Magnaporthe Blast of Pearl Millet in India

Present status and future prospects

S Chandra Nayaka, RK Srivastava, AC Udayashankar, SN Lavanya, G Prakash, HR Bishnoi, DL Kadvani, Om Vir Singh, SR Niranjana, HS Prakash and C Tara Satyavathi

All India Coordinated Research Project on Pearl Millet

(Indian Council of Agricultural Research)

Mandor, Jodhpur – 342 304, Rajasthan, India

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Correct Citation:

Chandra Nayaka S,[1] Srivastava RK,[2] Udayashankar AC,[1] Lavanya SN[1], Prakash G,[3] Bishnoi HR,[4] DL Kadvani,[5] Om Vir Singh,[4] Niranjana SR,[1] Prakash HS,[1] and Tara Satyavathi C.[4] 2017. Magnaporthe Blast of Pearl Millet in India - Present status and future prospects. All India Coordinated Research Project on Pearl Millet (Indian Council of Agricultural Research), Mandor, Jodhpur - 342 304, 51pp.

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Abstract

Pearl millet leaf blast is caused by the fungus *Magnaporthe grisea*. In India, blast disease was first reported during 1953 on a few pearl millet cultivars and later reported sporadically on many hybrids and varieties during 1980s. From 2000 onwards the disease is widespread in pearl millet growing states of India and many hybrids and varieties have shown susceptible reaction to the disease. Hybrids and varieties of pearl millet are showing wide range of disease reaction to the blast disease from susceptible, moderate to resistant reaction. The current status, biology of the pathogen, geographic distribution, host range, epidemiology, disease management strategies covering cultural practices, chemical control, biological control, and host resistance have been reviewed. Screening techniques, disease rating system in the host genotyping, breeding strategies are presented and molecular breeding approaches are also discussed. Not much research data is available on pearl millet blast. A comprehensive account of biology and management of blast disease of pearl millet will give a new impetus for future research and also help mitigate the disease situation in India. Identifying hotspots for screening pearl millet genotypes against blast disease and to start basic and applied research to tackle this problem is the need of the day.

This book also emphasises the future direction of research necessary for better understanding of the biology, host-pathogen interaction and management of pearl millet blast.

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the most widely grown millet and an important crop in India and Africa, extensively cultivated in arid and semi-arid regions after rice, wheat and sorghum. India is the single largest producer of pearl millet in the world, Rajasthan state being the largest producer of pearl millet in India. The crop is best suited for areas with low soil fertility, drought, high temperature, low pH or high salinity.

In comparison to maize and sorghum, pearl millet has a higher level of heat tolerance and is more efficient in utilization of soil moisture. In United States of America, Australia, Southern Africa and South America, pearl millet is extensively grown as a forage crop. Across India, pearl millet cultivation is dispersed mainly during Kharif season. It is also grown to a lesser extent during Rabi season in Andhra Pradesh, Karnataka and Tamil Nadu. Only about 8% of pearl milletcultivated area is irrigated across India (http://agropedia.iitk.ac.in/content/economic-importance-pearl-millet).

Around 90 million poor people are dependent on pearl millet crop for food and income. The crop is grown in 31 million hectares worldwide. Among the millets produced worldwide, 50% is contributed by pearl millet with a 130% increase in production in Central and West Africa since 1980. India is the largest producer of pearl millet in terms of both area (9.3 million hectares) and production (8.3 million tons). The Central and West Africa has 15.7 million hectares under millets, of which more than 90% is pearl millet (http://exploreit.icrisat.org/page/pearl_millet/680). During the past three decades, single-cross hybrids of pearl millet have shown 25-30% grain yield advantage over open-pollinated varieties developed based on cytoplasmic-nuclear male-sterility systems have significantly contributed to increased pearl millet productivity in India (Rai *et al*., 2006).

The importance of pearl millet as food crop is increasing. Pearl millet plays an imperative role in the food and energy security to the rural people especially in the rainfed areas thereby supplants other major cereals which are not otherwise feasible to cultivate.

Diseases of pearl millet

Pearl millet is affected by a number of diseases caused by fungus, bacteria, virus, oomycetes and nematodes, among which few are economically very important, namely downy mildew, blast, rust, ergot and smut (Table 1).

Among various constraints in attaining high grain yield potential of improved pearl millet cultivars in India and in Western and Central Africa, downy mildew disease is considered as one of the major problem. Nevertheless, in last decade, blast disease of pearl millet has emerged as a very serious threat. The severity of rust disease has also amplified; most likely this alteration is mainly attributed to changing climate. The use of host plant resistance is the most suitable approach in managing these diseases as pearl millet is grown on the resource poor soils under ruthless climatic conditions and by resource-poor farmers of western Africa and India.

Due to commercialization of new hybrids in the past two decades in India, the status of downy mildew infestation has changed, resulting in new virulent strains. In the meantime, severe outbreaks of *Magnaporthe* blast disease have been reported in pearl millet growing states of India during the past five years. Rust disease, which was reported limited to post rainy sowings, has turned out to be severe in rainy and summer crops. These findings indicate the need to recognize novel sources of resistance to be employed in the pearl millet breeding program.

Table 1: List of economically important diseases of pearl millet

Disease	Pathogen
Downy mildew	*Sclerospora graminicola* (Sacc.) Schroet.
Blast	*Magnaporthe grisea* (T.T. Hebert) M.E. Barr
Rust	*Puccinia substriata* var. *indica* Ramachar & Cumm
Ergot	*Claviceps fusiformis* Lov.
Smut	*Moesiziomyces penicillariae* Bref. Vanky

During the past pearl millet workshop, many delegates from the private seed companies highlighted the increased occurrence of *Magnaporthe* blast in the seed production plots and the damage caused to the crop. In continuation of this discussion the scientific group felt the need to consolidate the available information on pearl millet blast disease and to give more importance to understand the biology of the pathogen, epidemiology of the disease and disease management aspects. Accordingly, the present information has been collected and compiled based on the research already carried out under the All India Coordinated Pearl Millet Improvement Project and elsewhere.

Geographic distribution

Central African countries namely Burkina Faso, Nigeria, Upper Volta, represent parts of the Africa which are reported to be centres of origin of pearl millet and also the blast pathogen (Wilson *et al*., 1989). Center of origin of host and the pathogen are also the source of resistance for the host as well as virulence of the pathogen and their co-evolution existence. Pearl millet spread to other countries like India, South East Asian countries, USA by subsequent traders. Along with the host, pathogens might have also spread to pearl millet growing countries of the world today. The pathogen *Magnaporthe grisea* infecting pearl millet has been reported from Tifton, Georgia (Wells *et al*., 1965), Singapore (Buckley and Allen 1951) and India (Mehta *et al*. 1953).

Current status of *Magnaporthe* blast on pearl millet in India

Blast disease has been prevalent in pearl millet growing states of India since 1970; its increased incidence has been observed recently in most pearl millet growing states like Gujarat, Madhya Pradesh, Uttar Pradesh, Delhi, Maharashtra, Rajasthan and Karnataka (Fig. 1).

The disease incidence data from 2002-2016 indicates that the disease is becoming more and more widespread (AICPMIP Annual Reports, 2002-2016). The disease has been reported by Dhule, Jaipur, Jamnagar and Gwalior centers of AICRP - PM for over a decade and is recorded on almost all the entries that have been evaluated under pathological trials.

During 2016, in some pearl millet growing regions of Rajasthan, Uttar Pradesh, Delhi and Maharashtra, *Magnaporthe* blast incidence had become very high and the private seed companies who had supplied the seeds had recommended treating seeds with Carbendazim combined with Metalaxyl. The disease is becoming serious under warmer and humid conditions.

Over the years Magnaporthe blast incidence has been recorded both in AICRP - PM trials and also during the field surveys conducted by the pathologists of AICRP - PM coordinating centers and ICRISAT. It has been noticed that blast disease is widespread in both AICRP - PM coordinated trails and also in the farmers field surveys (Tables 2 & 3).

In 2002-2003 the incidence of blast disease ranged from 1 to 52.8%, the average being 13.4%. During 2003-2004, the incidence increased to 19.3% (range 2.5-100%). In the year 2004-2005, the mean blast incidence was 16.8% (range 0.5-70%). During 2005-2008, blast screening was also conducted by Madhya Pradesh, in addition to Maharashtra and Gujarat. During 2006, 2007 and 2008 the average blast incidence was 9.8, 9.2 and 8.7% respectively and the range of disease incidence was 0.5-37.5, 0.5-32.5, and 0.5-42.6% respectively.

In 2009 the disease ranged from 1.3 to 54%. The data also indicated that cultivars like ICMB 95444, ICMV155, PUSA 334, PUSA 283, PUSA 23 and CZE9802 have shown susceptible reaction to *Magnaporthe* blast. Cultivars RHB121, GHB555, GHB 538, NM5 20A, I2440, ICMB 93222 have shown resistance to *Magnaporthe* blast disease under natural disease incidence conditions.

During the year 2009-2012 the average blast disease ranged 7.2 to 24% and the incidence ranged from 01 to 99%. In the XII plan period the disease marginally increased in majority of the pearl millet hybrids, disease ranging from 13.8 to 24.7% and the incidence in individual field ranged from 0.1 to 92%. The data also indicated that cultivars such as ICTP8203, ICMH356, RHB121, RHB173, PUSA23, ICMV 221, ICMV155, ICMV 356, RAJ 171, HHB67Imp, 86M64, cultivars such as GHB732, GHB744, Kaveri Super boss, HHB223, ICMV155, NBH5061, MP7792, KBH108, and 86M86 have shown resistance to *Magnaporthe* blast disease under natural disease incidence conditions.

Incidence of *Magnaporthe* blast disease in farmers fields has also gradually increased over the years. During 2000-2001 the mean incidence of blast was 13.2% and in 2007-2008 it was 47.1% and the number of states reporting blast incidence have also increased. From 2000-2003 only Gujarat recorded the incidence of blast during farmers' field surveys and the incidence was ranged from 13.2- 32.1% . It was also observed that blast incidence was specifically noticed in Anand region of the Gujarat and the rest of the farmers fields surveyed were free from blast incidence. During 2002, 2003 and 2004 the blast disease was high because, during those years only one center recorded the blast incidence and also the climate might have been very favourable for blast disease spread. During 2000-2001 to 2003-2004 BK 560 and Nandi cultivars were highly susceptible and RHB 121 and GHB 558 were highly resistant to blast disease. During 2004-2005 field surveys the average incidence of blast in Gujarat and Madhya Pradesh increased to 37.6% with disease ranging from 0.5-44%. During 2005-2006, 2006-2007 and 2007-2008 disease was recorded by Gujarat, Madhya Pradesh and Karnataka and the average blast disease incidence was 39.2, 40.6 and 47.1% respectively and the disease ranged from 0.5 to 100%. In the year 2009 the disease ranged from 1-50%. In farmers fields PUSA 23, Nandi, SONA, Proagro 9330, NK 1602 and some local cultivars were highly susceptible to blast and GHB 558 and PUSA 38 were highly resistant to blast over the years. During 2010-2011 and 2011-2012 disease was recorded by Rajasthan, Madhya Pradesh, Maharashtra, Tamil Nadu, Gujarat which is ranged from 1-80%.

During the XII plan period, Rajasthan, Madhya Pradesh, Maharashtra, Tamil Nadu, Gujarat, Karnataka recorded high incidence of blast incidence which is ranged from 1- 90% disease incidence. Based on this information it is observed that the incidence and spread of Magnaporthe blast is on the rise (Tables 2 & 3).

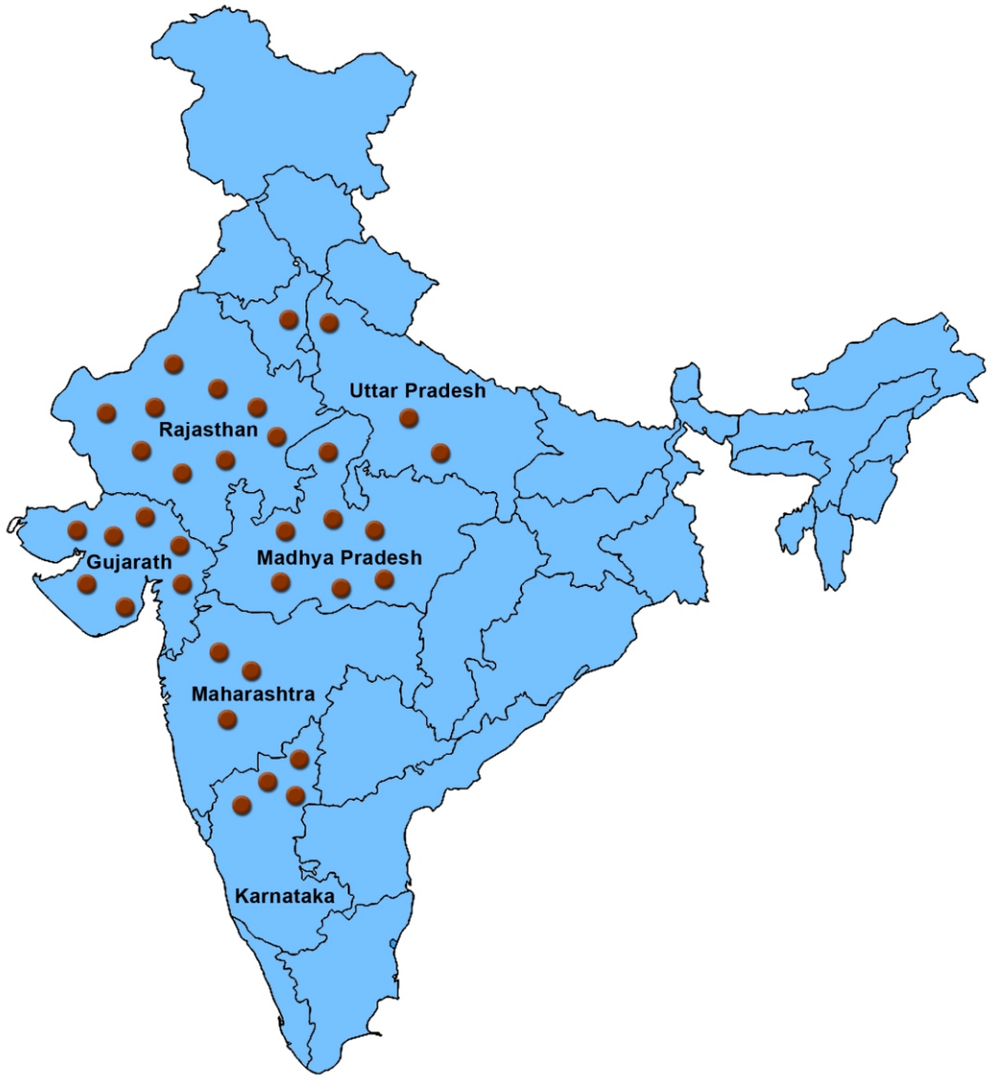


Figure 1: Hot spots of *Magnaporthe* blast of pearl millet in India

Table 2: Incidence of *Magnaporthe* blast disease on pearl millet in the AICRP - PM trials during 2002-03 to 2015-16

PMPT trials					
Year/ Trial	**Mean Blast % Incidence (All India)**	**Range (%)**	**AICPMIP Coordinating Centres recording blast**	**Susceptible cultivars**	**Resistant cultivars**
			2002-2003		
PMPTI PMPTII PMPTIII	3.84 9.76 26.82	1-18.20 3.1-50 0.1-52.8	Aurangabad (Maharashtra)	ICMB 95444, ICMV155, Pusa 334	RHB 121 GHB 555
			2003-2004		
PMPTI PMPTII PMPTIII	21.62 14.97 17.5	3.5-60 3.5-32.5 2.5-100	Jamnagar (Gujarat)	ICMV155, Pusa 283, Pusa 334, PUSA 23, MH 429, ICMV 221, MP 437, ICTP 8203	RHB 121 MH 1189 MH 1219
			2004-2005		
PMPTI PMPTII PMPTIII	16.0 14.6 19.8	0.5-70 3.5-60 10-41.3	Jamnagar (Gujarat) Gwalior (Madhya Pradesh)	ICMV155, Pusa 283, Pusa 334, MH 1324, MH 1333	RHB 121, RAJ 171, PB 106
			2005-2006		
PMPTI PMPTII PMPTIII	9.8 9.6 9.9	0.5-25.5 3-37.5 1.3-16.3	Jamnagar (Gujarat) Gwalior (Madhya Pradesh)	ICMV155, Pusa 283, Pusa 334	RHB 121, RAJ 171, PB 106
			2006-2007		NMS 20A,
PMPTI PMPTII PMPTIII	8.6 9.0 9.9	0.5-32.5 0.5-25 1.5-22.3	Jamnagar (Gujarat) Gwalior (Madhya Pradesh)	ICMB 95444, Pusa 283, Pusa 334	J2440, J2340, ICMB 93222
			2007-2008		
PMPTI PMPTII PMPTIII	4.2 9.0 12.9	0.5-42.6 3.5-25 0.5-32.5	Jamnagar (Gujarat) Gwalior (Madhya Pradesh)	PUSA 23, CZP 9802	PB 106, PUSA 38, GHB 558
			2008-2009		
PMPTI PMPTII PMPTIII	16.6 27.6 29.7	4.8-41.6 8.9-53.6 10.3-56.7	Jamnagar (Gujarat) Gwalior (Madhya Pradesh)	PUSA 23, CZP 9802	PB 106, PUSA 38, GHB 558

Contd.

Table 2: Incidence of *Magnaporthe* blast disease on pearl millet in the AICRP - PM trials during 2002-03 to 2015-16

PMPT trials					
Year/ Trial	**Mean Blast % Incidence (All India)**	**Range (%)**	**AICPMIP Coordinating Centres recording blast**	**Susceptible cultivars**	**Resistant cultivars**
			2009-2010		
PMPTI PMPTII PMPTIII	11.3 6.2 15.2	1.1 to 45.0 0.0 to17.5 0.0 to 57.0	Dhule/ Gwalior/ Jamnagar/ Jaipur/ Aurangabad	ICMV221, PUSA383 HHB197	GHB538, Raj171, JBV-2
			2010-2011		
PMPTI PMPTII PMPTIII	6.6 7.5 8.6	0.0 to 35.0 0.5 to 48.0 0.0 to 32.5	Jamnagar/Gwalior/ Jaipur/ Dhule/Aurangabad/ Punjab	ICMV 221, ICTP 8203, ICMV 155	GHB744 PB106 JBV-2
			2011-2012		
PMPTI PMPTII PMPTIII	22.1 23.7 28.2	0.0 to 99.0 1.9 to 99.0 2.5 to 99.0	Jamnagar/Gwalior/ Jaipur/ Dhule/Aurangabad	ICTP 8203 ICMH 356, RHB 121	GHB732, PB106, JBV-2
			2012-2013		
PMPTI PMPTII PMPTIII	15.95 19.05 25.45	0.0 to 92.5 0.0 to 60.0 0.0 to 64.5	Jamnagar/Gwalior/ Jaipur/ Dhule/Aurangabad	ICTP8203, ICMH356, RHB121	GHB732 PB106,JBV-2 GHB744
			2013-2014		
PMPTI PMPTII PMPTIII	17.8 20.5 12.8	0.0 to 77.0 0.0 to 71.0 0.0 to 55.0	Jamnagar/Gwalior/ Jaipur/ Dhule/Aurangabad	ICTP8203 ICMH356, RHB121	GHB732,719 PB106,JBV-2 GHB744
			2014-2015		
PMPTI PMPTII PMPTIII	12.5 14.5 14.5	0.0 to 69.0 0.0 to 63.3 0.0 to 65.6	Jamnagar/ Gwalior/Jaipur/ Dhule/ Aurangabad	ICTP8203 RHB177, PUSA Comp.	GHB732,197 GHB744,558 Nandi 61
			2015-2016		
PMPTI PMPTII PMPTIII	14.5 14.9 13.3	0.0 to 65.0 0.0 to 55.6 0.0 to 55.2	Jamnagar/ Gwalior/Jaipur/ Dhule/ Aurangabad	RHB177,173, PUSA Comp. 383, ICMV 221, MBC-2	GHB732, GHB744, Nandi61

Table 3: Incidence of *Magnaporthe* blast disease on pearl millet in the field surveys during 2002-2016

Field Survey				
Year	**Range of Blast % Incidence**	**Pearl millet growing states recording blast**	**Susceptible cultivars**	**Resistant cultivars**
2002-2003	0.5-60	Gujarat Rajasthan	BK 560, Nandi, ICMH 356, Pioneer 7688, PUSA 23	MH 179, RHB121, GHB 558
2003-2004	2-80	Gujarat	BK 560, Nandi, Proagro 9330, Sona	RHB 121 GHB 555
2004-2005	2.5-65	Gujarat, Madhya Pradesh	PUSA 23, Nandi, SONA, Proagro 9332, ICMV221, NK 1602	PAC 938, JNBH 26, GHB 558, PUSA 38
2005-2006	1-69	Gujarat Madhya Pradesh Maharashtra	PUSA 23, JKBH 26, JH 676Nandi, SONA, Proagro 9444, Ankur 2226, Swaminath 2001	GHB 558, PUSA 38,
2006-2007	2-20	Gujarat Madhya Pradesh Karnataka	PUSA 23, Nandi, SONA, Proagro 9330, NK 1602	GHB 558, PAC 938 PUSA 38
2007-2008	2-15	Gujarat Madhya Pradesh Karnataka	MBH 163 and 86M32 PUSA 23, 9310, 931 TAC, Parash, JKBH 26	GHB 558, Pioneer 86 M 52, Kaveri Boss, Saburi, Shakti, NHB 2123
2008-2009	1.3-50	Gujarat Madhya Pradesh Karnataka	MBH 163 and 86M32 PUSA 23, 9310, JKBH 26	GHB 558, Boss, Saburi, Shakti, NHB 2123
2009-2010	1.0-20.0	Rajasthan, Gujarat Madhya Pradesh Maharashtra Karnataka	86 M 32, ProAgro 94444	86M52, JKBH26, 27, GHB558, 744,
2010-2011	2.0-80.0	Rajasthan, Madhya Pradesh Maharashtra Tamil Nadu	86M86, 86M32,EXCEL51, Tulja,BK560,Nirmal40, Ankur	JKBH26, Shanti, Nirmal 9, 40

Contd.

Table 3: Incidence of *Magnaporthe* blast disease on pearl millet in the field surveys during 2002-2016

Field Survey				
Year	**Range of Blast % Incidence**	**Pearl millet growing states recording blast**	**Susceptible cultivars**	**Resistant cultivars**
2011-2012	1.0 to 80.0	Rajasthan, Madhya Pradesh Maharashtra Tamil Nadu Gujarat	NBH234,163,86M32, 86M86, ICTP8203,Mahyco163, Yashodhara, 86M64, GHB558,	Nirmal1650,86M52, NBH1183,9330 (Bayer) GHB744
2012-2013	1.0 to 90.0	Rajasthan, Madhya Pradesh Maharashtra Tamil Nadu Gujarat	Mahyco204,318, 86M33, 86M86,Dhanshakti,Tilak Mahalaxmi, Kalyani 25, Super 40, MRB 204,	Bslwan, Mahodaya, Bayer 9330, Tulja, Nirmal, Dhanya7872, GHB558, 719, Kaveri Super Boss, ProAgro 9332
2013-2014	2.0 to 70.0	Rajasthan, Madhya Pradesh Maharashtra Tamil Nadu Gujarat, Karnataka	Pioneer 86M86, 86M33, M204, Rana, Mahodaya 318, Gauri	Kaveri Super Boss, Krishna 7201, Bayer 9444, ProAgro 9450, RBH177, Eknath, GHB558, Jk26, NBH 1118, 05
2014-2015	1.0 to 50.0	Rajasthan, Madhya Pradesh Maharashtra Tamil Nadu Gujarat, Karnataka	86M86, ICTP8203, 86M35, 85, Narmal, M-204, 9330, MRB204	Kaveri Super Boss, Krishna 9119, ProAgro 9450, Eknath301, GHB558, JK26, 36, NBH1118, Dhanya 7792, 7888,
2015-2016	1.0 to 60.0	Rajasthan, Madhya Pradesh Maharashtra Tamil Nadu Gujarat, Karnataka	Mahodaya318, Mahyco 204, XL51, 86M35	ProAgro9444, Krishna7201, NBH1118, 05, Kaveri Super Boss, GHB558, JK26, MBH183, Nirmal, Bayer 9450

Disease assessment

The incidence of *Magnaporthe* blast on pearl millet has been mentioned by some workers without any definite proof. No scientific literature is available on assessment of disease incidence, severity, disease rating scale, crop loss in terms of grain yield, fodder yield loss, and dry matter content loss, photosynthetic efficiency of the foliage, effect on the metabolic activities and respiration aspects have not been done. Wilson and Hanna (1992) provided some information on the lesion length, width and areas of infected pearl millet leaf and analyzed by the general linear model procedure.

Magnaporthe blast

During recent years, Pearl millet blast caused by *Magnaporthe grisea* (T.T. Hebert) M.E. Barr is a major contemporary disease in the country. Magnaporthe blast disease in pearl millet was first observed during 1952 at the Government Research Farm, Kanpur, Uttar Pradesh (Mehta *et al.,* 1953). Once it was considered a minor disease in India, Pearl millet blast (*Magnaporthe grisea*) disease incidence has increased at an alarming rate predominantly on commercial hybrids in several states of India (Thakur *et al*., 2009). Lately the disease is causing chronic yield losses and has emerged as a serious disease affecting pearl millet forage and grain production in India.

Yield loss

Substantial yield losses of pearl millet grain (Timper *et al*., 2002) and forage (Wilson and Gates, 1993) has been reported due to *Magnaporthe* blast disease of pearl millet. The blast disease becomes more severe during humid weather conditions especially with dense plant stand. The productivity and quality of the pearl millet crop is affected by *Magnaporthe* blast and has been found to be negatively correlated with green-plot yield, dry matter yield and digestive dry matter (Wilson and Hanna, 1992).

The blast yield loss estimation with reference to grain and fodder yield due to the incidence and severity of the *Magnaporthe* blast disease in pearl millet needs to be worked out.

Taxonomy

The name *'Pyricularia'* refers to the pyriform shape of the fungus conidia. The type species '*Pyricularia grisea*' which originally was described from crab grass (*Digitaria sanguinalis* L.) was established by Saccardo (1880). Cavara (1892) subsequently described *P. oryzae* (Cav.) from rice *(Oryza sativa* L.) with similar morphology to *P. grisea*. Despite the lack of obvious morphological differences these two have been maintained as separate species.

Rossman *et al*., (1990) argued that *P. oryzae* should be synonymous with *P. grisea* and grouped these two anamorphs under the teleomorph *Magnaporthe grisea* (Hebert) Barr. Recent molecular genetic analysis however have indicated that Pyricularia species isolated from different hosts are genetically distinct (Asuyama, 1965; Webster and Gunnel, 1992; Borromeo *et al*., 1993; Shull and Hamer, 1994; Kato *et al*., 2000). The blast disease is reported on a wide range of gramineous hosts, including cultivated rice and other grass species by members of the *Magnaporthe grisea* species complex (Choi *et al*., 2013). The genus *Magnaporthe* consisting of five species (*M. grisea*, *M. oryzae*, *M. salvinii*, *M. poae* and *M. rhizophila*) have shared morphological character such as three-septate fusiform ascospores and black nonstromatic perithecia (ascocarp) with long hairy necks (Krause and Webster,1972). To delimit species, discontinuities in morphological characters have been employed. *M. rhizophila* and *M. poae* generate 'Phialophora-like' conidiopores and infect only roots of hosts while *M. oryzae*, *M. grisea* and *M. salvinii* form 'Pyricularia-like' (or sympodial) conidiophore and infect leaves or stems of hosts (Sesma and Osbourn, 2004; Besi *et al*., 2009; Zhang *et al*., 2011). Sclerotium is produced in tissues of host plants that release conidia by *M. salvinii* whereas *M. oryzae* and *M. grisea* do not produce any sclerotium. Nevertheless, no detectable morphological character is reported between *M. oryzae* and *M. grisea* (Besi *et al*., 2009; Zhang *et al*., 2011).

Taxonomic tree

Domain: Eukaryota
Kingdom: Fungi
Phylum: Ascomycota
Subphylum: Pezizomycotina
Class: Sordariomycetes
Subclass: Sordariomycetidae
Family: *Magnaporthaceae*
Genus: *Magnaporthe*
Species: *Magnaporthe grisea*
(http://www.cabi.org/isc/datasheet/46103)

Host range

Pennisetum is a diverse genus with over 100 species (Oliver, 1934). It is not clear whether all the species of Pennisetum are susceptible to *Magnaporthe grisea* infection. The available information indicates that the pathogen infects principally *Pennisetum glaucum*, *P. squamulatum*, *P. macroforum*, *P. pedicellatum* (Saikai *et al*., 1983), *P. ciliare* (Perrott and Chakraborty, 1999), *P. purpureum* (Buckley and Allen 1951). It can also survive on the other graminaceous hosts such as *Agrostis palustris*, *Brachiaria mutica*, *Cyperus rotundus*, *Eleusine indica*, *Eragrostis* sp., *Panicum miliaceum* (Lanoiselet and Cother, 2005).

Members of the *Magnaporthe grisea* complex infects quite a few cereal crops, including pearl millet, wheat, foxtail millet, rice, finger millet and other grass sp. *M. grisea* complex is highly specialized in its host range but highly variable. Hence, *M. grisea* infecting rice strains or any other hosts do not infect pearl millet and vice versa.

Disease symptoms

Magnaporthe blast symptoms in pearl millet are commonly referred to as grey leaf spot on leaves and stem. Blast typical symptoms initially begin with tiny specks or lesions that broaden and turn necrotic, resulting in widespread chlorosis and untimely drying of young leaves (Fig. 2 & Table 4). Lesions usually start near the leaf tips or leaf margins or both and extend down the outer edge(s).

Young lesions are pale green to greyish green, later turning yellow to grey with age. Consequently, during humid weather conditions particularly with crowded plant stands, blast disease becomes severe. Foliage lesions are elliptical or diamond-shaped; approximately 2.5-3.5 x 1.5-2.5 mm. Centers of lesion are grey and water-soaked while fresh but turn brown often surrounded by a chlorotic halo which will turn necrotic, giving the appearance of concentric rings (Fig. 4) (Kato, 2001). Such symptoms appear from seedling to flowering stage on leaf, stem and boot- leaf (Fig. 3).

Table 4: Magnaporthe blast symptoms on pearl millet

Inflorescence	**Leaves**
Discoloration panicle	Abnormal colours
Lesions on glumes	Abnormal forms
Rot	Abnormal leaf fall
Seeds	Fungal growth
Lesions on seeds	Necrotic areas
Rot	Rot
Stems	**Whole plant**
Internal red necrosis	Damping off
Mould growth on lesion	Plant dead; dieback
Stunting or resetting	

Figure 2: A pearl millet field severely infected by blast disease

Figure 3: Typical symptoms of pearl millet blast disease. **A.** In pearl millet plants, blast disease symptoms appear as initially small necrotic lesions, which become larger and coalesce. **B.** In older pearl millet plants, the disease can spread to the neck and panicle, causing devastating symptoms and severe yield losses. Up to 80 % yield losses can occur in severe neck and panicle blast. **C.** Severe blast symptoms observed on leaves covering > 75% of leaf area.

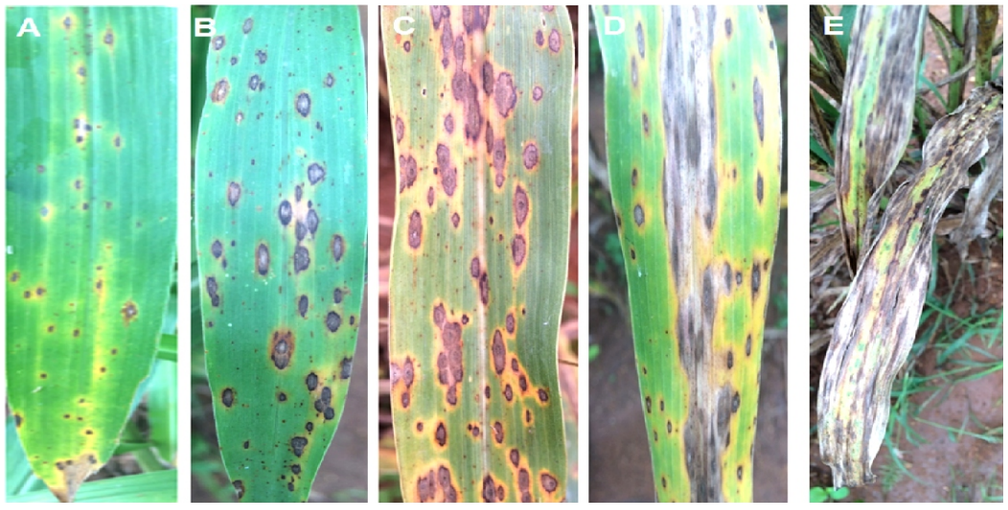


Figure 4: Different degree of Magnaporthe blast disease symptoms on pearl millet leaves. A = dark brown flecks, B = small flecks or scattered large brown necrotic lesions generally 0.5-3 mm long, C = moderately large to large water-soaked lesions (longer than 2-3 mm), D= large spindle or elliptical shaped lesions with necrotic gray centers and often associated with chlorosis, and E = lesions coalesced, often killing one or more leaves.

Biology and epidemiology of *Magnaporthe grisea*

Morphology

Asexual conidia of *M. grisea* are pyriform, hyaline, mostly 3-celled with a small appendage on the base cell. Conidia measure approximately 17.5-30.8 x 5.9-8.8 µm. (Mehta *et al.,* 1953). The conidiophores of the pathogen are produced in clusters from each stoma. They are rarely solitary with 2-4 septa. The basal area of the conidiophores is swollen and tapers toward the lighter apex. The conidiophores are produced in clusters from each stoma. They are rarely solitary and have 2-4 septa. The basal area of conidiophores is swollen and tapers towards the lighter apex. *M. grisea* produces light, inconspicuous, grey to greenish growth on large lesions on leaf, consisting of short delicate conidiophores carrying clusters of conidia at their tip. Conidia are typically obpyriform, hyaline, 2-septate, with protuberant hilum bearing acute apex, 20-25 x 9-12 µm (Fig. 5).

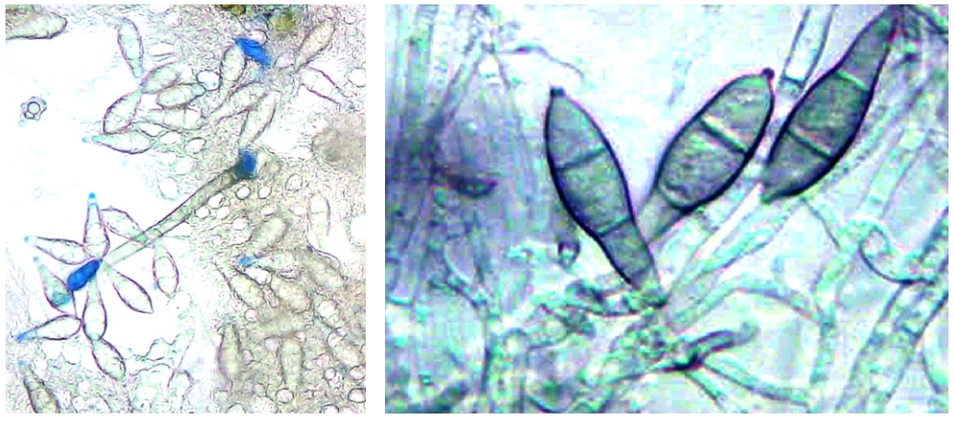


Figure 5: Conidia of *Magnaporthe grisea*

Factors favouring disease development

The seedling and tillering stage of the crop are most vulnerable to the disease. Presence of *M. grisea* conidial spores in the air, overcast skies, recurrent rain, drizzles, high RH (90% and higher), wet leaf temperature from 25-28 °C and high nitrogen fertilizer application predispose the crop for infection (Kato, 2001).

Epidemiology and transmission

Plant pathogens have their own lifestyles to gain access to nutrients in plants; biotrophs obtain their requirements from living host cells, while necrotrophs destroy host tissue for their self nourishment (Lewis, 1973; Glazebrook, 2005). *Magnaporthe* grisea belongs to hemibiotrophic pathogens, an intermediate class. Characteristically, hemibiotrophs primarily grow biotrophically and subsequently transform to necrotrophic growth, thus killing the infected tissues (Perfect and Green, 2001; Munch *et al*., 2008). Interestingly both the stages are maintained simultaneously by *M. oryzae* for invading foliar tissue (Kankanala *et al*., 2007).

Presence of blast conidial spores in the air, cloudy sky, frequent rain, and drizzles, high relative humidity (90% and higher) and wet leaves, temperature from 25-28 °C and high nitrogen fertilizers application predispose the crop for infection (Kato, 2001).

Initial infection by *M. grisea* is initiated from the conidia on seed/soil as well as internally in the form of mycelia harbouring on the surface of infected seeds and crop residues such as diseased straw and stubbles. Germinating conidium produces germ tube which develops to a melanized appressorium through which a penetration peg pierces the epidermal cell by mechanical piercing of the cell surface (Wilson and Talbot, 2009). Within the cell lumen, bulbous and intensively dividing invasive hyphae (IH) become bordered by a plant-derived membrane which bifurcates the IH and host cytoplasm, a distinguishing feature of biotrophy.

The inoculum present on the crop residues also act as source of inoculum and infect the young seedlings. The infected leaf act as source of reservoir of inoculum in eight days of establishment of the pathogen on leaf tissues and sporulates by liberating large number of conidia into the air.

The air-borne conidia act as secondary source of inoculum which falls on the surface of seedlings or the plants from tillering to flowering stage that infect large number of plants. Under dry conditions (at room temperature) conidia can survive for a year and mycelium for almost three years (Shetty *et al.,* 2009 ; Kato, 2001).

Life cycle

Up to 20,000 conidia can be discharged by a single leaf lesion carrying multiple conidiophores (Over a period of 20 days during night). Conidia require free water to germinate. A short germ tube emerges and forms an appressoria at the tip. Appressoria is melanized and pushes a fine penetrating peg through the cuticle of the host cell which develops hyphae and spreads in the host tissues to cause the lesion. This stage is followed by the lesions becoming necrotic and starts coalescing. Epidemic disease is caused by the large number of conidia formed from the diseases lesions. The disease is polycyclic with a spore to spore cycling time of 7 days (Fig. 6).

Asexual cycle- The conidia after release germinate forming a short term tube. The germ tube further elongates and a appressorium is formed with aid of which the hyphae establishes and manifests within the host tissue (Fig. 6).

Sexual cycle- The conidia sporulate and forms a germ tube. The sexual phase in initiated when there are stains of opposite mating types which come into contact. This mating contact results in the formation of bulbous structure with an elongated neck called as perithecium. Inside the perithecium specialized spore forming structure called as ascus is developed. Within each ascus there are several ascospores formed. The ascospores germinate and form the hyphal structures (Fig. 6).

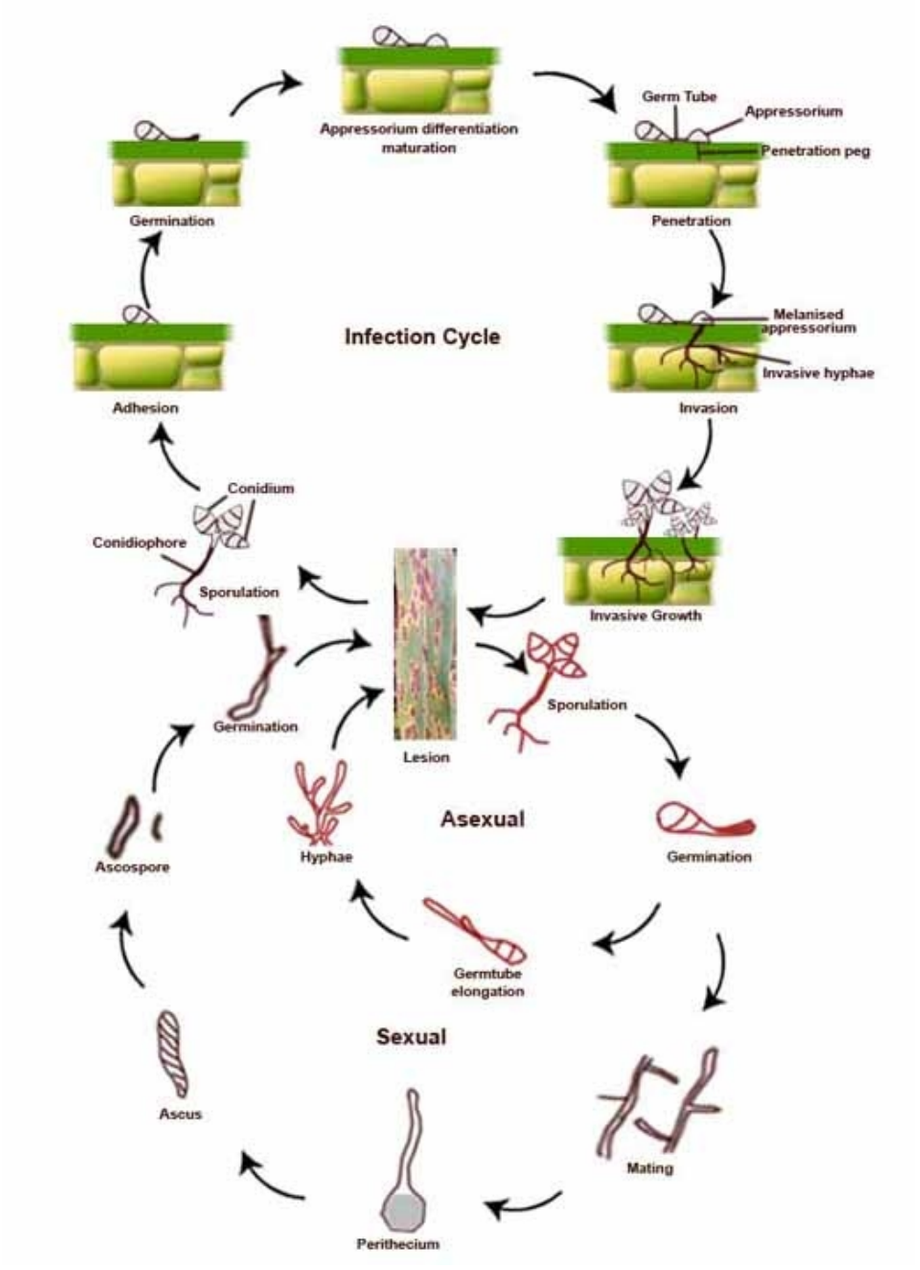


Figure 6: Life cycle of *Magnaporthe grisea*

Detection and identification

Asexual conidia are pyriform, hyaline, and mostly three-celled with a small appendage on the base cell. Conidia measure approximately 17.5-30.8 x 5.9-8.8 µm (Mehta *et al*., 1953). The conidiophores of the pathogen are produced in clusters from each stoma. They are rarely solitary with 2-4 septa. The basal area of the conidiophores is swollen and tapers toward the apex. The conidia of the fungus measure 20-22 x 10-12 µm (Fig. 5). The conidia are translucent and slightly darkened. They are obclavate and tapering at the apex.

Molecular detection of *M. grisea*

During high temperature and abundant moisture, symptoms of gray leaf spot or blast can be confused with those caused by other fungal diseases that also are common during these periods. A polymerase chain reaction (PCR)-based method to detect *M. oryzae* in infected perennial ryegrass tissue was developed by Harmon *et al*. (2003) based on primers pfh2a (5'-CGTCACACGTTCTTCAACC-3') and pfh2b (5'-CGTTTCACGCTTCTCCG-3') designed to amplify a 687-bp fragment of the Pot2 transposon gene of *M. grisea*. Based on polymerase chain reaction Chadha and Gopalakrishna (2006) developed a diagnostic assay for detection of *M. grisea* from blast infected rice seeds. Primers were designed for *mif* 23, an infection-specific gene of *M. grisea*.

The primers MIF-forward (5'-GGATCCATTGAGCATGCGTT-3') and MIF reverse (5' GGATCCAATACGATCACTC G -3') amplified target DNA from geographically and genetically diverse isolates of the pathogen. The lowest concentration of template DNA that led to amplification was 20 *p*g. With this PCR based seed assay, *M. grisea* was detected in rice seed lots with infestation rates as low as 0.2%.

Real-time quantitative polymerase chain reaction using SYBR Green I could detect *M. oryzae* at as low as 6.9×10^5 ng of genomic DNA targeting the 18S-28S region of ribosomal DNA of *M. oryzae w*ith forward, 5'-GGCATCGTTAGCGGTCTTC-3', and reverse 5'-CTACGAGGCTGCATAACGAC-3' primers (Sun *et al*., 2015).

Isolation and preservation of *Magnaporthe grisea*

Magnaporthe grisea can be isolated from the infected leaf tissue with typical blast symptoms. The freshly harvested infected leaf pieces (~1.5 x 2.5 cm^2) are surface disinfected for 1 min in 0.5% NaOCl solution and washed three times in distilled water. The leaf bit is placed on sterile petri plates with three blotter discs soaked in distilled water. Plates are incubated at 25 °C for 12 h NUV/12 h dark for 3-4 days. Incubated leaf bits were observed at 25-50 X with a stereo-microscope for typical morphological characters of *M. grisea* (Fig. 7a). Typical *M. grisea* conidia are borne in clusters at the tip of dark slender conidiophores.

Magnaporthe grisea single spore is transferred from the sporulating lesions to the separate synthetic media culture tubes. Pure culture of blast fungus can be grown and maintained on solid media namely, Oat meal agar, Carrot agar, host extract agar + 2% sucrose, Potato dextrose agar (PDA), V8 juice agar, Corn meal agar, Yeast extract agar and Czapeck's agar. Oat meal agar is best suited for culturing *M. grisea* as it supports good growth and sporulation of the fungus (Fig. 7b). For long term storage of blast fungus, freshly harvested leaf portions with typical blast symptoms can be placed in paper envelopes in the refrigerator, where they will slowly dry and fungus has been from such stores dried leaf samples up to three years. Pure cultures of blast fungus are also stored in 50% glycerol up to three years in a refrigerator (4 °C). *Magnaporthe grisea* is grown in oat meal broth or oat meal slant cultures. Broth cultures were cut into small pieces aseptically and stored submerged in 50% glycerol.

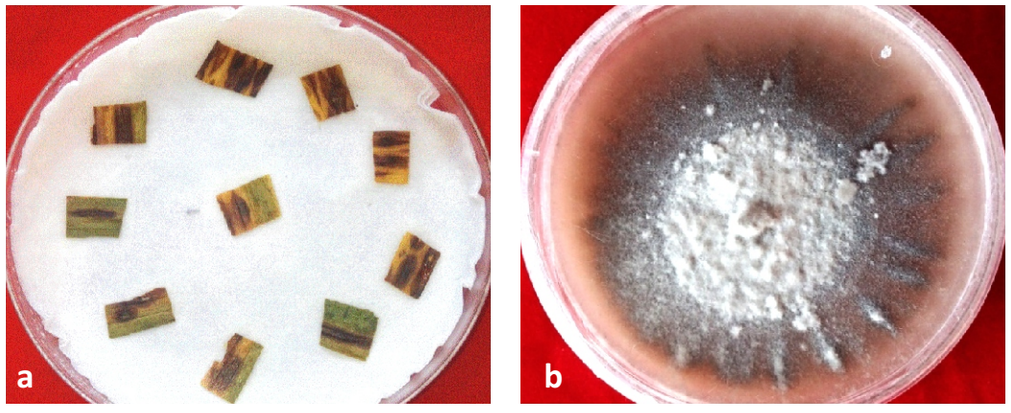


Figure 7: Culture of *M. grisea.* **a**) Standard blotter method for isolation of *M.grisea* **b**) Pure culture of *M. grisea* on oat meal agar

Media composition and preparation

1. Oat meal agar

Oat flakes	30 g
Agar	20 g
Distilled water (to make up volume)	1000 ml

Boil oat flakes in 500 ml distilled water for 30 min and filter through muslin cloth. Agar was melted in 500 ml water separately. Melt agar in 400 ml distilled water, mix thoroughly and make up the volume to 1000 ml and sterilize by autoclaving at 15 lbs pressure (121 °C).

2. Carrot agar

Carrot	200 g
Dextrose	20 g
Agar	20 g
Distilled water (to make up volume)	1000 ml

Peel carrot and cut into small pieces. Boil the carrot pieces in 500 ml distilled water. Filter the extract through muslin cloth. Dissolve dextrose (20 g) and agar (20 g) and make up volume up to 1000 ml. Sterilize by autoclaving at 15 lbs pressure (121 °C).

3. Host extract + 2 % sucrose agar

Sucrose	20 g
Pearl millet straw	100 g
Agar	20 g
Distilled water (to make up volume)	1000 ml

Boil 100 g of pearl millet leaves in 500 ml water for 30 min at ~100 °C. Filter the extract through muslin cloth and mix with the sucrose. Melt agar in 400 ml distilled water. Mix thoroughly and make up the volume up to 1000 ml and sterilize by autoclaving at 15 lbs pressure (121 °C).

4. Potato dextrose agar

Peeled potato	200 g
Dextrose	20 g
Agar	20 g
Distilled water (to make up volume)	1000 ml

Cut peeled potatoes into small pieces and boil in distilled water. Filter the extract through muslin cloth. Dissolve dextrose and agar (20 g each) in potato extract and make up the volume to 1000 ml with distilled water. Sterilize by autoclaving at 15 lbs pressure (121 °C).

5. Czapeck's agar

Potassium dihydrogen ortho phosphate	1.10 g
Ferrous sulphate	0.01 g
Magnesium sulphate	0.50 g
Potassium chloride	0.50 g
Sodium nitrate	3 g
Sucrose	30 g
Agar	20 g
Distilled water (to make up volume)	1000 ml

Melt agar in 400 ml distilled water. Add other ingredients in a separate beaker containing 400 ml distilled water. Mix thoroughly and make up the volume to 1000 ml and sterilize by autoclaving at 15 lbs pressure (121 °C).

6. Yeast extract agar

Soluble starch	10 g
Yeast extract	1 g
Agar	20 g
Distilled water (to make up volume)	1000 ml

Mix all ingredients thoroughly in 400 ml of distilled water, except agar. Melt agar separately in 400 ml of water. Mix thoroughly and make up the volume up to 1000 ml and sterilize by autoclaving at 15 lbs pressure (121 °C).

7. Leaf extract oat meal agar media

Dried pearl millet leaves	100 g
Sucrose	20 g
Oat meal agar	20 g
Agar	10 g
Distilled water (to make up volume)	1000 ml

Leaf extract oat meal agar media was prepared by adding 20 g oat meal agar powder (Hi- media) and 10 g agar powder in 1000 ml Leaf extract Broth. The media was thoroughly mixed by warming in microwave oven before autoclaving at 15 lb pressure for 15 minutes at 121°C.

Diversity of *M. grisea*

Five *M. grisea* pathotypes *viz*., Pg118, Pg119, Pg56, Pg53 and Pg45 have been identified based on the reaction type (avirulent reaction = score ≤ 3.0 [no lesion to small necrotic spots] on a differential line and virulent reaction = score ≥ 4.0 [typical blast lesions] on 1 to 9 scale), isolates were grouped as pathotypes on ten pearl millet genotypes under greenhouse conditions (Fig. 8) (Sharma *et al*., 2013).

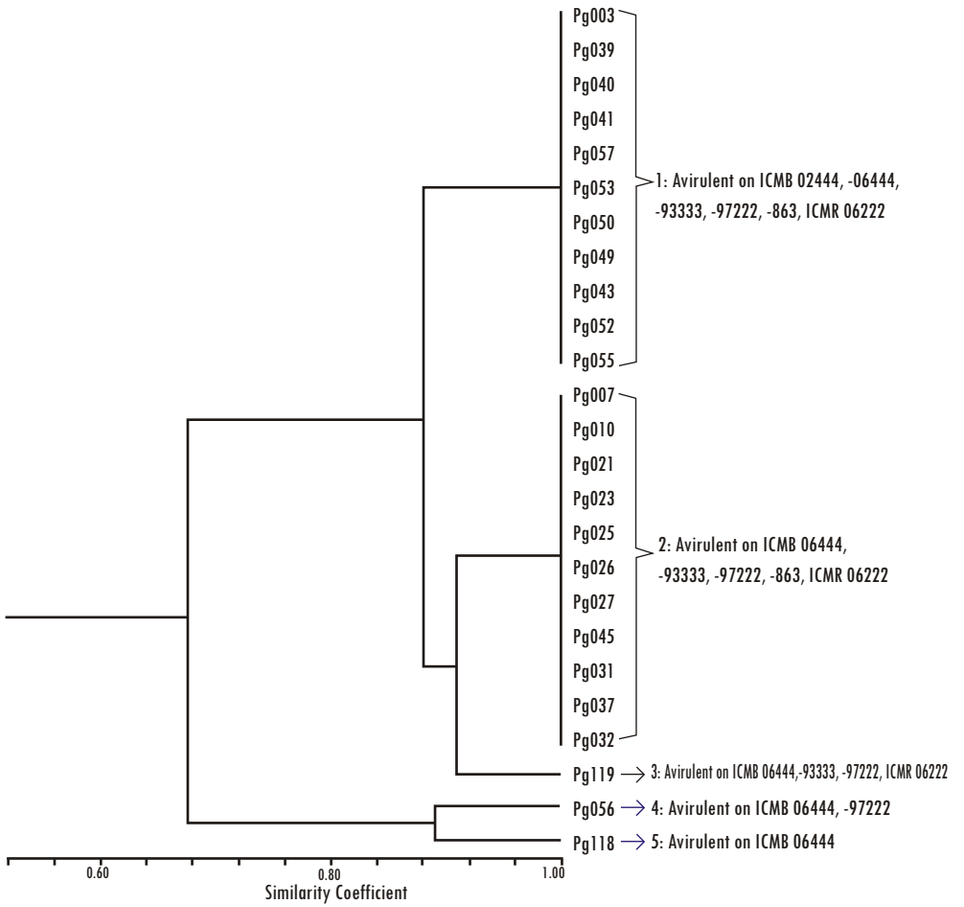


Figure 8: Pathogenic groups of *M. grisea* isolates based on reaction of 10 pearl millet genotypes (Sharma *et al*., 2013).

Disease management

Cultural practices

Preventive and low cost measures to control blast disease include burning of crop residues such as diseased straw and stubble, planting of disease-free seeds, avoiding excess nitrogen-based fertilizers and early sowing. Diseased straw and stubble must be burnt or composted; otherwise they can become inoculum sources for the next crop season.

Seeds must be harvested from the fields crops grown in disease-free areas located under unfavourable conditions for the pathogen and if necessary fungicide must be applied. Gravity separation method for selection of apparently healthy seeds is useful. Salt solution, 200 g l^{1} or ammonium sulfate solution, 230 g l^{1}, is used to separate sufficiently matured seeds, followed by chemical treatment for seed disinfection. Nitrogen and phosphorus content in plants affects disease proneness. Therefore the amount and type of fertilizer must be carefully decided upon according to the cultivar used, soil condition, climatic conditions and disease risk (Kato, 2001). However, these low impact control measures are rarely efficient under blast-favourable conditions.

Chemical control

Magnaporthe blast disease in field is reported to be controlled by a range of fungicides. For seed crops, large scale foliar application can be taken up at field level. However, at farmers' field for commercial grain production foliar application at field level is not economical and feasible. For the control of blast disease of pearl millet, two sprays of carbendazim 0.05% (ICBR 1:3.85) or one gram per one litre at 15 days intervals from the initiation of the disease are recommended (Singh and Pavgi, 1974). Many fungicides are used against blast disease, including benomyl, iprobenfos, pyroquilon, felimzone, diclocymet, carpropamid and metominostrobin (Kato, 2001).

The details of new generation fungicides effective against blast of rice and pearl millet leaf blast and their mode of action are given in table 5. Lately, some fungicides which are target specific to Magnaporthe blast are reported. In rice,

compounds like Tricyclazole (5-methyl-1,2,4-triazolo [3,4-b] [1,3] benzothiazole) has been extensively tested and recommended. It acts on melanin compound present in *M. grisea* conidia, germinating structures and inhibit its biosynthetic pathway (Kurahashi, 2001). Probenazole (3-allyloxy-1,2-benzothiazole1,1-dioxideor, 3-allyloxy-1,2-benz[d]isothiazole) which is reported to activate plant defense system is also effective against blast disease (Iwata, 2001). Isoprothiolane (diisopropyl 1, 3-dithiolan-2-ylidenemalonate) (Choline biosynthesis fungicides) which targets fungal membrane phosphatidlycholine synthesis is recommended for blast disease management (Uesugi, 2001). Azoxystrobin, a strobilurin fungicide which inhibit fungal respiration by binding to the cytochrome b complex III at the Q_0 site in mitochondrial respiration is also available in market to protect against blast disease.

Table 5: New generation of fungicides used against blast disease, their chemical name and mode of action

Fungicide	Chemical name	Mode of action
Probenazole	(3-allyloxy-1,2-benzothiazole1,1 dioxideor, 3-allyloxy-1,2 benz[d]isothiazole)	Activates plant defence system
Tricyclazole	(5-methyl-1,2,4-triazolo [3,4-b] [1,3] benzothiazole)	Inhibits melanin compound biosynthetic pathway in conidia & germinating structures
Azoxystrobin	(methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate)	Inhibitor of mitochondrial respiration at phenol sites of cytochrome b in the mitochondrial BC (1) complex
Isoprothiolane	(diisopropyl 1, 3-dithiolan-2-ylidenemalonate)	Targets fungal membrane phosphatidlycholine synthesis
Propiconazole	(1-[2-(2, 4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2-yl methyl]-1H-1, 2, 4-triazole)	Inhibits sterol biosynthesis in the fungus
Tebuconazole	1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol	Fungicidal triazole compound that acts as a demethylation inhibitor (DMI) of fungal sterol biosynthesis
Trifloxystrobin	methyl (2E)-2-methoxyimino-2-[2-[[(E)-1-[3 (trifluoromethyl) phenyl]ethylideneamino]oxymethyl] phenyl]acetate	Interferes with respiration of fungi. It is a potent inhibitor of fungal spore germination and mycelial growth
Picoxystrobin	methyl (E)-3-methoxy-2-[2-[[6-(trifluoromethyl)pyridin-2-yl]oxymethyl]phenyl]prop-2-enoate	Is a strobilurin analogue which inhibits fungal respiration
Hexaconazole	2-(2,4-dichlorophenyl)-1-(1,2,4-triazol-1-yl)hexan-2-ol	Effective systemic triazole fungicide with protective and eradicative action
Difenoconazole	1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	It is a sterol demethylation inhibitor which prevents the development of the fungus by inhibiting cell membrane ergosterol biosynthesis

Biological control

Pyricularia blast disease was controlled by using biocontrol agents like *Trichoderma harzianum* (Gouramanis, 1995) and *Pseudomonas fluorescens* (Krishnamurthy and Gnanamanickam, 1998), PGPR strains like *Bacillus subtilis, Bacillus pumilus* have been found to control blast pathogen both via biocontrol and induction of resistance (Yoshihiro *et al*., 2003). Streptomyces species were also found to be promising for the management of blast disease (Zarandi *et al*., 2009).

Studies have also been conducted on management of pearl millet *Pyricularia* blast using biological control agents like *T. harzianum*, *P. fluorescens, B. Subtilis* and *B. pumilus* by treating the seeds under greenhouse conditions and the results are promising.

Host Resistance

Expression of resistance to Magnaporthe blast tends to be dominant or partially dominant in pearl millet and is not affected by cytoplasm (Wilson and Hanna 1992). Three independent dominant resistance genes have been reported from wild accession of *P. glaucum* subsp. *monodii* (Hanna and Wells 1989) and four landraces each with independent dominant resistance genes from Burkina Faso (Wilson *et al*. 1989). Resistance to *M. grisea* (Indian isolate) was reported to be governed by a single dominant gene (Gupta *et al*., 2012).

Host cultivar resistance to leaf blast disease is most widely used method of disease control (Fig. 9). Observations made on the pearl millet blast disease incidence in the All India Coordinated Research Project on Pearl Millet and the farmers field surveys since 2002 to 2016 indicated that there are different types of host-cultivar reaction.

In an attempt to identify sources of resistance in pearl millet mini-core collection to different pathotypes, accessions were evaluated in greenhouse conditions against five *M. grisea* pathotypes. Among 238 accessions, 32 were reported to be resistant to at least one pathotype. Three accessions (IP 7846, IP 11036 and IP 21187) exhibited resistance to four among five pathotypes. Twenty one of these accessions originated in India; therefore, germplasm accessions from India appear to be promising sources of blast resistance and could be evaluated against diverse pathotypes of *M. grisea* to discover supplementary sources of blast resistance (Sharma *et al*., 2013).

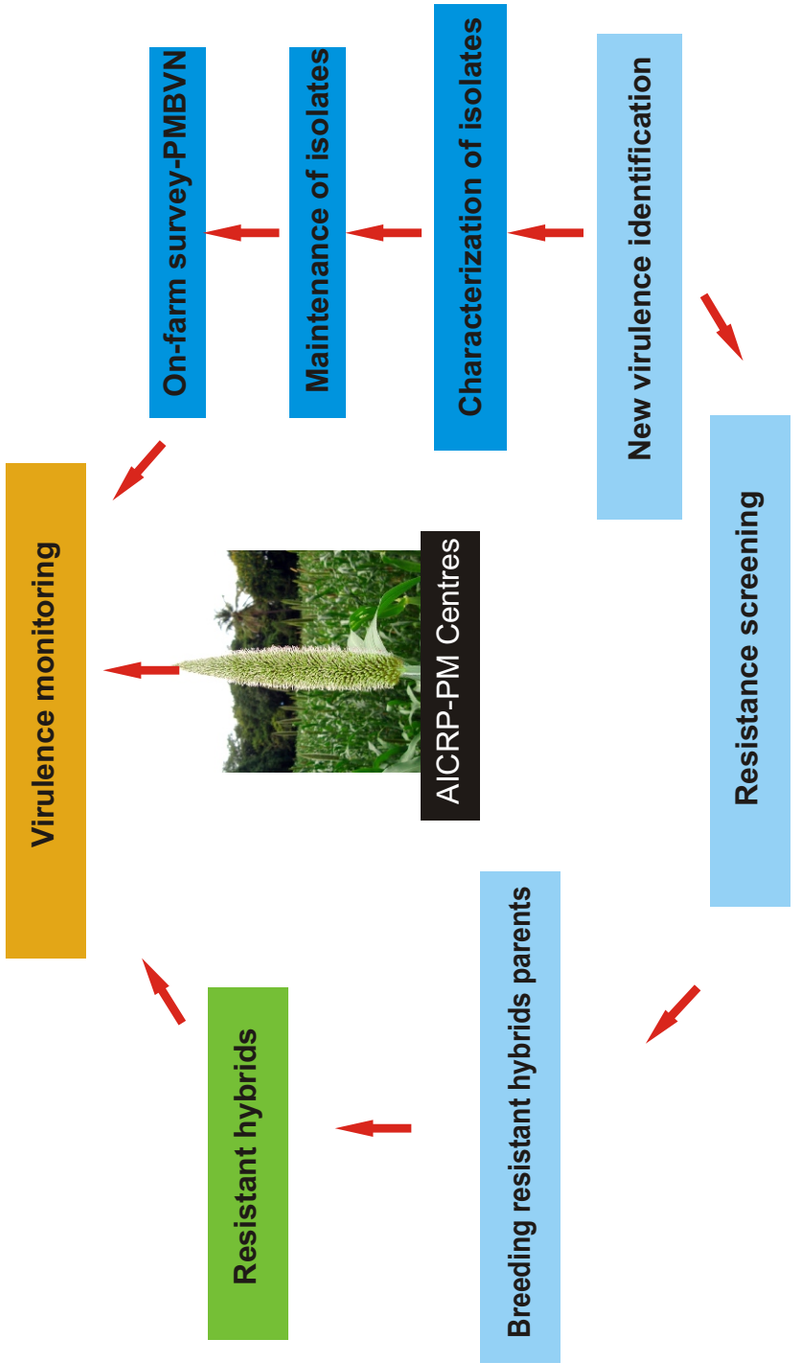


Figure 9: Strategy for managing pearl millet blast through host plant resistance

In United States of America, sources of blast resistance in pearl millet have been recognized and efforts have been made to integrate resistance into improved cultivars and elite breeding lines (Hanna *et al*., 1987; Wilson and Hanna, 1992). Wild species of *Pennisetum* may be useful sources of genes for disease resistance. Pearl millet land races also provide an abundant source of genetic diversity for resistance. Resistance to leaf blast in pearl millet was derived from *P. glaucum* subsp. *monodii* accession from Senegal. Resistance to Magnaporthe leaf spot in pearl millet was derived from the same *P*. *glaucum* sp. *monodii* accession in which the *Rr1* rust gene was found (Hanna *et al*. 1987).

Blast resistance in *P. glaucum* subsp. *monodii* was found to be controlled by three independent dominant genes (Hanna and Wells 1989), although *Tift* 85DB, with resistance derived from *P. glaucum* subsp. *monodii*, was shown to have a single resistance gene (Wilson *et al*. 1989). This resistance was efficient against diverse isolates tested in USA. However, *Tift* 85DB has been reported to be susceptible to Indian isolate (Gupta *et al*., 2012), indicating that the pearl millet-infecting populations of *M. grisea* are different in India from those reported from USA. Resistance in an elite parent line (ICMB 06222) from pearl millet fields at ICRISAT, Patancheru, India to isolate Pg45 is reported to be governed by a single dominant gene (Gupta *et al*., 2012).

Magnaporthe grisea is extremely variable, with several strains specialized in their host selection and hence strains infecting rice is reported not to infect pearl millet and vice versa. Nevertheless, unlike rust and downy mildew pathogens, *M. grisea* does not pass through a sexual stage to survive from season to season, implying there is fewer probability of developing novel genetic recombinants.

Consequently, breeding for robust resistance to blast in pearl millet might be easier than that to rust or downy mildew (Thakur *et al*., 2009).

Several other sources of Magnaporthe leaf spot resistance have been identified from Burkina Faso landraces. Each has been characterized as having dominant, single-gene resistance that is independent of the '*monodii*' resistance gene.

The wild subspecies, *P. glaucum* subsp. *monodii* has been significant germplasm source for improving pearl millet. Genetic resistance to rust (*Puccinia subtriata* Ellis and Barth; var. indica Ramachar and Cummins), *Magnaporthe grisea* leaf blast and smut (*Moesziomyces penicillariae* (Bref) Vanky (Wilson and Hanna, 1992) has been identified in 'monodii' accessions and incorporated into inbred lines to develop and release commercial pearl millet forage (Hanna *et al*., 1988, 1997) and grain hybrids (Hanna, 1993).

Wilson and Hanna (1992) evaluated Magnaporthe blast disease against alleles used for improvement of forage or grain pearl millet including the d2 alleles for dwarf stature, the 'e1' alleles for earliness and 'tr' alleles conferring trichome characters. The data indicated that inbreds homozygous for the 'e1' alleles were more resistant at anthesis than inbreds without the allele. The reduced leaf blast susceptibility at anthesis and increased resistance to *P. grisea* with 'e1' alleles may be an additional advantage for its use in breeding grain varieties of pearl millet.

Thakur *et al*. (2009) developed field and greenhouse screening techniques to identify sources of hybrid parental lines with resistance to blast disease. Among 211 elite hybrid parental lines, which include 126 designated B-lines, 65 potential R-lines and 20 designated R-lines were evaluated for blast resistance in disease nursery.

Forty five lines recognized as blast resistant (score ≤3.0 on 1-9 scale) were further screened through greenhouse screening technique. Twenty five (8 designated B-lines, 14 potential R-lines and 3 designated R-lines) of the 45 lines were found resistant to blast disease under greenhouse screening.

Fifteen pearl millet genotypes were screened against foliar blast under artificial conditions. Three lines PPMI 1087, PPMI 1089 and PPMI 660 showed high resistance (score of 0.0 to 0.4 on a 0-9 scale) and two entries (PPMI 1084 and J 108) were resistant (score of 1.0 to 1.3) (Prakash *et al*. 2016).

Breeding strategies for disease resistance

Screening Techniques

For identifying sources of resistance effective and efficient screening and evaluation technique is a pre-requisite. For *M. grisea* infecting rice a systematic screening technique and evaluation procedure has been developed (IRRI, 2002). The screening and evaluation techniques have also been developed for pearl millet against leaf blast disease pathogen (Wilson and Hanna, 1992). The same technique is being adopted currently in the AICPMIP with modifications.

The inoculum is prepared by isolating *M. grisea* from the pearl millet blast infected leaf lesions collected from naturally infected plants from the field. The leaf samples (about 1.5x2.5 cm^2) are surface disinfected for 1 min in a 0.5% NaOCl solution and plated on Oat meal agar. Leaf pieces are incubated at 24 $^{\circ}C$ under continuous fluorescent lighting (Fig. 10). Fungi growing from leaf pieces are identified microscopically 3-5 days after plating and are sub-cultured.

For sub-culturing four plugs of 5 mm diameter each from the colonies are placed on Oat meal agar in petri plates. After 7 days, the plates are placed in a dirt-free room and lids are removed for four days until the agar has dried. Conidia and mycelia are scraped off the dried agar. Conidia are suspended in deionized water with a drop of Triton B and the final concentration is adjusted to $1x10^5$ conidia/ml with a haemocytometer (Fig. 11).

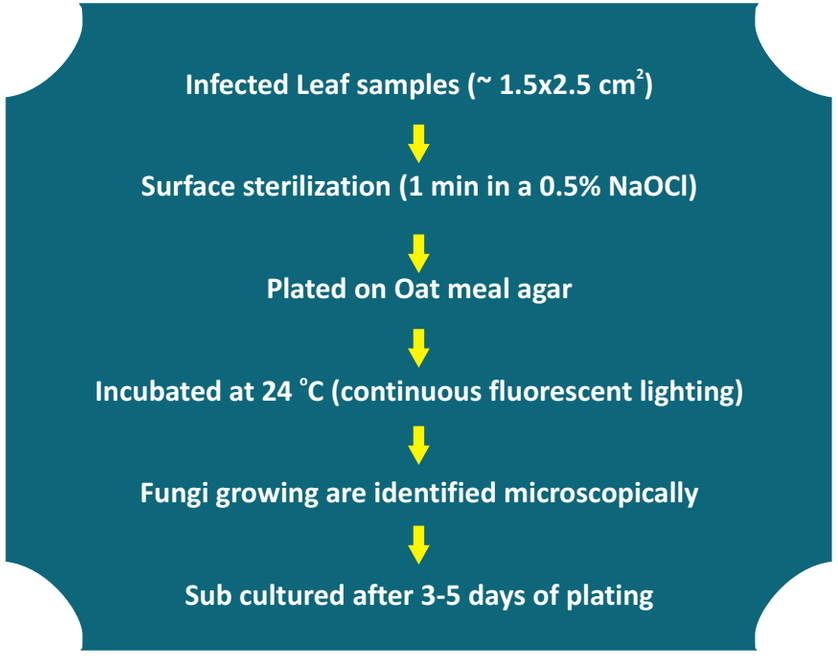


Figure 10: *Magnaporthe grisea* inoculum preparation

For single spore isolations the infected parts of leaf are surface sterilized with 1% sodium hypochlorite and incubated at 26 °C for 24 h in sterile petri dish in moistened blotting paper. Single spore is transferred from the sporulating lesions to the separate culture tube of Oatmeal Agar (Oatmeal 60 g/l and agar 12.50 g/l) and Potato Dextrose Agar (PDA) (200 g potato, 20 g dextrose, 20 g of agar, and 40 mg of streptomycin/l). Then the tubes are incubated at 25 ± 2 °C for 3-4 days. Mycelial growth of *M. grisea* from both the culture tubes is used for mass sporulation of conidia in oat meal agar medium (Fig. 12).

Pearl millet seedlings are raised in clay pots filled with field soil, inside the green house. In each pot, 20-25 seeds are sown and spaced 1-1.5 cm apart. Then seeds are covered with a thin layer of soil and watered regularly.

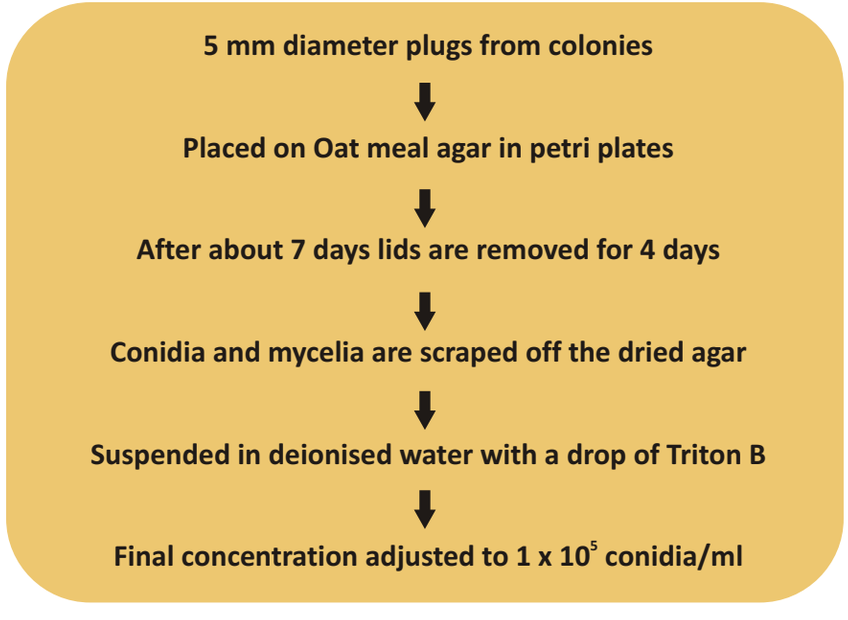


Figure 11: Schematic representation for sub-culturing *M. grisea*

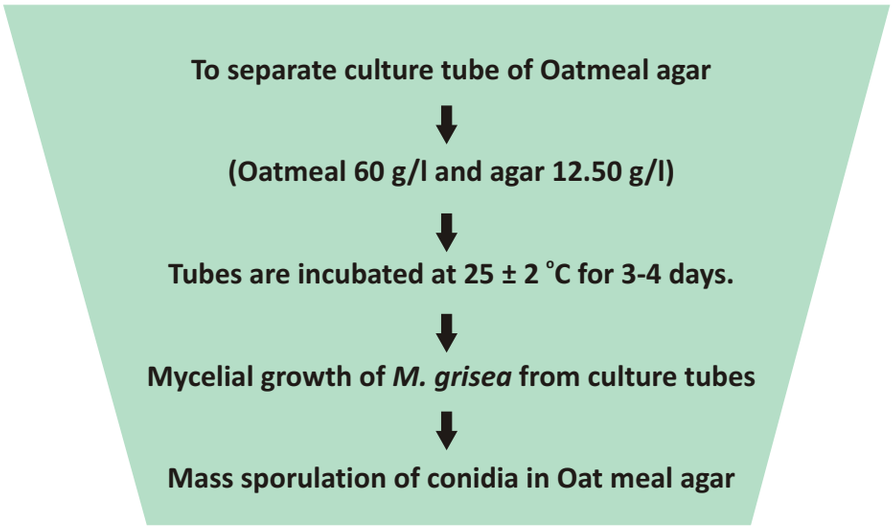


Figure 12: Schematic representation of single spore isolation and culturing of *M. grisea*

Plants maintained in pots are inoculated when the seedlings are in 2-3 leaves stage (7-8 days old). Plants are misted (sprayed) to dripping with inoculum concentration of 10^4 spores/ml with an atomizer. Immediately after inoculation, seedlings are covered with polythene bags for 24 h in 95-100% relative humidity and 25-26 °C temperature to facilitate spore germination and penetration (Correa-Victoria and Zeigler, 1993) (Fig. 13).

Seedlings are in 2-3 leaf stage (7-8 days old)

↓

Plants are sprayed to dripping with inoculum concentration of 10^5 spores/ml with an atomizer

↓

Seedlings are covered in polythene bags for 24 h (95-100% RH and 25-26 °C)

↓

Facilitates spore germination and penetration

Figure 13: Schematic representation of *M. grisea* inoculation to pearl millet plants

Field screening

Grow test lines in the central four rows and a highly susceptible line in the first row and every fifth rows as infector/indicator rows, Spray-inoculate seedlings at pre-tillering and/or pre-flowering stages with aqueous spore suspension to run-off. Provide high humidity (>90% RH) by operating perfo- or sprinkler irrigation twice a day 30-60 min each in the morning (between 10:00 and 11:00) and in the afternoon (between 17:00 and 18:00) on rain-free days, Record disease severity at the hard-dough stage using a progressive 0-9 scale developed for pearl millet blast. The disease severity rating scale has been modified to group the genotypes into different disease reaction classes (Table 6 & Fig. 14).

Table 6: Foliar blast severity rating scale (0-9)

Rating Scale	Symptoms and lesions	Disease reaction
0	No lesion	Highly resistant
1	No lesion to small brown specks of pinhead size without sporulating center	Resistant
2	Large brown specks	
3	Small roundish to slightly elongated necrotic gray spots (~ 1-2 mm in dia.), with brown margin	
4	Elliptical lesions, 1-2 cm long, frequently confined to the area between main veins, covering < 2% of the leaf area	Moderately resistant
5	Typical blast lesions covering < 10% of the leaf area	
6	Typical blast lesions covering 10-25% of leaf area	Susceptible
7	Typical blast lesions covering 26-50% of leaf area	
8	Typical blast lesions covering 51-75% of the leaf area with many leaves dead	Highly susceptible
9	> 75% leaf area covered with lesions and most leaves dead	

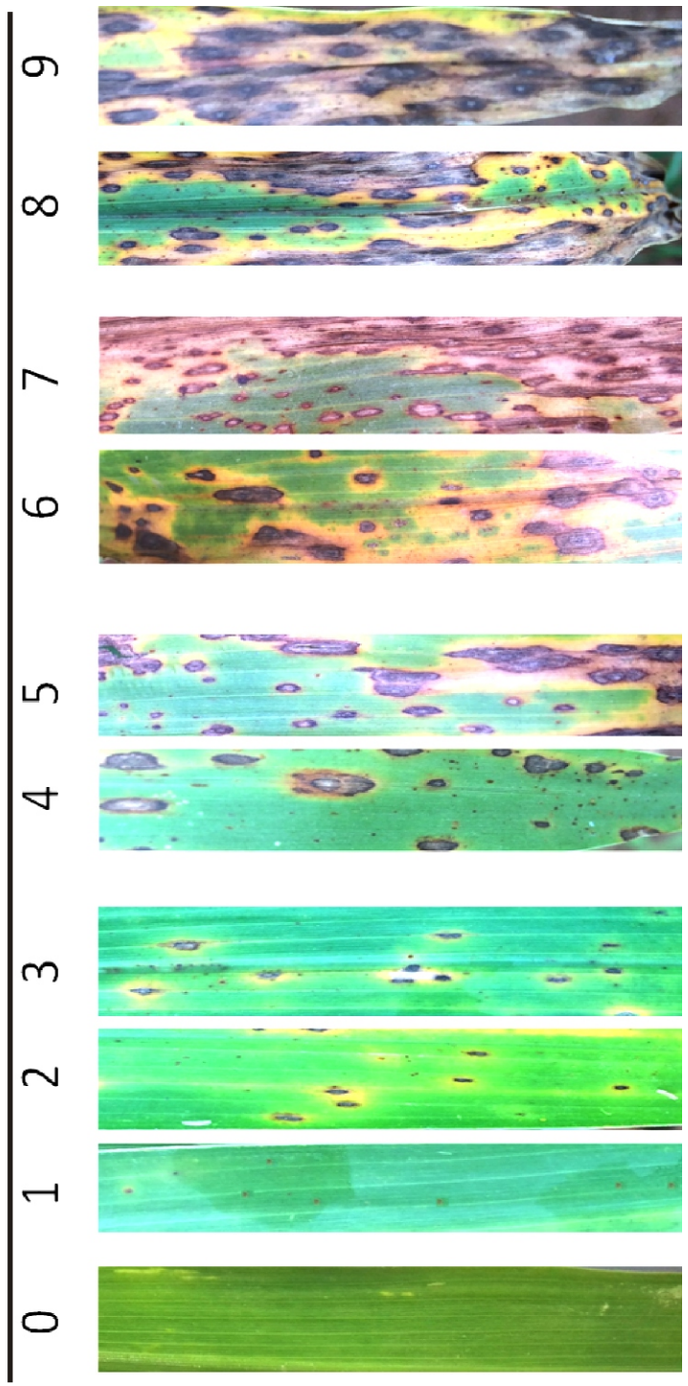


Figure 14: Magnaporthe blast severety rating scale. Scale: 0 = no lesion; 1= no lesion to small brown specks of pinhead size; 2 = larger brown specks; 3 = Small round to slightly elongated necrotic gray spots (~ 1-2 mm in dia.), with brown margin; 4 = Elliptical lesions, 1-2 cm long, frequently confined to the area between main veins, covering < 2% of the leaf area; 5 = typical blast lesions covering <10% of the leaf area; 6 = typical blast lesions covering 10 to 25% of the leaf area; 7 = typical blast lesions covering 26 to 50% of the leaf area; 8 = typical blast lesions covering 51 to 75% of the leaf area and many leaves dead; and 9 = all leaves dead.

The blast disease development is growth-stage dependent (Wilson and Hanna 1992). Hence**,** early-maturing varieties will express severe leaf blast than late-maturing varieties at any given evaluation, so severities of pearl millet with widely differing maturities should be corrected for maturity or rated at a similar growth stage.

Greenhouse artificial screening

Isolation of *Magnaporthe grisea*

The isolation of *M. oryzae* from blast infected samples should be carried out under aseptic conditions. For isolation of the pathogen from leaf blast samples, typical ashy grey lesions are selected and cut into 3-5 mm bits. The bits are surface sterilized in 0.5 % sodium hypochlorite solution for 30 seconds and rinsed with sterile distilled water. Surface sterilized leaf blast lesions should be placed over sterilized moist cotton set-up in separate petri-dishes. Infected lesions are incubated for 24-48 h at 25 °C temperature for leaf blast samples. Later, incubated samples are transferred to pertriplates which is having oat meal agar medium and sealed with parafilm. The set-up plates are incubated at 25 °C for 3 days or more until the colony forms. A portion of the mycelial disc is macerated in 1 ml leaf extract broth in sterilized petriplate and just molten warm leaf extract oat meal agar media should be poured and mixed thoroughly. 3-4 days after incubation at 25 °C, individual fungal colonies developed on oat meal media with *M. grisea* spores. Single spore colony is transferred to OMA slants for short term storage and for long term preservation sterilized filter paper disc were colonized on growing culture as per protocol of Parker *et al.* (2008).

Mass multiplication of *Magnaporthe grisea* cultures

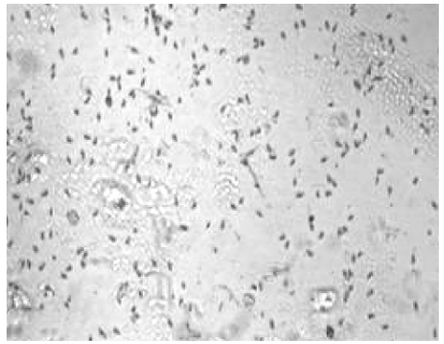
To obtain conidia for inoculations, the slants containing pure culture revived on leaf extract oat meal agar plates. The mycelia from slants or petriplates are macerated in 1 ml leaf extract and oat meal agar poured on to the macerated mycelia bits and allowed for solidification. The inoculated plates are incubated at 28 °C for 7-10 days to allow the fungal mycelium to grow and cover the entire plate.

Inoculum preparation

Inoculum is prepared from the profusely growing fungal mycelium. The plates with active fungal growth are flooded with 10-15 ml of sterile water and fungal mass containing mycelium and spores gently removed by scrapping with sterile glass slide and suspension transferred to 100 ml beaker. The fungal masses are gently mixed with a glass rod and suspension filtered with a muslin cloth. The spore concentration is adjusted to $1x10^5$conidia/ml with the help of haemocytometer. Tween 20 (0.02%) is added to spore suspension just before inoculation as adhesive agent.

Growing seedlings and inoculation

Pearl millet seeds are sown in 20 well plastic trays filled with a mixture of 3:1 field soil and farm yard manure. Inoculation is done at three to four leaf stage (approx. 15 days old plants) of the test seedlings. Inoculum is sprayed over the leaves thoroughly with the help of glass atomizer till there is run-off. The inoculated plants are kept in the polystyrene chamber under dark condition for 24 h at 25 ± 1°C and above 90% RH. After incubation period, the test plants are provided with proper illumination and regular watering for maintaining high humidity inside the chamber. The environmental conditions are standardized for disease establishment; *viz.,* temperature; 25 ± 1 °C, relative humidity; >90% and light duration: 10 h light (10000 Lux units) & 14 h darkness. Symptoms appear between 5 to 7 days after post inoculation (Prakash *et al.,* 2016). Blast severity is recorded 7 days after inoculation using a 0-9 scale given by IRRI and being followed by the All India Coordinated Research Project on Pearl Millet (Table 6 ; Fig. 14 & 15).



***Magnaporthe grisea* spores**

Seedlings ready for inoculation at three/four leaf stage

Environmental conditions for disease development

Temp. : 25°C / Humidity : >90%/Light duration: 10 h light & 14 h darkness

Disease scoring 7 days after inoculation by 0-9 scale (IRRI)

Figure 15: Pearl millet blast phenotyping under artificial condition

Strategies for breeding resistance

Conventional breeding

Breeders and pathologists should work together to screen and breed for resistance to blast disease. Elite lines and pearl millet germplasm serve as donors. Such lines should be used as parents in hybridization program. Donors showing partial resistance for blast are preferred. F1 seeds are increased to produce F2s. Breeders and pathologists evaluate the lines for blast resistance. Selection begins at F2, and continued from F3 to F6 or F7 generation (Fig. 16).

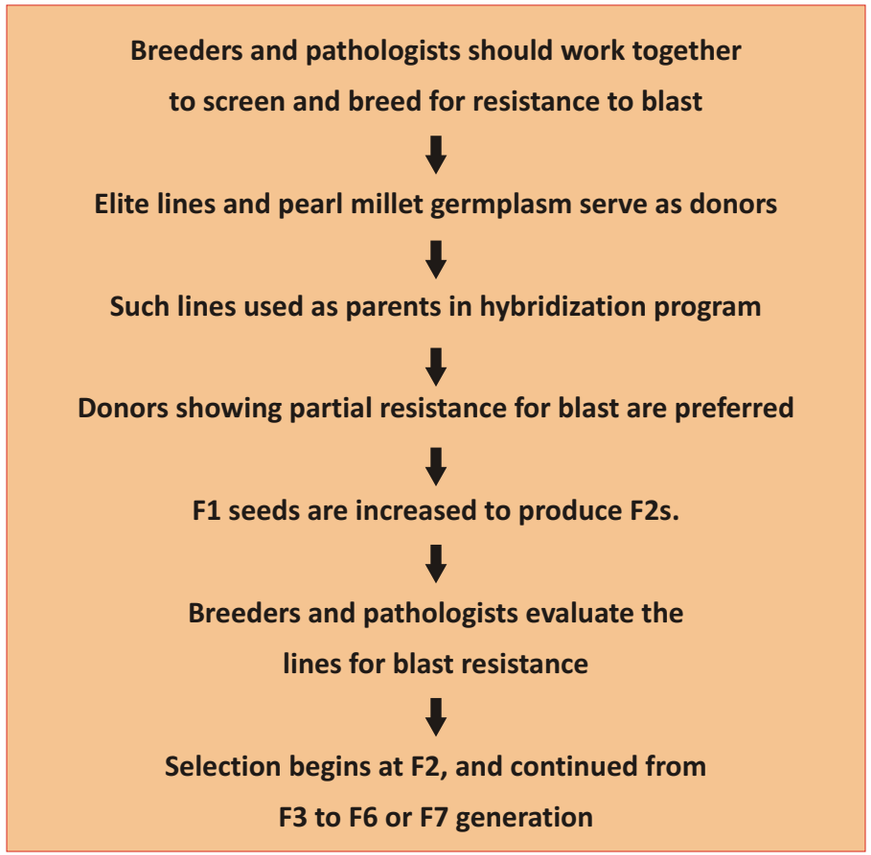


Figure 16: Schematic representation of conventional breeding strategy for pearl millet

Molecular breeding

Unfortunately, the genetic variation available for pearl millet blast resistance is relatively narrow. We need to look for additional sources of resistance such as screening the core or mini-collections maintained at ICRISAT and other AICRP centres against major isolates/pathotypes. We need to generate genetic and genomic resources for mining and mapping of the blast resistance QTLs/genes. At ICRISAT a pearl millet inbred germplasm association panel (PMiGAP) has been developed from pearl millet core collection of over 2000 accessions, landraces, cultivars and breeding lines representing major global diversity of pearl millet.

This panel is an outcome of a Biotechnology and Biological Sciences Research Council- Department for International Development (BBSRC-DfID) funded project in collaboration with the Institute of Biological, Environmental & Rural Sciences (IBERS) of the Aberystwyth University, UK. This panel has recently been sequenced using whole-genome re-sequencing (WGRS) strategy, resulting in over 25 million SNPs. Phenotyping of the association mapping panel at the blast hot-spot locations will help carry out precise genome wide association mapping study (GWAS) and mine novel alleles for blast resistance. Bi-parental and multi-parental QTL mapping of the blast resistance genes can be carried out as a complimentary approach (Fig. 17). We are also in a position to apply knowledge generated in other related crop species to explore synteny pearl millet genome. The world reference genotype Tift 23D2B1-P1-P5 has been sequenced to severe as a quality reference genome globally. These available genetic and genomic resources can be further enhanced and used to mine, map and deploy effective blast resistance genes (Sehgal *et al.,* 2015).

Molecular markers for resistance

Genes for disease resistance to both pathogens have been transferred into agronomically acceptable forage and grain cultivars. A study was undertaken to identify molecular markers for three rust loci and one *Magnaporthe* resistance locus in pearl millet by Morgan *et al*., (1998). Three segregating populations were screened for RAPDs using random decamer primers and for RFLPs using a core set of probes detecting single-copy markers on the pearl millet map. Only one RAPD marker (OP-D11700, 5.6 cM) was linked to *Magnaporthe* leaf spot resistance. A plant carrying the *Rr1* resistance gene from Tift 89D2, and *Pyricularia* resistance

from *P. glaucum* sp. *monodii*, could be identified with a good probability using three molecular markers (SCAR-G8, OP-K19, and OP-D11) in the PCR reaction.

Continued research is needed for pearl millet to map the currently deployed Magnaporthe resistance gene to a linkage group and to develop more efficient markers for this locus, as well as to search for other Magnaporthe resistance loci.

Incorporating quantitative blast resistance to popular hybrids using the advanced backcross QTL approach. Resistant donor crossed to a popular pearl millet variety. Analyze backcross lines (BC2F3) for candidate defense genes and blast resistance association. Selections made based on partial resistance to blast at disease hotspots. Genotyping for candidate defense genes conferring partial resistance and gene pyramiding of major genes for resistance (Fig. 18).

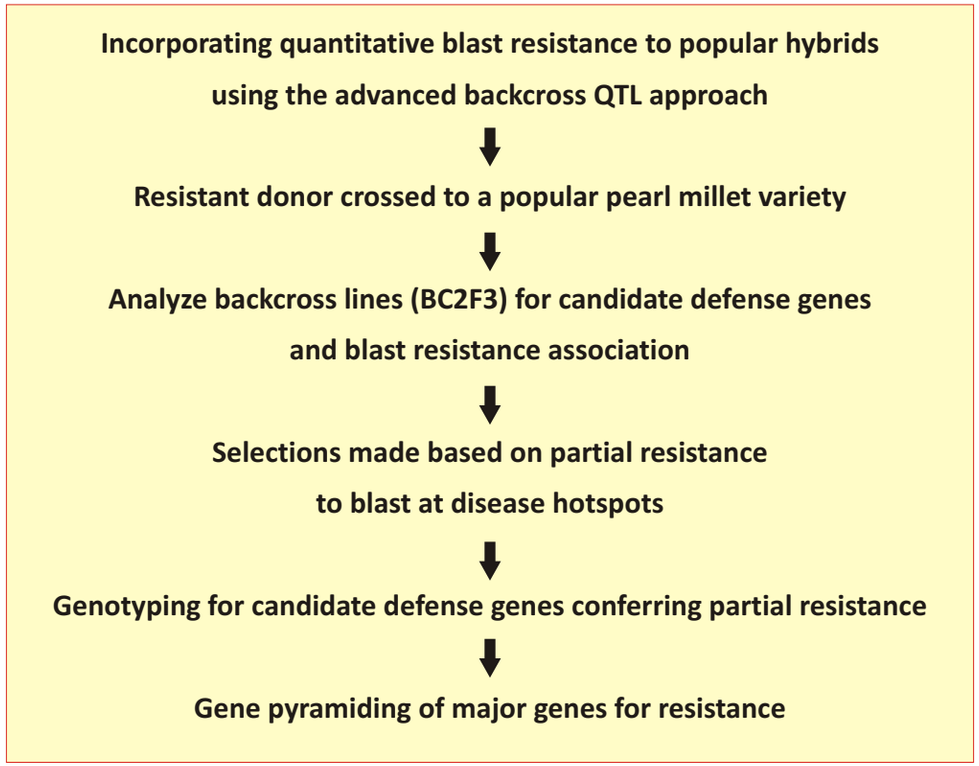


Figure 17: Schematic representation of molecular breeding strategy for pearl millet blast resistance

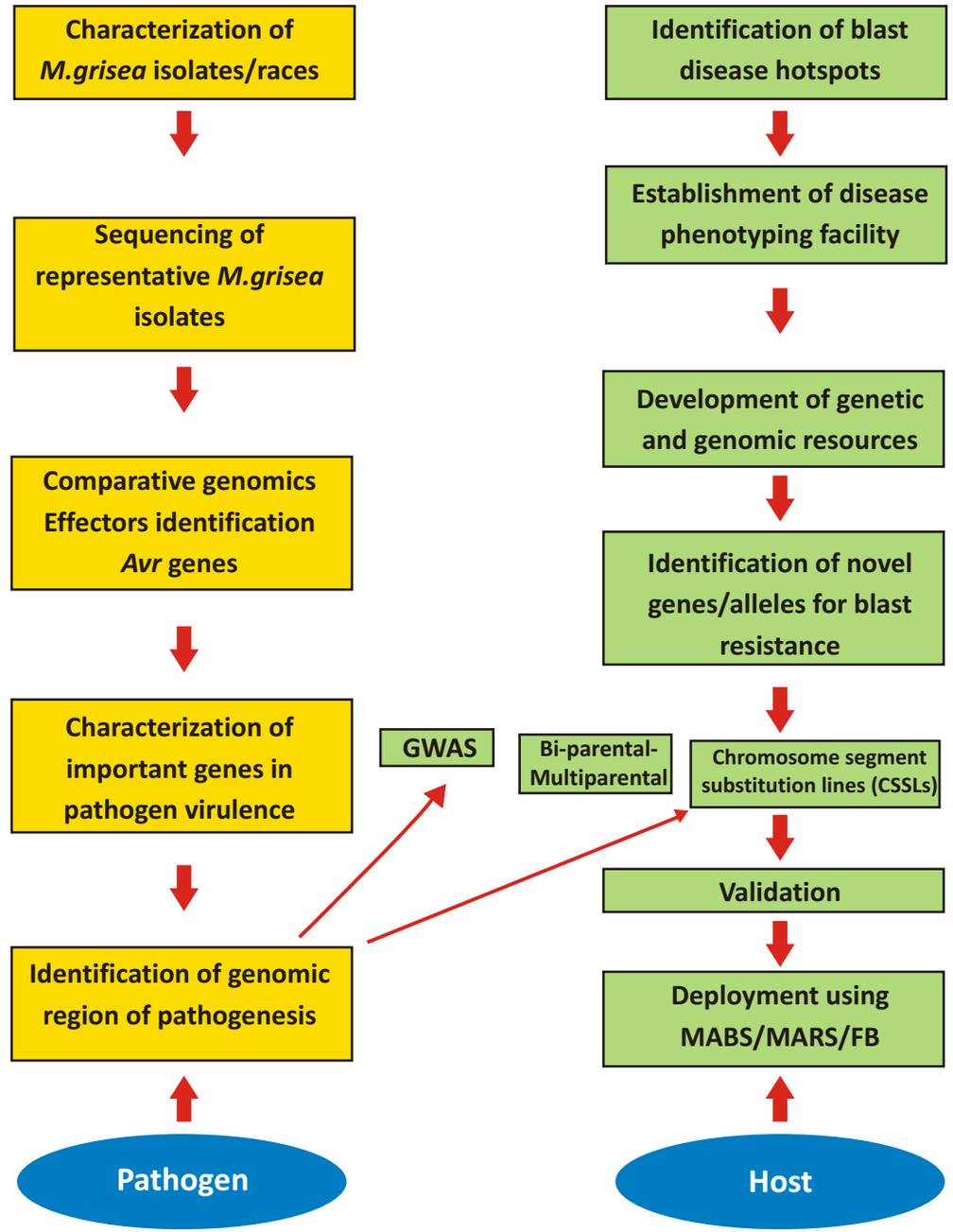


Figure 18: Scheme for blast disease management using genetic and genomic interventions

Conclusion

Lately pearl millet blast disease pressure is increasing, because, on one hand most of the popular pearl millet varieties are becoming susceptible to blast disease and on the other hand pathogen is fast evolving and adapting. Therefore, in the near future pearl millet blast will become a serious and recurrent problems in the pearl millet growing areas and this situation warrants devising technologically sound, highly effective, eco-friendly, economical and integrated management strategies.

One of the main concern is the appropriate recognition of the symptoms to minimize the damage, and this is an important area in which the extension departments play a crucial role so that the farmers take up suitable remedial measures in time. Disease forecasting based on the soil, disease history, pathogen virulence and weather pattern and suggesting suitable measures to tackle the disease remains vital for the prevention of epidemic and a remarkable progress is envisaged on this front. Latest software-based disease forecasting models help in establishing an effective risk management system. More importantly, keeping in view of the impending climate change scenario, strategies for breeding pearl millet blast resistance lines that can adapt to climate change is to be more emphasized and prioritized.

Several new generation fungicides are and being developed and formulated which are eco-friendly with desired fungitoxicity. The latest trend of nanotechnology can also be effectively integrated into pearl millet blast management program. Development of next-generation bio-based nanomaterials and nano-based antimicrobials and intelligent nano-delivery systems must be thoroughly researched for minimizing chemical application.

Deciphering the transcriptome dynamics for analyzing the functionally important genomic elements, their expression patterns and their regulation in different developmental stages, tissues, particularly under blast stress plays an important role in understanding pearl millet host tolerance towards the blast pathogen. Blast pathogen is known to be highly variable with an extraordinary ability to mutate. The blast fungus genetically evolves rapidly and breaks down the resistance of hybrids in regular intervals; therefore, a continuous search for the resistance genes/allelic reservoirs/sources becomes extremely important for

resistance breeding. Proper understanding of the genetic diversity and dynamics of the blast pathogen helps in analyzing the population structure and diversity and the *Avr* gene pool in the blast affected areas which ultimately can be used for blast resistance breeding projects.

Conventional breeding for blast resistance presents its own shortcomings and therefore molecular breeding will take a central role in future blast disease management. Large pearl millet germplasm collections provide allelic diversity required for evolution of *R* genes which serve as novel sources for pearl millet improvement. Identification of blast disease resistance genes and QTLs and marker-assisted pyramiding of disease resistance genes will serve as a big step forward in developing blast resistance pearl millet hybrids. The available pearl millet genome sequencing information must be efficiently utilized for allele mining for identification and isolation of novel and superior alleles is vital for the development of improved blast resistant pearl millet cultivars. Genetic dissection of the resistance mechanisms by QTL analysis and subsequent application of marker assisted selection aid quick development of blast resistant varieties through gene pyramiding. With the increasing availability of the latest and state of the art molecular technologies including the allele mining, genome-wide association studies (GWAS), single nucleotide polymorphism (SNP) genotyping etc., novel *R* genes could be identified, cloned, pyramided into the elite pearl millet lines. Cisgenic breeding could also be an option for breeding high yielding blast tolerant pearl millet varieties in less time and more research in this line is required for blast alleviation. Double haploid breeding might also serve as an interesting option which saves time, labour and cost.

Over dependence on one particular mode of control may not suffice blast management and perhaps combinations of host resistance, chemicals, induction of resistance, biological and cultural methods is necessary. A vast body of research and extension is under progress in various facets for pearl millet blast disease management involving strategies like cultural, chemical, host resistance; induction of resistance, biological control etc., and an integration of all these approaches seems to be the best approach forward. Integrated use of all these effective strategies which complement each other will result in a broad-based, environment-friendly approach for mitigating pearl millet blast damage leading to a cost-effective and sustainable pearl millet farming.

Future perspectives and consideration

The major difficulty in controlling pearl millet blast is the durability of genetic resistance. Enhancing the host plant resistance is being considered as the best approach to handle the pearl millet blast disease.

From a pathological perspective future thrust should be on :

- screening and identifying sources of genetic resistance for blast disease in pearl millet
- Analysing the shift in virulence of the pathogen for the blast disease.
- Development of environmental safe fungicides, bioagetns, plant growth promoting rhizobacteria, and disease resistance inducing components.
- Response of the host and pathogen interaction to changing climatic situation in the years to come.

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