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First report of leaf spot disease on ridge gourd caused by *Fusarium solani*

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Abstract
Ridge gourd (*Luffa acutangula* Roxb.) is a widely cultivated vegetable crop of sub-Himalayan West Bengal. During June to August 2022, a disease looking like leaf spot disease was found in Coochbehar district. The disease incidence was 25%. The leaves of ridge gourd showed symptoms of large brown necrotic lesions that appear on leaves, especially on the tip of basal leaves. In PDA medium profuse white aerial mycelium was seen. Macroconidia having 3 to 4 septa were 15-20 µm long. To confirm the identity of this fungus, the internal transcribed spacer, 28S rRNA regions and TEF (Translation Elongation Factor) gene of RG/DE/F were amplified and sequenced. GenBank accession numbers are OR026030, OR782923 and OR800599 respectively. All the sequences showed 99%, 97% and 100% resemblance with sequences of *Fusarium solani* in GenBank. Pathogenicity tests showed positive results with disease symptoms similar to those of natural symptoms. Thus, the pathogen has been identified as *Fusarium solani*. The isolate RG/DE/F (*F. solani*) is being reported as a pathogen of ridge gourd for the first time.

Keywords: *Fusarium solani*, Leaf spot, *Luffa acutangula*

Introduction
Ridge Gourd or *Luffa acutangula* Roxb. belongs to family cucurbitaceae, is one of the important vegetable crops of sub-Himalayan West Bengal. *Luffa acutangula* is mostly cultivated in the tropical parts of India, China, Korea, Japan and Central America for their nutritional food values (Dhilon *et al*., 2016) [7]. In India, ridge gourd is broadly cultivated in Uttar Pradesh, Karnataka, Tamil Nadu, Kerala, Andhra Pradesh, Odisha and West Bengal (Jaysingrao and Sunil 2014) [11]. Fruits of ridge gourd contain high amounts of edible fiber and essential nutrient elements like vitamin C, zinc, iron, riboflavin, magnesium, thiamine and other necessary amino acids (Swetha *et al*., 2016) [18]. This gourd is used to treat diseases like diabetes, jaundice, ulcers and hypoglycemia (Manikandaselvi *et al*., 2016) [12]. Like many other plants, *Luffa acutangula* is also infected by various foliar fungal pathogens that cause huge yield losses. It was reported from Odisha (Nayak and Bandamaravuri, 2018) [13] that *Podridosphaera xanthii* causes powdery mildew in ridge gourd. Fruit rot of *Luffa acutangula* caused by *Curvularia hominis* was recorded in Tamil Nadu (Balamurugan *et al*., 2019) [3]. It was the first time reported from Jammu & Kashmir, India (Bhat *et al*., 2010) [4] that *Didymella bryoniae* causes Didymella Blight diseases in ridge gourd. Both premature and mature plants are vulnerable to attack by fungal pathogens. Due to the economic importance of ridge gourd as a vegetable and due to substantial crop loss, there is an earnest need for investigation of its causal pathogens and also for their control. Hence, the present work has been taken into consideration to identify the primary fungal pathogens and also to know their mode of infection to combat the disease.

Materials and Methods
Isolation of pathogen: Infected plant leaf samples showing necrotic spots were randomly collected from the crop fields of ridge gourd in Coochbehar, West Bengal, India. Diseased ridge gourd leaves were dissected into small pieces of about 10 mm in diameter, sterilized with 0.1% mercuric chloride solution for 40 seconds and washed in sterile distilled water. The sterilized samples were then transferred aseptically to the potato dextrose agar (PDA) medium and were incubated at 28±1 °C for 6 days. Purified fungal mycelia were obtained from the 8-day old culture and stored at 28±1 °C for further studies.

Microscopic observation: The fungal isolate (RG/DE/F) was visualized under the Leica Application Suite V4.4 microscope. PDA culture plates of RG/DE/F isolate was also observed after 9 days of incubation. Detailed morphological properties of the fungi such as septation of hyphae, type and shape of spore were observed and recorded as proposed by Amadi *et al*. (2014) [2].

Pathogenicity test: Pathogenicity of the fungal isolate was tested following whole plant inoculation techniques as suggested by Dickens and Cook (Dickens *et al*., 1989) [8]. At the onset of the whole plant inoculation technique, 10^6 conidia/ml conidial suspension was prepared from 10-day old culture. For the experiment, four plants were taken along with two control sets. Conidial suspension (concentration used10^6 conidia/ml) was sprayed on the surface of the leaves of the plants. The control plants were sprinkled with autoclaved distilled water. All the plants were covered with plastic bags to maintain relative humidity and kept in the experimental garden under normal temperatures. After nine days, some infected parts of leaves of the plants were cut into pieces and the organism was re-isolated from the symptomatic leaf spots after proper sterilization. After seven days of growth, the organism was examined under the microscope.

Isolation of fungal DNA: The mycelium of fungal isolate was grown in 25 ml of potato dextrose broth and incubated for 15 days at 28±1 °C. Mycelium was gathered by refining through Whatman No.1 filter paper, washed over again with distilled water, desiccated and ground to powdered in liquid nitrogen using sterile mortar and pestle. The CTAB method (Haible *et al*., 2006) [10] was used in case of DNA extraction of RG/DE/F isolate. Plant tissue (1g) was homogenized with 2% CTABDNA extraction buffer of 60 ˚C for 1hour and mixed with chloroform-isoamyl alcohol (24:1) and then centrifuged. The aqueous phase was subjected to precipitation by using 0.6 volume of isopropanol and precipitates were washed with 70% ethyl alcohol for drying overnight. DNA was treated with RNase and was dissolved in buffer [TE, pH 8] and stored at -20˚C for further use. All extracted DNAs were diluted 10-fold in sterile distilled deionized water and quantified by use of ethidium bromide (EtBr) fluorescence.

Polymerase chain reaction (PCR) and sequencing: The total genomic DNA of RG/DE/F isolate was extracted from 12-15 days old potato dextrose broth cultures following the CTAB method (Haible *et al*., 2006) [10]. The DNA sample was subjected to PCR using two independent primer sets. The ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers were used for amplification of the internal transcribed spacer region (White *et al*., 1990) [21]. The 28S rRNA large subunit was amplified using NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) primers (O'Donnell, 1993) [14]. The fungal TEF (Translation Elongation Factor) gene was again characterized by another primer set EF1-728F CATCGAGAAGTTCGAGAAGG and EF1-986R TACTTGAAGGAACCCTTACC (Carbone *et al*., 1999) [5]. Amplification reaction was performed in a 25 µL reaction mixture containing 2 µL DNA template, 5 µL 5× Taq DNA buffer (containing 100 mM KCl, 10 mM Tris-HCl pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween 20 and 50% glycerol; Promega, USA), 1.5 µL 25 mM MgCl2 (Promega, USA), 1 µL 10 mM dNTPs (Promega, USA), 0.5 µL 10 µM each forward and reverse primers (Sigma, USA) and 0.125 µL 5u µL−1 Taq DNA polymerase (Promega, USA). PCR protocol for the amplification with the ITS 1 and ITS 4 primers was denaturation at 95 °C for 5 min, followed by 34 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min with a final extension at 72 °C for 12 min. PCR conditions for the NL-1 and NL-4 primers were 2 min at 94 °C followed by 38 cycles of 1 min at 94 °C, 1.5 min at 53 °C and 2 min at 72 °C and a final extension at 72 °C for 15 min. PCR protocol for the amplification with the EF1-728F and EF1-986R primers was denaturation at 95 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min with a final extension at 72 °C for 10 min. The amplicons were resolved in 1% agarose gels and were observed on a UV-transilluminator. The PCR products after purification were cloned in pGEM-T easy vector (Promega, USA) following the method of Sambrook & Russel (2001) and were sequenced by BioKart India Pvt. Ltd. (Bengaluru, India). The nucleotide sequences of the amplicons were submitted to GenBank after BLASTn analysis (Altschul *et al*., 1997) [1]. Phylogenetic trees were created by the neighbor-joining method through the Kimura two-parameter in MEGA 6.0 (Tamura *et al*., 2013) [19] following alignment with Clustal W 1.6 (Thompson *et al*., 1994) [20].

Phylogenetic analysis: The sequence data were analyzed using BLAST at the NCBI website. The sequence data from three amplified PCR products were assembled and analyzed using CLUSTAL W from MEGA 6.0 version software. The sequence was submitted to GenBank with proper annotations. The sequences were differentiated with interrelated sequences from a range of other plant-infecting fungus present in GenBank. Multiple sequence alignment was executed using the software CLUSTAL W in MEGA 6.0 version (Tamura *et al*., 2013) [19]. An evolutionary tree was constructed using the Neighbor-Joining method (Saitou and Nei 1997) [15].

Results and Discussions

Study of disease incidence based on visible symptoms: Disease symptoms were commonly observed on mature older leaves in the field. Ridge gourd leaves when infected produced yellowish-brown spots with concentric rings. Leaf spots often have a yellow halo and can crack through the middle. In some fields mature leaves show disease symptoms as white small circular to oval spots, which later enlarge and finally become necrotic (Fig 1A & Fig 1B). On average disease incidence was measured to be 25% in the cultivated field of Coochbehar district of West Bengal.

Morphology and identification: The fungal pathogen shows white creamy in color in the PDA plate after 7 days (Fig. 2A). These pathogenic fungi contain 2 types of conidia such as macro and micro-conidia. Microconidia were oval to ellipsoid in shape. The length and breadth of microconidia were 2.4-4.9 µm and 0.9-1.5 µm respectively. Macroconidia were sickle-shaped and contained 3-4 septa. The length and breadth of macroconidia were 15-20 µm and 1.6-2 µm respectively (Fig 2B). The morphological features of the fungus resembled to *Fusarium solani* (Hafiji *et al*., 2013) [9].

Establishment of the fungal culture as a pathogen following Koch's postulates: During pathogenicity test of the isolated fungus (code: RG/DE/F), symptoms were found to be started from 9 days post-inoculation (Fig 1C) in contrast to the control set plants where plants were symptomless even after 15 days. Then the fungus was re-isolated and compared with the original fungal isolate and the organism found was morphologically similar to that of the original inoculums. Thus, Koch's postulations were confirmed as the present organism (*Fusarium solani*) is associated with the disease development.

Molecular identification: The fungal isolate associated with leaf spots was detected by PCR using ITS specific primers, ITS 1/ ITS 4. The amplified PCR products showed an expected amplicon of ~500 nt (Fig 2C). For further confirmation, another portion of 28S rRNA large subunit was also amplified using NL1 and NL4 primers. The amplified PCR products showed an expected amplicon of ~600 nt (Fig 2C). The primer pair, EF1-728F and EF1-986R i.e., the amplified TEF gene products showed ~400 nt PCR product (Fig 2D).

The sequencing product of ITS region showed 99% nt similarity with *Fusarium solani* from India (Acc. No. MW774361), China (Acc. No. OP709878), Japan (Acc. No. LC002783), Malaysia (Acc. No. KF897898). The sequence comprised of partial 18S rRNA, complete ITS-1, 5.8S rRNA, ITS-2 and partial 28S rRNA genes. In BLAST analysis the partial 28S rRNA also confirmed 97% similarity with *Fusarium solani* from Malaysia (Acc. No. KF918591), China (Acc. No. OQ509677). The sequencing product of the TEF gene showed 100% similarity with *Fusarium solani* from China (Acc. No. MN650109), New Zealand (Acc. No. MG857343), Korea (Acc. No. JX277036). A phylogenetic tree (Fig 3) was created by using ITS sequences, where our isolates have shown similarities with that of *F. solani* strains isolated from other plants.

Fresh fruits of ridge gourd are rich in minerals, vitamins and other essential nutritional components. Ridge gourd is a major cultivated crop of sub-Himalayan West Bengal. In this study area, ridge gourd is predominately grown for its fruits and it has been found that ridge gourds are highly susceptible to fungal attack causing necrotic spots on both mature and pre-mature ridge gourd leaves. In northeast India *Fusarium solani* responsible for die-back diseases in tea plants, causes heavy losses in tea production (Sarmah *et al*. 2016) [16]. It was reported from Sikkim (Srivastava 1995) [17] that *F. solani* causes dry rot diseases in ginger. In the above mentioned reports, the identification of the fungi was done by cultural characteristics of the respective fungi, microscopic structural properties and molecular identification methods. For molecular identification two universal conserved primers from ribosomal DNA gene i.e. ITS1 and ITS4; 28S rRNA large subunit i.e. NL1 and NL4; TEF gene i.e. EF1-728F and EF1-986R were used. The RG/DE/F isolate of the present study (from ridge gourd leaf samples) was subjected to a pathogenicity test. Among them the fungal isolate RG/ DE/F was able to render re-occurrence of the large necrotic spot successfully.

Fig 1: (A) and (B) Naturally infected ridge gourd leaves showing leaf spot disease; (C) Disease symptoms on inoculated ridge gourd plant exhibiting some lesions of leaves after12th day of inoculation

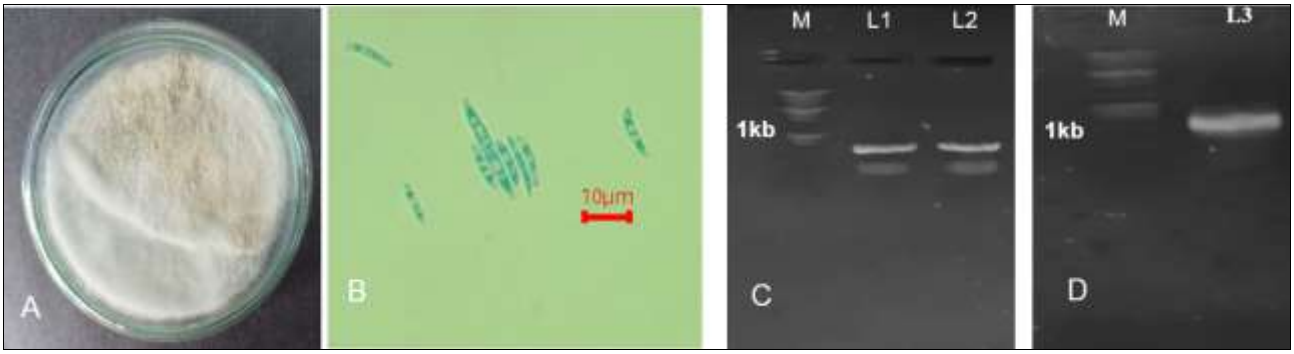

Fig 2: (A) Mycelia mat of *Fusarium solani* on PDA plate- after 7 days of inoculation; (B) Mature conidia of *F. solani* under light microscope; (C) Amplified PCR product of ITS gene (L1) and amplified PCR product of 28S gene (L2) of *F. solani* on 1% agarose gel under UV transilluminator; (D) Amplified PCR product of TEF gene (L3) of *F. solani* on 1% agarose gel under UV transilluminator [M=1 kb DNA ladder]

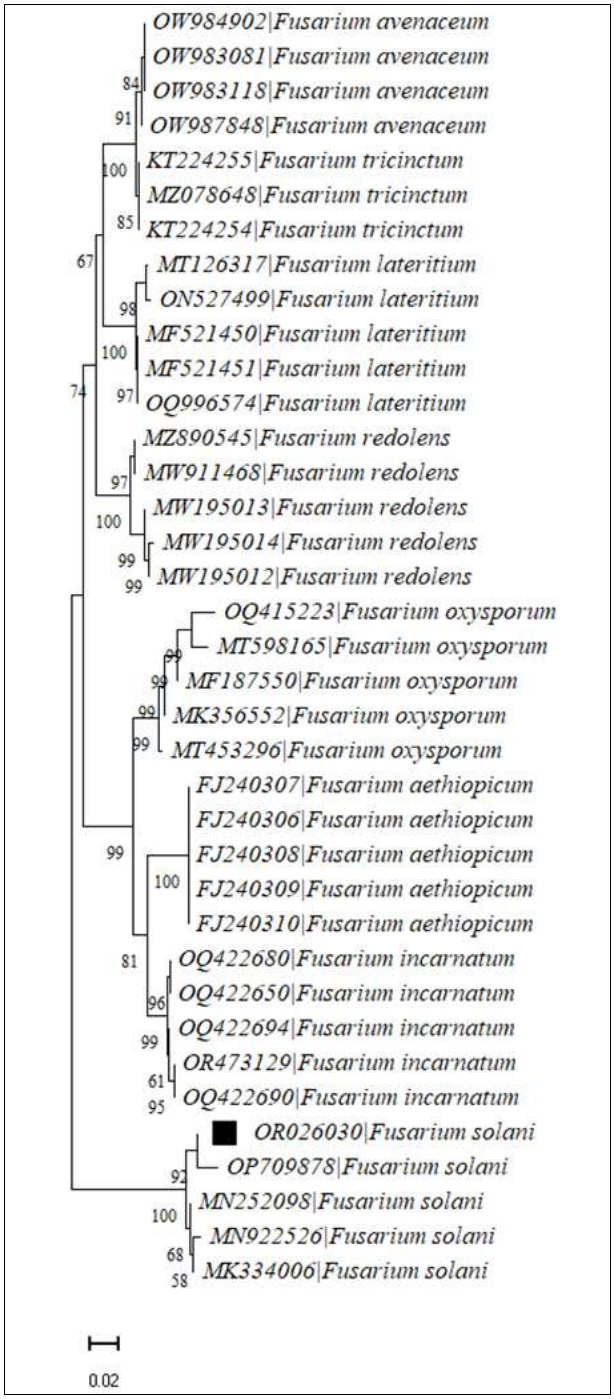

Fig 3: Phylogenetic tree of the ITS region of *Fusarium solani* [indicated in the figure by ■] and other *Fusarium* isolates obtained from GenBank. The tree was generated by neighbour joining method using Kimura-2-parameter model. Values at the nodes indicate percentage of bootstrap support out of 1000 replicates. GenBank accession numbers have been indicated at the end of each branch

Conclusion

From this study one new fungal pathogen has been identified. The identification of the pathogen has been done by morphologically as well as by molecular procedure. Pathogenicity of the fungus has also been ascertained by confirmation of Koch's postulates. Thus, the pathogen was identified as *Fusarium solani*. The fungus causes large necrotic spots on the leaf surface of ridge gourd. It is the first report of *Fusarium solani* to cause leaf spot disease in ridge gourd.

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References

1. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, *et al*. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Research. 1997;25:3389-3402. http://dx.doi.org/10.1093/nar/25.17.3389
2. Amadi JE, Nwaokike P, Olahan GS, Garuba T. Isolation and identification of fungi involved in the post-harvest spoilage of guava (*Psidium guajava*) in Awka metropolis. International Journal of Engineering and Applied Science. 2014;4:7-12.

3. Balamurugan A, Krishnan S, Aundy K, Malaiyandi M. First report of *Curvularia hominis* inciting fruit rot of ridge gourd (*Luffa acutangula*) in Tamil Nadu, India. Journal of Plant Pathology. 2020;2:529-529. https://doi.org/10.1007/s42161-019-00417-0
4. Bhat ZA, Dar GH, Anhanger MA, Bhat MA. First report of Didymella blight (*Didymella bryoniae*) on ridge gourd (*Luffa acutangula* (L.) Roxb.) from Jammu & Kashmir. International Journal of Applied Agricultural Research. 2010;3:335-336. http://www.ripublication.com/ijaar.htm
5. Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 1999;91:553. https://doi.org/10.1080/00275514.1999.12061051
6. Debbarma R, Kamil D, Bashyal BM. First report of crown and stem rot of tomatoes (*Solanum lycopersicum* L.) caused by *Fusarium solani* in India. Journal of Plant Pathology. 2021;103:1373. https://doi.org/10.1007/s42161-021-00938-7
7. Dhillon N, Sanguansil S, Singh SP, Masud MAT, Kumar P, Latchumi LK, *et al*. Gourds: Bitter, bottle, wax, snake, sponge, and ridge. Genetics and Genomics of Cucurbitaceae. 2016;20:155-172. https://doi.org/10.1007/7397_2016_24
8. Dickens JSW, Cook RTA. *Glomerella cingulata* on camellia. Plant Pathology. 1989;38:75-85. https://doi.org/10.1111/j.1365-3059.1989.tb01430.x
9. Hafizi R, Salleh B, Latiffah Z. Morphological and molecular characterization of *Fusarium solani* and *F. oxysporum* associated with crown disease of oil palm. Brazilian Journal of Microbiology. 2013;44:959-968. https://doi.org/10.1590/S1517-83822013000300047
10. Haible D, Kober S, Jeske H. Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. Journal of Virological Methods. 2006;135:9-16. https://doi.org/10.1016/j.jviromet.2006.01.017
11. Jaysingrao JS, Sunil CN. Nutritional assessment of fruits of *Luffa acutangula* var. *Amara*. International Journal of Scientific Research. 2014;10:2205-2207.
12. Manikandaselvi S, Vadivel V, Brindha P. Review on *Luffa acutangula* L.: Ethnobotany, phytochemistry, nutritional value, and pharmacological properties. International Journal of Current Pharmaceutical Research. 2016;7:151-155.
13. Nayak AK, Bandamaravuri KB. First report of powdery mildew caused by *Podosphaera xanthii* on *Luffa acutangula* in Odisha State, India. Archives of Phytopathology and Plant Protection. 2018;51:795-802. https://doi.org/10.1080/03235408.2018.1504642
14. O'Donnell K. *Fusarium* and its near relatives. In: The fungal holomorph: mitotic, meiotic, and pleomorphic speciation in fungal systematics. CABI, Wallingford, UK. 1993;225-233. https://www.researchgate.net/publication/224900762
15. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1997;4:406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
16. Sarmah SR, Dutta P, Bhattacharyya PN, Payeng B, Tanti AJ. Growth habit of tea pathogens and evaluation of relative susceptibility of selected tea cultivars. International Research Journal of Biological Sciences. 2016;5:1–9.
17. Srivastava LS. Review of ginger diseases. In: Ginger Disease Workshop: Proceedings and Strategic Plan, Gangtok, Sikkim; c1995. http://dx.doi.org/10.5772/intechopen.88839
18. Swetha MP, Muthukumar SP. Characterization of nutrients, amino acids, polyphenols, and antioxidant activity of ridge gourd (*Luffa acutangula*) peel. Journal of Food Science and Technology. 2016;53:3122-3128. https://doi.org/10.1007/s13197-016-2285-x
19. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution. 2013;30:2725-2729. https://doi.org/10.1093/molbev/mst197
20. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Research. 1994;22:4673-4680. http://dx.doi.org/10.1093/nar/22.22.4673
21. White TJ, Bruns T, Lee S, Taylor J. PCR protocols: A guide to methods and applications. Academic Press Inc. 1990;38:315-322.