Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 2195-2200

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 03 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.276

Amplification of Housekeeping Genes in *Magnaporthe grisea* causing Blast Disease in Foxtail Millet

M. Dhivya[1*], S. Thiruvudainambi[1], G. Senthilraja[2], N. Senthil[3] and C. Vanniarajan[4]

[1]Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625 104, TN, India
[2]Regional Research station, Tamil Nadu Agricultural University, Vriddhachalam 606 001, Tamil Nadu, India
[3]Department of Plant Molecular Biology and Bioinformatics, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[4]Department of Genetics and Plant Breeding, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625 104, Tamil Nadu, India

**Corresponding author*

Keywords

Magnaporthe-housekeeping genes- PCR

Article Info

Accepted:
25 February 2021
Available Online:
10 March 2021

A B S T R A C T

The genus *Pyricularia* (anamorph)/*Magnaporthe* (teleomorph) causes blast disease on a broad range of plants including rice, foxtail millet and other species of the family Poaceae. The infected leaves of foxtail millet with typical spindle shaped lesions of light tan in colour, dusty gray appearance with necrotic borders were collected from various regions of Tamil Nadu during 2017 and 2018 and the pathogen was isolated from the samples. The collected isolates were named as TNFxM1 to TNFxM32. All the 32 blast isolates were characterized by their colony morphology and spore morphology. Amplification of housekeeping genes *viz.*, Actin, ß- tubulin, and Calmodulin were done using the specific primers at 336 bp, 536 bp and 512 bp respectively.

Introduction

Magnaporthe grisea, a major plant pathogenic fungus has been regarded as nationally important hemi - biotroph. It invades more than fifty plant species of the family Poaceae which includes rice, wheat, barley, finger millet, pearl millet and foxtail millet (Talbot 2003, Tanweer *et al.*, 2015, Sharma *et al.*, 2013, Prabhu *et al.*, 1992). The hemi- biotroph caused food grain losses ranging from 30 to 40% especially in northern china and India (Nagaraja 2007). Eukaryotes are a diverse group of microbial community which possess

several conserved regions. Among the regions, housekeeping genes are involved in various cellular processes. In general, conserved region of a known gene has been used for PCR based analysis and phylogenetic map construction. The cytoskeleton is an extremely well organized, complex and dynamic network that present in cells of all domains of life, including archaea, bacteria, and eukaryotes. In eukaryotic cells cytoskeleton is mainly composed of microfilaments, microtubules, and intermediate filaments. Actin, the widely distributed protein in the most eukaryotic cells involves in cell motility, cell shape maintenance and secretion of proteins (Li *et al.,* 2020). Tubulin is categorized into two class *viz., α*-tubulin and ß- tubulin. Both the tubulins are involved in the formation of microtubules and it is imperative component of the eukaryotic cytoskeleton. In addition, microtubules were involved in the cellular process like mitosis(Gunning *et al.,* 2015). Calcium (Ca^{2+}) is an important element which essential to numerous biological process. It plays a crucial role to maintain the cell integrity and membrane system. The calmodulin (CaM) group is a primitive class calcium sensor proteins which play a major role in signaling among the cells through the regulation of numerous target proteins. CaM is the important class of conserved proteins distributed in all eukaryotic cells (Ranty *et al.,* 2006). It plays a major role in cell growth and cell differentiation (Ma *et al.,* 2009). The present study was undertaken to isolate the blast pathogen from foxtail millet that could be employed for the amplification of housekeeping genes.

Materials and Methods

Collection and isolation of the pathogen

A total of 32 isolates of the blast pathogen were collected from different foxtail millet growing areas of Tamil Nadu which includes both plain and hill areas. The infected leaves and sheath portions were cut into small pieces and surface sterilized with 0.1 per cent mercuric chloride solution for 60 seconds. Then the leaves were continuously washed with sterile distilled water for two times and dried in sterile filter paper. The affected tissues was placed on potato dextrose agar medium and incubated at 25±2 °C for 7 days. Pure culture of the fungi were obtained by single spore isolation method (Ou 1985).The pathogen was confirmed by their spore morphology.

Genomic DNA extraction

Genomic DNA was extracted from the dried mycelial mats of *M. grisea* using a CTAB method (Murray and Thompson 1980). 100 mg of the dried mycelial mats were macerated using liquid nitrogen. The mycelial powder was transferred to 1.5ml ependorff tubes and 600 µl of CTAB was added and vortexed for 2 min. Then the tube was incubated at 65°C for 30 min. After incubation, the tube was centrifuged at 10,000 rpm for10 min. After centrifugation, supernatant was collected in to a new tube. 750 µl of chloroform and isoamyl alcohol (24:1 v/v)was added, mixed and then centrifuged at 10,000 rpm for10 min.

The upper aqueous phase was transferred to a new microfuge tube and re-extracted with an equal volume of chloroform and isoamyl (24:1)and centrifuged at 10,000 rpm for 10 min. The upper aqueous phase (300 µl) was mixed with 0.5 volume of 5M NaCl and 2 volume of ice-cold isopropanol and incubated at -20 °C for overnight. The contents were centrifuged at 13,000 rpm at 4 °C for 10 min and the DNA pellet was air dried and dissolved in 50 µl of Tris-EDTA buffer and storedat -70°C. The genomic DNA was checked by running 0.8per cent agarose gel electrophoresis and the DNA concentrations of the samples were determined using

Nanodrop (NanoDrop products, Thermo Scientific, Wilmington, DE, USA).

PCR amplification of housekeeping genes

Amplification of housekeeping region (Actin, ß- tubulin, Calmodulin) was done by PCR assay (table 1). PCR reactions were carried out in 20 µl reaction which contained 100 ng of template DNA, 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 U *Taq* DNA polymerase, 200 nm of primers and 0.2 mM of each dNTP. Thermal cycling conditions involved an initial denaturation at 94°C for 1 min, followed by 30 cycles of 94°C for 30 s, 65°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 1 min. PCR products were resolved in 1.5% agarose gel, stained with ethidium bromide, electrophoresed at 100v and photographed under gel documentation unit (Bio rad, USA).

Results and Discussion

A total of thirty two isolates of *Magnaporthe grisea* collected from different locations of Tamil Nadu were tested to identify actin, β-tubulin and calmodulin and all the isolates were successfully amplified with 560, 336, 536, and 512 bp long respectively (Plate 1). Actin and beta tubulin, a primitive source for fungal cytoskeleton were involved in pathogenesis. Calmodulin was involved in the process of conidial formation, germ tube elongation and appressorium formation (cell differentiation).

PCR product was amplified at 336 bp to identify the presence of actin gene. Similar result was found by Abed-Ashtiani *et al.,* (2016). Presence of actin gene in *M. grisea* may involve in the process of pathogenicity, hyphal growth and to maintain cell shape of the pathogen. The present results concordance with Takeshita *et al.,* (2014) who found that, actin rings involved in septum formation and reported as crucial element for cytokinesis in budding yeast and actively participated in pathogenesis process of *M. oryzae* (Ryder *et al.,* 2013).

Similar findings observed by González-Rodríguez *et al.,* (2016) revealed that, actin and actin related proteins were mainly participated in the virulence and secretion of proteins in fungi. The F-actin capping protein has been proved its crucial role in hyphal development and pathogenicity of *Botrytis cinerea.* Deletion of actin gene was responsible to the loss of cellular development and virulence (Li *et al.,* 2020).

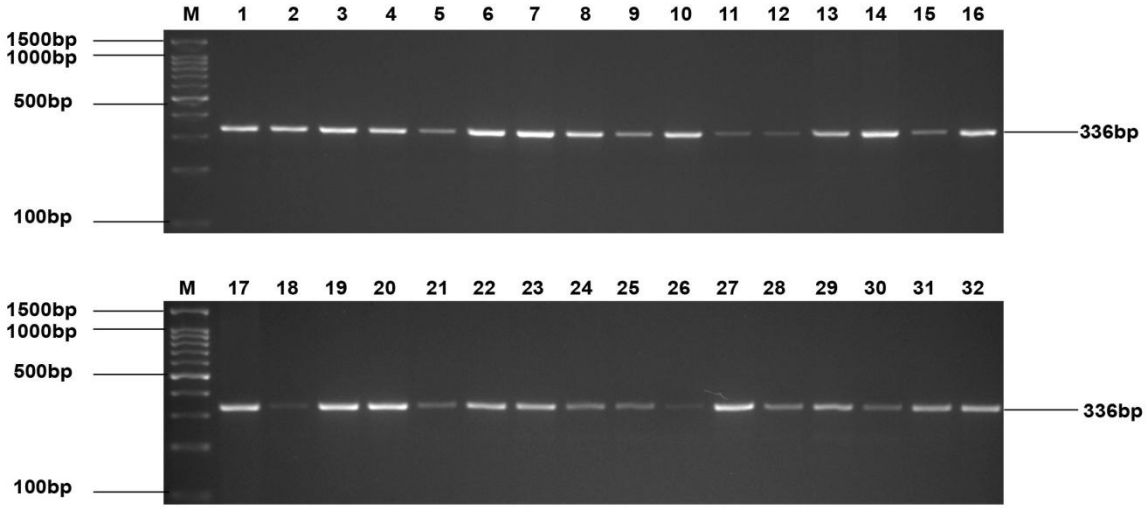
Liu and Kolattukudy (1999) found that self-inhibitors were existed in the spores of *M. grisea*. So, the early expression of calmodulin gene in blast pathogen leads to the formation of infection morphologies *viz*., (conidia, germ tube and appressorium). Early expression of calmodulin gene in *Colletotrichum gloeosporioides* (Kim *et al.,* 1998) and *Colletotrichum trifolii* (Buhr and Dickman 1997) involved in the process of conidial morphogenesis

Table.1 List of primers used in this study

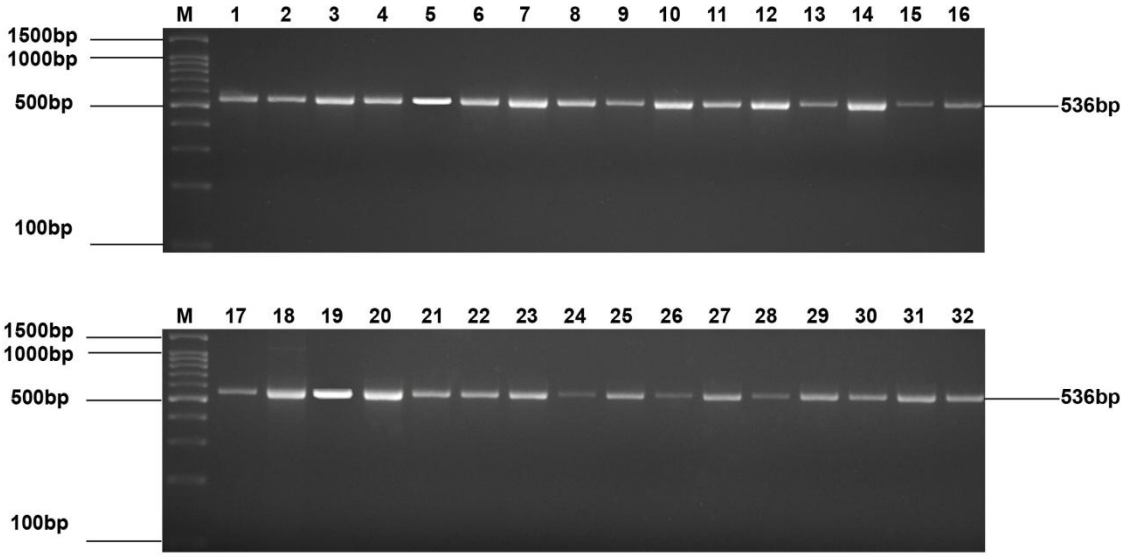
Gene	Primer	Primer sequence(5'-3')	Reference
Actin	ACT- 512F	ATGTGCAAGGCCGGTTTCGC	(Carbone and Kohn 1999)
	ACT- 783R	TACGAGTCCTTCTGGCCCAT	
ß- tubulin	Btla	TTCCCCCGTCTCCACTTCTTCATG	(Glass and Donaldson 1995)
	Btlb	GACGAGATCGTTCATGTTGAACTC	
Calmodulin	CAL- 228F	GAGTTCAAGGAGGCCTTCTCCC	(Carbone and Kohn 1999)
	CAL- 737R	CATCTTTCTGGCCATCATGG	

Plate.1 PCR amplification of house keeping regions

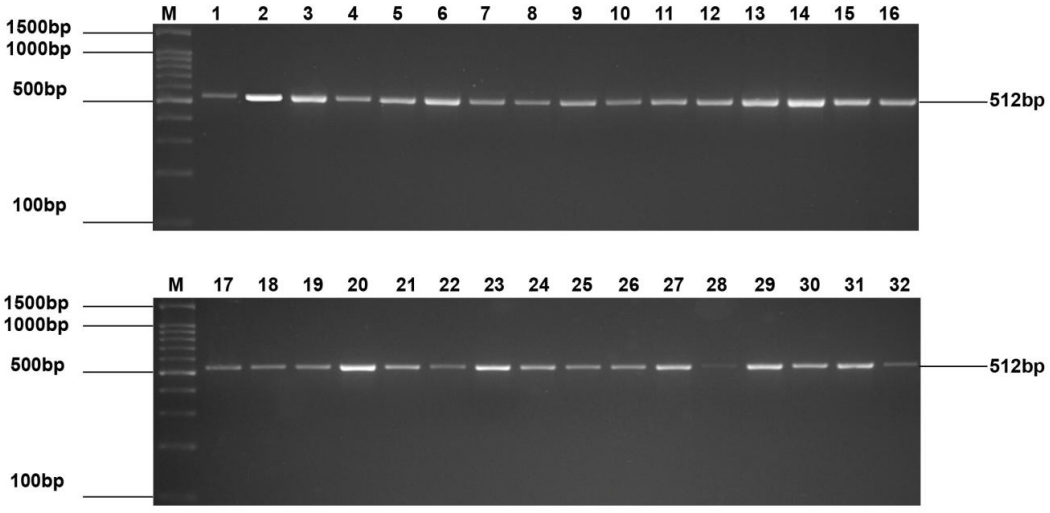
a) actin



b) Beta-tubulin



c) Calmodulin



Lane

1. TNFxM1	7. TNFxM7	13. TNFxM13	19. TNFxM19	25. TNFxM25	31. TNFxM31
2. TNFxM2	8. TNFxM8	14. TNFxM14	20. TNFxM20	26. TNFxM26	32. TNFxM32
3. TNFxM3	9. TNFxM9	15. TNFxM15	21. TNFxM21	27. TNFxM27	M - 100bp ladder
4. TNFxM4	10. TNFxM10	16. TNFxM16	22. TNFxM22	28. TNFxM28	
5. TNFxM5	11. TNFxM11	17. TNFxM17	23. TNFxM23	29. TNFxM29	
6. TNFxM6	12. TNFxM12	18. TNFxM18	24. TNFxM24	30. TNFxM30	

References

Abed-Ashtiani, Farnaz, Jugah Kadir, Abbas Nasehi, S-R Hashemian-Rahaghi, Ganesan Vadamalai, and S-K Rambe. 2016. "Characterisation of Magnaporthe oryzae isolates from rice in peninsular Malaysia." *Czech Journal of Genetics and Plant Breeding* 52 (4):145-156.

Buhr, TL, and MB Dickman. 1997. "Gene expression analysis during conidial germ tube and appressorium development in Colletotrichum trifolii." *Applied and environmental microbiology* 63 (6):2378-2383.

Carbone, Ignazio, and Linda M Kohn. 1999. "A method for designing primer sets for speciation studies in filamentous ascomycetes." *Mycologia* 91 (3):553-556.

Glass, N Louise, and Gary C Donaldson. 1995. "Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes." *Appl. Environ. Microbiol.* 61 (4):1323-1330.

González-Rodríguez, Victoria E, Carlos Garrido, Jesús M Cantoral, and Julia Schumacher. 2016. "The F-actin capping protein is required for hyphal growth and full virulence but is dispensable for septum formation in Botrytis cinerea." *Fungal biology* 120 (10):1225-1235.

Gunning, Peter W, Umesh Ghoshdastider, Shane Whitaker, David Popp, and Robert C Robinson. 2015. "The evolution of compositionally and functionally distinct actin filaments." *Journal of cell science* 128 (11):2009-2019.

Kim, Yeon-Ki, Daoxin Li, and Pappachan E Kolattukudy. 1998. "Induction of Ca2+-calmodulin signaling by hard-surface contact primes Colletotrichum gloeosporioides conidia to germinate and form appressoria." *Journal of Bacteriology* 180 (19):5144-5150.

Li, Hua, Zhanquan Zhang, Guozheng Qin, Chang He, Boqiang Li, and Shiping Tian. 2020. "Actin Is Required for Cellular Development and Virulence of Botrytis cinerea via the Mediation of Secretory Proteins." *Msystems* 5 (1).

Liu, Zhi-Mei, and Pappachan E Kolattukudy. 1999. "Early expression of the calmodulin gene, which precedes appressorium formation in Magnaporthe grisea, is inhibited by self-inhibitors and requires surface attachment." *Journal of Bacteriology* 181 (11):3571-3577.

Ma, Zhi-bin, Jun-xia Zhao, Li-an Wang, and Xiao-bo Zheng. 2009. "Cloning, prokaryotic expression, and bioactivity of the calmodulin gene of Magnaporthe grisea." *FEMS microbiology letters* 300 (1):107-114.

Murray, MG, and William F Thompson. 1980. "Rapid isolation of high molecular weight plant DNA." *Nucleic acids research* 8 (19):4321-4326.

Nagaraja, A.; Kumar, J.; Jain, A.K.; Narasimhudu, Y.; Raghuchander, T.; Bijender Kumar and Hanumanthe Gowda, B.. 2007. "Compendium of small millets diseases." *Project Coordination Cell, All India Coordinated Small Millets Improvement Project, UAS, GKVK Campus, Bangalore. 80p.*

Ou, SH. 1985. "Rice Diseases: Commonwealth Mycological Institute." *Kew, Surrey, England*.

Prabhu, AS, MC Filippi, and N Castro. 1992. "Pathogenic variation among isolates of Pyricularia oryzae affecting rice, wheat, and grasses in Brazil." *International Journal of Pest Management* 38 (4):367-371.

Ranty, Benoit, Didier Aldon, and Jean-Philippe Galaud. 2006. "Plant calmodulins and calmodulin-related proteins: multifaceted relays to decode calcium signals." *Plant Signaling & Behavior* 1 (3):96-104.

Ryder, Lauren S, Yasin F Dagdas, Thomas A Mentlak, Michael J Kershaw, Christopher R Thornton, Martin Schuster, Jisheng Chen, Zonghua Wang, and Nicholas J Talbot. 2013. "NADPH oxidases regulate septin-mediated cytoskeletal remodeling during plant infection by the rice blast fungus." *Proceedings of the National Academy of Sciences* 110 (8):3179-3184.

Sharma, Rajan, HD Upadhyaya, SV Manjunatha, KN Rai, SK Gupta, and RP Thakur. 2013. "Pathogenic variation in the pearl millet blast pathogen Magnaporthe grisea and identification of resistance to diverse pathotypes." *Plant disease* 97 (2):189-195.

Takeshita, Norio, Raphael Manck, Nathalie Grün, Satur Herrero de Vega, and Reinhard Fischer. 2014. "Interdependence of the actin and the microtubule cytoskeleton during fungal growth." *Current opinion in microbiology* 20:34-41.

Talbot, Nicholas J. 2003. "On the trail of a cereal killer: exploring the biology of Magnaporthe grisea." *Annual Reviews in Microbiology* 57 (1):177-202.

Tanweer, Fatah A, Mohd Y Rafii, Kamaruzaman Sijam, Harun A Rahim, Fahim Ahmed, and Mohammad A Latif. 2015. "Current advance methods for the identification of blast resistance genes in rice." *Comptes rendus biologies* 338 (5):321-334.

How to cite this article:

Dhivya, M., S. Thiruvudainambi, G. Senthilraja, N. Senthil and Vanniarajan, C. 2021. Amplification of Housekeeping Genes in *Magnaporthe grisea* causing Blast Disease in Foxtail Millet. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 2195-2200.
doi: https://doi.org/10.20546/ijcmas.2021.1003.276