A new growth medium for identification and isolation of *Pyricularia grisea* from finger millet (*Eleusine coracana* L. Gaertn.)

Finger millet belongs to the family Poaceae, an important cereal crop. Finger millet is the third most important small millet among other millets after sorghum and pearl millet. It is widely grown in different parts of Africa and South Asia including India[1,2]. In India, finger millet, popularly known as 'Ragi', is a potential food crop due to its wide adaptability to diverse climatic and cultural conditions. It is generally called 'nutritious millet' and contains proteins, calcium, minerals and vitamins over other cereals. It contains 9.2% protein, 76.32% carbohydrates and 1.29% fat. It is rich in calcium, iron, protein, fibre and other minerals, which are the core ingredients of normal human diet[3]. In addition, finger millet is well-known for its drought tolerance and diverse pathogen resistance[2]. This crop is highly vulnerable to blast disease caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*)[4]. This disease is very destructive and mostly occurs during rainy and winter seasons[5,6]. The disease occurs at leaf, neck and finger blasts of plant growth. Leaf blast is the initial and most damaging stage. It affects vital traits such as length and number of fingers, number of productive tillers and quality of grains[7]. Besides, the fungus also attacks the neck and subsequently affects the entire plant which reduces the grain production drastically[8]. It is reported to undergo a yield loss of about 28–36% each year in Asia due to blast diseases[6] and it can go as high as 80–100% in certain areas[9].

Very scanty information is available on the biology of *Pyricularia* species of finger millet even though it has nutritional importance. Therefore, it is essential to study different aspects of disease initiation and development and determination of nutritional and physiological conditions of the growth and sporulation of the fungus. Isolates collected from different hosts differ in their response in media with regard to mycelium growth and sporulation. Hence, this study was conducted to know the physiological and biochemical requirements for initiation of growth and development of the pathogen and the need to identify the suitable media for growth and sporulation of *P. grisea* isolates from finger millet.

Infected leaf samples (blast disease) of *E. coracana* were collected from the conservation farm of Orissa University of Agriculture and Technology during *Kharif* season (Figure 1 *a*). The pathogen *P. grisea* was isolated using the standard tissue isolation method[10]. Blast-infected leaves were segmented into small pieces and subsequently washed thrice in sterile distilled water. The cut leaves samples were surface-sterilized with 0.1% (w/v) mercuric chloride solution for 60 sec followed by rinsing thrice in sterilized distilled water. The samples were blotted dry to remove the excess water. Well-dried leaves samples were used for culturing and isolation of pathogen on two growth media.

Two types of culture media with modification were prepared, the growth and sporulation evaluated and the stages of *P. grisea* identified. The pH of both the media was adjusted to 7.5 by using 1/10th HCl. Oat meal agar media composed of 72.5 g of oat meal agar were suspended in 800 ml of sterilized distilled water and boiled for 10–15 min to dissolve the ingredient completely. Final volume was made up to one litre with sterilized distilled water and autoclaved at 15 kg/cm^2 at 121°C for 15 min. The medium was cooled to 45–50°C and mixed well before pouring into sterile petri plates. Ragi yeast extract Mannitol Media (RYMA) composed of ragi seed flour (20 g/l) along with yeast extract (1 g/l), mannitol (5.50 g/l) and agar-agar (20 g/l) were taken and mixed well with 800 ml boiled sterile distilled water to dissolve the constituents properly. The final volume was made up to 1000 ml and autoclaved at 121°C for 15 min. The media was then cooled down to 45–50°C and mixed well before pouring into sterile petri plates. The pH of both the media was adjusted with 1/10th HCl to 7.5. After solidification, well-dried cut leaf samples were transferred to petri plates containing oat meal agar (OMA) and ragi yeast extract mannitol agar (RYMA) media aseptically. The petri plates were incubated at 25 ± 1°C with an alternate 8 h photoperiod for 12–14 days. The data was recorded on growth and sporulation of *P. grisea.*

The microscopic preparations of fungal samples were carried out using lacto phenol cotton blue (LPCB) wet mount straining[11]. The microscopic examinations of spore and mycelium characteristics were recorded. The preparation has three components, i.e. phenol, lactic acid and cotton blue. To observe the structure of fungi and subsequent identification of pathogen to the species level, the following standards described by Barnett and

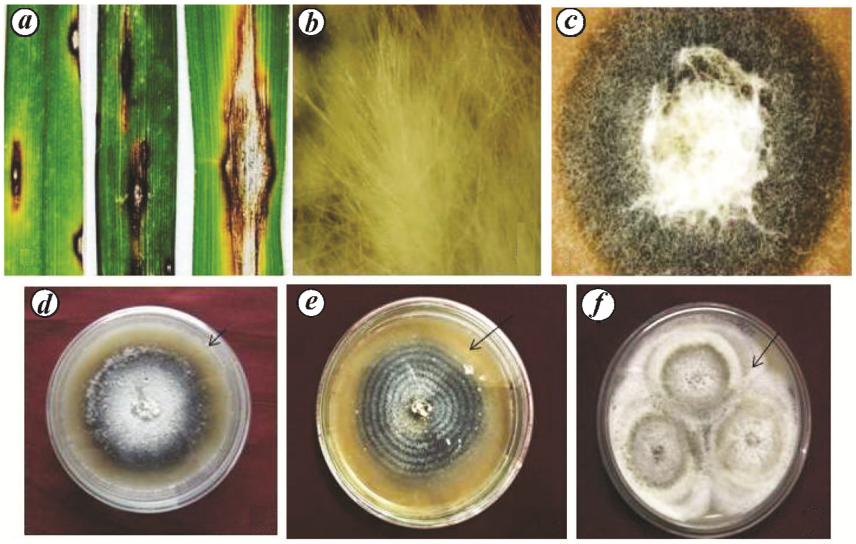

Figure 1. Growth of *Pyricularia grisea*: ***a***, Blast disease lesion; ***b***, Mycelium growth; ***c***, Initial colony growth colour; ***d***, Smooth and uniform growth in RYMA medium; ***e***, Concentric rings in RYMA medium; ***f***, Concentric rings in OMA medium

Table 1. Characteristics of *Pyricularia grisea* on culture media

Media	Colony colour	Growth type	Colony margin	Surface and topography	Sporulation	Colony diameter (mm) ± SE
OMA	Buff and greyish black	Uniform	Smooth	Raised mycelia growth with concentric rings	Low and incomplete	81.7 ± 1.7
RYMA	Black and light greyish in the centre	Uniform	Smooth	Minimum mycelia growth with concentric ring	High and excellent	79.5 ± 1.4

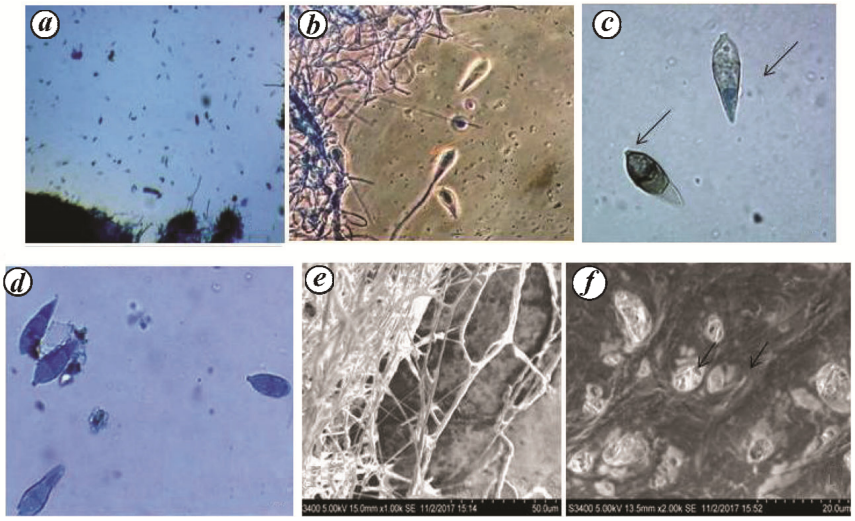

Figure 2. Light and scanning electron microscopic study of *P. grisea*. ***a***, Spores of isolate (10×); ***b***, Conidiophore of isolate (40×); ***c***, Compartment of conidia (100×); ***d***, 3-celled shape of conidia (100×); ***e***, Scanning electron image of mycelium (scale bar, 50 µm); ***f***, Scanning electron image of conidia (scale bar, 20 µm).

Hunter[12] were carried out. Light microscopy images were captured by visual scanning at 10×, 40× and 100× magnifications.

For SEM study, both mycelia and conidia of *P. grisea* were fixed with gold coating. All samples were imaged in a scanning electron microscope (Hitachi S3400N, Japan). A gold-coated specimen of mycelium was imaged under high vacuum at 5 kV, with 15.0 mm working distance and a 50 µm objective lens aperture. Image of conidia was obtained using the vacuum with working distance of 13.5 mm and 20 µm of objective lens aperture.

A new growth medium was formulated for better growth and sporulation of *P. grisea* collected from *Eleusine coracana*. There were distinct morphological differences in colour, growth and sporulation of *P. grisea* in the studied culture media (Table 1). In the case of OMA medium, the growth of *P. grisea* became buff and greyish black colour with concentric rings. Whereas, RYMA medium produces black colour margin with a light greyish colour in the centre. The growth of pathogen in both the media was smooth and uniform. It was also observed that the colony colour variation was due to the difference in spore production of *P. grisea* on various media[13]. A good and uniform mycelium growth with concentric ring pattern was observed in both the media. In OMAM medium, mycelium growth was different compared to RYMA media. OMAM favours the mycelium growth of *P. grisea* higher than RYMA medium as reported earlier by Susan and Ambika[14]. They also reported that the mycelium growth on both media was white to grey in colouration (Figure 1 *b* and *c*). Barnett and Hunter[15] and Getachew *et al.*[16] reported that the test fungus identified as *P. grisea* produces white to grey colour of aerial mycelium. The highest sporulation was observed in the RYMA medium. However, OMAM produces less sporulation and more vegetative growth compared to RYMA medium (Figure 1 *d* and *f*). Excellent sporulation was observed on RYMA medium, whereas incomplete sporulation occurred on OMAM media. On the basis of light microscopy and scanning electron microscopy, the study revealed that the shape of the conidia in all the isolates was typically pyriform, rounded base, narrowed apex, 2–3 septate and typical three-celled. The middle cells were broader than others (Figure 2 *c* and *d*). Getachew *et al.*[16] also found that the middle cell covers the larger area compared to other cells. In the present study, there were significant differences with respect to the source of carbon utilization by *P. grisea*. RYMA media containing mannitol was the most preferred carbon source as evidenced by higher sporulation and less vegetative growth compared to OMAM. All the isolates showed similarity in their ability to utilize mannitol as a carbon source. The higher preference for mannitol than oat meal might be due to biochemical differences. OMAM supported the maximum mycelium growth (81.7) whereas RYMA induced least mycelium growth (79.5). The findings agree with reports on utilization of different carbon sources by *P. grisea*, thereby indicating biochemical differences noted by Hossain[17]. Netam *et al.*[18] reported that glucose supported significantly more mycelia growth, followed by sucrose and galactose. Considering mean vegetative growth and sporulation value, RYMA medium was found to give best and uniform growth with excellent sporulation. RYMA appears to be a well-suited medium for vegetative growth and sporulation of *P. grisea* isolated from *E. coracana*.

1. Vietmeyer, N. D. (ed.), *Lost Crops of Africa*, Grains, National Research Council, National Academy Press, Washington, DC, 1996, vol. 1.
2. Goron, T. L. and Raizada, M. M., *Front. Plant Sci.*, 2015, **6**, 157.

3. Pandey, P. K. and Kumar, G. S., *Indian Farmers Digest*, 2005, **38**, 28–30.
4. Ignacimuthu, S. and Ceasar, S., *J. Biosci*., 2012, **37**, 135–147.
5. Nagaraja, A., Kumar, J., Jain, A. K., Narasimhudu, Y., Raghuchander, T. and Kumar, B., Compendium of small millets diseases, AICSMIP, UAS, GKVK, Bengaluru, 2007, pp. 1–80.
6. Nagaraja, A., Jagadish, P. S., Ashok, E. G. and Gowda, K. T. K., *J. Mycopath. Res*., 2007, **45**, 237–240.
7. Pall, B. S., *Food Farming Agric*., 1977, **9**, 55.
8. Sreenivasa Prasad, S., Finger millet blast in East Africa: Pathogen diversity and disease management strategies. DFID Crop Protection Programme, Final Technical Report, Horticultural Resources International, UK, 2004, p. 86.
9. Rao, A. N. S., *J. Agric. Sci*., 1990, **24**, 57–60.
10. Tuite, J., *Plant Pathological Methods, Fungi and Bacteria*, Burges Publishing Company, New York, 1969, p. 239.
11. Thomas, P. A., Kuriakose, T., Kirupashanker, M. P. and Maharajan, V. S., *Diagnotic Microbiol*., 1991, **14**, 219–224.
12. Barnett, H. L. and Hunter, B. B., *Illustrated Genera of Imperfect Fungi*, Burges Publications Company, UK, 1998, 4th edn.
13. Meena, B. S., Morphological and molecular variability of rice blast pathogen *Pyricularia grisea* (Cooke) Sacc. M Sc thesis, Dharwad University of Agricultural Sciences, Dharwad, 2005.
14. Susan Partridge-Metz and Ambika, C., *Cell Plant Sci*., 2011, **2**, 9–11.
15. Barnett, H. L. and Hunter, B. B., *Illustrated Genera of Imperfect Fungi*, Burgess Publishing Company, Morgantown, West Virginia, 1960, 2nd edn, p. 71.
16. Getachew, G., Tesfaye, A. and Kassahun, T., *J. Appl. Biosci.*, 2013, **73**, 5883–5901.
17. Hossain, M. M., Srikant, K. and Hegde, Y. R., *Karnataka J. Agric. Sci*., 2004, **17**, 851–853.
18. Netam, R. S., Bahadur, A. N., Tiwari, R. K. S. and Tiwari, U., *Res. J. Agric. Sci*., 2013, **4**, 83–86.

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New isolate of *Chilli leaf curl virus* on capsicum (Bell Pepper) under protected cultivation in Dharwad, Karnataka, India

During September 2017, capsicum (*Capsicum annuum* L. var. grossum Sendt.) plants in a poly-house at Hi-tech Horticulture, University of Agricultural Sciences, Dharwad showed typical leaf curl symptoms, viz. upward curling of leaf margins, yellowing, reduced lamina, short internodes (Figure 1). DNA was extracted from symptomatic and asymptomatic leaf samples of capsicum plants[1] and PCR amplification was done with *Chilli leaf curl virus* (ChiLCV) coat protein specific primers[2]. Gel electrophoresis on 0.8% agarose revealed ~500 bp size amplicons in all symptomatic samples and no amplification was observed in asymptomatic samples, confirming the presence of ChiLCV on capsicum (Figure 2). Sequencing of PCR products carrying *CP* gene of *Chilli leaf curl virus-Capsicum-Dharwad* (ChiLCV-Ca-DWD) and results of the BLAST (www.ncbi.nih.gov/BLAST) search performed to identify sequence homology clearly demonstrated that the CP gene sequence of ChiLCV-Ca-DWD matched with (95%, 96%, 97%, 98% and 99%) Pepper LCV-Varanasi (JN192448.1), Pepper LCV-Lahore (JN880419.1), ChiLCV-Amritsar (GU136803.1), ChiLCV-Jodhpur (HM007104.1) and ChiLCV-Sonipat (KJ649706.1) viruses respectively. It is clear from the results that ChiLCV is associated with leaf curl symptoms on capsicum. Cluster analysis grouped the ChiLCV-Ca-DWD isolate into two main clusters with similarity co-efficient from 0.000 to 0.035, indicating a good level of diversity. Grouping of the sixteen isolates on the basis of nucleotide relationship resulted in two major divergent groups I and II. ChiLCV-Ca-DWD infecting capsicum in Dharwad locality formed cluster I showing 35% diversity with cluster II. ChiLCV infecting capsicum in Dharwad was found to

Figure 1. Leaf curl symptoms, viz. upward curling, puckering, reduced leaf size, stunted growth on capsicum (Bell Pepper).