Original Research Article

Impact of different cultural media on growth and sporulation of *Magnaporthegrisea* causing blast of finger millet

Abstract

Finger millet (*Eleusine coracana*), a vital cereal crop, suffers significant yield losses due to blast disease caused by *Magnaporthegrisea*. Understanding the sporulation dynamics of the pathogen is crucial for elucidating its biology, infection mechanisms, and epidemiology, as well as for developing effective disease management strategies. This study investigated the influence of different cultural media on the vegetative growth and conidial production of *M. grisea*. Among the tested media, Oat meal agar, Corn meal agar, Rice bran agar and Rice straw extract agar supported the growth of the pathogen. Whereas, Oat meal agar demonstrated superior efficacy in promoting both mycelial proliferation and sporulation. The findings emphasize the role of optimized media in facilitating reliable and abundant spore production, essential for advancing research in understanding the multi dynamics involved in disease occurrence, pathogen management and resistance breeding. These results contribute to the development of integrative approaches to mitigate the impact of blast disease on finger millet production and ensure agricultural sustainability.

Introduction

Finger millet (*Eleusine coracana*), a vital cereal crop in subtropical and tropical regions, is a staple for millions due to its resilience to drought, high nutritional content, and adaptability to marginal soils and role in supporting food security among smallholder farmers [1]. It is rich in essential nutrients, including calcium, iron, and dietary fiber, making it a valuable food source for regions prone to food insecurity and malnutrition[2]. In addition, finger millet's ability to thrive in less fertile soils under limited water conditions makes it a key crop for sustainable agriculture in semi-arid and resource-limited areas. However, despite

its hardiness, finger millet faces a major threat from blast disease, caused by the fungal pathogen *Magnaporthe grisea*[3].

Blast disease in finger millet is primarily characterized by necrotic lesions on leaves, stems, and panicles, which disrupts the plant's photosynthetic activity, weakens structural integrity, and results in considerable yield losses. In severe cases, losses can reach up to 80% or even lead to total crop failure, posing a significant threat to food security in areas reliant on finger millet[1]. This pathogen can infect several economically important cereals, with blast disease severely impacting yield and quality. Given that *M. grisea* can spread rapidly under favorable conditions, it is crucial to understand the factors that promote its growth and sporulation, especially since its conidia (spores) serve as the primary inoculum for new infections. These spores are easily disseminated by wind, water, or mechanical contact, enabling the pathogen to infect healthy crops quickly, intensifying the disease spread across large areas[4].

A critical aspect of *M. grisea*'s pathogenicity is its ability to produce abundant spores, which are essential for its propagation, infection, and survival under diverse environmental conditions. Understanding factors that influence the growth and sporulation of *M. grisea* is vital for developing effective management strategies. Cultural media composition plays a crucial role in determining the fungus's growth rate, colony morphology, and sporulation capacity. By optimizing media that enhance spore production, researchers can facilitate in vitro studies on pathogen biology, host interactions, and fungicide screening[5]. Moreover, identifying suitable media for spore production can aid in laboratory studies, where a reliable supply of spores is necessary to study infection dynamics, test disease resistance in finger millet varieties, and develop integrated disease management strategies.

Moreover, insights into the sporulation patterns of *M. grisea* on different media can help in creating standardized protocols for disease assessment and pathogen quantification in laboratory settings. By studying the impact of various media on growth and spore production, researchers can gain knowledge to design efficient pathogen culture methods and screen disease-resistant finger millet varieties. Ultimately, this study aims to evaluate the impact of different cultural media on the growth and sporulation of *M. grisea* to identify optimal conditions for spore production. Understanding these conditions will support research efforts to advance our understanding of *M. grisea* biology and provide valuable resources for ongoing efforts to manage and mitigate the effects of blast disease on finger millet

production, ensuring greater food security and sustainability for communities that rely on this essential crop.

Materials and Methods

The pathogen was isolated from finger millet showing typical symptoms of blast disease using the standard spore drop technique described by [6]. Blast-infected tissues were cut into small pieces, surface sterilized in a 1% sodium hypochlorite solution for 30 seconds, and rinsed with sterile distilled water three times. The surface-sterilized blast lesions were placed on sterilized moist cotton in separate Petri plates and incubated for 24-48 hours at 25±1 °C. After incubation, the blast-infected tissues were transferred to sterilized moist cotton attached to the inner surface of the upper lid of a Petri plate containing rice straw extract agar medium in the bottom portion. The plates were sealed with parafilm tape and incubated at 25±1 °C for three days or until tiny fungal colonies appeared on the medium. A portion of the mycelial disc from a single colony was transferred to a sterile Petri plate containing 1 mL of RSE broth, macerated with a sterile glass rod, and single spore isolation was performed. Three to four days after incubation at 25±1 °C, individual fungal colonies developed on RSEA medium.

The pathogen was identified as *M. grisea* based on cultural, mycelial and conidial morphology followed by molecular confirmation (ITS region) by sequencing. Pathogenecity assays also proved the obtained culture as the pathogen, *M. grisea*.

The influence of eight culture media viz., Ragi yeast lactose agar, Potato dextrose agar, Oat meal agar, Corn meal agar, Sabouraud dextrose agar, Water agar, Rice bran sucrose agar and Rice straw extract agar on growth and sporulation of *M. grisea* were studied. The composition of the above media was obtained from "Ainsworth and Bisby's Dictionary of the Fungi" by [7] and the media prepared by following standard procedures[8] . Table 1. represents the composition of the different media used in the study.

Seven milli meter diameter disc of pure culture of the fungus was placed in the center of plates and incubated at 27±1 ^{0}C for 15 days. There were three replicates of each treatment. Observation related to cultural characters like colony colour, texture, surface, type of margin, lusture, pigmentation and sporulation of the blast pathogen were recorded.

Table 1: Composition of different media

Sl. No.	Media	Compound	Quantity
1	Ragi yeast lactose agar	Ragi powder	20 g
		Lactose	5 g
		Yeast powder	1 g
		Agar agar	20 g
		Sterile distilled water	1000 mL
2	Potato dextrose agar	Potato dextrose broth	24 g
		Agar agar	20 g
		Sterile distilled water	1000 mL
3	Oat meal agar	Oat meal powder	40 g
		Agar agar	20 g
		Sterile distilled water	1000 mL
4	Corn meal agar	Corn meal infusion	50 g
		Agar agar	20 g
		Sterile distilled water	1000 mL
5	Sabouraud dextrose agar	Sabouraud dextrose agar	65 g
6	Water agar	Agar agar	20 g
		Sterile distilled water	1000 mL
7	Rice bran agar	Rice bran	40 g
		Sucrose	20 g
		Agar agar	20 g
		Sterile distilled water	1000 mL
8	Rice Straw Extract Agar	Rice straw	150 g
		Sucrose	20 g
		Agar agar	20 g
		Distilled water	1000 mL

Results and Discussion

The pure culture of *M. grisea* was successfully isolated on Rice straw extract agar medium and identified based on the morphological and molecular characteristics as well as pathogenicity of the fungal culture.

The results of the cultural studies on solid media indicated that corn meal agar, oat meal agar, rice bran agar and Rice straw extract agar supported significantly the maximum colony growth (90.0 mm) followed by Potato dextrose agar (82.3 mm). Similar trend was observed when growth rate was considered with highest being 6.33 mm/day. The pathogen produced differently coloured colonies on various media. On Sabouraud dextrose agar, it showed a whitish colony with a grey center, while on potato dextrose agar, the colonies were light grey with whitish margins. *M. grisea* generally formed cottony textured colonies except on water agar, where immersed colonies were noticed. All media tested resulted in colonies with regular margins. The colony topography ranged from flat to raised, with only water agar showing immersed colonies. Pigmentation was noted across all media, varying from dark to concentric patterns.

Good sporulation was recorded only in Oat meal agar medium after ten days post inoculation. Among all cultural media, the Oat meal agar medium was found to be most suitable for culture growth and sporulation (Table 2, Plate 1 and Plate 2).

The following results are in line with the findings of [9], [10] , [11] and[12]. They documented that, by the seventh day, sporulation decreased to 0.5×10^5 spores per ml in RSEA, while in OMA, it increased to 7.4×10^5 spores per ml. Oatmeal provides a rich source of carbohydrates, proteins, and essential nutrients that support fungal growth and sporulation [13] and [14].

Conclusion

In conclusion, this study underscores the critical importance of understanding *M. grisea* sporulation for effective management of blast disease in finger millet. The research highlights the significant influence of cultural media on fungal morphology, growth rate, pigmentation, and sporulation. Among the media tested, Oat Meal Agar (OMA) was identified as the most suitable for both growth and sporulation of *M. grisea*, while Corn Meal Agar (CMA), Rice Bran Agar (RBA), and Rice Straw Extract Agar (RSEA) also supported its growth. These findings are essential for producing spores, facilitating pathogen-host interaction studies, screening of resistant cultivars and evaluating fungicides efficacy against the pathogen, thereby enhancing our understanding of *M. grisea* and contributing to effective disease management and sustainable finger millet cultivation.

Table 2:Cultural and morphological characteristics of *M. grisea* on different agar media

Sl. No.	Media	Mycelial growth (mm)	Growth rate (mm/day)	Colony colour	Colony texture	Surface and topography	Margin	Pigmentation character	Sporulation index	Days to sporulate
1	Ragi yeast lactose agar	77.3 *(61.54)	5.52 (13.59)	Grey	Cottony	Flat	Regular	Dark	-	-
2	Potato dextrose agar	82.3 (65.12)	5.88 (14.03)	Light Grey with whitish Margin	Cottony	Raised	Regular	Concentric	-	-
3	Oat meal agar	90.0 (71.54)	6.43 (14.68)	Light Grey	Cottony	Raised	Regular	Dark	+++	10
4	Corn meal agar	90.0 (71.54)	6.43 (14.68)	Grey	Cottony	Flat	Regular	Concentric	-	-
5	Sabouraud Dextrose agar	74.0 (59.32)	5.29 (13.29)	Whitish Colony with Grey center	Cottony	Raised	Regular	Concentric	-	-
6	Water agar	48.0 (43.84)	3.43 (10.67)	Light Grey	Immersed	Immersed	Regular	Concentric	-	-
7	Rice bran sucrose agar	90.0 (71.54)	6.43 (14.68)	Grey	Cottony	Flat	Regular	Dark	-	-
8	Rice straw extract agar	90.0 (71.54)	6.43 (14.68)	Grey	Cottony	Flat	Regular	Dark	-	-
	CD (1%)	1.51	0.20							
	S. Em	0.36	0.05							

Note: *arc sine transformation values; -= no sporulation, ++++= excellent (>30 spores/400x) sporulation

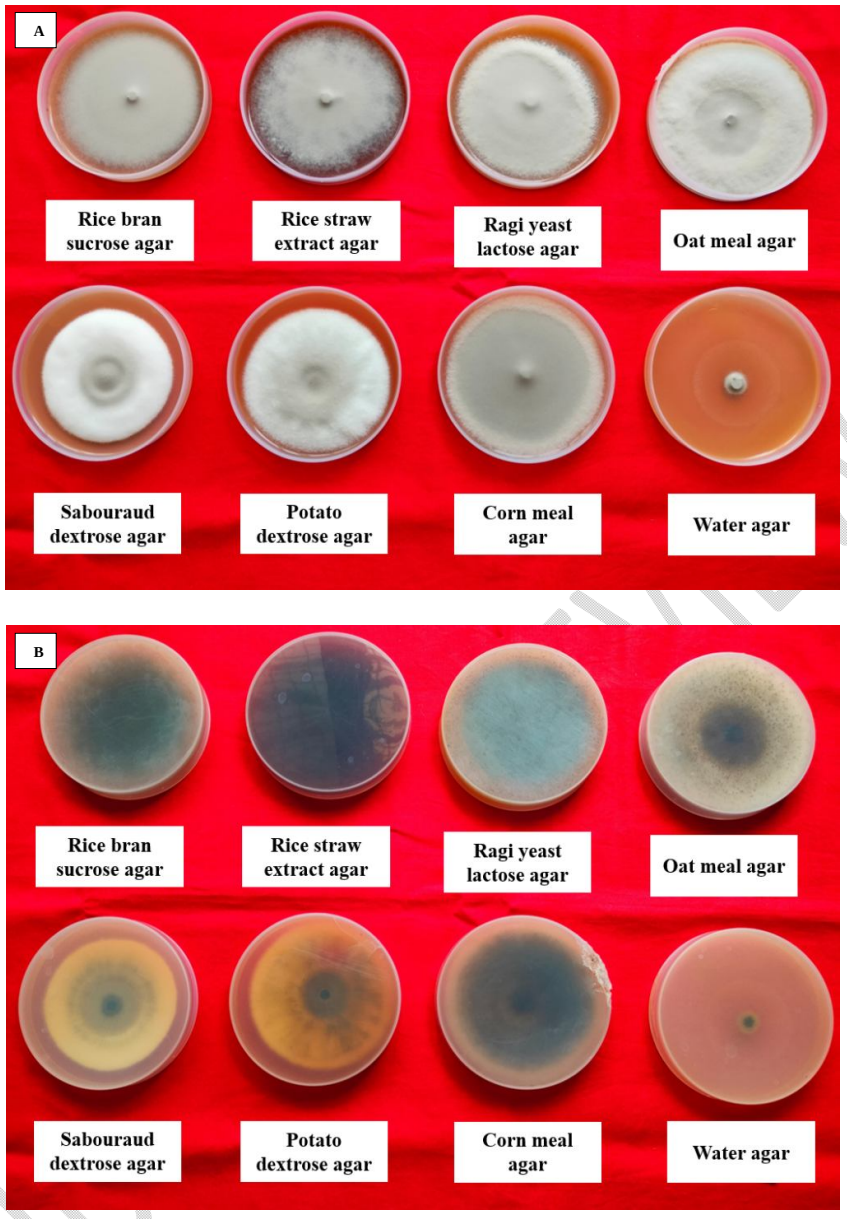
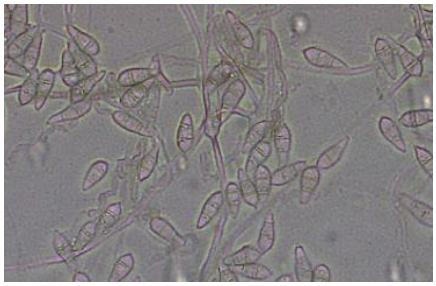


Plate 1: Effect of different media on growth of *M. grisea* A) upper surface B) lower surface

Plate 2: Conidia of *M. grisea* on Oat meal agar medium



References

1. Poonacha, T. T. *et al.* Blast disease of millets: present status and future perspectives. *Millets-Rediscover Ancient Grains* (2023).
2. Maharajan, T., Ceasar, S. A. & Ajeesh Krishna, T. P. Finger Millet (*Eleusine coracana* (L.) Gaertn): Nutritional Importance and Nutrient Transporters. *Critical Reviews in Plant Sciences***41**, 1–31 (2022).
3. Palanna, K. B. *et al.* Current Scenario and Integrated Approaches for Management of Finger Millet Blast (Magnaporthe grisea). in *Blast Disease of Cereal Crops* (eds. Nayaka, S. C., Hosahatti, R., Prakash, G., Satyavathi, C. T. & Sharma, R.) 27–49 (Springer International Publishing, Cham, 2021). doi:10.1007/978-3-030-60585-8_3.
4. Palanna, K. B. *et al.* Geographic distribution, host preference and phylogenetic relationships among *Pyricularia* species inciting millet and rice blast disease in India. *Plant Pathology***73**, 692–705 (2024).
5. Jadhao, K. R. & Rout, G. R. A new growth medium for identification and isolation of Pyricularia grisea from finger millet (Eleusine coracana L. Gaertn.). *Current Science***116**, 1955–1957 (2019).
6. Rajashekara, H. *et al.* An efficient technique for isolation and mass multiplication of Magnaporthe oryzae from blast infected samples. *Indian Phytopathol***69**, 260–265 (2017).
7. Ainsworth, G. C. *Ainsworth & Bisby's Dictionary of the Fungi*. (Cabi, 2008).
8. Tuite, J. Plant pathological methods. Fungi and bacteria. (1969).
9. Kulmitra, A. K. *et al.* Growth of Rice blast fungus Pyricularia oryzae (Cav.) on different solid and liquid media. *International Journal of Current Microbiology and Applied Sciences***6**, 1154–1160 (2017).

10. Tripathi, S. K. Effect of media, pH, nitrogen, carbon sources and light on the growth and sporulation of Pyricularia grisea. *Annals of Plant Protection Sciences***14**, 166–168 (2006).

11. Farooqkhan, K. B., Tharana Poonacha, T. & Anusha, M. N. Beyond nourishment: The impact of micronutrients on Magnaporthe grisea sporulation infecting finger millet.

12. Molinari, C. & Talbot, N. J. A Basic Guide to the Growth and Manipulation of the Blast Fungus, *Magnaporthe oryzae*. *Current Protocols***2**, e523 (2022).

13. Dagar, B. *et al.* Morphology and Influence of Abiotic Factors on Magnaporthe grisea. *International Journal of Plant & Soil Science***34**, 176–187 (2022).

14. Aravind, K., Babu, T. K., Rajeswari, B. & Pushpavalli, S. Precise Methods for Single Spore Isolation and Controlled Sporulation in Magnaporthe oryzae Isolates. *International Journal of Environment and Climate Change***13**, 2411–2418 (2023).