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Disease incidence of anthracnose, stem rot and gray blight of Dragon fruit in Assam: A new report

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Abstract

The experiment was carried out in the Department of Plant Pathology at Assam Agricultural University, Jorhat during the year 2020 to 2023 on Dragon fruit (*Hylocereus* spp.) plant to identify diseases associated with the crop in Assam. Survey and sampling for diseased dragon fruit plant samples were carried out in eight districts of Assam during November, 2020 until October, 2021. Prevalence of stem anthracnose, stem rot and stem gray blight with highest per cent disease incidence (30%) of stem anthracnose was recorded from Gogamukh followed by KVK Chirang (12%), KVK Jorhat (10%), Paanjan Jorhat (10%), KVK Borpetta (10%), AAU-ZRS Gossaigaon (10%) and KVK Nalbari (5%). Stem rot disease incidence of anthracnose was recorded from six surveyed areas with highest incidence of 35 per cent from Gogamukh, followed by AAU-ZRS Gossaigaon (25%), KVK Jorhat (12%), KVK Borpetta (10%), KVK, Chirang (10%) and Paanjan Jorhat (5%). Stem gray blight disease of dragon fruit was recorded from three surveyed areas with highest disease incidence (25%) was recorded from Rongdoi Jorhat, followed by KVK Jorhat (12%) and Tinsukia (10%). Morphological and molecular study including pathogenicity test confirmed the pathogens as *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Fusarium dimerum* and *Diaporthe phaseolorum* causing stem anthracnose, stem rot and stem gray blight disease of dragon fruit. The diseases reported in the present study are new disease and to the best of our knowledge, this is the first report of anthracnose, stem rot and stem gray blight disease of dragon fruit in Assam.

Keywords: Dragon fruit, anthracnose, stem rot, gray blight, *Colletotrichum*, *Fusarium*

Introduction

Dragon fruit (*Hylocereus* spp.) is a perennial, climbing cactus vine with a triangular fleshy jointed stems that belongs to the family Cactaceae. It is a tropical fruit native to South America, Central America and Mexico). In recent years, cultivation of dragon fruit is also gaining popularity in Assam that pave the way of its demand for commercialization of cultivation due to its low maintenance and profitability with many success stories of farmers from different regions of the state. The crop can be easily cultivated in parts of degraded land and drought prone areas and prefers slightly acidic soil of pH 5.5-6.5 for its optimum growth and hence, a potential fruit crop for Assam too. Though dragon fruit is a low-maintenance crop, several diseases have arisen with significant impact, likely due to increases in planting acreage and the consequent buildup of pathogen inoculums. Among these, losses due to plant diseases play a significant role in fruit yield reduction and profitability. Considering the aforementioned facts and to identify the pathogens associated with the crop for sustainable cultivation in Assam the present investigation was undertaken as effective, sustainable and practical management strategies are yet to be developed.

The present investigation was carried out in the Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam during the year 2020-2023. A roving survey and sampling of diseased dragon fruit plant were concurrently carried out in eight districts of Assam viz., Dhemaji, Lakhimpur, Tinsukia, Jorhat, Nalbari, Borpetta, Chirang and Kokrajhar during November, 2020 to October, 2021. Longitude and latitude data of the surveyed areas were recorded (Table 1).

Table 1: GPS Coordinates of surveyed locations

Sl. No	Location	GPS coordinates		Agro climatic Zone
		Latitude	Longitude	
1.	Tinsukia	27.496156°N	95.35381°E	UBVZ
2.	Paanjaan, Jorhat	26.4963° N	94.2051° E	UBVZ
3.	KVK, Jorhat	26.8356° N	94.4620° E	UBVZ
4.	Rongdoi, Jorhat	26.8242° N	94.3090° E	UBPZ
5.	Gogamukha, Dhemaji	27.448459°N	94.322191°E	NBPZ
6.	Jengrai Singimari, Lakhimpur	27.109326°N	94.251931°E	NBPZ
7.	KVK, Nalbari	26.469438° N	91.43212° E	LBVZ
8.	KVK, Borpeta	26.441439° N	90.981408° E	LBVZ
9.	KVK, Chirang	26.528967° N	90.502354° E	LBVZ
10.	AAU-ZRS Gossaigaon, Kokrajhar	26.447445° N	89.942061° E	LBVZ

KVK: Krishi Vigyan Kendra, AAU: Assam Agricultural University, ZRS: Zonal Research Station, UBVZ: Upper Brahmaputra Valley Zone
NBPZ: North Bank Plain Zone, LBVZ: Lower Brahmaputra Valley Zone

During the survey, dragon fruit plants were closely inspected for their characteristic disease symptoms and disease incidences (DI) were recorded and calculated by the equation given by Cooke (2006) [2] as

$$DI (\%) = \frac{\text{No. of infected plant units}}{\text{Total no. of plant units assessed}} \times 100$$

The occurrence of stem anthracnose on dragon fruit was recorded from seven surveyed areas with highest per cent disease incidence of 30% recorded from Gogamukh followed by KVK Chirang (12%), KVK Jorhat (10%), Paanjaan Jorhat (10%), KVK Borpeta (10%), AAU-ZRS Gossaigaon (10%) and KVK Nalbari (5%).

External symptoms of the disease appeared as reddish-brown spots with yellow halos in the stem. As the disease progresses, these spots become darker with brown centres and coalesced to rot. The pathogen sporulate to form a salmon-coloured spore masses on the black acervuli formed in a concentric pattern around the necrotic tissue and can also be seen as black flecks in the infected tissue (Plate 1a). Typical symptoms of anthracnose with reddish brown spot measuring a 20 mm to 30 mm diameter, having chlorotic haloes on stems of dragon fruit with black acervuli has been

reported (Vijaya *et al.* 2014) [19]. The disease is regarded as the most severe fungal disease which can reduce production of dragon fruit (Crane and Balerdi, 2009; Masyahit *et al.* 2009) [3, 9].

Stem rot of dragon fruit was recorded from six surveyed areas with highest incidence of 35% from Gogamukh, followed by AAU-ZRS Gossaigaon (25%), KVK Jorhat (12%), KVK Borpeta (10%), KVK, Chirang (10%) and Paanjaan Jorhat (5%). External symptoms of the disease appeared as yellowish-brown water-soaked lesions on the affected stems, which gradually extends to upper and lower stems and finally became soft and watery and disintegration of the infected tissue occurs (Plate 1b).

Stem gray blight incidence was recorded from three surveyed areas with highest disease incidence (25%) from Rongdoi Jorhat, followed by KVK Jorhat (12%) and Tinsukia (10%). The infected plant observed had whitish irregular lesions on the affected stems and as the disease progresses these lesions became sunken and turned darker and become apparently dry and turned to grayish in colour. At later stage, the infected lesions expand resulting in the appearance of large blighted areas on the stem with formation of tiny black pycnidia (Plate 1c).

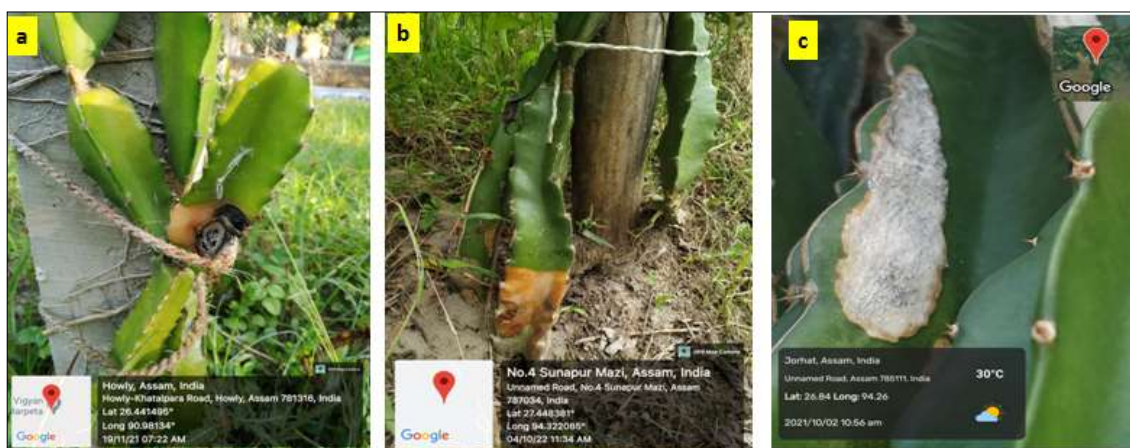


Plate 1 (a-c): External disease symptoms observed in the surveyed dragon fruit growing areas of Assam

A: Anthracnose b: Stem rot c: Stem gray blight

Diseased dragon fruit plant samples showing various symptoms were brought to the Plant Pathology laboratory of Assam Agricultural University, Jorhat for identification and characterization of associated pathogens. Diseased plant parts were excised aseptically from the lesions consisting of diseased and healthy parts. The cut samples were surfaced

sterilized in 1% sodium hypochlorite solution for 1 minute, rinsed in sterile water for three times and then air-dried on a blotting paper and then inoculated on to a Petri plate containing Potato dextrose agar (PDA) media. The plates were then incubated at 25±2 °C and examined daily for the growth of the microbes. Purification of the pathogen was

done by hyphal tip culture method (Tutte, 1969) [18] with slight modification.

Morpho-cultural identification of the DF-CG pathogen isolated from stem anthracnose produced initially dense white mycelial colonies on PDA, later became gray with reverse distinct gray concentric rings. Conidiophores produced were simple, short, erect and formed from a densely clustered mycelial cushion. Conidia were hyaline, one-celled and cylindrical with obtuse ends and guttulate (Containing an oil-droplet-like body). The size of the conidia ranged from 9.26-16.63 μm length \times 3.2-3.4 μm in width.

Anthrachnose pathogen with size of conidia 6.0-10 \times 2.0-2.5 μm , 9.0-24 \times 3.0-4.5 μm and 12.1-18.1 \times 3.6-8.2 μm on PDA was reported on white-fleshed spp. of dragon fruit (Masyahit *et al.* 2009; Taba *et al.* 2006 and Takahashi *et al.* 2008) [9, 16, 17]. Various species of *Colletotrichum* causing diseases in dragon fruit have been reported such as, *C. truncatum*, *C. aenigma*, *C. karstii* and *C. gloeosporioides* and *C. siamense* (Guo *et al.* 2014; Vijaya *et al.* 2014; Masyahit *et al.* 2009; Meetum *et al.* 2015; Palmateer *et al.* 2007; Zhao *et al.* 2018 and Takahashi *et al.* 2008) [4, 19, 9, 10, 13, 23, 17]. Out of which *C. gloeosporioides* is widely reported and hosted by three popular cultivated dragon fruit species viz. *H. undatus*, *H. polyrhizus* and *H. megalanthus* (Takahashi *et al.* 2008) [17]. Occurrence of anthracnose cause by *C. siamense* is reported on dragon fruit from Andaman Islands in India and *C. karstii* in Brazil (Nascimento *et al.* 2019) [12].

DF-FO and DF-FD were isolated from stem rot disease of dragon fruit. DF-FO pathogen formed pinkish white cottony mycelium and a light-orange undersurface on PDA media, later produced yellowish pigmentation on the surface of the cottony mycelium. Conidiophores were short, single, lateral monophialides in the aerial mycelium with macro conidia fusiform, slightly curved, pointed at the tip and had 3-5 septa, 22.5-24.3 μm length \times 2.5-3 μm in width. Micro conidia were oval to ellipsoid single celled, 3.2-3.6 μm in length \times 1.5-2 μm in width, false head formed on short monophialide and abundant chlamydo spores in chain.

DF-FD produced orange to deep apricot surface colonies on PDA media. Later aerial mycelium produced floccose and whitish growth on the surface of the media. Conidiophores produced were short, branched with swollen phialides. Macro conidia were curved, pointed at the apex, one median septa and 6-8.2 μm in length \times 1.6-1.8 μm in width in size. Micro conidia were ellipsoidal, single celled 2.2-2.4 μm in length \times 1.2-1.6 μm in width. Chlamydo spores produced were single, mostly intercalary and spherical in shape.

Stem rot of dragon fruit caused by *Fusarium oxysporum* from Argentina, Malaysia and Bangladesh has been reported (Wright *et al.* 2007; Mohd Hafifi *et al.* 2019 and Mahmud *et al.* 2020) [21, 11, 8]. The association of *F. solani* in stem rot of dragon fruit has also been reported (Rita *et al.* 2013) [14]. Other *Fusarium* spp. associated with stem rot of dragon fruit are *F. fujikuroi*, *F. proliferatum*, *F. semitectum* (Hawa *et al.* 2017 and Hawa *et al.* 2013) [5, 6]. Association of *F. dimerum* Penzig on stem rot of dragon fruit is reported from China (Yingying *et al.* 2016) [22].

DF-DP pathogen associated with stem gray blight of dragon fruit produced white aerial mycelium that covered the Petri plate containing PDA medium with brownish concentric rings on the lower surface. The pathogen formed conidiomata in black stromatic structures that contains

globose pycnidia with alpha and beta conidia. Conidiophores were hyaline, branched and straight to slightly curve. α -conidia produced were unicellular, hyaline, ellipsoidal to fusiform and guttulate, size of 6.33-6.40 μm in length \times 2.35-2.57 μm in width. β -conidia were aseptate, hyaline, filiform to hamate and were a size of 23.41-24.38 μm in length and 1-1.2 μm in width.

The sexual stage of *Phomopsis* (*Diaporthe phaseolorum*) producing α -conidia with size 6.43 \pm 0.55 \times 2.38 \pm 0.21 μm and β -conidia 24.57 \pm 2.77 \times 1.33 \pm 0.29 μm in PDA media has been reported (Shakirah *et al.* 2021) [15]. Isolate from stem blight of dragon fruit in Bangladesh with colony characteristics of white aerial mycelium, production of conidiomata in black stromatic structures, consisting of pycnidia with alpha and beta conidia and confirmed through molecular studies as *D. phaseolorum* (Karim *et al.* 2019) [7]. *Diaporthe* spp. associated with stem gray blight of dragon fruit are *D. arecae*, *D. eugeniae*, *D. hongkongensis*, *D. phaseolorum* and *D. tectonendophytica* (Shakirah *et al.* 2021) [15] and *D. ueckerae* in Taiwan (Wang *et al.* 2022) [20]. Pathogenicity test of the isolates DF-CG, DF-FO, DF-FD and DF-DP were carried out on healthy detached symptomless stems of dragon fruit. Detached healthy stems were surface-disinfected with one per cent sodium hypochlorite solution and artificial wounds approximately 2 mm deep on the stems were aseptically made using sterile cork borer. On each detached stem, two points were used to inoculate the pathogen and one point for control. The stems were then inoculated with a 5mm diameter disk culture of the fungal pathogen. Control stems were inoculated with a PDA disk without the fungus. Inoculated stems were placed into moisturized filter paper-layered plates and kept in an incubator at 25 \pm 2 $^{\circ}\text{C}$ and observed daily for development and progression of the disease symptoms for 7 days. The pathogens were re-isolated from symptomatic inoculated stems and re-identified by morphological characteristics.

The result of *in vitro* pathogenicity test of DF-CG, DF-FO, DF-FD and DF-DP isolates were proven to be pathogenic on inoculated detached dragon fruit stems while no symptom developed on control points.

DF-CG pathogen showed typical characteristics symptoms of stem anthracnose on the inoculated stems as reddish-brown spots with yellow halos around the spots. As the disease progresses, these spots become darker with brown centres and coalesced to rot on the 7th day after inoculation. The pathogen sporulate to form a salmon-coloured spore masses on the black acervuli formed in a concentric pattern around the necrotic tissue and can also be seen as black flecks in the infected tissues similar to those found in the field.

Isolates DF-FO and DF-FD showed typical symptoms on the 2nd day of inoculation as yellowish-brown water-soaked lesions which gradually extends to upper and lower stems and finally became soft and watery and disintegration of the infected tissue occurs on the 7th day similar to those found in the field.

Stem gray blight, isolate DF-DP showed typical symptoms as whitish irregular lesions on the inoculated stems and as the disease progresses these lesions became sunken and turned darker and become apparently dry and turned to grayish in colour.

For further confirmations of the pathogens, molecular approaches through genomic DNA amplification of the pathogens using ITS1 and ITS4 universal primers. Agarose gel amplification showed amplification products of size ~ 507 bp, 485 bp, 267 bp and 661 bp with universal primers.

PCR amplified products of the pathogens were sequenced at the Bio-serve, Bangalore, India and percentage identity and query coverage were used to identify probable phylogenetically closest species to our query sequences.

Phylogenetic analysis revealed that DF-CG pathogen from stem anthracnose of dragon fruit belongs to *Colletotrichum gloeosporioides* accession no. KR259525 isolate from Pakistan.

Phylogenetic analysis of DF-FO and DF-FD pathogens from stem rot of dragon fruit revealed that it belongs to genus *Fusarium*. DF-FO pathogen showed highest similarity to *Fusarium oxysporum* accession no MN959997 isolate from China while phylogenetic analysis of DF-FD showed highest similarity to *Fusarium dimerum* accession no KR139925 isolate of China.

Similarly phylogenetic analysis revealed that DF-DP pathogen from stem gray blight of dragon fruit belongs to *Diaporthe* genus and showed highest similarity to *Diaporthe phaseolorum* accession no MT043765 isolate from Malaysia respectively. The accession numbers of the pathogens are OQ274997 for *Colletotrichum gloeosporioides*, OQ274999 for *Fusarium oxysporum*, OQ274998 for *Fusarium dimerum* and OQ275000 for *Diaporthe phaseolorum*. Hence, based on the morpho-cultural and molecular characterization studies including pathogenicity test (Koch's postulates), the pathogens DF-CG, DF-FO, DF-FD and DF-DP were identified as *Colletotrichum gloeosporioides* causing stem anthracnose disease, *Fusarium oxysporum* and *Fusarium dimerum* causing stem rot disease and *Diaporthe phaseolorum* causing stem gray blight disease of dragon fruit in Assam.

Hence, from the above studies it can be concluded that *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Fusarium dimerum* and *Diaporthe phaseolorum* caused stem anthracnose, stem rot and stem gray blight disease of dragon fruit in Assam and to our knowledge, this study through morpho-cultural and molecular identification of the pathogens could be likely considered as the first scientific report pertaining to occurrence of stem anthracnose, stem rot and stem gray blight disease of dragon fruit in Assam.

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