



Isolation and Molecular Characterization of Collar Rot Causing Pathogen *Aspergillus niger* on Groundnut in Rajasthan, India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Groundnut, a major pulse crop also grown as an oil seed over 90 countries worldwide, is important to the Indian economy. The states of Andhra Pradesh, Gujarat, Rajasthan and Tamil Nadu are the main producers of groundnut. In 2021, disease incidence with symptoms similar to collar rot was reported from Jaipur district of Rajasthan. The fungal strain was isolated from the collar region of susceptible groundnut variety TAG-24. White colonies with mycelia were observed in 3-4 days which eventually turned dark brown in color. Pathogenicity tests were performed on healthy groundnut and collar rot symptoms developed within 7-8 days of inoculation. Sequencing of PCR products amplified with the 18S rRNA (NS1F-NS8R) and ITS (NS1F-ITS4R) primers indicated 99.4% and 100% identity respectively with a reference strain of *Aspergillus niger* CBS 554.65 (ATCC 16888) in the NCBI-Genebank database. Based on the morphological and molecular characteristics, the fungal pathogen was identified as *Aspergillus niger* and submitted to Gene Bank with the accession nos. ON954789 and OQ653131. The culture is submitted to NCMR-NCCS (accession number MCC 9892).

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1. INTRODUCTION

Collar rot disease caused by *Aspergillus niger* is a major fungal disease of groundnut in the tropical and sub-tropical regions of the world. It increases in severity during the rainy season with high temperatures and moisture. In Rajasthan, a major groundnut growing state of India, collar rot disease was first reported in 1926 [1] and is widespread [2]. Many groundnut cultivars are highly susceptible or moderately susceptible to this disease. *Aspergillus* blight of groundnut was reported in India by Jain [3]. In the 1980's, high incidence of the disease (25-50%) was reported from Rajasthan [4]. A comprehensive investigation of disease incidence in agricultural fields of Sikar and Jaipur districts of Rajasthan was carried out during the rainy season of 2014-15 [5]. The disease incidence was recorded at the range of 13-52% with blackening symptoms that went from roots to stem and affected the vascular system, complete wilting and plant death.

Aspergillus sp. is widespread in the soil and is often reported in indoor environments where its black colonies can be confused with those of *Stachybotrys* sp. due to similar morphological appearance [6]. Culture methods are time consuming and often inconclusive. Molecular detection such as PCR and/or together with C-probe, RSIC, direct single strand/double strand detection are more accurate for the identification of the pathogen [7].

A survey conducted in the rainy season of 2021, in groundnut fields of Jaipur district of Rajasthan, revealed collar rot disease incidence. This paper describes the isolation and molecular characterisation of the collar rot disease-causing microorganism based on morphological and molecular characteristics and pathogenicity tests.

2. MATERIALS AND METHODS

2.1 Isolation of Fungal Pathogen

Disease incidence in the groundnut varieties grown in the agricultural fields in Newai Tehsil and Jaipur district viz., *Arachis hypogaea* L. TAG-24, RG-510, RG-578, RG 559-3, RG 632-1 and HNG 123 were monitored for Collar rot disease incidence through personal visits and information obtained from Krishi Vigyan Kendra (KVK),

Banasthali Vidyapith and Rajasthan Agriculture Research University, Rajasthan during the rainy season of 2021.

Collar rot disease symptoms were reported in field growing the TAG-24 variety at Jaipur district and the diseased plants were obtained and brought to the laboratory for further isolation and characterisation of the pathogen.

Isolation and characterization of the fungal pathogen was done following standard procedures [8]. Diseased plant parts were washed in running tap water, surface sterilized in freshly prepared sodium hypochloride solution (0.4%) for 2min and rinsed with sterile distilled water thoroughly. Excised pieces (1-2 cm) of the diseased surface sterilised plant samples were placed in centre of solidified potato dextrose agar media (PDA) amended with rifampicin and kanamycin and incubated for 5-10 days at 28±2°C in dark. Pure culture was obtained and the morphological characteristics noted. Pathogenicity test was performed to confirm the disease and then molecular characterization was done.

2.2 Morphological Characterization

The fungal culture was observed for 5-10 days and the colony characteristics noted. Slides of fungal mycelia in the reproductive stage was prepared using lactophenol and cotton blue and observed under the microscope [9].

2.3 Pathogenicity Test

Pathogenicity test was performed using 15 days grown fungal culture [10,11]. Actively growing mycelium was collected with a sterile punch and a 5mm disc of mycelium was inoculated near the collar region of *Arachis hypogaea* cv. TAG-24 seedlings (15 days after sowing). After 7 days of inoculation, disease development in infected plants was observed. The pathogen was isolated as mentioned in the above section.

2.4 Molecular Characterization

Fungal DNA was isolated by the phenol-chloroform extraction method [12]. PCR amplification was done using 18S rRNA (NS1F-NS8R) and ITS (NS1F-ITS4R) primers (Table 1) following an optimised programme (Table 2). The amplified PCR product was purified by

Table 1. Universal primers and their combinations

Primer name	Sequence (Forward/Reverse)	
18S rRNA	NS1	Forward: 5'-GTAGTCATATGCTTGTCTC -3'
	NS8	Reverse: 5'-TCCGCAGGTTACCTACGGA-3'
ITS	NS1	Forward: 5'-GTAGTCATATGCTTGTCTC -3'
	ITS4	Reverse: 5'-CTTCCGTCATTCTTTAAG -3'

Table 2. Reaction conditions for PCR amplification

Gene	PCR Primers (forward/ reverse)	PCR - thermal cycle (annealing in bold)
18S r RNA	NS1/NS8	95°C: 2 min, (95°C: 30s, 52°C: 30s, 72°C: 2 min) x 35
ITS	NS1/ITS4	cycles, 72°C: 2 min

PEG-NaCl precipitation and sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) at the National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune, India. Similarity to related fungi were evaluated using BLAST and analysed using NCBI tools (<https://blast.ncbi.nlm.nih.gov>).

3. RESULTS AND DISCUSSION

Collar rot disease is frequently observed in groundnut cultivated area of Rajasthan (Sikar and Jaipur district) and there are maximum possibilities of seed born infection in groundnut seeds [5 & 13]. The groundnut cultivar TAG-24 was evaluated in terms of yield against eleven groundnut cultivars in AICORPO (All India Coordinated Research Project on Oilseeds) summer/rabi varietal trails from 1987 to 1997 and it showed highest pod yield [14]. TAG-24 has maximum harvest index [15], however, it is a collar rot susceptible variety [16]. In the present study, Collar rot disease incidence was reported

in *Arachis hypogaea* cv. TAG-24 from Jaipur district in the rainy season (July) of 2021 and the infected plants were obtained. The collar region of infected plants were covered with black spores. Usually, the infected plants died within a few days (Fig. 1a).

Fungal strain was isolated from the collar rot region of TAG-24 groundnut cultivar. Eight out of the ten isolates recovered from the diseased plants collected displayed the same morphology on PDA. White colonies were observed during initial growth and later it developed dark brown in color (Fig. 1b, c). Under the microscope, fungal mycelium was observed with spores and conidiophores (Fig. 1d). Hyphae were septate, brown in color and the diameter of hyphae were 2.9-6.6µm wide. Conidial heads were dark brown color with globose structure (~40-65µm diameter) and spherical conidia were around 4-5µm in diameter with irregular spines. These morphological characters observed in the isolated fungal strain are similar to those reported in *Aspergillus* sp. [17].

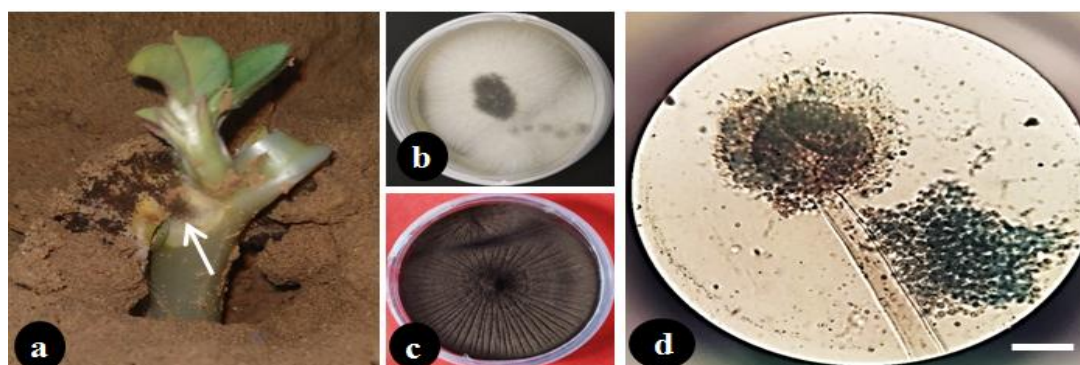


Fig. 1. (a) Collar rot infected (arrow) groundnut cultivar (TAG-24), (b) fungal mycelium growing in PDA medium, (c) fungus in reproductive stage growing on PDA medium and (d) conidiophore, conidial head and conidiospores observed under microscope (bar = 10 µm)



Fig. 2. Pathogenicity test: appearance of disease symptom in inoculated TAG-24 seedling

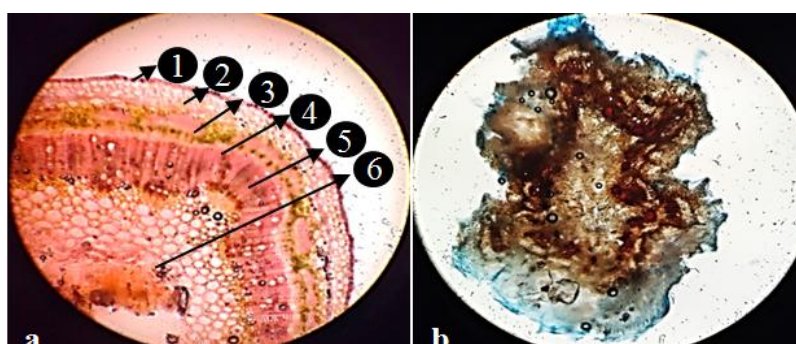


Fig. 3. Transverse section of stem (a) healthy plant (1: cuticle; 2: epidermis; 3: chlorenchyma; 4: endodermis; 5: bundle sheath; 6: pith) and (b) infected plant

During the pathogenicity tests, all inoculated seedlings displayed symptoms similar to those observed in the field after seven to ten days of inoculation. Disease symptoms were not observed on control set. These findings indicate that re-isolated strain was similar to the original isolate. Histopathology study of healthy and infected plant was performed and well-defined tissues of healthy plants and ruptured tissues of infected plants were observed (Figs. 2 and 3).

Sequencing the PCR products obtained with *18S rRNA* (NS1F-NS8R) and *ITS* (NS1F-ITS4R) primers and subsequent Blast analysis confirmed that the isolated fungal strain had 99.4% and 100% similarity respectively with *Aspergillus niger* CBS 554.65 (ATCC 16888). The sequences were submitted to the NCBI library as *Aspergillus niger* isolate H with the accession nos. ON954789 and OQ653131 for the sequences obtained with *18S rRNA* (NS1F-NS8R) and *ITS* (NS1F-ITS4R) primers respectively.

ITS is a fastest evolving portion of rRNA cistron which is useful for the molecular identification of

fungi because of its easy amplification and widespread use. ITS can be used alone or with combination with other protein coding genes.

Several *Aspergillus niger* isolates causing collar rot disease in groundnut have been characterized with different primers [18]. It is reported that the aflatoxin producing fungi are more efficiently amplified with aflpF/aflpR primers rather than FLA1/FLA2 and PAR1/PAR2 from the ITS region [19]. Mixed and pure culture of fungi can be identified by using the aflR gene specific primer [20].

4. CONCLUSION

Morphological, molecular characterisation and pathogenicity studies of the fungal pathogen isolated from groundnut cultivar TAG-24 growing in the Jaipur district of Rajasthan confirmed that the Collar rot disease is caused by *Aspergillus niger* H (Gene Bank accession no. ON954789 and OQ653131; NCMR-NCCS, accession number MCC 9892). The TAG-24 is grown for its superior yield; however, increasing reports of its

vulnerability to fungal diseases like collar rot necessitates employing corrective measures during cultivation or a shift to other varieties.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Jochem SC. *Aspergillus niger* on groundnut. Indischculturen (Teysmannia). 1926;11:325-326.
- Kumari M and Singh M. Morphological and pathogenic variabilities among *Aspergillus niger* isolates associated with groundnut (*Arachis hypogaea* L.) Annals of Plant Sciences. 2016;24(2): 364-368.
- Jain AC, Nema KG. *Aspergillus* blight of groundnut seedlings. Science and Culture. 1952;17:348-349.
- Bakhetia DR. Control of white grub (*Holotrichia consanguinea*) and collar rot (*Aspergillus niger*) of groundnut sown in different dates in Punjab. Indian Journal of Agricultural Sciences. 1983;53: 846-850.
- Kumari M, Singh M. Investigation of disease occurrence and pathogenicity test of *Aspergillus niger* causing collar rot of groundnut in Rajasthan. International Journal of Chemical Studies. 2017;5: 909-913.
- Samson Ra, HoubrakenJA, Kuijpers AF, Frank JM, Frisvad JC. New ochratoxin A or sclerotium producing species in *Aspergillus* section Nigri. Studies in Mycology. 2004;50:45-69.
- Abdin MZ, Ahmad MM, Javed S. Advances in molecular detection of *Aspergillus*: an update. Archives of Microbiology. 2010; 192:409-425.
- Wilson AA, Leopold TN, Michel JDP, Priya P, Mariya A, Manilal VB, Krishnakumar B, Henri AZP. Isolation and molecular identification of fungi in stored maize (*Zea mays* L.) and groundnuts (*Arachis hypogaea* L.) in Ngaoundere, Cameroon. American Journal of Microbiological Research. 2016;4:85-89.
- Sowley ENK, Adam L. Growth and yield of groundnut (*Arachis hypogaea* L.) plants in soil inoculated with *Aspergillus niger* van Tieghem. Archives of Phytopathology and Plant Protection. 2013;46:340-347.
- Zheng B, He D, Liu P, Wang R, Li B, Chen Q. Occurrence of collar rot caused by *Athelia rolfsii* on soybean in China. Canadian Journal of Plant Pathology. 2020;43:43-47.
- Tejaswini GS, Mahadevakumar S, Sowmya R, Deepika YS, Meghavarshinigowda BR, Nuthan BR, Sharvani KA, Amruthesh KN, Sridhar KR. Molecular detection and pathological investigations on southern blight disease caused by *Sclerotium rolfsii* on cabbage (*Brassica oleracea* var. capitata): A new record in India. Journal of Phytopathology. 2022;170:363-72.
- Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd Edn. NewYork Cold Spring Harbour Laboratory Press; 1989.
- Bajaya T, Bajaya M, Ghasolia RP, Shivran M. Incidence of Collar Rot of Groundnut in Rajasthan and its Management. Legume Research. 2022;1:6.
- Kale DM, Badigannavar AM, Murty GS. Groundnut variety, TAG 24, with potential for wider adaptability. International Arachis Newsletter. 1999;19:12-13.
- Badigannavar AM, Murthy GS, Kale DM. Evolution of Trombay groundnut varieties through mutation and recombination breeding. Plant Mutation Breeding. 2007; 1(3):46-51.
- Palaiah P, Narendrappa T and Mallesh SB. Screening of groundnut varieties and germplasm against collar rot, stem rot and dry root rot diseases. International Journal of Current Microbiology Applied Science. 2019;8(6):2321-2328.
- Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. Minneapolis, MN: Burgess Publishing Co; 2006.
- Divya RV, SudiniH, Reddy NP, Kumar VKK, Devi UG. Resistance screening of groundnut advanced breeding lines against

- collar rot and stem rot pathogens. International Journal of Pure and Applied Bioscience. 2018;6:467-74.
19. Ahmad MM, Ahmad M, Ali A, Hamid R, Javed S, Abdin MZ. Detection of *Aspergillus flavus* and *Aspergillus parasiticus* from aflatoxin-contaminated peanuts and their differentiation using PCR-RFLP. Annals of Microbiology. 2014;64(4):1597-1605.
 20. Manonmani HK, Anand S, Chandrashekar A, Rati ER. Detection of aflatoxigenic fungi in selected food commodities by PCR. Process Biochemistry. 2005;40: 2859-2864.

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