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Isolation and Identification of *Pyricularia* sp. the Incitant of Pearl Millet Blast in Tamil Nadu

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ABSTRACT: Pearl millet (*Pennisetum glaucum*), often called as Bajra which is cultivated mostly in marginal agricultural regions with unpredictable annual rainfall. Though it is cultivated in larger areas its production is hampered due to many foliar diseases among which leaf blast disease caused by *Magnaporthe grisea* is one of the most significant foliar diseases causing grain and forage yield losses. A total of four isolates of *Magnaporthe grisea* were isolated from the infected samples of pearl millet collected from eight districts of Tamil Nadu, India. The varied symptoms including spindle shaped lesions, eye shaped spots which had greyish centre with brown borders were taken for the isolation of the pathogen. The main aim of this particular research was to look at the various morphological traits in the culture media, pathogenicity assay to identify the virulent isolate, isolate confirmation using molecular level and generate phylogenetic analysis. Morphological characters like colony appearance and colour, mycelial characters, conidia structure, shape, growth pattern and virulence assay were evaluated both *in vitro* as well as in pot culture studies. The universal primers ITS 1 and ITS 4 have been used for amplification of the internal transcriber spacer and the isolates were amplified at 560bp (appx.). The findings revealed that all isolates are *Pyricularia* spp. and phylogenetic analysis done comparing eight ITS sequences of reference *Pyricularia* isolates indicated that the nucleotide sequences of the collected isolates showed sequence similarity with *Pyricularia grisea* and *Pyricularia pennisetigena* which have been deposited in NCBI Genbank database (*Pyricularia grisea* -OM883863 and *Pyricularia pennisetigena* ON116174). The main course of the research was to isolate and characterize the blast pathogen, *Pyricularia* grisea infecting the pearl millet crop.

Keywords: Pearl millet, blast, Pyricularia grisea, isolation, characterization, phylogenetic tree.

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the most widely grown arid and semi-arid crops in India and African sub-continents after rice, wheat and sorghum. It has a good energy source of carbohydrate, fats(5-7%), protein (9-13%), fibre (1.2 g/100 g)and contains antioxidant such as coumaric acids which helps in better digestion (Patni and Agrawal 2017). Pearl millet blast which was originally documented in 1942 from Kanpur, UP, India (Mehta *et al.*, 1952) has become a serious threat in the last decade causing rampant in India's pearl millet-growing areas. In India, the incidence of Pearl millet blast (*Magnaporthe grisea*) disease, which was once considered a minor disease, but now it has been increased at an alarming rate, primarily on commercial hybrids, in numerous states (Thakur *et al.*, 2009). Severe outbreaks of *Pyricularia* leaf spot known as blast disease was reported in major pearl millet producing states, including Gujarat, Rajasthan, Uttar Pradesh, Madhya Pradesh, Karnataka and Delhi were shown an upsurge in the occurrence of this disease both inpearl millet leaves and grains (Timper *et al.*, 2002). The forage production of pearl millet (cumbu) causing chronic reductions in the last few years (Wilson and Gates 1993). The quality and productivity of the pearl millet crop are impacted by *Magnaporthe* blast, which has been found to be negatively connected with green-plot yields, dry matter production and digestion dry matter yield. The severity of the blast disease is exacerbated by humid weather conditions and dense plant stands. The fungus may infect plants at any stage of development, from seedling to adult, lowering grain and forage yields in varying degrees with occasionally dramatic negative consequences. The symptoms of *Magnaporthe* blast in pearl millet are most generally known as grey leaf spot.

Initially, the symptoms appear as minute lesions or specks that expand and develop, necrotic elliptical greyish brown lesions, leading to extensive chlorosis and premature drying of leaves. The typical symptoms of the plants infected by *Pyricularia pennisetigena* showed small, brown, pinpoint, elliptical lesions with greying of the center in the later stages (Martinez *et al.,* 2021). The *M. grisea* group is specific in its host range, but it is highly versatile in its ability to adapt to new environments. Molecular identification of the pathogen, which has shown to be a solid alternative to older approaches, is one of the rapid and easy ways for detecting the species diversity of fungal communities. To distinguish between the isolates, morphological characters and molecular by through internal transcribed spacer sequencing by using primers ITS 1 and ITS 4 were performed. The main intention of this research was to analyse different morphological characters of the fungi and confirming the pathogen using both morpho and molecular methods. Since, infectivity of the pathogen *Pyricularia pennisetigena* on pearl millet is not reported in Southern parts of India, this study paved a way for better understanding of the pathogen characters.

MATERIALS AND METHODS

Collection and Isolation of the blast pathogen: Leaves with typical spindle shaped lesions, associated with blast disease were collected from the pearl millet field from 8 districts of Tamil Nadu which were designated with the code as PMBT (Tirupur), CO-10 (Coimbatore), DP1 (Dharmapuri), KR2 (Krishnagiri), ERT (Erode), ALR (Ariyalur), SLM (Salem),and TVM (Thindivanam). The pathogen was isolated on potato dextrose agar medium. Infected leaves were washed using sterile water and the infected leaf portions were cut into small bits using sterile scalpel blade. Then the leaf bits were sterilized using 1% sodium hypochlorite solution for about 30 seconds followed by washing in sterile distilled water for three times and placed in Whatman no. 40 filter paper. Using sterile forceps the leaf bits were placed in petri plates containing sterilized PDA medium which was amended with streptomycin (50µg/ml) under aseptic conditions. The plates were incubated at room temperature (25^{o}C). After initiation of growth is observed in the petri plates, single hyphal tip was transferred into new petri plate containing 15ml of sterilized PDA medium to obtain the pure culture of the fungi. For sporulating the fungus, the stem of the main host were cut into small bits (3-5 cm) and added to conical flasks, sterilized in an autoclave at 121^{o}C for 20 minutes at 15 lbs pressure. The mycelial disc (9mm) of 7 days old culture was added to each flask. After 15 days the stem bits were taken out under sterile conditions and added to eppendorf tube containing distilled water, shaked vigorously to dislodge the spores and the conidial morphology was observed under the light microscope (Vanaraj *et al.,* 2013). For additional research, the culture was maintained on PDA slants at 4 °C and stored at -20 °C for medium to long term preservation.

Examination of cultural and morphological characteristics. Pearl millet blast infected isolates were grown at 25°C for 7 days on PDA medium, following which mycelium discs were transferred to the middle of a fresh PDA medium, within 10-14 days, fungal mycelium had covered the whole plate. All of the isolates on PDA media were evaluated daily for 5 to 10 days for their colony shape (morphology), texture and colour. The mycelial characters, colour and septations were observed.

Pathogenicity assessment under *in vitro* and glasshouse conditions. Fresh leaves were collected and washed under running tap water. The leaves were then surface sterilized with 1% sodium hypochlorite and subsequently washed with sterilized distilled water for three times and placed on sterilized tissue paper for drying. The leaves were placed in petri plates which has been basally layered with cotton on which 4-5 drops of sterile water was added to maintain humidity, the leaves were placed on blotter paper placed above the cotton which was cut to fit the shape of the petri plate. A 9 mm actively growing mycelial disc was placed on the centre of the leaf. The plates were covered and incubated at a temperature of 22-24^{o} C. After 7-10 days, the lesions were developed on the region around the disc. For conducting the pathogenicity assay under glass house conditions, Pearl millet seeds were sown in plastic pots filled with a combination of three parts soil to one part farmyard manure. The isolates grown on PDA plates with actively growing fungal mycelia were flooded with 10-15 ml of sterile water and fungal mass containing mycelia were scrapped with sterile scalpel blade. It was filtered through a muslin cloth and concentration of spores were adjusted using haemocytometer to 5×10^4 conidia/ml. Tween 20 (0.02%) was added as an adhesive agent to spore suspension before inoculation. Inoculation was performed on test seedlings at the three to four leaf stages (about 15 days old plants) by spraying the inoculum till run-off under glass house conditions. Polythene bags were placed over the infected plants for 24 hours. Following the incubation phase, the test plants were watered on a daily basis to maintain high humidity levels favourable for disease development (>90% RH). Symptoms occurred 5-7 days post-inoculation.

Isolation and quantification of genomic DNA. Total DNA was extracted by using modified CTAB method. Isolates PMBT and CO-10 were cultured in PDA plates and 5 days after the cultures were transferred to potato dextrose broth (100 ml). The fungal mycelia (100-150 mg)were collected and ground to fine powder in a pestle and mortar by using liquid nitrogen. The ground and powdered mycelium was transferred to an Eppendorf tube (2 ml) which consists of a mixture of 1 ml of extraction buffer consisting of (2% CTAB buffer, 4M NaCl, 0.5M EDTA, 1M Tris-Cl, 0.02% β-Mercapto- ethanol). After 1 hour of incubation at 65°C, an equal amount of phenol, chloroform, and isoamyl alcohol (25:24:1) was added and the mixture was centrifuged at 12000 rpm for 10 minutes. After mixing well, the clear supernatant was put into an Eppendorf

tube and an equal amount of chloroform and isoamyl alcohol (24:1) was added. This mixture was mixed and the tube was spun at 12000 rpm for 10 minutes. Then, ice cold isopropanol was added into the supernatant, mixed thoroughly and refrigerated at -20°C for overnight. After centrifugation the supernatant was discarded and the DNA pellet was washed with 100% ethanol, air dried after centrifugation again and resuspended in 100 µl sterile distilled water. The quality and amount of fungal DNA were determined using gel electrophoresis and the Nanodrop Spectrophotometer. For use in the PCR process, the DNA samples were diluted to a concentration of 30-50 ng/µl after nanodrop quantification. (Amplification via polymerase chain reaction and documentation) (Doyle *et al.,* 1990).

Amplification via polymerase chain reaction and gel documentation. With the help of fungal universal primers, the ITS region of rDNA was successfully amplified using ITS 1 ((5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGTTGATATGC-3') primers (White *et al.,* 1990). Amplification of genomic DNA was carried out using a thermocycler (Master Eppendorf) using the following PCR conditions. The reaction mixture consisting of 10 µl of master mix, 4 µl of sterile water, each 2 µl of forward and reverse primers and genomic DNA 2 µl was used for the amplification process. The PCR was performed by initial denaturation at 94°C for 5 min which is followed by denaturation at 94°C for 60 sec, annealing at 58°C for 60 sec, extension at 72°C for 60 sec followed by a final extension at 72°C for 10 min for over 35 cycles (White *et al.,* 1990). Agarose gel prepared at a concentration of 1.2 percent of agarose in TAE (tris acetic acid) buffer containing ethidium bromide at a concentration of 2µl/100 ml and allowed to solidified to form agel with place of comb to form a well. The amplified PCR products were loaded in the wells and run by electrophoresis at a voltage of 80 for 1 hour followed by documentation and assessment using a gel documentation Transilluminator system (GelDoc, BIO-RAD, Canada). The PCR product size was compared to the standard 1kb ladder. The DNA got amplified around the region of 560 bp.

ITS region sequencing and phylogenetic tree construction. The PCR results were subsequently sequenced to authenticate the species. The resulting nucleotide sequences were analysed by using BLAST. Accession numbers were obtained after depositing the isolates in Gen Bank. Mega X Software was used to align the sequences of the isolates, and similar ITS1-ITS4 sequences were obtained from the NCBI gen bank in order to discriminate between the isolates. The neighbour-joining technique was used in the construction of a phylogenetic tree (Kumar *et al.,* 2016). The resilience of clades was assessed using a 1000 bootstrap replication.

RESULTS AND DISCUSSIONS

Collection and Isolation of *Magnaporthe grisea*. Blast infected pearl millet leaf samples were collected from eight districts of Tamil Nadu among which the virulent isolates were pertaining to the samples collected from Tirupur and Coimbatore district (Table 1) (Fig. 1). The samples were collected based on the symptoms such as typical eye shaped lesion with dark brown borders (Yi *et al.,* 2022). The leaf blast prevalent in hot western arid regions of Rajasthan were found to belong to the species *Pyricularia pennisetigena* which was validated from multiple sequence alignment verified from the deposited sequences in USA (Solanki *et al.,* 2022). The host physical stimulus and hydration plays a key role in appressorium development of the pathogen (Sharma *et al.,* 2019). The diseased leaf samples were isolated on PDA medium and incubated at 25^{o}C (Fig. 2). The pure culture of the pathogen was obtained by single hyphal tip method. For sporulating the pathogen, stem bits of the host were autoclaved and the mycelial discs of the pathogen were inoculated. After 2 weeks the conidia were observed on the surface of the stem bits (Fig. 3). *Magnoporthe grisea* infects leaves with spores, the pathogen's most crucial stage for disease emergence, which tend to germinate on a hydrophobic leaf surface. After adhering firmly to the plant's surface, the fungus produces immense pressure within the melanized appressorium and enters the leaf's epidermal cell using a small penetration peg (Heath *et al.,* 1992). The culture was kept on PDA slants and stored at 4 °C and -20 °C, respectively, for the course of the experiment.

Fig. 1. Symptoms observed in leaves and stem portion.

Table 1: Survey on collection of pathogen from various locations in Tamil Nadu.

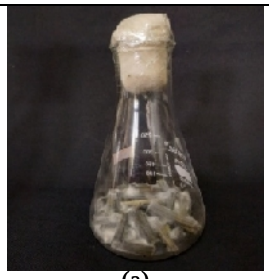
Sr. No.	Location	Village	GPS coordinates	Disease incidence(%)*
1.	Dharmapuri	Palakodu	12.2986° N, 78.0738° E	22.71[g] (28.46)
2.	Krishnagiri	Pochampalli	12.3780° N, 78.4117° E	31.43[e] (34.10)
3.	Salem	Vazhapadi	11.6555° N, 78.4013° E	26.56[f] (31.02)
4.	Erode	Thalavadi	11.8044° N, 76.9953° E	35.80[c] (36.75)
5.	Ariyalur	T. Palur	11.0944° N, 79.3699° E	33.16[d] (35.16)
6.	Tiruppur	Udumalaipet	10.5855° N, 77.2513° E	39.75[b] (39.08)
7.	Coimbatore	New area, Department of millets, TNAU	12.2986° N, 78.0738° E	38.24[b] (38.20)
8.	Villupuram	Thindivanam	12.2267° N, 79.6504° E	42.79[a] (40.85)
	CD(p=0.05)			1.636

*Mean of three replications; Values in the parenthesis are arc sine transformed values

(a) PMBT 1

(b) CO-10

Fig. 2. Morphology of the culture on PDA medium.



(a)

(b)

Fig. 3. Mycelial discs inoculated on autoclaved stem bits of the host and the conidia observed from the surface o the bits (a- mycelium of the pathogen covered on the surface of autoclaved stem bits, b - spores observed on the stem bits observed under light microscope (40 x).

Examination of cultural and morphological characteristics. The mycelial characters of the fungi including the colour, colony characters and morphology (pattern) on PDA medium were observed. The radial growth and texture of the colony varied substantially for the isolates. The growth and colour of the mycelia ranged from greyish white to blackish grey in colour on PDA medium (Table 2). On potato dextrose agar, blast fungus had greyish colonies with smooth circular margins and concentric rings (Sonah *et al.*, 2009). The pathogen was found to be *Magnaporthe* based on its conidial structures and colony characteristics (Ou, *et al.*, 1985). The fungal pathogen identified as *M. grisea* in this investigation was based on the main morphological and cultural features described by (Bourett *et al.*, 1990). The morphology of pathogenic cultures varied from one culture to others and based on medium also. The quantity of aerial mycelium varies greatly, ranging from a thin to a dense cottony mass. The cultural and morphological differences revealed among the pearl millet blast pathogen isolates matched prior results on *P. grisea* made by other researchers investigating on the same topic (Srivastava *et al.*, 2014). The spores were examined under the microscope. The shape, presence of septation, hilum etc. were observed.

Table 2: Morphological features of the pathogen observed on PDA medium.

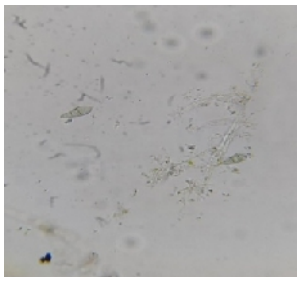
Sr. No.	Isolate	PDA Medium			Conidia	
		Colour	Hyphae	Radial mycelial growth (cm)	Length (µm)	Breadth (µm)
1.	VIR	Blackish grey	Aerial	7.5	9.26	3.65
2.	PMBT	Grey	Effuse	8.7	8.42	4.03
3.	CO-10	Greyish white	Aerial	8.5	9.01	3.99
4.	TVM	Whitish grey	Effuse	7.9	8.73	3.54

The isolate's conidiophores were discovered to be thin and straight, containing clusters of conidia that were generally pyriform or obclavate and 2-3 septate (Getachew *et al.,* 2014). The pyriform shape of the conidia indicates that *Pyricularia grisea* is an asexual anamorph of the *Magnaporthe* isolate.

Pathogenicity assessment under *in vitro* and glasshouse conditions. The virulent isolate among the two was finalized as virulent based on the assay conducted under *in vitro* in which fresh untreated leaves of the host were taken. The leaves were surface sterilized using 1% sodium hypochlorite solution followed by washing with sterile distilled water 3 times. The 9mm mycelial disc of 7 days old culture was placed in the leaf surface. The lesions were formed around the mycelial disc after a week (Fig. 4). The pathogen becomes necrotrophic at 48 hours after inoculation by producing thin-invasive hyphae which is followed by the development of peg which differentiates into lobed- bulbous infectious hyphae that will expand intracellularly and intercellularly causing blast lesions (Howard *et al.,* 1996). For conducting the pathogenicity test under glass house conditions, seeds were sown in pots containing mixture of FYM and soil. The spore suspension was sprayed at a rate of 5×10^4 conidia/ml with tween 20 (0.02%) which was used as an adhesive (Fig. 5). Based on the symptom development the isolates PMBT and CO-10 showed virulent nature compare to other isolates. In accordance with Koch's postulate, pathogens were determined to be related with disease at all stages of crop development and were able to produce disease when re-inoculated into the same host. Hence study was conducted by testing the pathogenicity of the detected field strain on the same pearl millet variety from which the organism was obtained. The re-isolation of the pathogen was done and it was found to be the same kind as the previously isolated one. Thus, Koch's postulate was demonstrated.

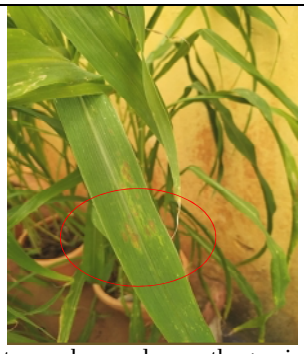
(a). Lesion around the mycelium disc placed on the cut host leaves

(b). prominent spindle shaped lesions formed on the leaf

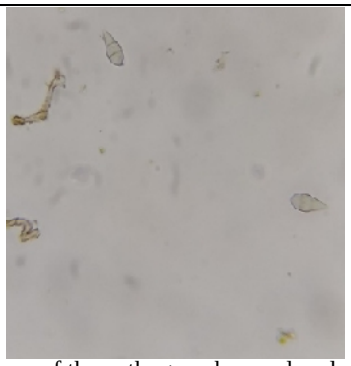


(c) Spore of the pathogen observed under light microscope

Fig. 4. Pathogenecity test conducted *in vitro*- detached leaf assay.



(a) Symptoms observed on pathogen inoculated pearl millet leaves after 7 days



(b) Spore of the pathogen observed under light microscope (40x)

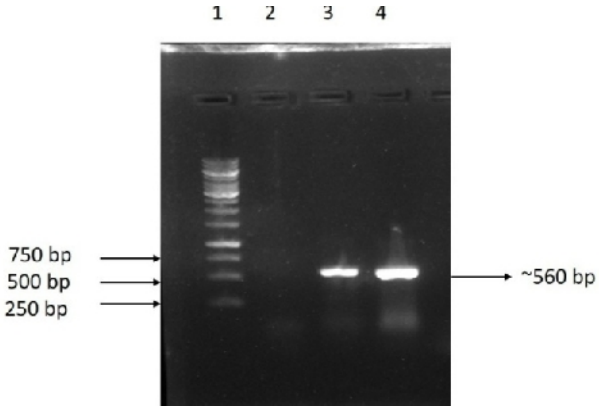
Fig. 5. Pathogenecity test conducted under glass house conditions.

Molecular characterization of the pathogen:

Amplification via polymerase chain reaction and gel documentation. DNA extraction, PCR amplification using ITS PCR primers, and Sanger sequencing were used to identify and validate the molecular identity of the field sample. ITS rDNA sequencing and molecular analysis were done. Two isolates *viz*., PMBT and CO-10 which were virulent among others were opted for performing molecular approaches. Molecular detection of *Pyricularia grisea* and *Pyricularia pennisetigena* using the primers ITS 1 and ITS 4 for amplification of isolates were done (Fig. 6). The amplification values were found to be approximately 560bp which was similar to our experiments (Chuwa *et al.,* 2013). The amplicon of size 520 bp was observed in course of amplifying, *Magnaporthe oryzae* isolates from Eastern India using universal primers ITS 1 and ITS 4 (Priyanka *et al.,* 2021). To confirm the pathogen at species level, the PCR products were purified up and sequenced.

ITS region sequencing and phylogenetic tree construction. Phylogenetic relationship of the isolates

with other nucleotides available in NCBI are done to know the category in which the isolates are genetically related with (Fig. 7) Isolates of *Pyricularia* infecting different hosts were taken for phylogenetic analysis (Klaubaf *et al.*, 2014). The *Pyricularia grisea* isolate showed 98.90% and *Pyricularia pennisetigena* isolate showed 100% identity when compared with sequences available in NCBI and these isolates sequenced were deposited NCBI genbank (Accession number- *Pyricularia grisea* -OM883863 and *Pyricularia pennisetigena* ON116174) (Table 3). Phylogenetic tree constructed by using MEGA X software revealed that the isolate *Pyricularia grisea* showed 26% similarity and *Pyricularia pennisetigena* showed 91% similarity with the isolates in various clades taken for phylogenetic tree construction.



1. 1Kb ladder, 2. none, 3. PMBT, 4. CO-10

Fig. 6. Gel electrophoresis of amplified DNA fragments.

Fig. 7. Phylogenetic tree constructed using the MEGA X software using neighbour joining method.

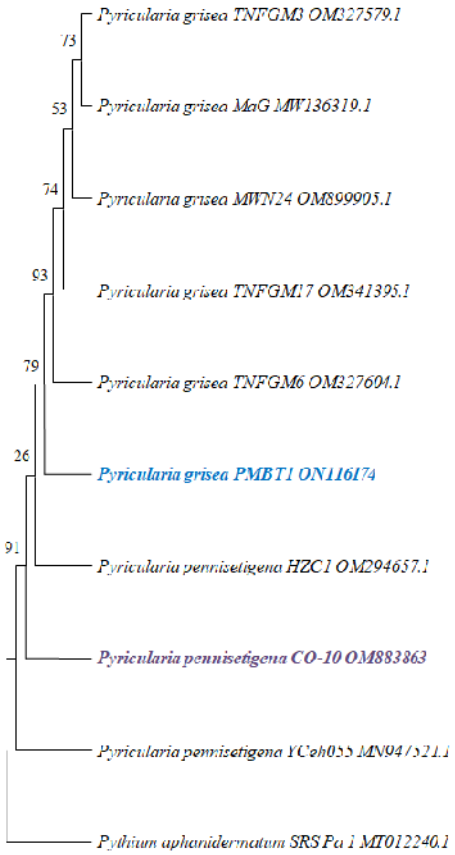


Fig. 7. Phylogenetic tree constructed using the MEGA X software using neighbour joining method.

Table 3: Accession number of the isolates obtained by depositing in NCBI database.

Sr. No.	Location	Isolate code	GenBank Accession number
1	Tirupur	PMBT	OM883863
2	Coimbatore	CO-10	ON116174

CONCLUSION

The findings of this study validated the symptomatology, isolation, morphological characterization and molecular characterization of the *Pyricularia* isolates collected from 8 districts of Tamil Nadu. Species level confirmation of virulent isolates and submission of the sequences in NCBI GenBank database and acquisition of accession number were obtained. Phylogenetic tree was constructed to know the closely related species in relation to the isolate.

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Conflict of Interest. None.

REFERENCES

Bourett, T. M., & Howard, R. J. (1990). In vitro development of penetration structures in the rice blast fungus *Magnaporthe grisea*. *Canadian Journal of Botany*, *68*(2), 329-342.

Cazal-Martínez, C. C., Reyes-Caballero, Y. M., Chávez, A., Pérez-Estigarribia, P. E., Rojas, A., Arrua, A., & Kohli, M. M. (2021). First report of a leaf blight caused by *Pyricularia pennisetigena* on *Cenchrus echinatus* in Paraguay. *Plant Disease*, *105*(11), 3758.

Chuwa, C. J., Mabagala, R. B., & Reuben, M. S. O. W. (2013). Pathogenic Variation and molecular characterization of *Pyricularia oryzae*, Causal agent of rice blast disease in Tanzania. *Int. J. Sci and Res*, *4*(11), 1131-1139.

Getachew Gashaw, G., Alemu, T., & Tesfaye, K. (2014). Morphological, physiological and biochemical studies on *Pyricularia grisea* isolates causing blast disease on finger millet in Ethiopia. *Journal of Applied Biosciences*, *74*, 6059-6071.

Heath, M. C., Howard, R. J., Valent, B., & Chumley, F. G. (1992). Ultrastructural interactions of one strain of *Magnaporthe grisea* with goosegrass and weeping lovegrass. *Canadian Journal of Botany*, *70*(4), 779-787.

Howard, R. J., & Valent, B. (1996). Breaking and entering: host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. *Annual review of microbiology*, *50*, 491-513.

Klaubauf, S., Tharreau, D., Fournier, E., Groenewald, J. Z., Crous, P. W., De Vries, R. P., & Lebrun, M. H. (2014). Resolving the polyphyletic nature of *Pyricularia* (Pyriculariaceae). *Studies in mycology*, *79*(1), 85-120.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, *33*(7), 1870-1874.

Mehta, P. R., Babu, S., & Mathur, S. C. (1952). A new leaf spot disease of bajra (*Pennisetum typhoides* Stapf and Hubbard) caused by a species of *Piricularia*. *Indian Phytopathology*, *5*(2), 140-143.

Ou, S. H. (1985). *Rice diseases*. IRRI.

Patni, D., & Agrawal, M. (2017). Wonder millet–pearl millet, nutrient composition and potential health benefits–a review. *International Journal of Innovative Research and Review*, *5*(1), 6-14.

Priyanka, P., Kumar, Y. M., Prabhukarthikeyan, S. R., Raghu, S., Keerthana, U., Umakanta, N., & Pramesh, D. (2021). Morpho-molecular characterization of Indian population of *Magnaporthe oryzae*, an incitant of blast disease in rice. *Research Journal of Biotechnology Vol*, *16*, 1.

Serba, D. D., Perumal, R., Tesso, T. T., & Min, D. (2017). Status of global pearl millet breeding programs and the way forward. *Crop Science*, *57*(6), 2891-2905.

Sharma, N., & Gautam, A. K. (2019). Early pathogenicity events in plant pathogenic fungi: A comprehensive review. In *Biol. Forum* (Vol. 11, No. 1, pp. 24-34).

Solanki, R. K., Kakani, R. K., Singh, S. K., Sharma, R., & Gupta, S. K. (2022). Identification of pearl millet genetic resources having resistance against leaf blast caused by *Pyricularia pennisetigena* for hot and arid environments of Western India.

Sonah, H., Deshmukh, R. K., Parida, S. K., Chand, S., & Kotasthane, A. (2009). Morphological and genetic variation among different isolates of *Magnaporthe grisea* collected from Chhattisgarh. *Indian Phytopathology*, *62*(4), 469.

Srivastava, D., Shamim, M. D., Kumar, D., Pandey, P., Khan, N. A., & Singh, K. N. (2014). Morphological and molecular characterization of *Pyricularia oryzae* causing blast disease in rice (*Oryza sativa*) from North India. *International Journal of Scientific and Research Publications*, *4*(7), 2250-3153.

Thakur, R. P., Sharma, R., Rai, K. N., Gupta, S. K., & Rao, V. P. (2009). Screening techniques and resistance sources for foliar blast in pearl millet. *Journal of SAT Agricultural Research*, *7*, 1-5.

Timper, P., Wilson, J. P., Johnson, A. W., & Hanna, W. W. (2002). Evaluation of pearl millet grain hybrids for resistance to *Meloidogyne* spp. and leaf blight caused by *Pyricularia grisea*. *Plant Disease*, *86*(8), 909-914.

Vanaraj, P., Saveetha, K., Ramalingam, S. A. R., & Sabariyappan, R. (2013). Variability in *Pyricularia oryzae* from different rice growing regions of Tamil Nadu, India. *African Journal of Microbiology Research*, *7*(26), 3379-3388.

Wilson, J. P., & Gates, R. N. (1993). Forage yield losses in hybrid pearl millet due to leaf blight caused primarily by *Pyricularia grisea*. *Phytopathology*, *83*(7), 739-744.

Yi, R. H., Huang, Y., & Pang, R. (2022). Leaf blast, a new disease on *Pennisetum sinese* caused by *Pyricularia pennisetigena* in China.

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