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First Report of *Rigidoporus Vinctus*, a Fungal Pathogen Causing Wilt Disease of Dragon Fruit in Nagaland, India

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ABSTRACT

As dragon fruit is one of the ornamental fruits and it is of immense important now a days because of highly nutritious and medicinal values. Dragon fruit are sensitive to disease infection but the crop has gained less attention in disease management practices which causes high yield reduction. So, knowledge on the causal organisms associated with the diseases of dragon fruit is the foremost priority to develop disease management practices. This research study aims to investigated the characteristics of fungal wilt disease of dragon fruit in three major dragon fruit growing districts of Nagaland, India. A systematic survey was conducted in Chumoukedima, Niuland and Dimapur districts for the symptomatologic study and disease sample collections. The wilt disease symptoms were characterised by yellowing of stems, stem rot, vascular discolouration, wilting and shrivelling and death of the plant. Fungal pathogens were isolated from the disease samples and pathogenicity studies confirmed the causal agent of the wilt disease of dragon fruit plants. The isolate was identified as *Rigidoporous vinctus* through morphological studies and molecular characterization. This study provides an information on the causal organism of wilt disease of dragon fruit which helps in developing effective disease management strategies and future research on the causal pathogen.

Keywords Fungal wilt, Morphological, Molecular, *Rigidoporous vinctus*, Symptomatologic.

INTRODUCTION

Hylocereus undatus, commonly known as dragon fruit, belongs to the cactaceae family and is prized for its distinctive appearance and delicate texture (Nerd *et al.* 2002). The *Hylocereus* spp. is among the most significant members of this family due to their nature, and they have been consumed as part of the human diet for more than 9000 years. The *Hylocereus* spp. is native to South America, Central America and Mexico (Nobel 2002). Dragon fruits are abundant in vitamins, antioxidants and natural pigments that can serve as colorants in both the food and textile industries (Wybraniec *et al.* 2007 ; Tenore *et al.* 2012 ; Ortiz-Hernandez and Carillo-Salazar 2012). In India,

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dragon fruit was first introduced in the late 1990s, and its cultivation has been expanding since then (Karunakaran *et al.* 2019). The major dragon fruit growing states in the country include Tamil Nadu, Karnataka, Mizoram, Andhra Pradesh, West Bengal, Nagaland, Maharashtra, Gujarat, Odisha and the Andaman and Nicobar Islands. Globally, four species of *Hylocereus* are widely cultivated : *Hylocereus polyrhizus* (red flesh with red rind), *Hylocereus undatus* (white flesh with red rind), *Hylocereus megalanthus* (white flesh with yellow rind) and *Hylocereus costraricencis* (deep red flesh with red rind) (Esquivel *et al*. 2007).

Diseases represent one of the major challenges in dragon fruit production, leading to significant yield losses and consequently reducing growers' income (Balendres and Bengoa 2019). Fungal diseases are widely reported worldwide. Out of the many fungal pathogens, *Rigidoporous vinctus* is also reported to be a causal agent affecting dragon fruit. *Rigidoporous vinctus* is a basidiomycete fungus belonging to the genus *Rigidoporous*, a group of wood decaying fungi that comprises numerous plants associated pathogens. Species within this genus are well known for causing white rot by degrading lignin rich plant tissues, and *Rigidoporous* spp. have long been documented as pathogens on a variety of woody host (Nunez and Ryvarden 2001).

Recently, *Rigidoporous vinctus* was identified as the causal agent of wilt disease affecting dragon fruit (*Hylocereus* spp.), an economically important cactus crop valued for its fruit and horticultural potential.

MATERIALS AND METHODS

A field survey was conducted in three districts of Nagaland, India where dragon fruit were cultivated viz. Chumoukedima, Niuland and Dimapur.

Symptoms were observed directly in the field and compared with descriptions available in recent literature. Thereafter, identification was carried out by matching field with documented photographs and references. Suspected plant samples were then collected for further confirmation under laboratory conditions. The pathogen was identified by isolating it from the diseased plant tissues collected from the field. Infected dragon fruit stems exhibiting the symptoms were cut into small segments (5 mm × 5 mm × 5 mm). These segments were surface sterilized using 1% of *Sodium hypochlorite* for one minute, followed by rinsing three times with sterile distilled water for one minute each. The sterilized pieces were then placed onto sterile potato dextrose agar (PDA) medium and incubated at 25±2°C for seven days. A pure fungal culture was subsequently obtained using the single hyphal tip culture method (Rangaswamy 1972). The entire experiment was conducted in the Department of Plant Pathology, School of Agricultural Sciences (SAS), Medziphema Campus, Nagaland University. Identification was carried out both morphological and molecular basis. Molecular characterization was performed by using universal primers ITS 1 and ITS 4 of 28S rRNA. The causal organism was confirmed through a pathogenicity test conducted on young cladodes of dragon fruit in three replications. The stems were wounded using a sterile needle and the pathogens were inoculated to the wounded lesions using agar plug method. The controls were processed similarly but agar plug of pathogen was substituted to agar plug of non-colonize PDA. The emergence of external symptoms was observed every day for two weeks. For pathogenicity test confirmation, morphological characteristics were used to re-identify after re-isolation of the fungal isolates.

RESULTS

Symptomology

The symptoms observed at the initial stages are the appearance of small, water-soaked lesions on stems especially near the base along with slight yellowing or discoloration of infected areas and tissue becomes soft and weak. The infected tissue turns pale brown to wheatish due to fungal colonization and the lesions expand rapidly under humid conditions.

Localized round or irregular dark brown lesions appear on the margin of the stem (cladodes) which progressively expanded into large, elongated dark brown necrotic areas. The symptoms progress from the tips towards the margin having a yellow halo Fig. 1.

(A) (B) (C) (D)

Fig. 1. Progress of the disease symptoms from (A) to (D).

Pathogenicity study

Isolation and identification of the pathogen

The infected cladodes showing the characteristic symptoms were collected from the dragon fruit farms in Chumoukedima, Dimapur and Niuland districts of Nagaland and the fungus was isolated from the parts of the stem showing the infection. The varying morphological characteristics observed were mycelial growth which varies from thin, pale and circular cottony growth to thick, milky white and fluffy growth due to abundant aerial mycelium and shows rapid radial growth when incubated at 28±1ºC. Upon microscopic evaluation, the hyphae are hyaline, septate and profusely branched forming an interwoven network. Prominent clamp connection was present at the septa (Fig. 2).

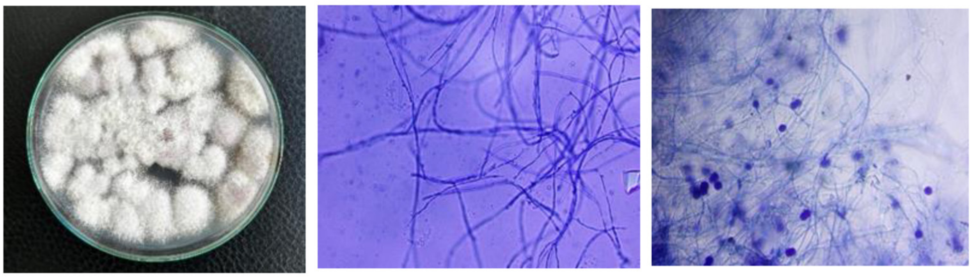

Fig. 2. Culture and microscopic view of *Rigidoporous vinctus*.

The isolate was further characterized molecularly using ITS 1 and ITS 4 Universal primers. Blast and phylogenetic analysis of ITS region revealed 100% similarity to *Rigidoporous vinctus* available in Gene bank (Table 1). The sequence of *R. vinctus* was deposited in the Gen bank (Acc. No. PZ593223).

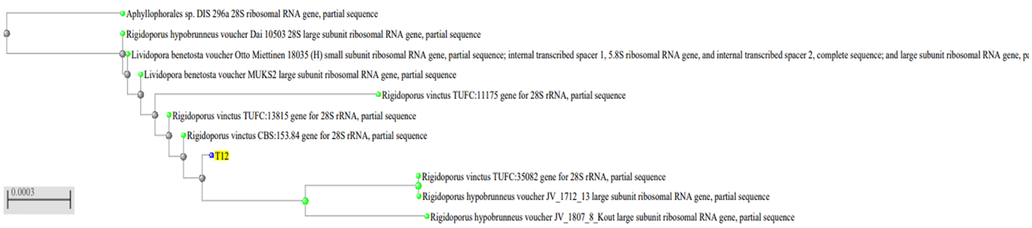

Table 1. Sequence comparison of the *R. vinctus* with the similar sequences available in the Gen Bank.

Sl. No.	Organism Name	Accession No.	% Match	Country
1	*Rigidoporus vinctus* CBS:153.84 gene for 28S rRNA.	LC770106.1	100.00%	Japan
2	*Rigidoporus vinctus* TUFC:13815 gene for 28S rRNA.	LC770097.1	100.00%	Japan

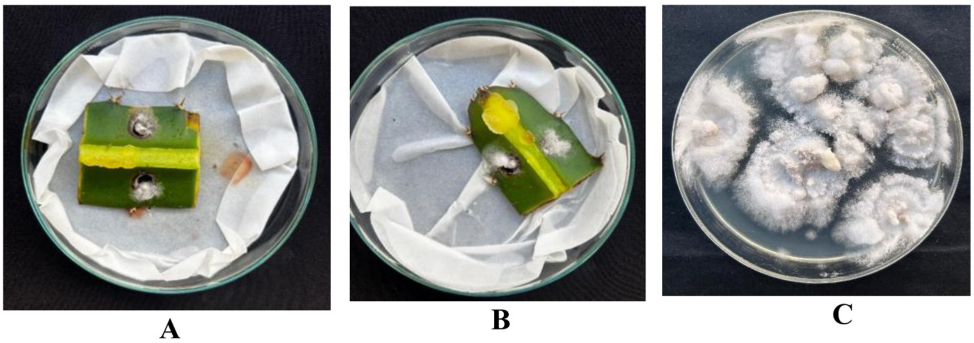

Fig. 3. Pathogenicity study by Agar plugs method (A & B) and Pure culture of *R. vinctus* (C).

Pathogenicity test was carried out on healthy cladodes of dragon fruit using mycelial plug method in three replications. The cladodes were wounded using a sterile needle and the pathogens was inoculated to the wounded lesions using mycelial plug method (Chuang *et al.* 2012). The controls were processed similarly but mycelial plug of pathogen was substituted with agar plug of non-colonize PDA. The inoculated stems were monitored daily for a period of two weeks for the development of disease symptoms. Subsequent re-isolation and re-identification of fungal pathogen confirmed that the symptoms observed on the infected cladodes were caused by *Rigidoporous vinctus* (Fig. 3).

DISCUSSION

Ann *et al.* (1999) also reported the occurrence of similar disease symptoms in rubber plants caused by a fungus belonging to the genus *Rigidoporous*. This symptom includes yellowing of leaves, progressive wilting and eventual defoliation. Although such wilt related symptoms had not been previously been documented in dragon fruit, the findings of the present study indicate otherwise. It was observed that dragon fruit plants exhibit comparable disease characteristics, particularly in plantations that had been cultivated with rubber trees. This suggests a possible persistence of the pathogen in the soil or planting environment, enabling it to infect subsequent crops. The occurrence of the disease in these previously used rubber plantations highlights the likelihood of cross host infection or carryover of the pathogen, thereby emphasizing the importance of understanding land use history and implementing proper management practices. Reddy *et al.* (2024) also described that the symptoms initially began as yellowing of cladodes along with water-soaked lesions which further progressed to browning and eventually the plant starts wilting as a result of the infection.

Based on their microscopic and macroscopic characteristics with reference to the literature documented earlier the organism was identified as *Rigidoporous vinctus* using the species description given by (Schwarze *et al.* 2000). The results were also similar to the reports given by (Ryvarden and Melo 2014) where they described the hyphae as hyaline, having septations and extensively branched. Also, prominent clamp connections are observed at the septa (Kirk *et al.* 2008).

Similar observations were made by Schwarze *et al.* (2000) who conducted pathogenicity test using agar plug method and proved pathogenicity.

CONCLUSION

Based on the present findings, it can be concluded that the wilt disease of dragon fruit plant is caused by *Rigidoporous vinctus*. Further confirmation was performed experimentally through pathogenicity study, where the same pathogen was successfully re-isolated from artificially inoculated plants under laboratory conditions. These results clearly establish the causal role of the fungus in disease development. Therefore, gaining a thorough understanding of the biology and characteristics of *Rigidoporous vinctus* essential as a first step towards developing effective and sustainable management strategies for the disease in dragon fruit plants.

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