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Host response in pearl millet after challenging inoculation of *Magnaporthe grisea*

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Abstract
The present study entitled "Studies on *Magnaporthe grisea* incitant of blast disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.]" was conducted during the *kharif* 2019 at research farm, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar. Pearl millet blast disease is a devastating fungal disease causing considerable yield losses. Blast of pearl millet incited by *Magnaporthe grisea* is the most widespread and destructive disease of pearl millet in India and other pearl millet growing area of the world. In the present investigation, Incubation period as the measure of infection efficiency of fungus, was found about 5 days. Latent period as the measure to decide the disease epidemic, was found about 3 days. Both, lowest mean lesion length and lesion number were found in resistant (18-0035) genotype. Six pearl millet genotypes were screened against blast disease under field conditions for lesion length, latent period, number of lesions, disease severity and found that lowest mean lesion length, lowest mean lesion number were found in resistant (18-0035) genotype. The terminal disease rating was observed highest in 18–0114 genotype and lowest in 18-0035 genotype and consider as highly susceptible and resistant genotype respectively.

Keywords: *Magnaporthe grisea,* blast disease, epidemic, disease severity and latent period

1. Introduction
Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is one of the most important crops that can be grown in the areas having adverse agro – climatic conditions *viz.,* hot, dry weather and less fertile sandy soils with low moisture, hence called nutritious poor man's crop. Pearl millet [*Pennisetum glaucum* (L.) R. Br)] belonging to family *Poaceae* is one of the assured *Kharif* crops domesticated in an area with the annual rainfall of 150 mm to 1000 mm in India. Pearl millet is a rainfed crop which can survive well in the rainfall as 250 mm on relatively poor soils. It is a highly cross-pollinated small-seeded cereal crop which is protogynous in nature. Bajra is cultivated in over 30 countries of Africa, America and Asia where dry land system is possible. India and Africa are together occupying 90 % area of total pearl millet producing area in the world (Yadav *et al.*, 2012) [1]. Pearl millet ranks sixth among cereal crops based on world production, next to rice, wheat, maize, barley and sorghum and also more tolerant to harsh and water scarcity conditions. India is the largest producer of pearl millet as largest area also in the world, Rajasthan being the largest producer of pearl millet in India.

This millet is one of the most important with greatest potential of all the millets which provide staple food for millions of people in semi- arid tropics regions. This coarse grain is known as poor man's food particularly for working class. Due to its wider adaptability under agro-climatic conditions, this crop is mostly grown in the states of Rajasthan, Madhya Pradesh, Gujarat, Haryana, Karnataka, Andhra Pradesh, Tamil Nadu and some parts of Delhi, Uttar Pradesh and Punjab. It is cultivated over an area 6.98 million hectares with a production of 8.06 MT in India (GOI, 2016). In Haryana, it is grown in 4.78 lakh hectare area with 9.64 lakh metric tones production (GOH, 2018).

In pearl millet, several diseases caused by fungi, bacteria, viruses and nematodes have been recorded. Economically important are only a few that include blast, downy mildew, ergot, smut and rust (Thakur *et al*., 2011) [4]. In India 60 per cent (Sharma *et al.*, 2012) or more pearl millet is sown with single-cross hybrids that are particularly vulnerable to Pyricularia leaf spot disease or blast disease of pearl millet caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*) and downy mildew disease caused by *Sclerospora graminicola*. Pyricularia leaf spot, also known as blast disease, is important in pearl millet grain and forage cultivars.

The disease causes chronic yield losses of forage (Wilson and Gates, 1993) [6] and grain (Timper *et al*., 2002) [7].

The pathogen can be easily isolated from infected leaf tissue and grown on synthetic media. The freshly harvested leaf portions with infection can be placed in glassine or paper envelopes in the refrigerator, where they will slowly dry and remain flat. No or very less scientific literature is available on assessment of disease symptoms, severity, effect on photosynthetic efficiency of the foliage, effect on the level of biochemical and other metabolic activities. Therefore, present investigation will be carried out with following objectives. The plant responds both non-specifically and specifically to the invading pathogen and its virulence factors. After establishment of the relationship plants under go several physical and biochemical changes, few among them are Incubation period, Latent period, Size and numbers of spot and Severity of the disease.

Incubation period, i.e. the time between inoculation and symptom expression as a measure of infection efficiency for isolates of leaf blast pathogen in pearl millet is an important parameter to testify host specificity as well as resistance against the pathogen. Latent period is the time between infection and sporulation from that infection or time between infection and production of secondary inoculum (Pariaud et al., 2009) [14]. Peral millet blast is a polycyclic disease generally with a spore to spore cycling time of seven days (Chandra et al., 2017) [15]. A latent period of slightly more than four days has been recorded by Roumen and Boef (1993) [9] in case of rice blast, no or very less information is available in case of pearl millet blast. Lesion development is the component of infection process and it is the key determinant of blast epidemics. According to Turechek and Stevenson (1988) [16] conidia production was positively correlated with lesion size. Lesion expansion may attain a length of 25 and reaches upto 35mm (Kato and Kozaka, 1974) [11]. Castejon-munoz (2008) [12] observed that as the plant developed, number of lesions also increased.

2. Materials and Methods

The present investigation entitled, "Studies on *Magnaporthe grisea* incitant of blast disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.]" was conducted in the Laboratory and Research Area, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar during *Kharif* 2019.

2.1 Pearl millet response to blast pathogen

To study the reactions of different inbred line of pearl millet in terms of incubation period, latent period, spot size, number of spots, disease severity and acquired changes in level of biochemicals, a field experiment was conducted. The Hisar isolate was used for inoculation at 30 DAS.

Detail of experiment given below:

No. of cultivars/lines: 6

2.1.1 Recording of data

Data were recorded for incubation period, latent period, spot size, number of spots and disease severity.

2.1.2 Incubation period

Inoculated plants were observed daily for lesion count till 10 days. From inoculation to development of 50 % of the lesion has been considered as incubation period (IP_{50}) for blast.

2.1.3 Latent period

The lesions with grey center were selected to find latent period. These were transferred to Petri dish already provided with moist filter paper and incubated at 25±1°C. Formation of spore was judged by using sticky tape at one day interval and observing under microscope.

2.1.4 Spot size and number of spots

The lesion number and length of the lesions was measured eight days after inoculations with the help of metric scale.

2.1.5 Disease severity

Disease rating to the genotypes was recorded at the hard-dough stage. The disease severity was assessed by using foliar blast severity rating (1-9) scale (Thakur *et al*., 2011) [4].

Rating scale	Symptoms and lesions	Disease reaction
1.	No lesion to small brown specks of pin head size	Highly Resistant
2.	Large brown specks	Resistant
3.	Small, roundish to slightly elongated, necrotic gray spot, about 1-2 mm in diameter with a brown margin	
4.	Typical blast lesions, elliptical, 1-2 cm long, usually confined to the area between main veins, covering <2% of leaf area	Moderately Resistant
5.	Typical blast lesions covering <10% of the leaf area	
6.	Typical blast lesions covering 10 – 25% of the leaf area	Susceptible
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	Highly Susceptible
9.	75% leaf area covered with lesions and most leaves dead	

3. Results

3.1 Pearl millet response to blast pathogen

3.1.1 Incubation period

After 24 hour of inoculation, blast lesions were observed to development of 50 % of the lesions which considered as incubation period (IP_{50}) for blast.

Table 1: Incubation period (IP_{50}) of *Magnaporthe grisea* on pearl millet genotypes

Days	No. of spots (average)	Cumulative frequency	Cumulative frequency %
1.	0	0	0
2.	0.5	0.5	3.57
3.	0.9	1.4	10.00
4.	2.5	3.9	27.85
5.	3.1	7.0	50.00
6.	3.3	10.3	73.57
7.	3.7	14	100

Based on the lesion number developed up to 7 days after inoculation, incubation period (IP_{50}) in pearl millet was estimated to be about 5 days.

3.1.2 Latent period and 3.1.3 Spot size and number of spots

After 24 hour of incubation period, blast lesions were observed to sporulation of 50 % of the lesions which considered as latent period (LP_{50}) for blast. Based on the

sporulating lesions number developed 5 days after incubation period in pearl millet, the LP_{50} was estimated to be about 3 days.

Table 2: Latent period (LP_{50}) of *Magnaporthe grisea* on pearl millet genotypes

Days	No. of spots (average)	Cumulative frequency	Cumulative frequency %
1	1.7	1.7	9.66
2	2.8	4.5	25.57
3	4.3	8.8	50
4	5	13.8	78.40
5	3.8	17.6	100

3.1.3 Lesion length

Six genotypes were taken for this experiment out of which very two genotypes were resistant (18-0035, 18-0109), moderately resistant (18-0426, 18-0549) and susceptible (18-0060, 18-0114). Lesion length was increased continuously with increase in time period of all the six genotypes. The highest mean lesion length was found (4.78 mm) in susceptible (18-0114) genotype but lowest mean lesion length was found (4.06 mm) in resistant (18-0035) genotype.

3.1.4 Number of lesions

Number of lesions was increased continuously with increase in time period upto 60 hours but further decrease upto 72 hours in all the six genotypes. The highest mean lesion number was found (36.46) in susceptible (18-0114) genotypes but lowest mean lesion length was found (14.67) in resistant (18-0035) genotype.

Table 3: Effect of artificial inoculation on lesion length of pearl millet blast disease development

Name of genotypes	Lesion length (mm)						
	12 h	24 h	36 h	48 h	60 h	72 h	Mean
18 – 0035	2.58	2.95	3.64	4.14	5.12	5.95	4.06
18 – 0109	2.79	3.21	3.78	4.34	5.19	6.22	4.25
18 – 0426	2.74	3.12	3.60	4.58	5.34	6.27	4.28
18 – 0549	2.78	3.36	3.97	4.80	5.57	6.48	4.49
18 – 0060	2.85	3.44	4.15	5.07	5.86	6.62	4.67
18 – 0114	2.80	3.52	4.42	5.27	5.92	6.74	4.78
Mean	2.76	3.27	3.93	4.70	5.50	6.38	
C.D (P=0.05)	Genotype	0.07					
	Time	0.07					
	Genotype x Time	0.17					

Table 4: Effect of artificial inoculation on lesion number of pearl millet blast disease development

Name of genotypes	No. of lesions per leaf						
	12 h	24 h	36 h	48 h	60 h	72 h	Mean
18 – 0035	7.03	10.45	14.25	17.42	20.36	18.49	14.67
18 – 0109	10.52	16.57	21.45	25.92	30.21	27.24	21.09
18 – 0426	10.64	16.69	21.15	25.18	29.45	26.87	21.66
18 – 0549	11.78	20.78	29.07	35.45	43.56	37.48	29.69
18 – 0060	13.54	22.56	32.72	43.32	52.78	45.23	35.03
18 – 0114	13.68	23.26	34.18	44.52	54.57	48.56	36.46
Mean	11.19	18.39	25.47	31.97	38.49	33.98	
C.D (P=0.05)	Genotype	0.28					
	Time	0.28					
	Genotype x Time	0.69					

3.1.5 Disease severity

Disease rating of genotypes was recorded at hard-dough stage. Disease rating was found to be maximum (8.40) in susceptible genotype (18-0114) and lowest (3.00) in resistant genotype (18-0035).

Table 5: Effect of artificial inoculation on severity of pearl millet blast disease

Name of genotypes	Disease rating scale	Disease Reaction
18 – 0035	3.00	Resistant
18 – 0109	4.80	Moderately Resistant
18 – 0426	5.00	Moderately Resistant
18 – 0549	6.20	Susceptible
18 – 0060	8.20	Highly Susceptible
18 – 0114	8.40	Highly Susceptible

4. Discussion

In the present investigation, pearl millet genotypes were tested to determine their incubation period and found that incubation period of pearl millet was about five days which are in agreement with findings of Kumar *et al.* (2018) [8] who reported incubation period of pearl millet about 4.98 days. At the same time, latent period of pearl millet genotypes also determined and was found about three days which are in contradiction with Roumen *et al.* (1993) [9] who found latent period of rice on same fungus (*Magnaporthe grisea*) slightly more than four days. The highest mean lesion length was found (4.78 mm) in susceptible (18-0114) genotype but lowest mean lesion length was found (4.06 mm) in resistant (18-0035) genotype. The highest lesion length was found (6.74 mm) at 72 hours while minimum (5.95 mm) at 72 hours in (18-0114) and (18-0035) genotype respectively.

Results are in consistent with the finding of Goud, 2015 who reported that the highest lesion length was (5.91 mm) at 72 hours duration of wetness followed by (5.31 mm) at 60 hours wetness duration. Results are not in agreement with findings of Kato and Kozaka, 1974 [11] reported that expansion of lesion may attain a length of 25-35 mm. The highest mean

lesion number was found (36.46) in susceptible (18-0114) genotype but lowest mean lesion number was found (14.67) in resistant (18-0035) genotype. The highest lesion number was (54.57) at 60 hours followed by minimum (20.36 mm) at 60 hours in (18-0114) and (18-0035) genotype respectively. Results are in agreement with that Castejon-munoz (2008) [12] who reported that with the development of plant, number of lesions also increased.

The results of study also in consistent with the observance of Goud, 2015 who reported that mean lesion number increased upto maximum from 1.37 to 41.30 at 48 hours leaf wetness duration and then decreased to 37.25 and 33.86 at 60 hours and 72 hours leaf wetness duration, respectively. Disease severity can be defined as the area of the sampling unit showing infection symptoms, expressed as a proportion or percentage. Highest disease severity rating of 8.40 was observed in susceptible genotype (18-0114). No scientific information has been available in literature regarding assessment of disease severity of pearl millet blast in Haryana or India of these genotypes. These results of present investigation are more or less similar to Sharma *et al.* (2013) [13] grouped 25 isolates into five different pathotypes based on their reaction types (virulent = score > 4 and avirulent = score < 3 on a l to 9 scale).

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Conflict of Interest: None.

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