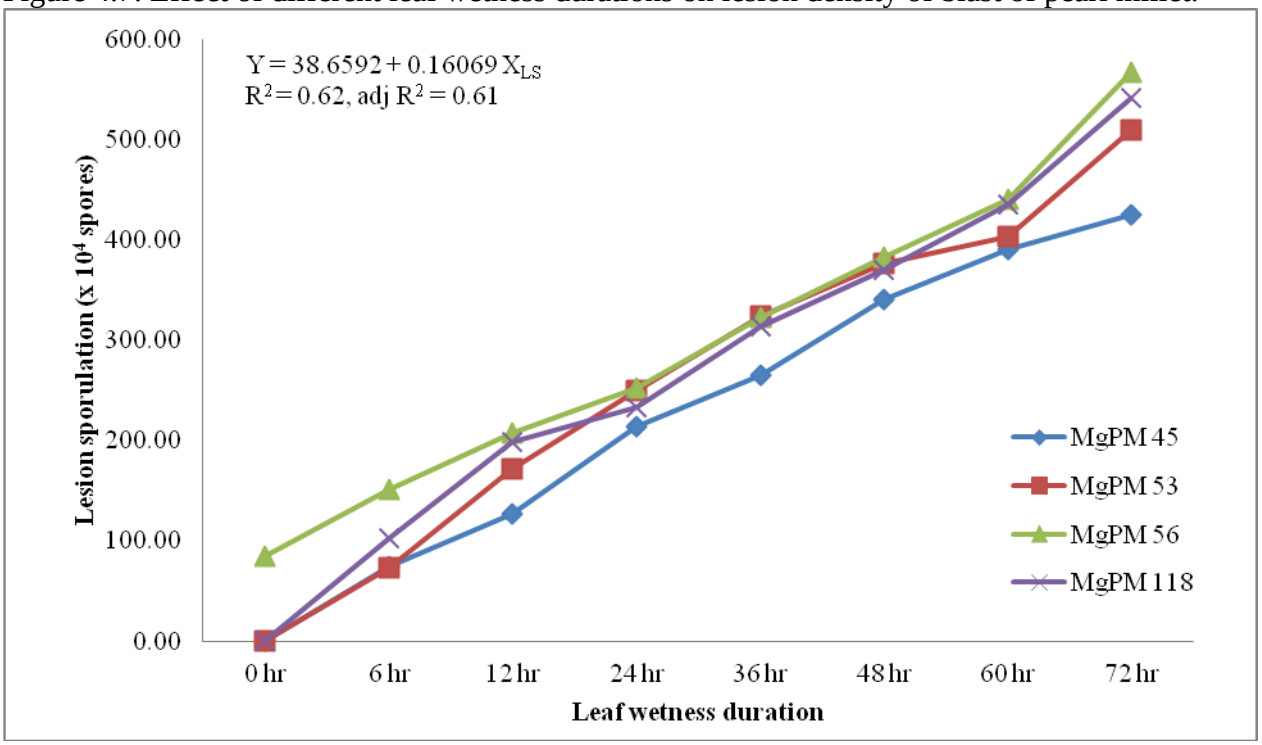


Figure 4.8. Effect of different leaf wetness durations on lesion sporulation of blast on the leaves of pearl millet.

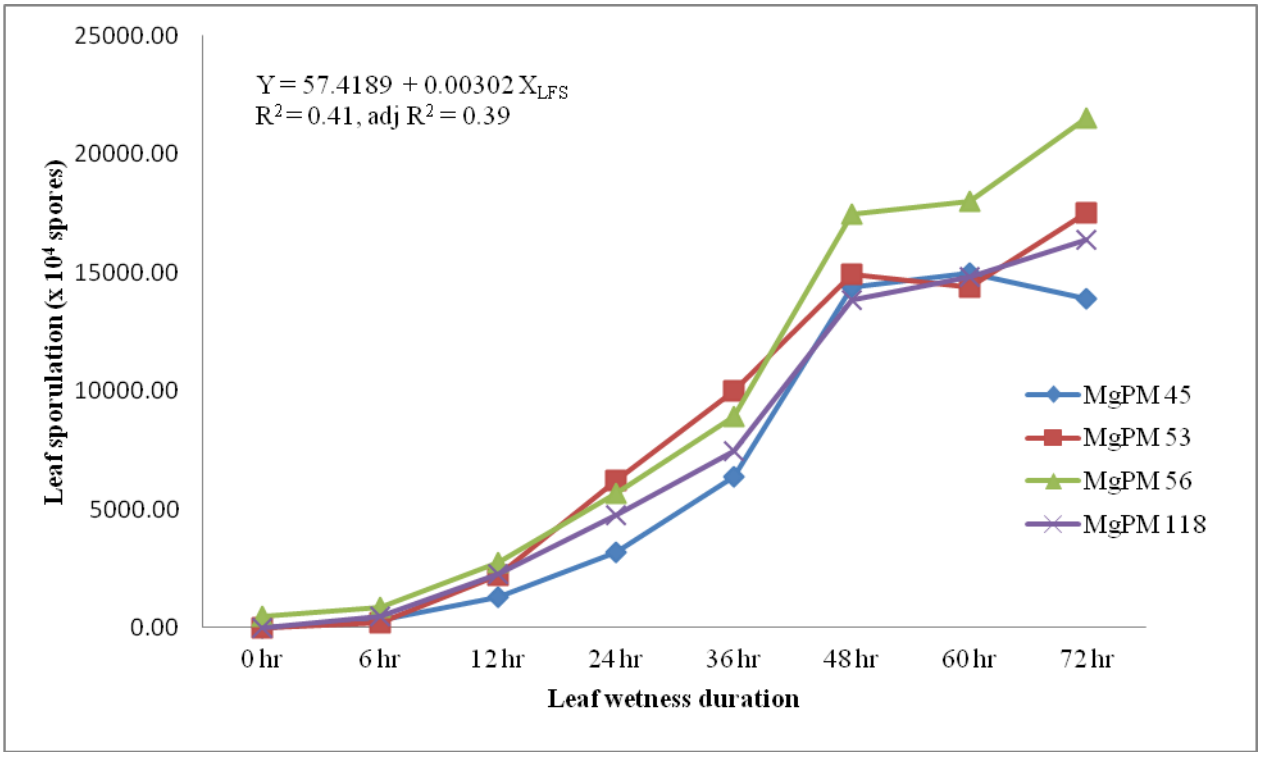


Figure 4.9 Effect of different leaf wetness durations on blast of pearl millet leaf sporulation.

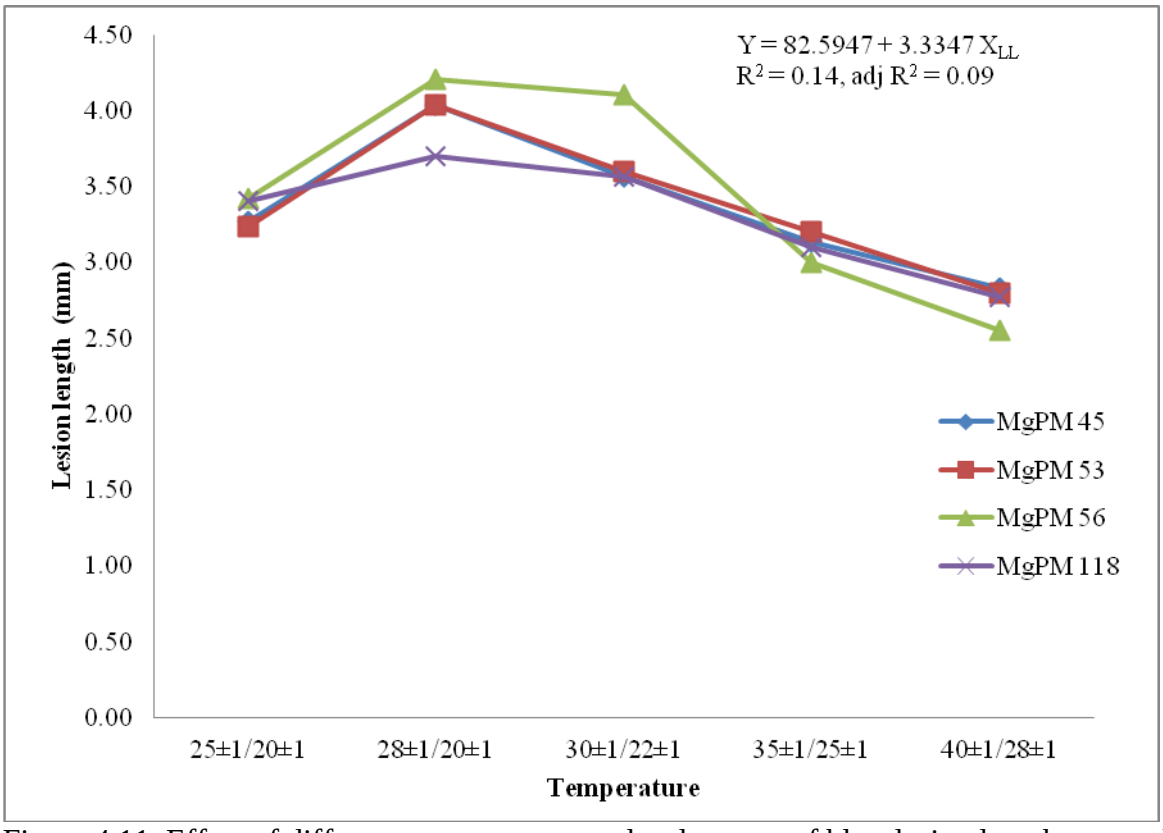


Figure 4.11. Effect of different temperatures on development of blast lesion length on pearl millet.

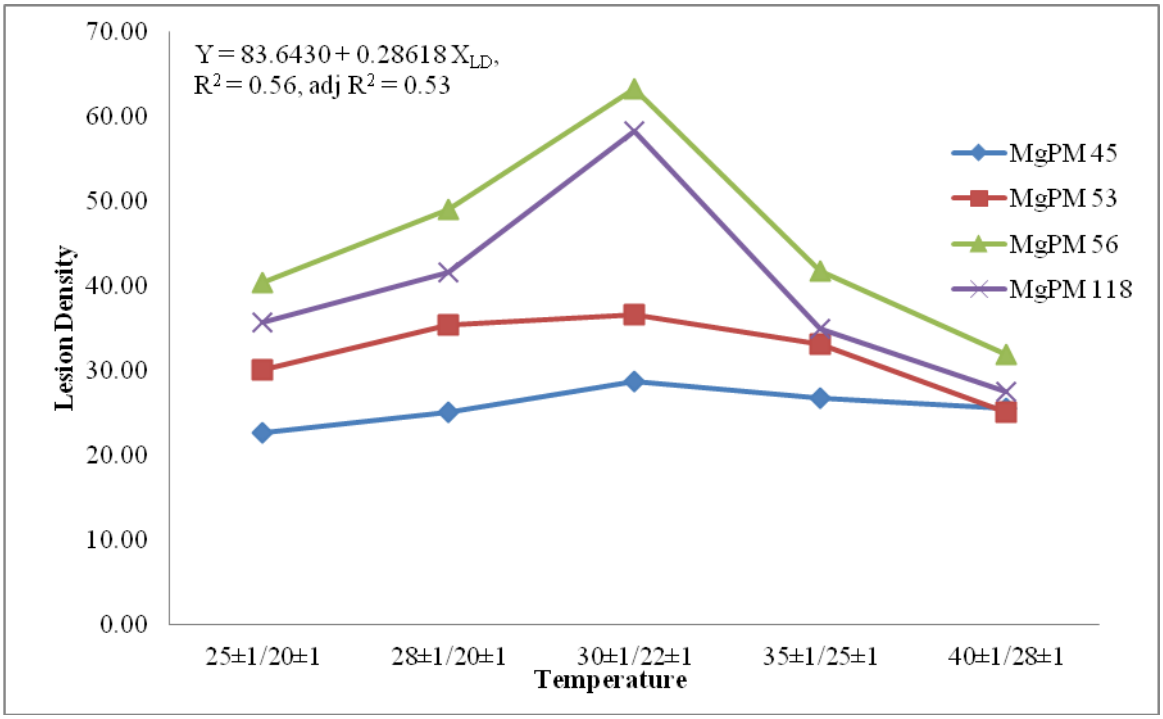


Figure 4.12. Effect of different temperatures on lesion density of blast of pearl millet.

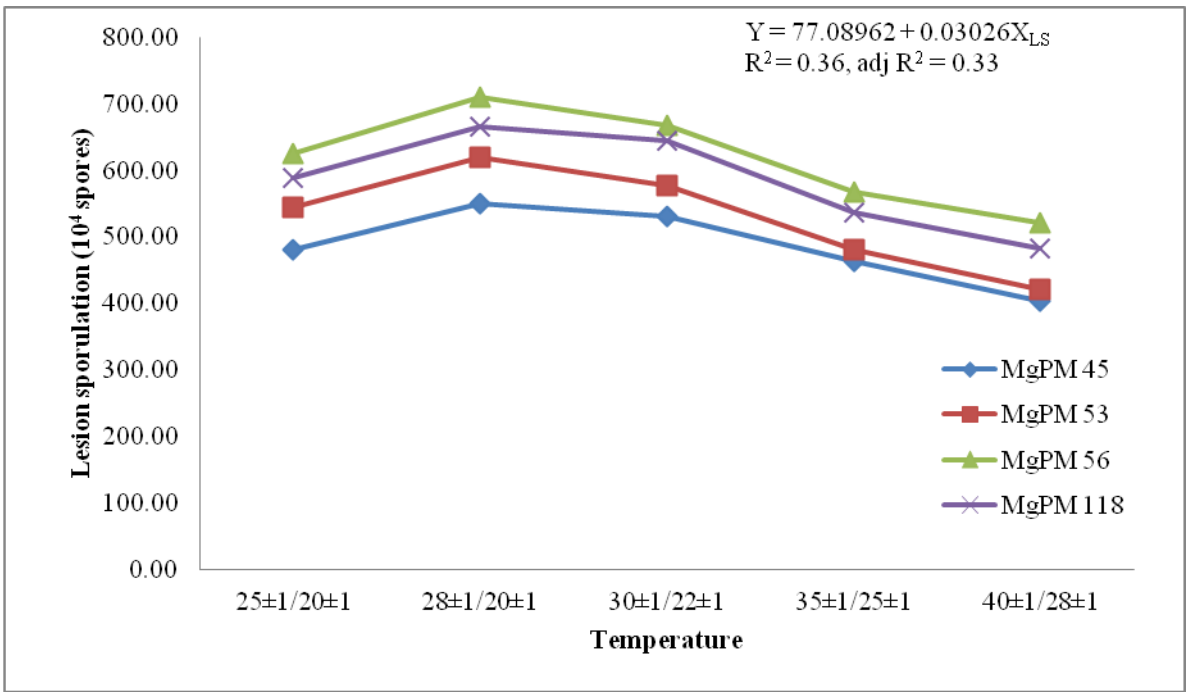


Figure 4.13. Effect of different temperatures on sporulation of blast lesions of pearl millet.

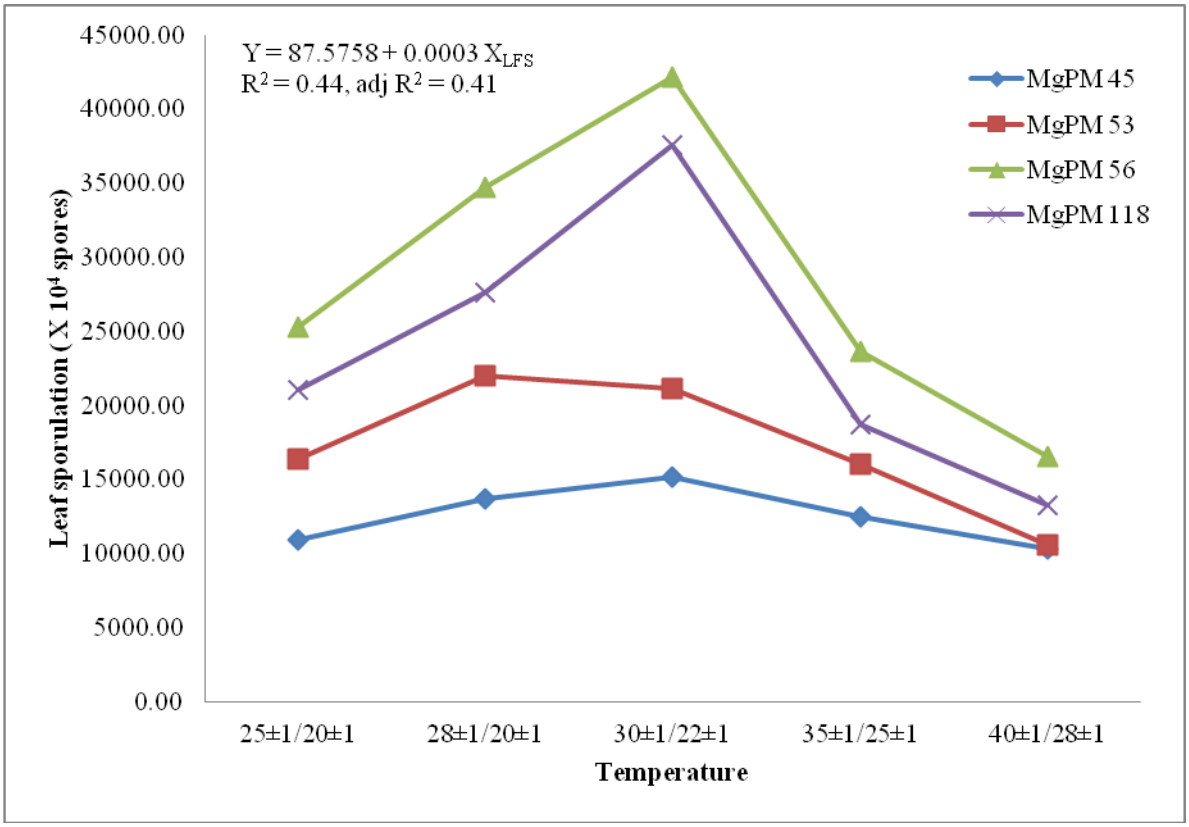


Figure 4.14. Effect of different temperatures on blast sporulation on leaves of pearl millet.

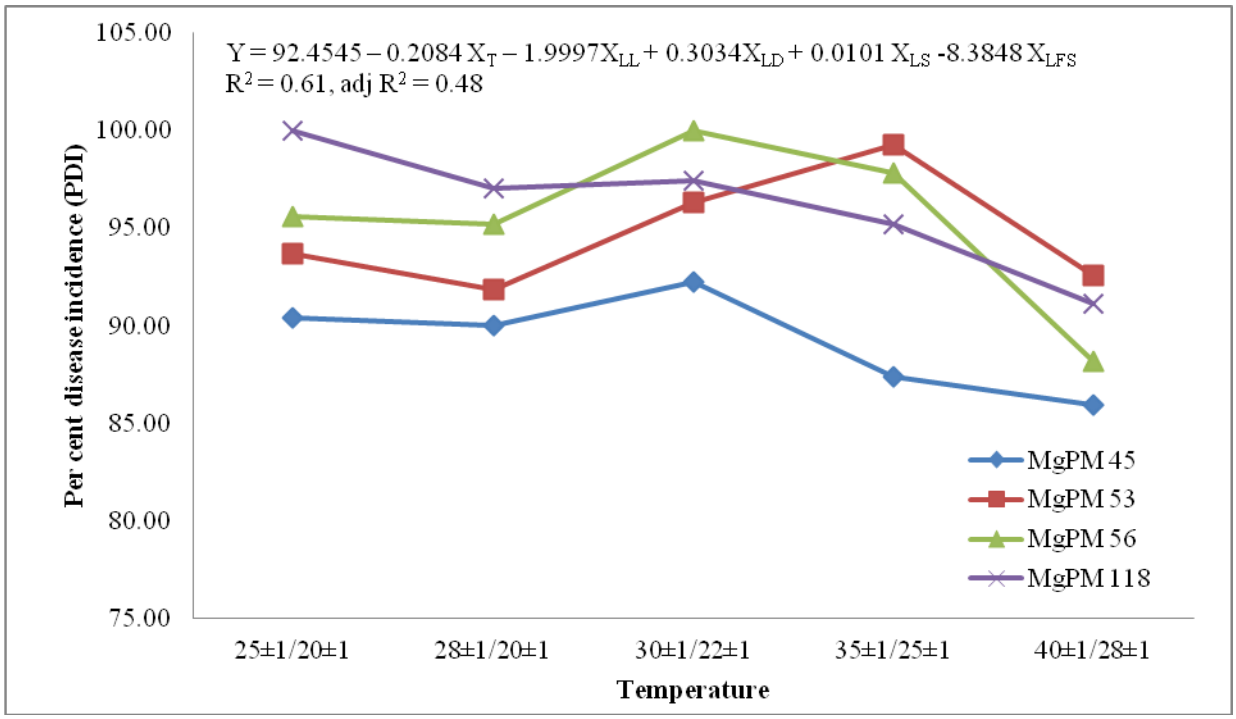


Figure 4.10. Effect of different temperatures on per cent disease index (PDI) of blast of pearl millet.

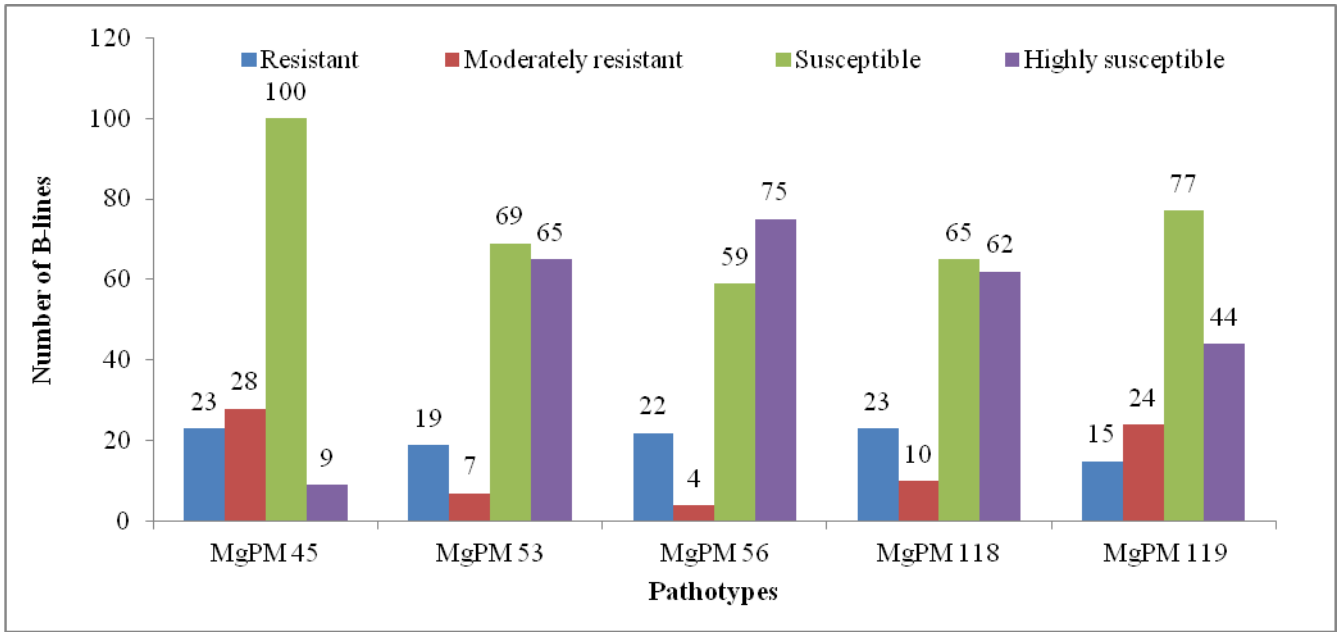


Figure 4.15. Number of designated B-lines (n=160) exhibiting resistant to highly susceptible reaction against five pathotypes of *Magnaporthe grisea*.

CHAPTER V
SUMMARY AND CONLUSIONS

The present study reports to cultural, pathogenic and molecular diversity in the *M. grisea* isolates infecting pearl millet, effect of temperature and leaf wetness duration on blast development and screening of pearl millet lines for resistance to blast disease. The study was carried out at ICRISAT, Patancheru, Telangana, India. The field experiment was conducted at six locations and the results obtained in these investigations are summarized below.

A total of 102 blast disease samples from pearl millet were collected from almost all pearl millet growing regions of India during the Kharif season 2010 and 2012. Out of 102 pearl millet isolates, 65 monoconidial isolates were established and maintained for further studies.

To identify the optimum pH for the growth of *M. grisea*, culture characters of four monoconidial isolates of *M. grisea* MgPM 45, MgPM 53, MgPM 56 and MgPM 118 were tested on OMA and PDA medium at different pH ranging from pH 5.5 to pH 8.0 (pH 5.5, pH 6.0, pH 6.5, pH 7.0, pH 7.5 and pH 8.0) and different incubation temperatures 22^{o}C, 24^{o}C, 26^{o}C, 28^{o}C and 30^{o}C.

Irrespective of differences in composition and pH levels of culture media, good and uniform mycelial growth was observed on both the media and significant variation was observed. The maximum growth was observed on both the media at 28^{o}C. It is concluded from this experiment that about neutral pH of the medium (pH 6.5 to 7.0) and 28^{o}C incubation temperature are optimum to support the growth of this fungus. Similar results were obtained for sporulation of four isolates on OMA and PDA. In general, profuse sporulation was observed on OMA, whereas it was sparse on PDA. As observed for radial growth, maximum sporulation was observed at pH 6.5 and temperature 28^{o}C to 30^{o}C. This further indicated that pH of about 6.5 and temperature of about 28^{o}C is ideal for the growth and sporulation of M. grisea adapted to pearl millet.

The variability in cultural and morphological characteristics of 65 isolates of *M. grisea* was carried out on OMA medium at pH 6.5 by incubating at 28^{o}C. Culture morphology varied significantly among isolates. A range of colour variation in the medium was also observed from buff colour to black among field isolates with smooth or rough margin. In most of the cases the colour of the media turned brownish black following growth of the *M. grisea* isolates and in some cases it was found brown, black or buff colored. For most of the isolates (61) texture of mycelial growth was rough while some isolates had smooth surface with either raised or smooth margins.

The radial growth of the *M. grisea* isolates varied significantly; maximum radial growth of 4.25 cm was recorded for isolates MgPM 125 and MgPM 162 whereas minimum radial growth was recorded for the pearl millet isolate MgPM 148. Correspondingly, large variation was

observed for sporulation ability of the field isolates. It was observed that isolates with grayish black and brownish black growth with sector formation produced more spores. Colony texture or surface of all the isolates was rough to smooth with trace to abundant sporulation. In majority of the isolates, maximum sporulation was confined to sectored region.

Sixty five *M. grisea* isolates were evaluated for pathogenic variation on a set of 10 host differentials (ICMB 93333, ICMB 95444, ICMB 97222-P1, ICMB 01333, ICMB 02444, ICMR 06444, 863B-P2, ICMR 06222 ICMR 11003 and IP 21187). The mean blast severity across the differentials was maximum for isolate MgPM 138 collected from Jaipur, Rajasthan (score 7.4 on 1-9 scale) and minimum severity was observed for MgPM 132 (score 2.4) collected from Jodhpur, Rajasthan across the differentials. On the basis of the reaction type (avirulent/virulent), the 65 isolates were grouped into 28 different pathotypes. Of these groups, least virulence was exhibited by pathotype G2 which had seven less virulent isolates viz., MgPM 127, MgPM 129, MgPM 132, MgPM 149, MgPM 158, MgPM 159 from Rajasthan and one isolate MgPM 39 from Haryana. These isolates were virulent only on ICMB 95444. Pathotype G22 comprising isolates MgPM 121, MgPM 137, MgPM 138, MgPM 145, MgPM 148 from Rajasthan and MgPM 173 and MgPM 174 from Uttar Pradesh appeared as most virulent as it could infect all the 10 host differentials.

Molecular diversity among these 65 isolates of *M. grisea* was studied with 12 URP markers. Five isolates of *M. grisea* from other hosts (two from rice and one each from foxtail millet, Cenchrus and Dicanthium) were included for comparison. Cluster analysis with UPGMA clearly separated the isolates into five main clusters. Among them cluster I (32) and III (31) included more than 95 per cent isolates whereas cluster II, IV and V contained 2-3 isolates. Large variation was also observed with in the clusters as well. Bayesian model analysis revealed the presence of distinct population structure in *M. grisea* isolates from different hosts. *M. grisea* isolates could be grouped into 2-4 sub populations (clusters).

To understand the effect of temperature and leaf wetness duration on the blast development, different leaf wetness durations (0, 6, 12, 24, 36, 48, 60 and 72 hours) and different incubation temperatures (25±1/20±1^{o}C, 28±1/20±1^{o}C, 30±1/22±1^{o}C, 35±1/25±1^{o}C and 40±1/28±1^{o}C) were tested using a susceptible line ICMB 95444. In the effect of leaf wetness duration on disease development studies, there was an overall trend of increase in leaf blast severity, lesion length (mm), number of lesions per plant, lesion sporulation and leaf sporulation with the increase in leaf wetness duration (LWD). It can be concluded based on the results of both leaf wetness duration and temperature experiments that blast on pearl millet becomes more

severe at longer wetness durations beyond 48 hours during optimum day/night temperature ranges from 25±1/20±1^{o}C to 30±1/22±1^{o}C.

For the identification of blast resistance, 160 designated B-lines (designated till 2009 at ICRISAT's hybrid parent breeding program) of pearl millet were screened for blast resistance under greenhouse conditions along with a resistant designated restorer parent (ICMR 06444) and a susceptible designated seed parent (ICMB 95444) check. The screening was carried out against five pathotype-isolates viz., MgPM 45 collected from Telangana, MgPM 53 and MgPM 56 from Rajasthan, and MgPM 118 and MgPM 119 from Haryana. Most of the B-lines exhibited susceptible to highly susceptible reaction against five pathotypes used in this study. However, we could identify 23 lines having resistance to 3-5 pathotypes. Eight lines (81B, ICMB 88004, ICMB 92444, ICMB 97222-P1, ICMB 02111, ICMB 07111, ICMB 09333 and ICMB 09999) were found resistant to all the five pathotypes. Nine lines had resistance against four pathotypes and six lines were found resistant to any three pathotypes. Similarly for the identification of stable sources of adult plant resistance, 28 lines were evaluated in the disease nursery (PMBVN) at six locations, Aurangabad, Dhule, Durgapura, Gwalior, Jamnagar and Patancheru during 2013 and 2014. None of the entries in the blast nursery was resistant at all the test locations. However, ICMR 06444 was found resistant at three (Gwalior, Jamnagar and Patancheru) locations and showed moderate resistance at other three locations and recorded mean minimum blast severity (2.99 score) across locations. ICMB 01333, ICMR 11009 and HHB 146 improved (a hybrid) were resistant at Gwalior and Jamnagar. Ten lines showed resistance at any one location. The variation in the pathogen population was also evident from the mean severity levels at different locations. Considering the blast severity at hard dough stage, pathogen population at Dhule, Aurangabad and Durgapura appeared highly virulent as none of the entry was found resistant at these locations. Pearl millet lines identified in this study that are resistant at 2-3 locations can be a potential source for the pearl millet breeding programs aiming to develop blast resistant hybrids.

CONCLUSIONS AND FUTURE STRETEGIES

Distinctive patterns of pathogenicity and genetic diversity observed in the present investigation emphasizes the variability in *M. grisea* populations infecting pearl millet in India. A well-planned strategy to monitor virulence changes in the pathogen population and resistance breakdown in host cultivars, and identification and incorporation of novel resistance genes will help in reducing the chances of epidemics and losses from blast in pearl millet.

Ten genotypes identified in the present study could serve as differential hosts for the pearl millet blast system. More work should be done by screening the identified host differentials in the present study against representative isolates from India or elsewhere.

Bayesian model analysis revealed the presence of distinct population structure in *M. grisea* isolates from different hosts. A combination of molecular and pathological assays is required to trace out the role of host-pathogen interaction. Further, *M. grisea* isolates from different hosts should be collected from the same locations for the studying the possible gene flow between the isolates from different hosts.

Results pertaining to the effect of leaf wetness duration and temperature studies showed that blast of pearl millet becomes more severe at longer wetness durations during optimum day/night temperature ranges. More work should be done on the effects of environmental factors could provide a better understanding of the disease and lead to improved disease management.

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