Mysore J. Agric. Sci., 57 (1) : 52-58 (2023)

Antagonistic Effect of Bacterial Endophytes Isolated from Landraces of Finger Millet Against Blast Disease

K. B. Bhagyashree[1], N. Umashankar[2], N. Earanna[3], L. Krishna Naik[4], H. R. Raveendra[5] and P. S. Benherlal[6]

[1,2,3&4]Department of Agril.Microbiology; [6]Plant Biotechnology, College of Agriculture, UAS, GKVK, Bengaluru-560 065
[5]All India Coordinated Research Programme (AICRP) on Small Millets, College of Agriculture, V. C. Farm, Mandya
e-Mail : bhagyakb03@gmail.com

Authors Contribution

K. B. Bhagyashree : Drafting the article, data collection, data analysis & interpretatioin;
N. Umashankar : Conception of the work, critical revision of the article and final approval;
N. Earanna, L. Krishna Naik, H. R. Raveendra & P. S. Benherlal : Design of the work and critical revision of the article

Corresponding Author :
K. B. Bhagyashree
Dept.of Agril. Microbiology, College of Agriculture, UAS, GKVK, Bengaluru

Received : August 2022
Accepted : September 2022

Abstract

Finger millet is an important food crop that is grown in arid and semi-arid parts of Africa and Asia and it serves as a staple food source for millions of people. Blast is the most devastating disease affecting it at every stage of growth and development. In recent years, endophytes received attention because of their potential to increase crop yield without massive application of synthetic chemicals. Landraces are traditional, regional ecotypes that have adapted to their natural environment, some possess resistance to the blast disease. In this study, 42 bacterial endophytes were isolated from fifteen landraces and were screened for their antagonistic activity against *Pyricularia grisea*, causal agent of finger millet blast. Seed constitutes high number of bacterial colonies with the landrace, thenemundaga ragi. The isolates HMS-1 (91.04%), GPRS-1 (92.54%) and GPRR (92.54%) were efficient in inhibiting the pathogen growth in both dual culture plate and biomass assay by means of antibiosis and hyper parasitism. Hence, one can explore the microflora of these landraces and can utilize them as best microbial inoculants for management of blast disease, to improve plant growth and crop yield.

Keywords : Antagonistic activity, Endophytes, Landraces, *Pyricularia grisea*

The Mysore Journal of Agricultural Sciences

Finger millet [*Elusine coracana* (L.)] is grown widely in different agroecosystems and is an important crop for its diverse nutritional value and well suited to adverse growing conditions. It is cultivated in more than 25 countries of Africa and Asia, occupies about 12 per cent of the total global millet area (Vetriventhan *et al*., 2015). It can withstand biotic and abiotic stresses, therefore it is considered as one of the important cereal crops grown under rainfed conditions (Chandra *et al.,* 2016).

Biotic constrains affecting the crop are mainly diseases such as blast, foot rot, smut, leaf blight *etc.* Among them blast is one of the predominant disease, caused by *Magnaporthe grisea* (anamorph: *Pyricularia grisea*), the fungus infects all stages of plant growth, attacking different plant parts like stem, leaf, neck and fingers, causing yield loss of about 28-36 per cent **(**Nagaraja *et al*., 2010).

There are many strategies to manage the blast disease, like use of resistant cultivars, fungicide application, optimum fertilizer application and appropriate planting dates (Bonman, 1992). Fungicides like carbendazim, mancozeb and tricyclazole are effective in controlling blast disease (Netam *et al*., 2014), but these synthetic fungicides are hazardous to human health, leaves a negative impact on the environment (Budnik and Baur, 2009) and also repeated application of chemicals may induce resistance in the pathogen. In this perspective, it is desirable to identify eco-friendly, economical and farmer-friendly approaches to control the blast, one of the methods can be use of microbial agents.

In recent decades, the novel research on biological control of plant diseases demonstrated the role of a particular class of microbes that colonize the internal tissues of the host plant, referred to as endophytes, and hypothesized that they confer host plant resistance against fungal pathogens (Mousa and Raizada, 2013). 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).

Wild plants grow and resist pathogens without the use of fungicides, a landrace is a dynamic population or populations of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems. Plant defences are stronger in landraces in modern varieties (Davila-Flores *et al*., 2013 and Camacho Villa *et al.*, 2005). 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 (Afroz *et al*., 2017). Thus in the present study we hypothesized that cross inoculation of endophytes from landraces to susceptible variety will impart resistance to the pathogen, in turn results in higher yield in an eco-friendly manner. Hence, study was taken up to isolate bacterial endophytes from landraces of finger millet and screen against blast disease caused by *Pyricularia grisea*.

TABLE 1

Landraces used for endophytes isolation

Sample	Abbreviation
Guppe Ragi Seeds	GPRS
Haaluguli Ragi Seeds	HGRS
Guppe Ragi Root	GPRR
Thenemundaga Ragi Seeds	TMRS
Hasirumundaga Ragi Seeds	HMS
Dodda Ragi Shoot	DRP
Hasirumundaga Ragi	HMS
Haaluragi Root	HRR
Biliragi Shoot	BRP
Keenya Ragi Root	KRR
Thenemundaga Ragi Seeds	TMRS
Hasirumundaga Ragi	HMS
Hasirumundaga Ragi	HMS
Keenya Ragi Seeds	KRS
Dodda Ragi Seeds	DRS

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. Landraces seeds were collected from All India Coordinated Research Programme on Millets (AICRP), Zonal Agricultural Research Station (ZARS), V. C. Farm, Mandya, Karnataka, as listed in Table 1 and Plate 1. The plant

Plate 1 : Finger millet landraces

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. Washing was repeated with sterile distilled water 5-6 times. The surface sterilized samples were blot-dried using sterile filter paper and were cut into 2 halves and each half was impregnated on nutrient 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 24 - 48 hours (Bacon *et al.,* 2002).

Isolation of Pathogen

Diseased plant samples were collected from finger millet fields located in ZARS, UAS, GKVK, Bangalore. Samples were thoroughly washed and infected specimen was cut into small pieces measuring 2-3 cm in size with sterile blade. These bits were surface sterilized by dipping them in 2 *per cent* sodium hypochlorite solution (Riker and Riker, 1936) for 30 seconds and then washed thrice in sterilized distilled water for 45 seconds each to remove the traces of sodium hypochlorite. These bits were incubated in a humid chamber at 28 ºC for 24 h to induce sporulation. The spore mass from individual lesion was streaked on 4 per cent water agar and then transferred to ragi yeast lactose agar (RYLA) medium under aseptic conditions and incubated at 25±2 ºC (Jabbar and Nagaraja, 2018).

Pathogenicity Test

Pathogenicity test was performed on finger millet plants (Variety - udurumallige), by using modified procedure described by Jia *et al.* (2003). Plants were grown in controlled conditions at 24-30 ºC with sufficient day light and high humidity. Plants were inoculated with 2 ml of spore suspension with sufficient spore density by piercing slightly with sterile needle and were sealed with plastic bag as high humidity was required for fungal penetration in to host plant. Disease symptoms were monitored daily and plants were maintained till symptoms were noticed (Laxman, 2017).

Pure axenic form of fungus was obtained and morphological characteristics of blast fungus showed greyish colony with circular smooth margin and concentric ring pattern on RYLA (Plate 2). Isolated culture could infect the same variety of finger millet from where organism was isolated. Hence, the Koch's postulates were proved. The characteristic feature of pyriform shaped conidial pattern confirms the pathogen as *Pyricularia grisea* (Kulkarni and Peshwe, 2019).

Plate 2 : a) Culture of *Pyricularia grisea,* b) Microscopic view of *P. grisea* spores

Screening for Biocontrol Activity of Endophytic Bacteria Against Blast Pathogen

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 bacterial 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 24-hour old culture of endophytic bacteria (10^8 cfu/ml) was streaked at corner of the plate and incubated at 27 ºC for four to eight days in triplicates. Observations were recorded when there was a full growth of pathogen in the control plate. The *per cent* inhibition on growth of the test pathogen was calculated using formula as suggested by Vincent (1927).

$$I = \frac{(C-T)}{C} \times 100$$

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)

Biomass Method

Fungal growth inhibition by endophytic isolates was measured using mycelial mat weight determination method (Opgenorth and Endo, 1983). Both pathogenic fungus and the antagonistic bacterial endophyte were Co-inoculated to the culture flasks. To each flask containing 100 ml broth, 8 mm disc of the pathogenic fungi along with 1 ml of 24-hrs old endophytic bacterial culture (10^5 cfu/ ml) was inoculated and flasks inoculated with only pathogen was used as control. The flasks were incubated at 30 ºC for 10 days, then the contents were filtered through a pre-weighed Whatman filter paper and were dried in oven at 105 ºC for 48 hrs. The reduction in weight of co-inoculated flasks was determined in comparison with the control flasks.

Results and Discussion

Isolation of Endophytic Bacteria from Landraces of Finger Millet

Forty-two bacterial endophytes were isolated from the fifteen landraces and number of bacteria isolated were more from the landrace thenemundaga ragi (6), followed by keenya ragi (4), guppe ragi (4), mundaga ragi (4) and hasirumundaga ragi (4) the isolates from each landrace is represented in Fig. 1. Number of bacteria isolated from seeds (16) were more compared to other parts suggesting that a large population of endophytic bacteria reside inside the finger millet seeds. Higher number of endophytic bacteria indicates their possible contribution in promoting seed germination and establishment or modulating other physiological functions since a given plant species normally harbours 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). Different studies demonstrated that the seed microbiome plays an important role in seed germination and seedling development. Thus endophytes play a positive role in root and shoot development, in the formation of root hairs and help to increase the chlorophyll content of seedlings (Shearin *et al*., 2018).

Screening for Biocontrol Activity

The most commonly reported mechanism for biocontrol by rhizospheric bacteria and endophytic bacteria is antagonism through predation, competition, and production of enzymes or chemicals that are antagonist in nature. Unlike rhizospheric microbes, the endophytes have an alternative mechanism of biocontrol known as Induced Systemic Resistance (ISR) where bacterial metabolites affect the plant in such a way as to increase the plants resistance to pathogens (Kloepper and Ryu, 2006). Among 42 endophytic isolates, based on their different morphological and colony characteristics, 33 isolates were selected for antagonistic activity screening.

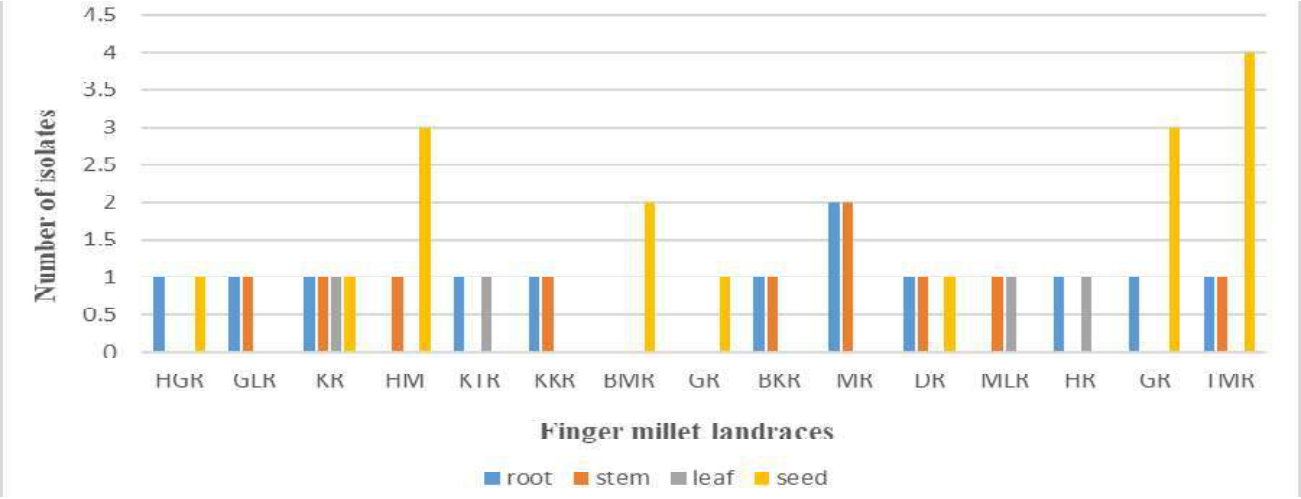

Fig. 1 : Endophytic bacterial isolates from different plant parts of finger millet landraces

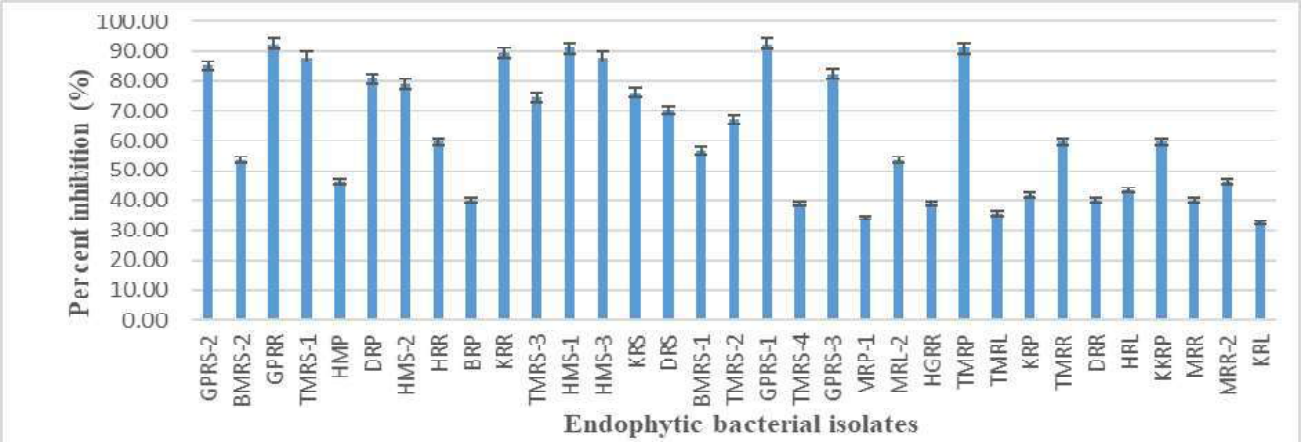

Fig. 2 : Antagonistic activity of bacterial endophytes in dual plate assay

In dual culture plate assay, zone of inhibition by isolates were measured and per cent inhibition was calculated by comparing control. The results are summarized in the Fig. 2. The per cent inhibition by bacterial isolates were between 32 and 93 per cent. Highest antagonistic activity was shown by isolates GPRR and GPRS-1 (92.54%) from landrace Guppe ragi followed by HMS-1 (91.04%) and lowest activity was reported in isolate KRL (32.84 %) of Keenya ragi.

It is clearly observed that the growth of fungal mycelium was inhibited by bacterial endophyte that is due to the hyper parasitism and production of antagonistic substances and also different locality and inherent microflora of landraces can be the reason for their difference in biocontrol activity (Misganaw *et al.,* 2019). Endophytes from GPU, blast resistant variety, *Stenotrophomonas maltophilia* and *Lactobacillus aerocolonigenes* were reported to hold a strong antagonistic activity against *M. grisea*. The production of alkaline serine proteolytic enzyme, induction of host systemic acquired resistance and thiobutacin production by *S. maltophilia* and *L. aerocolonigenes* respectively might play a key role in its biocontrol activity against oomycetes and some ascomycetes fungi. The high abundance of these species in GPU might have a potential role in the resistant reaction of this cultivar against the blast pathogen (Prasanna kumar *et al.,* 2020).

In biomass assay, the per cent reduction in dry weight of the mycelium mat of plant pathogens by the bacterial endophytes is presented in Fig.3. The highest per cent reduction of dry weight of mycelium was shown by isolate GPRS-1 (96.02%) isolated from seeds of landrace Guppe Ragi and lowest activity was found with the isolate GRS (24.77 %). Pathogen growth was inhibited due to the microbial activity by production of enzymes, secondary metabolites and by competing for limited nutrients or space (Suman *et al*., 2016). The filtrates of *S. globisporus* JK-1 suppressed the growth of *M. oryzae* in dual culture as well as inhibited conidial germination and appressorial formation as demonstrated by detached leaf assay. Kumar *et al*. (2020**)** evaluated the antifungal activity of bacterial seed endophytes showed inhibition of growth of tested fungal pathogens *Alternaria* sp., *Curvularia* sp., *Fusarium oxysporum* and *Rhizoctonia solani* by *Paenibacillus dendritiformis* with per centage inhibition 54.54, 58.33, 30.43 and 50 per cent respectively. Bacterial endophytic isolates from minor millets, KMS 5 (62.22 %) and PML 3 (82.05 %) were able to inhibit the growth of *Rhizoctonia solani* (Raveendra and Shivaprakash, 2018), the suppression of mycelial growth of fungal pathogen by bacterial endophytes may be due to the production of inhibitory allelochemicals, volatile and non-volatile compounds, hydrogen cyanide and cell wall degrading enzymes. .

Thirty-three bacterial isoloates were screened against blast pathogen under *in vitro* conditions.

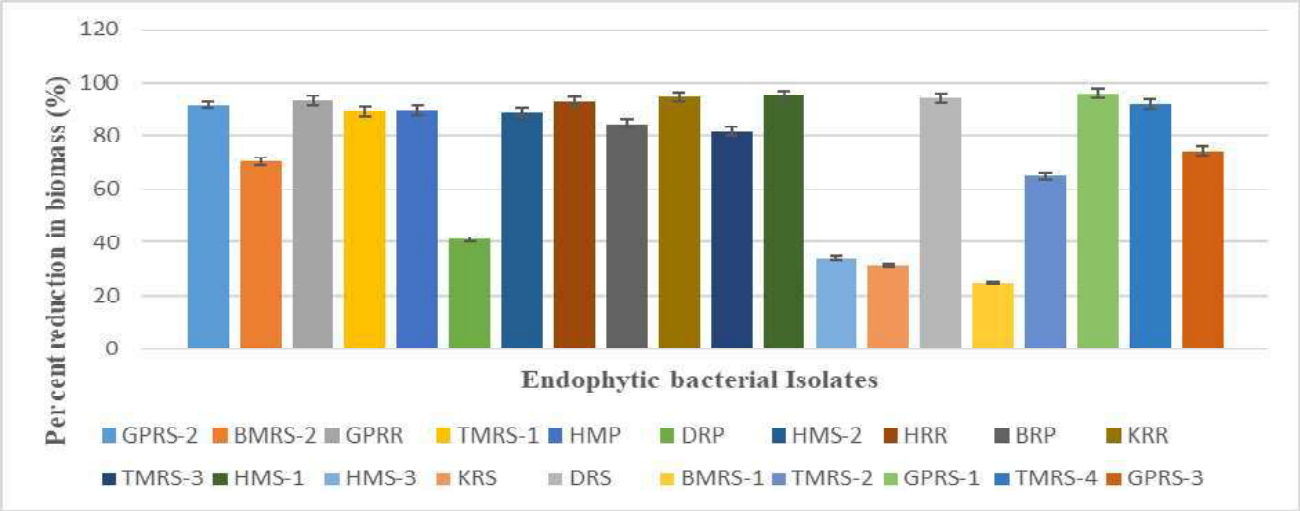

Fig. 3 : Antagonistic activity of bacterial endophytes in biomass assay

Based on dual culture assay, 20 isolates were selected and screened in biomass method. Among 15 landraces, thenemundaga ragi had harboured high endophytic bacteria and finger millet seed contributed for higher population. Three isolates HMS-1, GPRS-1 and GPRR were efficient in inhibiting growth of the pathogen *P. grisea*. Efficiency of these isolates from landraces can be exploited as a bio agent in controlling blast.

References

Afroz, R., Zahra, J., Faluk, S., Fauzia, Y. H., Muhammad and Nadeem, H., 2017, *Bacillus* spp., a bio-control agent enhances the activity of antioxidant defense enzymes in rice against *Pyricularia oryzae*. *Plos One*, **12** (11) : 0187412.

Bacon, C. W., Glenn A. E. and Hinton, D. M., 2002, Isolation, in planta detection and culture of endophytic bacteria and fungi. In: Hurst, C. J., Crawford, R. L., Mc Inroy, M. J., Knudsen, G. R. and Stetzenbach, L. D. (eds) Manual of Microbiology, 2nd edn. ASM, Washington D. C., pp. : 543 - 553.

Bonman, J, 1992, Durable resistance to rice blast disease - environmental influences. *Euphytica*, **63** : 115 - 123. https://doi.org/10.1007/BF00023917.

Budnik, L. T. and Baur, X., 2009, The assessment of environmental and occupational exposure to hazardous substances by biomonitoring. *Dtsch. Arztebl. Int*., **106** : 91 - 97.

Camacho, V., Maxted, N., Scholten, M. and Ford-Lloyd, B. V., 2005, Defining and identifying crop landraces. *Plant Genet. Resour.,* **3** (3) : 373 - 384.

Chandra, D., Chandra, S., Pallavi and Sharma, A. K., 2016, Review of finger millet [*Eleusine coracana* (L.) Gaertn]: A powerhouse of health benefiting nutrients. *Food Sci. Hum. Well*, **5** : 149 - 155.

Davila-Flores, A., Dewitt, T. and Bernal, J., 2013, Facilitated by nature and agriculture : Performance of a specialist herbivore improves with host-plant life history evolution, domestication and breeding. *Oecologi*a, **173** : 1425 - 1437.

Dennis, C. and Webster, J., 1971, Antagonistic properties of species groups of *Trichoderma* production of non-volatile antibiotics. *Trans. Br. Mycol. Soc*., **57** : 25 - 39.

Jabbar Sab and Nagaraja, A., 2018, Assessment of morphological and cultural variability of *Pyricularia grisea* (Cooke) Sacc. isolates associated with finger millet blast disease. *Mysore J. Agric. Sci*., **52** (2) : 391 - 401.

Jia, Y., Valent, B. and Lee, F. N., 2003, Determination of host responses to *Magnaporthe grisea* on detached rice leaves using a spot inoculation method. *Plant Dis.,* **87** : 129 - 133.

Kulkarni, K. and Peshwe, S., 2019, Screening, isolation and molecular identification of rice

pathogen *Magnaporthe oryzae*. *Int. J. Adv. Res.*, **7** (3) : 428 - 433.

Kumar, K., Pal, G., Verma, A. and Verma, S. K., 2020, Seed inhabiting bacterial endophytes of finger millet (*Eleusine coracana* L.) promote seedling growth and development and protect from fungal Disease. *S. AFR. J. Bot*., **134** : 91 - 98.

Kloepper, J. W. and Ryu, C. M., 2006, Bacterial endophytes as elicitors of induced systemic resistance. In: Schulz, B., Boyle, C., Sieber, T. N. (eds) Microbial root endophytes, Soil Biology, Springer-Verlag Berlin Heidelberg, **9** : 33 - 52.

Laxman Singh Rajput, T. S., 2017, Effect of temperature on rice blast infection process with emphasis on appressoria formation by *Magnaporthe oryzae. Int. J. Curr. Microbiol. Appl. Sci.,* **6** (4) : 1931 - 1939.

Misganaw, G., Simachew, A. and Gessesse, A., 2019, Endophytes of finger millet (*Eleusine coracana*) seeds. *Symbiosis,* https://doi.org/10.1007/s13199-019-00607-5.

Mousa, W. K. and Raizada, M. N., 2013, The diversity of anti-microbial secondary metabolites produced by fungal endophytes : An interdisciplinary perspective. *Front. Microbiol*., **4** : 1 - 18.

Nagaraja, A., Nanja, Y. A., Anjaneya, B., Patro, T. S. S. K., Kumar, B., Kumar, J. and Krishne, K. T., 2010, Reaction of finger millet recombinant inbred lines to blast. *Crop Res.,* **39** : 120 - 122.

Netam, R. S., Tiwari, R. K. and Bahadur, A. N. D. S., 2014, *In vitro* and *in vivo* efficacy of fungicides against *Pyricularia grisea* causing finger millet blast disease. *Int. J. Plant Prot*., **7** : 137 - 142.

Opgenorth, D. C. and Endo, R. M., 1983, Evidence that antagonistic bacteria suppress *Fusarium* wilt of celery in neutral and alkaline soils. *Phytopathol.,* **73** : 703 - 708.

Pablo, C., Maria, P. M., Rodriguez, J. A. N. and Rafael, M. J. D., 2015, Interactions of *Pratelenchus thornei* and *Fusarium oxysporum* f. sp. *ciceris* on Chickpea. *Phytopathol*., **88** : 828 - 836.

Pan, J. J., Baumgartenam and May, G., 2008, Effects of host plant environment and *Ustilago maydis* infection on the fungal endophyte community of maize (*Zea mays*). *New Phytol*., **178** : 147 - 156. https://doi.org/10.1111/j.1469-8137.2007.02350.x

Prasannakumar, M. K., Mahesh, H. B., Radhika, U., Desai, Bharath Kunduru, Karthik, S., Narayan, Kalavati Teli, Puneeth, M. E. and Rajadurai, R. C., 2020, Metagenome sequencing of finger millet associated microbial consortia provides insights into structural and functional diversity of endophytes. *Biotech.*, **10** : 15.

Raveendra Reddy, M. and Shivaprakash, M. K., 2018, Antagonistic activity of bacterial endophytes isolated from millets against *Rhizoctonia solani* in foxtail millet. *Mysore J. Agric. Sci.,* **52** (2) : 241 - 247.

Ricker, A. J. and Ricker, R. S., 1936, Introduction to research on plant disease. John S. S. wit Co., No.4, New York, USA.

Shearin, Z. R. C., Filipek, M., Desai, R., Bickford, W.A., Kowalski, K. P. and Clay, K., 2018, Fungal endophytes from seeds of invasive, non-native *Phragmites australis* and their potential role in germination and seedling growth. *Plant Soil*, **422** : 183 - 194. https://doi.org/10.1007/s11104-017-3241-x.

Suman, A., Yadav, A. N. and Verma, P., 2016, Endophytic microbes in crops : Diversity and beneficial impact for sustainable agriculture. In: Singh DP, Abhilash PC, Ratna P (eds) Microbial inoculants in sustainable agricultural productivity. Springer, Dordrecht, pp. : 117 - 143.

Vetriventhan, M., Upadhyaya, H. D. and Dwivedi, S. L., 2015, Finger and foxtail millets. Elsevier Inc., Amsterdam, pp. : 291 - 319.

Vincent, J. M., 1927, Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **59** : 850.