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Bio Control Efficacy of Fungal Endophytes of Finger Millet Landraces against Blast Pathogen (*Pyricularia grisea*)

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ABSTRACT: Plant diseases cause heavy losses to crop yield, which encourages effective disease management practices like use of chemicals, resistant varieties, etc. From the point of environmental safety, biological control of plant diseases is gaining importance. Endophytes are microbes that reside within the plant tissues without causing any harm to the host. Finger millet is considered a climate-resilient crop, able to resist harsh environmental conditions, while the crop is affected by a devastating disease, the blast caused by *Pyricularia grisea*. Landraces are well adapted to local conditions including biotic and abiotic stress factors and some possess resistance to the blast disease. In the present study, 34 fungal endophytic isolates from different landraces were screened for their antagonistic activity against blast pathogen. Isolates KRL (80.21 %) and HGRS-2 (78.45 %) were efficient in inhibiting the pathogen in dual culture assay by means of antibiosis and hyper-parasitism. Three isolates were positive for siderophore production and seven for ammonia production and most of them were positive for various cell wall degrading enzymes. It suggests that the microflora of these landraces possess antimicrobial activity and one can make use of them as the best microbial inoculants for management of blast disease, to improve plant growth and crop yield.

Key words: Endophytes, Landraces, Biocontrol activity, Blast disease, *Pyricularia grisea*.

INTRODUCTION

The plant-endophyte association is an mutualistic relation, these microbes help the host to combat a diverse array of biotic and abiotic stressful conditions. Endophytes are living entities which may be either fungi or bacteria living within the plant tissues for at least a part of their life cycle without causing any apparent symptoms with more beneficial effect on their host plants (Pablo *et al*., 2015). Presently, endophytic microbes are gaining importance both at scientific and commercial level, as they can improve the quality of plant by their close association with internal tissues of host plant. Finger millet belongs to the grass family Poaceae, with excellent nutraceutical properties, such as high dietary fiber content, amino acids (methionine, phenylalanine, tryptophan, cysteine, isoleucine, and leucine), calcium and iron. As a member of small millets, finger millet is the most climate-resilient crop which can be cultivated under a diverse range of climatic conditions. Biotic stresses include weeds, birds, pests and diseases (Grovermann *et al*., 2018). Blast disease caused by the fungus *Pyricularia grisea* (*Magnaporthe grisea*) is the most destructive disease of finger millet threatens its production worldwide as well as to other economically important cereal crops including rice and wheat (Chung *et al*., 2020). *Pyricularia grisea,* the fungus affecting different aerial parts of the plant at all stages of its growth like stem, leaf, neck, and fingers. Average yield loss of about 28-36 per cent is usually associated with kernel abortions and shriveled grains caused by damage of panicle during reproductive stage but could be as high as 80-90 per cent in endemic areas (Rao, 1990).

Landraces evolved in response to natural selection for the local environment by means of mutations, migrations and genetic drift. Consequently, they are well adapted to local conditions including biotic and abiotic stress factors. They are valuable sources of quality traits agro-ecological adaptation, abiotic stresses and resistance to pests and diseases (Villa *et al*., 2005; Sanchez-Martin *et al*., 2011). The crop's wild types, landraces, and ecotypes smartly choose their microbial partners through co-evolution processes (Pozo *et al*.,

2021). Consequently, the high-yielding cultivars depend heavily on external resources/inputs (chemical fertilizers, pesticides, *etc*.), while the landraces manage their needs through their microbial partners (Ray *et al*., 2020).Wild finger millet relatives as well as landraces that are abundant in eastern Africa can be utilized to improve blast disease resistance in finger millet, as they are generally more diverse and have co-evolved with the pathogen over the time under non-intensive cultivation conditions (Agarwal and Maheshwari 2016). Hence, exploring the microbiome of native landraces, wild types, and ecotypes and applying them as inoculant for high-yielding cultivars would be a novel and sustainable approach to improving crop growth and fitness. Plant growth promoting bacteria (PGPB) elicit induced systemic resistance (ISR) to secure plants against aerial pathogens, where chances of direct contact between the pathogen and biocontrol agent are very rare (Rais *et al*., 2017). Endophytic bacteria are able to lessen the deleterious effects of certain pathogenic organisms. Increased environmental awareness has prompted the development of biological alternatives to chemical crop protection agents (Dimock *et al*., 1989). Unfortunately, most of these biocontrol agents have not fulfilled their initial promise, their failure usually being attributed to poor rhizosphere competence and the difficulties associated with the instability of bacterial biocontrol agents in long-term culture. In the present study, fungal endophytes from finger millet landraces were tested against blast pathogen, *Pyricularia grisea*.

MATERIAL AND METHODS

Isolation of Endophytes from Landraces of Finger Millet. Isolation was carried from different parts of plant like root, shoot, leaf and seeds of landraces namely, Bennemundaga Ragi, Bilikaddi Ragi, Dodda Ragi, Gidda Ragi, Gulu Ragi, Guppe Ragi, Haalu Ragi, Haaluguli Ragi, Hasirumundaga Ragi, Kapputhene Ragi, Keenya Ragi, Kuntukulu Ragi, Mallige Ragi, Mundaga ragi and Thenemundaga Ragi. The plant samples were washed and cut into 2-3 cm and the pieces were surface-sterilized by immersing in 70 per cent ethanol for 1 minute, followed by dipping plant samples in 1.5 per cent sodium hypochlorite. The plant samples were washed with distilled water to remove traces of sodium hypochlorite and repeatedly washed with sterile distilled water for 5-6 times, were blot-dried using sterile filter paper and were cut into 2 halves and each half was impregnated on potato dextrose agar plates in triplicate and plating was done from the final wash, which serves as the control. The plates were incubated at room temperature for 4-7 days (Bacon *et al*., 2002).

Biocontrol Activities of Endophytic fungi. Dual culture plate assay: Endophytic isolates were screened for their antagonist activity against blast pathogen, *Pyricularia grisea*, according to dual culture plate assay (Dennis and Webster 1971), in which both endophytic fungal isolate and pathogen were inoculated on single potato dextrose agar (PDA) plate. The pathogen (5 mm diameter disc) was inoculated at the centre of PDA plate and endophytic fungal culture was placed at corner of the plate and incubated at 28 ºC for four to eight days in triplicates. The per cent inhibition on growth of the test pathogen was calculated using formula as suggested by Vincent (1927).

$$I = (C-T)/C \times 100$$

Where,

I = Per cent inhibition, C = Growth of fungal plant pathogens in control (mm), T = Growth of fungal plant pathogens in dual culture plate (mm)

Siderophore and ammonia production. Fungal isolates were streaked on to the CAS (cromoazurol solution) plates and were incubated at 28 ± 2 ºC for 72 hrs for growth of fungi, then plates were observed for the formation of yellow halo around the fungal colony, which confirms siderophore production (Schwyn and Neils (1987). For ammonia production, isolates were inoculated to peptone broth and after incubation with addition of 0.5 mL Nessler's reagent, change in the medium's colour from brown to yellow indicates positive reaction (Kumar *et al*., 2015).

Cell wall degrading enzymes activity. The hydrolytic enzymes activity like cellulase, amylase, protease and lipase was tested on CMC agar plates, starch agar plates, skim milk agar plates and peptone agar plates respectively. After incubation at room temperature, plates were flooded with 0.2 % aqueous Congo red and detained with 1M NaCl for 15 minutes to test cellulase activity and 1.0 % iodine solution for amylase activity and clear halo zone around the colony indicates positive reaction.

RESULTS AND DISCUSSION

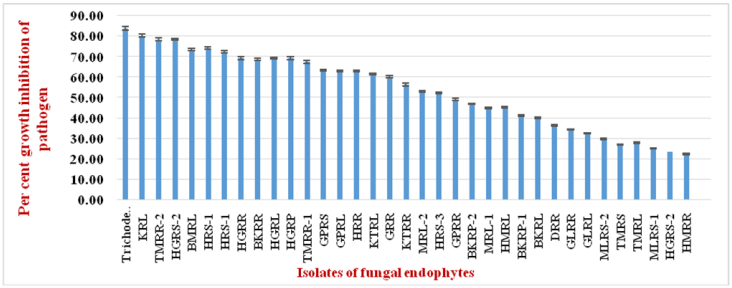
Isolation of Endophytes from Landraces of Finger Millet. From different plant parts, a total of thirty four fungal endophytes were isolated. Landrace haaluguli ragi recorded more number of isolates (5) followed by bilikaddi ragi (4), haaluragi (4) and the nemundaga ragi (4). More number of fungal isolates were from roots, followed by leaf and then the shoot part.

The number of endophytes in different parts of the plant systems vary as the micro environment may play a larger role in determining the endophyte community associated with the given host (Kotian *et al*., 2013). The presence of endophytic bacteria indicates their possible contribution in promoting seed germination and establishment or modulating other physiological functions since a given plant species normally harbors a wide range of microbial species, the specific composition of which is shaped by complex interactions with the host plant and the environment (Pan *et al*., 2008).It is suggested that the microenvironment may play a larger role in determining the endophyte community associated with a given host than supposed. Rodriguez *et al*. (2004) reported that a 'core' group of horizontally transmitted fungi (such as *Colletotrichum*, *Phyllosticta*) may not be influenced by host chemistry and can be isolated as endophytes from various tropical hosts irrespective of the environment in which the host grows.

Biocontrol Activities of Endophytic fungi

Dual culture plate assay. In dual culture assay, the isolate, KRL recorded higher growth inhibition of the pathogen (80.21 %) isolated from the landrace keenya ragi followed by isolates HGRS-2 (78.45 %) which were having significant difference with each other, while *Trichoderma* has shown inhibition of 83.64 per cent. Lowest inhibition was observed with the isolate HGRR (22.34 %) and the results are represented in the Graph 1 and Fig. 1. Some endophytes tend to slow the growth of the pathogen in the diffusion plate method, suggesting the production of antibiotic and activity of crude extracts by tested endophytes. Another mechanism of pathogen growth restriction by fungi is competition, where the pathogen growth was limited to its inoculation point by some endophytic fungi. This implies that diffusible antibiotics may be produced when they interact with pathogens. Various structures produced by fungal endophytes to parasitize the pathogen were observed under the high power of the light microscope and these might have a role in the control of the growth of pathogen (Atugala and Deshappriya 2015).

Fig. 1. Biocontrol activity of fungal endophytes against blast p pathogen in dual culture assay.



Graph 1. Biocontrol activity of fungal endophytes in dual culture assay against *Pyricularia grisea.*

Siderophore and ammonia production. Three isolates, KRL, BMRL and HGRS-2 were positive for siderophore production. Seven isolates were positive for ammonia production. The close linkage of endophyte and plant renders the endophyte to produce bioactive compounds by various mechanisms and pathways inside the plant which advantageously help the plant in growth and immunity. Endophytic fungi produce siderophore, HCN, hydrolyzing enzymes which positively influence the growth of the host plant (Hassan, 2017). Microbes will compete with the pathogen for nutrients like iron by producing siderophores. Siderophore directly influences biosynthesis of other antimicrobial compounds by increasing availability of metals like Fe, Zn and Cu to bacteria that suppresses the growth of pathogenic organisms *viz*., *Fusarium oxysporum* and *Rhizoctonia solani*, function as stress factors in inducing host resistance (Wahyudi and Astuti 2011). PGPR may also produce other fungal growth inhibitory compounds such as ammonia (NH4), easily expands and penetrates through soil pores, killing infectious propagules of targeted phytopathogens (Mota *et al*., 2017).

Cell wall degrading enzymes activity. For enzymatic activity, six isolates KRL, HRS-1, HRS-2, BMRL and HGRS-2 were positive for amylase and cellulase activity. Four isolates, KRL, HRS-2, BMRL and HGRS-2 were positive for lipase activity, none of them showed protease activity. The production of different hydrolytic enzymes such as chitinases, amylase, cellulase, protease, pectinase and lipase is another trait associated with PGPR enabling them to restrict fungal pathogens growth by disintegrating their cell wall (Dhar *et al*., 2018). These mycolytic enzymes induce structural and cellular disintegration causing hyphal lysis and subsequent fungal death *via* mycoparasitism. Further, mycoparaistism hinders pathogen development and virulence mainly *via* inhibiting spores' germination, germ-tube elongation and destroying oospores (Dukare *et al*., 2019).

CONCLUSIONS

Thirty four fungal endophytic isolates were isolated from fifteen landraces of finger millet and were screened against blast pathogen under in vitro conditions. Isolates, KRL and HGRS-2 were efficient in controlling pathogen growth in dual culture with siderophore and ammonia production. These fungal isolates were able to control the pathogen establishment with their enzymatic activity. Endophytic ability of these isolates will be further examined in pot culture to test their biocontrol efficacy.

FUTURE SCOPE

Management of plant diseases through use of biological agents is gaining importance, in this perspective

endophytes that live inside the plants are taking the upper hand. So, one has to exploit the efficiency of these bioagents for management of blast disease.

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Conflict of Interest. None.

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