

SHORT COMMUNICATION

Improved and efficient method of isolation of *Pyricularia angulata* Hashioka causing Blast and Pitting diseases of banana

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Blast like lesions on tissue culture plantlets of banana and Pitting symptoms on fruits of cv. Grand Naine were observed under Odisha condition during 2014-15 in a regular survey. Routine isolation from representative samples yielded fungal isolates which failed the pathogenicity test several times. In the present study, existing method commonly used for isolation of leaf and fruit spotting fungi, was modified which led to successful isolation and purification of banana blast pathogen *Pyricularia angulata* Hashioka. Further pathogenicity was also demonstrated successfully.

Key words : Banana blast, Pitting disease, *Pyricularia angulata*

Among different *Pyricularia* spp. known to cause Blast disease in several plants, *P. oryzae* is the most important species causing blast on crops like rice, wheat, perennial and annual ryegrass, finger millet, foxtail millet. Most species of *Pyricularia* are recorded from monocotyledonous plants especially those belonging to families Poaceae, Comelinaceae, Cyperaceae and Zingiberaceae while few *Pyricularia* species were also recorded from dicotyledonous plants. Banana, a monocotyledonous crop is attacked by *Pyricularia angulata* Hashioka causing Blast and Pitting disease leading to significant quality loss of fruits. It was reported to occur in Australia, Central and South America and in some Asian countries. Male *et al.* 2011). During, 1960 and 70's it was considered as one of the most important disease of banana in banana producing Central American countries, but later it has become less important. However, occurrence of *P. angulata*

on tissue culture banana plantlets as well as on maturing fruits of cv. Grand Naine has been documented recently in Eastern India. Even though *Pyricularia* sp. infecting banana was described as *P. grisea*.

Isolation of pathogens from field samples is essential for researchers involved in studying the biology and management of any plant disease. However for routine isolation of *P. angulata* from Blast and Pitting disease affected banana samples, efficient and user friendly method for single spore isolation was not available. The pathogen associated with the spotting symptoms could not be isolated from infected fruits and leaf tissue easily, partly due to the presence of other associated saprophytic and pathogenic microorganisms and partly due to the noncompetitive, slowgrowing growth habit of *P. angulata* on artificial media. Often the diseased tissues were occupied by numerous necrotrophic fungi. Hence need for preincu-

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bation of diseased tissues for 23 days was carried out before pathogen isolation to induce sporulation to ensure successful isolation. In the present study we have developed a userfriendly method to isolate and evaluate *P. angulata* from field samples for further studies. In the modified methodology for isolating the pathogen responsible from banana Blast and Pitting disease affected samples, the diseased leaves and fruits samples were first collected washed with running tap water. The diseased samples were cut into small pieces and rinsed with sterile distilled water thoroughly twice under aseptic condition. These pieces were kept on sterile microscopic slide and it was placed on Petri plates lined with two layers of moist filter paper. The Petri plates were covered with lid and kept under florescent light for 12 hrs followed by 12 hrs incubation in dark. This cycle continued for 23 days till the sporulation occurred on diseased tissue as small powdery heap of spores. The spores were examined under light microscope and after confirming the sporulation, the tissues were suspended on one side of double sided tape or on transparent cellophane tape. The tape was be pasted on lid of PDA (Potato Dextrose Agar) plate in such as way that the tissue can be suspended on it and the sporulating colonies faced down towards the medium. Care was taken not to allow the tissue to touch the media. After 23 days of incubation, the spores fell on the medium, germinated and formed individual fungal colonies. Each colony emerging from single spore was subcultured and used for short and longterm storage. The halfwhite, delicate, floccose, loosely interwoven pathogen colonies became light brown upon aging. The conidia, measuring 20-22 x 6-9 mm in size, were hyaline to pale brown, two septate, ovate to obpyriform in shape, thin walled, with small protuberent hilum. The another unique morphological feature of *P. angulata* is the formation of irregularly angulared appressoria, which is distinct from *P. grisea* infecting rice and other *Pyricularia* infecting grass species having mostly globular appressoria. Here it is pertinent to mention about the diamond spot of banana caused by complex of fungi viz., *F. pallidroseum*, *Fusarium solani* and other *Fusarium* spp. During the course of investigation, the routine method of isolation from symptomatic leaves and fruits yielded only *Fusarium* spp. but failed the pathogenicity test. However modified method of isolation consistently yielded *Pyricularia* species indicating the efficiency and specificity of the new method. The fruit symptoms caused by *P.*

angulata included, oval, dark brown depression with or without water soaked halo which is referred as Pitting disease since it causes small pits on fruits. *P. angulata* also incites blast lesions on tissue culture banana plantlets, which appeared as minute oval brown lesions. The matured lesions had zonate appearance with light and dark brown rings, which further enlarged, coalesced with adjacent lesions to produce large necrotic tissue. The blast lesions were also noticed on mid rib, petioles, peduncle and crown portion but rarely on matured leaves. The new method was found to be highly efficient for isolation of *P. angulata* irrespective of the tissue or type of the symptom noticed. In the present study, we presented an improved method to isolate *P. angulata* from banana samples. In our experience, surface sterilization of diseased tissue eliminated the viable mycelia inside plant cells, as a result, isolation using surface sterilization has not been effective for *P. angulata*. Further, single spore isolations were time consuming tedious microscopic process. In the present study, the existing method of isolation commonly used for isolation of leaf and fruit spotting fungi has been modified leading to successful isolation and purification of banana blast pathogen. It is expected to aid the efforts to understand the biology of *P. angulata* and devise management methods against Blast and Pitting disease of banana.



Fig. 1 : Symptoms of Blast and Pitting disease of banana

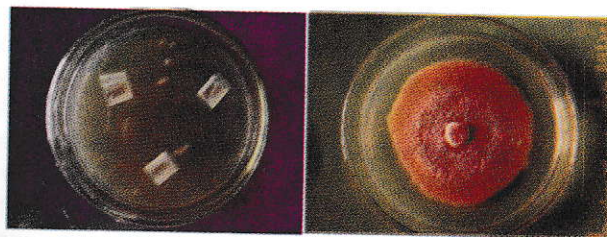


Fig. 2 : Improved isolation technique of *P. angulata* and pure culture of *P. angulata*

REFERENCE

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