Histopathology of Banana Infected with
*Fusarium oxysporum* f. sp. *cubense* (E. F. Sm.) Synd. & Hans.

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ABSTRACT

Fusarium wilt is a severe disease of banana plants caused by the fungus *Fusarium oxysporum* f. sp. *cubense*. The objective of this study was to trace the pathway and colonization in roots and rhizome of the highly susceptible banana cultivar, ‘Nanjangud Rasabale’. We observed that the entry of the fungus into the roots is primarily through the rootlet, progressing upwards to the rhizome and pseudostem. External symptoms of characteristic yellowing of the lower leaf sheath were observed only after 90 days after inoculation though internally the colonization of the fungus had occurred as early as 45 days after inoculation. Longitudinal splitting of the outer leaf bases just above soil level was seen only after 150 days after inoculation. Formation of chlamydospores, microconidia were observed in root and rhizome sections. Macroconidial formation was observed in only preserved root and rhizome samples.

Keywords: fungal pathway, macroconidia, Nanjagud Rasabale, vascular browning

Abbreviations: DAL, days after inoculation

INTRODUCTION

Bananas constitute the fourth largest fruit crop of the world, and India stands to be the world’s biggest banana grower, with an annual production of 10.4 million tonnes accounts for 20% of total world output of 48.9 million tonnes in 2010 (Anon. 2011). *Fusarium* wilt of banana is the most significant vascular wilt disease causing immense loss of crop (Stover 1962; Ploetz 1990, 2000). Fusarium vascular wilt disease of banana is caused by soil-borne parasitic strains of the soil fungus *Fusarium oxysporum* Schlecht. f. sp. *cubense* (Foc). The causal organism is a member of the *Elegans* section of *Fusarium* (Fungi Imperfecti), owes its original name to *F. cubense* to Smith (1910), who isolated it in culture from diseased vessels in material from Cuba. The fungus is mainly intracellular found typically in the vascular vessels. The mycelium is small septate white, usually with a purple tinge, darker at the edge. Microconidia are abundant hyaline irregular shape oval ellipsoid to cylindrical straight to curve mostly one cell borne 5-12 × 2.2-3.5 μm borne on short, sparsely branched conidiophore. Macrocystidia hyaline borne on branched conidiophores and are 3-5 septate, fusoid-sulcate with pedicellate base 27-46 × 3.5 μm. Chlamydospores are oval to spherical, 7-13 × 7-8 μm. The fungus survives in the soil mainly as chlamydospores formed by the hyphal and conidial cells.

The disease was originally reported from southeast Queensland, Australia in 1874 (Bancoff 1876), in Honolulu by Higgins (1904), in Costa Rica and Panama in 1904 (McKenny 1910). In India, it was first reported from Chinsurah in 1911 (Stover 1962) and then became widespread in regions of Tamil Nadu, Karnataka, Bihar and Assam.

The entry of the fungus into the host plant is mainly at the point of emergence of the secondary and tertiary roots as reported in banana (Stover and Simmonds 1987), pea (Bishop and Cooper 1984), tomato (Hutson and Smith 1983), cotton (Rodriguez-Galvez and Mendgen 1995), lentil (Bhalla et al. 1992), chick pea (Gupta and Khare 1992), lily (Baayen and Rijkenberg 1999), oil palm (Flood 2006), gerbera (Li et al. 2010) and pine (Ahangar et al. 2011). Pathogen-induced tracheary obstructions lead to wilting of the plant. The infected host plant shows characteristic yellowing of the leaf blades followed by buckling of the petiole wherein the pseudostem stands with dead leaves hanging around it. Four to six weeks after the appearance of the first symptoms, longitudinal splitting of the outer leaf sheath begins from the base and progresses upwards. Internally when the affected rhizome is cut transversely, vascular discoloration, commonly proceeding from yellow to red to brown, a generally recognized feature of vascular infection is observed. Roots become blackened and decayed. Root bases show characteristic red strands passing into the rhizome stele. The diseased rhizomes and pseudostems emit a characteristic odour. Early infected plants fail to produce inflorescence. However late infection leads to improper development of bunches and clump formation in fruits. Beckman et al. (1961) suggested that the infection of the root xylem was accomplished by movement of macroconidia upwards in the vascular stream. The disease expression is observed to be severe at the time of fruiting. The main aim of the present study was to understand the mode of infection and the pathway of fungal movement inside the host. Thus histopathological studies were under taken at different levels of infection.

MATERIALS AND METHODS

Tissue-cultured banana cv. ‘Nanjangud Rasa bale’ saplings were procured from the Biotechnology Centre, Department of Horticulture, Government of Karnataka, Bangalore and raised in the Botanical Garden of University of Agricultural Sciences, Bangalore with a spacing of 2 m × 2 m according to the Package of practices for Horticultural crops (Anon. 2000). Cultures for inoculum were grown for 21 days on corn seed meal mixture was prepared as follows. Sand and maize meal in the proportion of 95:5 w/v mixture
was prepared and moistened with sterile water to 20% (v/v). About 125 g of mixture was placed into 250-ml conical flask and was sterilized at 1.1 kg/cm² for 20 min. Aseptically the flask was inoculated with actively growing seven days old mycelial disc cultures of 5 mm diameter. The flasks were incubated at 27 ± 1°C for 30 days. The flasks were shaken regularly to facilitate uniform growth of fungus. Thus obtained inoculum was applied to the field in a 1: 9 (corn seed mixture: sand) ratio by making a furrow close to the rhizosphere of the plant, after planting. Two experiments were set up. In the first experiment, the pathogenicity test of the isolate of Fusarium was carried out. Foc culture multiplied on maize meal was thoroughly mixed with sterilized field soil separately at 1:20 w/v ratio. This mixture was filled in big earthen pots (1.5 × 1 feet), previously surface sterilized by two per cent formalin solution. The sterilized pots filled with the mixture were incubated for ten days under glass house conditions. On the 11th day, tissue-cultured Nanjangud Rasa Bale plantlets drenched in streptomycin solution overnight were planted in pots. Pots were watered regularly so as to maintain 50% holding capacity of the soil. After 45 days of inoculation (DAI) root bits was placed on PDA and incubated, reisolated to prove Koch’s postulates and presence of Fusarium was confirmed by the second author, who is a mycologist.

After confirmation, in the second set of experiment, plants were infected in the same manner as in the first experiment. Plants at an early stage of infection were sampled once a week until 30 days and thereafter once in 30 days for 6-7 months.

The healthy plants were planted in big earthen pots containing sterilized field soil autoclaved at 1.1 kg/cm² for 30 min and this sterilized soil is buried in soil next to the inoculated plants to create the same environmental conditions.

Samples for microtome sections consisted of few roots and a portion of the rhizome of healthy and infected plants. Root and rhizome bits of 1 cm² were cut and fixed in Carnoy’s B fixative (ethyl alcohol: chloroform: acetic acid; 6: 3: 1) for 3 hrs. The fixed sections were dehydrated in an alcohol: tertiary butanol: xylene (ethyl alcohol: chloroform: acetic acid; 6: 3: 1) for 3 hrs. The fixed and stained sections were observed under a research microscope (Olympus BX50) and photomicrographs were taken.

RESULTS

External symptoms

The external symptoms manifested in the form of characteristic yellowing of the lower leaves after 90 DAI, followed by the buckling of the petiole and hanging of the leaves. The roots when longitudinally split open exhibited red discolored vascular strands traversing into the rhizome stele through the root-rhizome plexus. Extensive necrosis of cortical cells led to gap formation between the cortical and stelar region. The small gaps formed initially in cortical cells, enlarged eventually to encompass the entire transverse area of infected xylem tissue leading to the separation of the cortical region from the stelar region and thus facilitate the release of abundant microconidia formed in the xylem vessels. The fungus by this had progressed into the rhizome.

In the rhizome, the fungus has thoroughly established in the wood vessels and there is little doubt that the fungus will succumb to wilt disease. The hyphae grow through the continuous system of vessels into the upper most part of the rhizome. Hyphae are found in the cells of the phloem and adjoining parenchyma cells. The mycelium continues its growth through the continuous system of vessels, entering the stele, hence passing again through the cortex at the upper part of the rhizome, where the vascular bundles form

Histology of infected plants

The entry of the fungus into the roots is primarily through the rootlet, progressing upwards to the rhizome and pseudostem (Fig. 1A, 1B). Vascular discoloration is a generally noted phenomenon of infection was evident with the entry of the fungus into the host. By moving intercellularly the fungus invades the entire xylem elements and the content of the host cells is evidently absorbed. The fungus initially being confined only to the xylem vessel elements and tracheids later invades the xylem parenchyma cells and inner phloem cells in the immediate vicinity of affected xylem vessels (Fig. 1C).

Traces of mycelial bits were evident from 8 days old infected root sample sections. At 30 DAI, the portion of the xylem tissue showing histological changes was very restricted and was macroscopically recognizable. After 45 DAI, profuse growth of mycelium in the roots, and its entry being made into the rhizome was evident after 90 DAI. The fungal entry being made into the fructification in the roots was evident with the production of abundant microconidia (Fig. 1D). Formation of chlamydospores (resting spores) was seen in longitudinal sections of roots (Fig. 1E). Externally, rotting of the cortex region of the roots was observed initially. Extensive necrosis of cortical cells led to gap formation between the cortical and stelar region. The small gaps formed initially in cortical cells, enlarged eventually to encompass the entire transverse area of infected xylem tissue leading to the separation of the cortical region from the stelar region and thus facilitate the release of abundant microconidia formed in the xylem vessels. The fungus by this had progressed into the rhizome.
leaf traces, and so on up into the leaves, but apparently always being confined to the vessels. These internal changes are responsible for the typical wilting, discoloration and final breakdown of the leaves.

Macroconidial formation was evident in rhizome when preserved for 2 days and then fixed and sectioned (Fig.1F, 1G). At no stage macroconidial formation was observed in fresh sections. The macroconidia occurrences were more on the cut surface of the infected material.

Hypertrophy and Hyperplasia occurred in the xylem and phloem elements of both infected rhizome and roots. The adjoining cells of xylem and phloem also were found hypertrophied. Concurrently at no time were gums, gels or tylose formations observed in any of the root sections.

DISCUSSION

Foc prevails as mycelium in the xylem vessel elements. It leads to disintegration of xylem parenchyma cells in infected areas, hyperplasia and hypertrophy of xylem parenchyma cells and formation of cavities in the cortex region. The presence of microconidia and chlamydospores has been found in the infected tissue. The yellowing and wilting of the entire plant is observed subsequently (Beckman and Roberts 1995).

The fungus enters the roots either by direct penetration roots or indirectly through wounds (Lucas 1998). The most common sites of direct penetration are located at or near the root tip of both tap roots and lateral roots (Lucas 1998). The fungus penetrates the root cap in banana while in pea at the root tips and root-hair zone (Ter Haak 1970), in cabbaged Fusarium oxysporum, f. sp. conglutinans (Smith and Walker 1930), Mexican fan palm and queen palm the entry site is through the zone of elongation (Elliot et al. 2010) and in barley roots through the root hairs (Strunnikova et al. 2010). The fungus enters the apical region of the root where endodermis is not fully differentiated and grows to enter the developing protoxylem. Inside the host tissue, the mycelium grows through the xylem vessel elements, establishes itself and invades the adjoining vessel elements as reported in tomato infected with Fusarium oxysporum f. sp. lycopersici (Chambers et al. 1963), in carnation infected with Fusarium oxysporum f. sp. dianthi (Barbara and Paul 1972) and in chicory (Garibaldi et al. 2011).

Gel and gum formation, hypertrophy of xylem parenchyma cells including tylose formation and vascular discoloration are the histological changes found as response of the host to infection.

Vascular gelation and gum formation are the first visible structural changes in the sequence of vessel occluding processes in response to the pathogen infection (Beckman and Halmos 1962). Vascular gelation as earlier mode of defense mechanism to the invasion of the host has been reported in Fusarium wilt fungus (Chambers et al. 1963). The Foc conidia were transported into the vascular stream as reported in tomato (Scheffer and Walker 1953) and date palm (Elliott 2011). Macropodial occurrence in stellar and cortical region was observed in preserved rhizome section inferring that the production of macroconidia in banana is only in dead material.

The cortical cell necrosis in the roots facilitated the release of microconidia and chlamydospores into the soil as reported in mimosa wilt (Phipps and Stipes 1976).

Proliferation of parenchyma cells adjacent to infected cells has been noticed in tomato infected with Fusarium wilt fungus (Deese and Stahmann 1962b; Chambers et al. 1963) and in staghorn sumac (Ouellette et al. 2006). Such proliferation is probably due to the action of auxins (Diamond 1970). Hypertrophy and hyperplasia was found in the xylem parenchyma cells of rhizome of banana. Disintegration or tearing of these cells may be due to the weakened cell walls.

Distortion of the cortex cells and endodermis leading to the exposure of the vascular bundles were the final response of banana roots to Foc. Bickerton (1942), Pennypacker and Nelson (1972) and Baayen and Elgermsa (1985) reported similar formation of cavities within the stems of susceptible variety of carnation in advanced stages of pathogenesis and also in guava (Gupta 2010). It has been shown that several Fusarium wilt pathogens produce pectinase (Heitefuss et al. 1960; Edginton et al. 1961; Deese and Stahman 1962a) which attack the cell walls and the middle lamellae leading to cavity formation.

The protective mechanism such as formation of gel, gums and tyloses did occur in banana roots and rhizomes of ‘Nanjangad Rasabale’ variety which however failed to play the role of resistance. This is the first report of histopathological studies conducted showing the pathway of fungal movement inside the host at different levels of infection.

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