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Status of Rhizome and Pseudostem Wet Rot Diseases of Banana

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A B S T R A C T

Rhizome rot and pseudostem rot diseases of banana are caused by bacterial pathogens mainly of *Pectobacterium carotovorum* or *Dickeya chrysanthemi* individually or together and *D. paradisiaca* respectively. The bacterial rot diseases are reported to cause 40-70% incidence in severely infected fields and their occurrence has been reported worldwide. Diagnosis of the disease is based on presence of the characteristic symptoms such as brown rotting in rhizome and foul odor in advance stages in case of rhizome rot while wet brown rotting on pseudostem in initial stage followed by breakage of pseudostem from the point of wet rot in later stage for pseudostem wet rot. The pathogens are characterized by morphology, gram staining, biochemical and molecular bases. Management of the diseases is focused by use of chemicals, cultural practices and beneficial microbes.

Introduction

Banana is the major fruit crop grown mainly in tropical and subtropical continents. The cultivation of the crop is mainly affected by biotic factors. Among the biotic factors, *Fusarium* wilt, bacterial wilt caused by *Ralstonia* or *Xanthomonas* and soft rot or rhizome rot caused by *Pectobacterium* or *Dickeya* are the major ones which affect the crop. *Fusarium* wilt disease is distributed worldwide including India and considerable studies on molecular characterization and management practices have been carried out (Dita *et al.*, 2018). Similarly, though the *Ralstonia* and *Xanthomonas* wilts are not encountered in India their occurrence and such studies have been documented elsewhere (Blomme *et al.*, 2014; Blomme *et al.*, 2017a; 2017b).

Soft rot or rhizome rot is a serious disease in countries of American (Fernandez, 1967; Rivera, 1978), African and Asian continents including India (Chattopadhyay and Mukherjee, 1986; Snehalatharani and Khan, 2010; Gokul *et al.*, 2019). The soft rot or rhizome rot disease causes >75% incidence in severely infected garden (Dita *et al.*, 2013; Blomme *et al.*, 2017a). The yield loss due to

the bacterial soft rot was 82% in Guangzhou, China during 2009 (Lin *et al.,* 2010). Bacterial pathogens such as *Pectobacterium carotovorum, Dickeya chrysanthemi*, *D. zeae* and *D. paradisiaca* have been reported as causal agents of the bacterial rot diseases in different parts of world. The disease development is associated with single or two causal agents together or intermediate group which depend on cultivars (genotype) and place (country) of occurrence. For an instance, *Pectobacterium carotovorum* pv. *carotovorum* has been reported as causal agent of rhizome rot in banana cv., Grand Naine (AAA) in India (Rajamanickam *et al.*, 2018) while it was *Dickeya* sp. (earlier *Pectobacterium chysanthemi*) in *Musa sapientum* (cv. ABB) in China (Lin *et al.*, 2010). Hence, it indicates, a thorough understanding on characterization of causal agent and etiology is required to classify the pathogen(s) and also to develop suitable management practices for combating the disease.

Hitherto, only few biocontrol, cultural and chemical based management practices have been attempted to manage bacterial rhizome rot or soft of banana (Arun *et al.,* 2012; Patel *et al.,* 2011; Rajamanickam *et al.,* 2018). Keeping the above background, updates on characterization and management of rhizome rot or soft rot and pseudostem wet rot have been summarized in this document.

Symptoms

The bacteria produce two different symptoms *viz*., rhizome rot and pseudostem wet rot. Soft rot is a common symptom observed in both the types and it forms due to secretion of pectinases which degrade the middle lamella and primary cell walls followed by tissue maceration, wet and foul smell rotting (Hugouvieux-Cotte-Pattat *et al.,* 2014). The pathogen attacks both rhizome and succulent tissue pseudostem.

Rhizome rot

It weakens the rhizome or corm. The affected young plants show leave yellowing and heart rot symptoms. The infected rhizome is cut open, yellow or brown coloured water soaked bigger spots with dark brown margin can be seen. Severely infected rhizomes may have characteristic symptoms such as decay of corm tissues, cavity formation and brown ooze with foul smell. Severely affected plants may topple down at maturity (Stover, 1972)

Pseudostem wet rot

The disease attacks pseudostem of young and pre-flowering plants. The pathogen enters into leaf sheath through wounds made during pruning or other cultural operations and multiplies in pseudostem. Initially it produces translucent spots at the base of the leaf sheath and pseudostem and later they become enlarged brown to dark brown spots cover large area of pseudostem. It may spread upward and down ward (Rivera and Ezavin, 1980) and interior part of leaf sheath causes rotting. Amber colour ooze comes out when the rotten area is pressed. Severely affected plant pseudostem may break due to wind or bunch weight.

Causal agents

Rhizome rot/soft rot

Different species or genus of causal organisms such as *Pectobacterium carotovorum* (earlier it was known as *E. carotovora* subsp. *carotovora*) and *Pseudomonas cichorii* in Korea (Chio *et al.,* 1988) and intermediate group of *E. carotovora* and *E. chrysanthemi* in Iran (Hassanzadeh, 1990) and *Dickeya* sp. (earlier *Erwinina chrysanthemi* then *Pectobacterium chrysanthemi*) in China (Lin *et al.,* 2010) and Papua New Guinea (Tomlinson *et al.,* 1987)

were reported. Besides, with in a country also the causal agents vary, in India, the causal agents of both *P. carotovorum* (*E. carotovora* subsp. *carotovora*) and *Dickeya* sp (*E. chrysanthemi),* and an intermediate group of those two in Karnataka and Andhra Pradesh (Snehalatharani and Khan, 2010), and *P. carotovorum* in Tamil Nadu and Kerala (Rajamanickam *et al.*, 2018: Gokul *et al.*, 2019) were reported.

Pseudostem wet/soft rot

Dickeya paradisiaca previously named as *E. chrysanthemi* pv. *paradisiaca* was reported as causal agent of pseudostem wet rot (Dickey and Victoria, 1980; Samson *et al.*, 2005). Besides, in one case *D. zeae* was isolated from rotten area of pseudostem (Zhang *et al.*, 2014).

Characterization of causal agents

Taxonomy of pathogen is important for studying epidemiology and to frame management strategies. Both *Pectobacterium* and *Dickeya* are the two major genera which cause rot diseases in banana and they belong to family Enterobacteriaceae and class Gammaproteobacteria.

P. carotovorum

The bacterium is non sporulating, gram negative rod with peritrichous and aerobic bacterium. It produces grayish white or cream to yellowish and mucoid raised colonies on Nutrient Agar and it forms characteristic pits on crystal violet pectate medium (Cuppels and Kelman, 1974).

D. paradisiaca

Bacterium is aerobic, rod, gram negative, peritrichous flagella and non sporulating type. Various growth patterns and colony colours were observed when the bacterium was grown on different media. On nutrient agar, it produces fine granular, irregular colonies initially and they turn to grey colour after 48 h and the colony centre rises after 96 h. On Kings B and YDC, it produces diffusible brown and non diffusible blue pigments respectively while MNT medium was found to be selective medium for *D. paradisiaca* (Hevesi *et al.,* 1981). Pathogenicity variation also observed among *D. paradisiaca* isolates as the isolates obtained from rhizome cortex could infect both rhizome and pseudostem whereas isolates recovered from pseudostem could only infect pseudostem (Rivera and Ezavin, 1980; Rivera *et al.,* 1980).

D. zeae

Bacterial strains produce opaque, dull, wrinkled, slight convex, round or nearly round gray coloured colonies on nutrient agar medium while cultured at 32°C for 24-48 h but subsequent sub-culturing on the medium the gray colour disappeared. The strain *D. zeae* was distinguished from *D. paradisiaca* as the former one able to grow at 39ºC and to catabolize *cis*-aconitate, myo inositol, mannitol and D-melibiose but the later one failed in the tests (Zhang *et al.,* 2014). Though *D. zeae* infects both banana and paddy, phylogenetic analysis revealed *D. zeae* infecting banana in Guangdong, China was different from the strain infecting paddy collected in the same region (Zhang *et al.*, 2014). The isolates of *D. zeae* could be distinguished by sequencing of 16S rDNA, and genes of dnax, gry B and rec A (Zhang *et al.*, 2014).

Dickeya sp. (earlier, *Erwinia chrysanthemi* then *Pectobacterium chrysanthemi*)

It is a straight rod with peritrichous flagella, facultatively anaerobic and gram-negative bacterium (Dickey, 1979).

Although molecular characterization helps in identification of bacterial pathogens, comprehensive parameters including utilization of various biochemicals and growth at different temperature and salt concentration etc., as summarized in Table 1 are important for classification of banana bacterial rot pathogens at and beyond species level (Rivera, 1978; Dickey, 1979; Dickey and Victoria, 1980; Rivera and Ezavin, 1980; Tomlinson *et al.,* 1987; Zhang *et al.,* 2014; Liu *et al*., 2016; Blomme *et al*., 2017a).

Pathogenicity of soft rot pathogen

Injection of *Dickeya* sp., suspension (10^8 CFU/ml) in rhizome of 40-60 d old plants established the characteristic symptoms of yellowing and wilting in ABB genome banana *M. sapientum* (Lin *et al.,* 2010). *In vitro* inoculation of *Pectobacterium carotovorum* bacterial disc on rhizome bits and covering the disc with moist cotton and keeping the cotton covered inoculated rhizome bits in polythene bag to create humidity was able to produce rotting in Nendran cultivar within 8 d (Gokul *et al.,* 2019). Similarly, inoculation of the *P. carotovorum* bacterial disc in rhizome of three month old Nendran and on pseudostem of two month old tissue culture Nendran produced the complete rotting symptoms on 37 d and 16 d after inoculation respectively (Gokul *et al.,* 2019). As both *Pectobacterium* and *Dickeya* are having same strategy to cause disease and they share common host and geography, they tend to occur together in an environment. Also, several species of *Dickeya* are reported to cause soft rot. Hence possible combinations of different species of *Dickeya* and *Pectobacterium* causing rot diseases are yet unanswered.

Etiology

Bacterial rot pathogens enter via wounds caused due to pruning and detachment of senescent leaves, and natural openings. Poor sanitation, water deficit and dry spell for long time are also associated with severity of the disease. The bacteria can spread through irrigation water, tools, equipments and propagation materials without expressing symptoms (Charkowski, 2018). Banana is having different genome groups (ploidy levels) and response of the genome to the pathogens also varied. Banana cultivars of cooking (ABB) and plantain (AAB) were susceptible to *D. paradisiaca* than Cavendish (AAA) group (Blomme *et al.,* 2017a). Cultivars with diploid genome (AA) were susceptible to *E. chrysanthemi* (*Dickeya* sp.) while ABB genome group was free from the disease and AAA showed reaction in between AA and ABB genome groups (Tomlinson *et al.,* 1987).

Management

Earlier, soft rot was not a serious problem for banana cultivation while it was severe in vegetables, tubers, post harvest horticultural produces etc. However, of late, rhizome rot or soft rot become a major problem in tissue cultured derived banana seedlings planted gardens (Loganathan *et al.,* 2018). Hence here possible management practices hitherto developed for controlling rhizome rot or soft rot have been documented.

Frequent visit to field at least once in two weeks is advised for early diagnosis of bacterial disease symptoms so as to take up sanitation measures to curtail further spread (Lehmann-Danziager, 1987) or to take up control measures in time. A sanitation programme through ELISA based indexing of planting materials for management of soft disease in Cavendish banana in Cuba was effective and successful (Pérez-Vicente, 2003). However, in case of *D. paradisiaca*, several stages of indexing were required as it could multiply in meristem tissue and it was reported as much as six indexing in different

stages of mass multiplication were required to exclude the disease (Hernández *et al.*, 1994). Also continuous disinfection of tools in sodium hypoclorite (3.5%) during different stages of field operations especially during pruning was effective to control the disease up to 80% in plantain bananas (Fernández *et al.*, 2013).

Table.1 Biochemical based differentiation of bacterial rot pathogens at species level

Test	*E. chrysanthemi* (then *Dickeya* sp.)	*E. carotovora* pv. *carotovora* (then *P. carotovorum*)	*E. chrysanthemi* pv. *paradisiaca* (then *D. paradisiaca*)	*D. zeae*
Polypectate degradation	+	+	+	+
Soft rot on potato	+	+	NA*	+
Gelatin liquefaction	V	+	-	+
Phosphatase activity	+	-	+	NA
Gas from D glucose	+	-	+	NA
Growth at different temperature	36°C	36°C	V	39°C
Growth in 5% NaCl	V	+	-	NA
Acid from maltose	-	V	-	+
Erythromycin sensitivity (60 µg)	+	-	NA	NA
Acid from Ethanol	+	-	+	NA
Acid from Trehalose	-	+	-	-[a]

*NA: Not available; + most positive; - most negative; V: Variable results; a-fail to utilize.

Several treatments involving chemicals such as combination of 0.15% acephate dip and 1% Bordeaux mixture or mancozeb 0.3% (Patel *et al.*, 2011) and series of treatments with COC (0.3%,), antibiotic 600 mg/l (Streptomycin sulphate 9%+Tetracycline hydrochloride 1%), 0.2% solution of Methyl Ethyl Mercuric chloride (MEMC), mono ammonium phosphate (0.2%) and 50 g carbofuran/pit (Arun *et al.*, 2012) have been advocated to manage the rhizome rot of banana. However, use of chemicals especially streptomycin and MEMC are having several demerits hence they could not be listed in recommended chemicals for use in banana (Central Insecticide Board and Registration Committee as on 31.05.2018: http://ppqs.gov.in/ contactus/central-insecticide-board-and-registration-committee-cibrc). Information on biocontrol based management of bacterial rot of banana is very scanty except few reports. Mini scale application of *Pseudomonas fluorescens* cells, *Bacillus subtilis* and VAM fungus (*Glomus fasciculatum*) (Nagaraj *et al.*, 2012) and bio-priming of banana with plant growth promoting bacterial strains (*B. subtilis* PP and CL3) during primary and secondary hardening (Rajamanickam *et al.*, 2018) showed considerable level of disease control which indicates that there is an urge in developing field deliverable bioformulation(s) or consortium for management of the rot diseases.

In conclusion, a holistic approach which reveals about the causal agents responsible for rhizome rot or Pseudostem wet rot which infect across the cultivars or genome groups is

lacking in India and elsewhere. Weather parameters which are essential for development of soft rot in banana have not been worked out. Management of soft rot diseases through biocontrol agents' showed significant disease control in other crops such as vegetables and potato etc., hence similar or improved management practices can be attempted in banana.

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