

Full Length Research Paper

Isolation and identification of some pathogenic fungi associated with cassava (*Manihot esculenta* Crantz) root rot disease in Cameroon

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Root rot diseases constitute a major constraint to cassava production in Cameroon. However, not much is known about the identity of pathogens associated with them. It is in this light that this study was realized with the aim of characterizing the various root rot diseases and identifying their associated fungal causal agents in Cameroon. Sixty four cassava stems with root rot symptoms were sampled in the Littoral, Southwest and West Regions of Cameroon. Results revealed that cassava root rot (CRR) is either wet (soft) or dry depending on the region of study. Isolation was done on PDA medium enriched with Chloramphenicol. After purifying thrice on the PDA medium, 20 isolates were collected. Identification with the help of the Barnett and Hunter key revealed the existence of seven fungi including *Colletotrichum* sp., *Fusarium* sp., *Pestalotia* sp., *Geotrichum* sp., *Sphaerostilbepens*, *Trichoderma viride* and *Botryodiplodia theobromae*.

Key words: Cassava root rot, pathogen, sustainable agriculture, disease control.

INTRODUCTION

The actual crop yield as percentage of potential yield is more than 60% for North America, Western and Central Europe, but is less than 50% for South America and North Africa and it is about 30% for Central America and the Caribbean, Eastern Europe and sub-Saharan Africa

(FAO, 2012; Valipour, 2014; Valipour et al., 2015). One of the world's most important food crops is cassava (*Manihot esculenta* Crantz) which belongs to the family *Euphorbiaceae*. Cassava roots and leaves serve as an essential source of calories and income throughout the

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tropics. Most people in Africa, Asia and Latin America depend on the cassava crop for their food and income. A significant progress has been observed in cassava production in recent times. In 2005, cassava occupied the 5th rank in the world production of food crops after maize, rice, wheat and solanum potatoes. World production of fresh cassava tubers increased from 189,099,633 tons to 232 million tons in 2008 with Sub-Saharan Africa alone producing 118 million tons per annum constituting about half of the world's production (FAOSTAT, 2010). In Cameroon where roots and tubers account for 70% of the total cultivated area and 46% of food crop production, total cassava production was estimated at 2,882,734 tons in 2008 (Agristat, 2009). In Cameroon, 80% of urban households consume cassava products on a daily basis, and about 90% of small-scale producers market at least a small part of the cassava they produce.

Despite the relative progress observed in world annual yield of cassava, its cultivation is faced with several pests and diseases. The fungal root rot disease of cassava which affects the tubers, is a disease caused by infection of the roots by fungi found in humid or poorly drained soil (Silvestre and Arrandeu, 1983). It is characterized by browning and wilting of leaves, accompanied by loss of water which may eventually lead to the death of the plant. The other symptoms are swelling of roots and a light brown coloration observed when the roots split in the soil or when they are cut open. In Africa, CRR causes enormous yield losses. It actually hinders the synthesis and storage of nutrients in the roots. This consequently limits plant development, reduces number of roots and their ability to form tubers and become mature, hence limiting production (Msikita et al., 2000).

In Cameroon, about 36% of farmers classify CRR as the second cause of reduced yields in the cassava sector. However, proper identification of pathogenic fungi associated with this disease is yet to be done (Messiga et al., 2004). In order to address this situation, this study was realized in the Littoral, South-west and West Regions of Cameroon with the aim of identifying the pathogenic agents associated with CRR through their isolation from infected plants and morphological description of their fructifications.

MATERIALS AND METHODS

Study sites

Visits and observations were done between March and June 2010 in cassava farms in eight localities, namely Douala, Dibombari, Souza, Mondoni, Batoke, Ekona, Kumba and Dschang, which are found in the Littoral, West and Southwest Cameroun. These three main agro-ecological zones are constituted principally of the humid forest with monomodal rainfall for the Littoral and South-West, characterized by an average temperature of 25°C, 4000 mm of annual rainfall with ferrallitic sandy or sandy clay soil. The western highland zone on its part has an average temperature of 20°C and about 1500-2600 mm rainfall with reddish ferrallitic soil formed on basalt. Samples were collected from infected cassava plants in these

regions and carried to the Crop Protection Laboratory of the Department of Plant Protection, Faculty of Agriculture, University of Dschang where fungal pathogens were isolated.

Collection of samples

Two farmers' farms per locality were surveyed for the presence of plants with external symptoms such as leaf browning and discoloration of the lower part of the stems, and generalized wilting, which are most often indicative of root infections. Of all the plants identified, four were off-rooted from each farm and observations were done on the phytosanitary status of their root system. Samples of partially rotten tubers were taken from these infected plants and carried to the laboratory where fungal isolations were done using potato dextrose agar (PDA) medium.

Isolation and purification of fungi

Fungal isolation was done on PDA enriched with chloramphenicol (150 ppm) in order to avoid bacterial growth. The culture medium was poured in Petri dishes and allowed to solidify. Tubers were washed with tap water. With the help of a sterilized scalpel, 1 cm fragments were collected from the necrotic front and disinfected with 95°C alcohol with the help of a sterile pincer. The fragments were then washed with distilled water and dried with sterile blotting-paper. This exercise was undertaken under a laminar flow hood in the presence of a Bunsen burner in order to assure aseptic conditions. These sterilized explants were cultured on PDA-chloramphenicol medium at 25°C in the dark. After ten days of incubation, fungal colonies which emerged from the explants were sub cultured individually on new PDA simple culture medium. This action was repeated thrice until pure cultures were obtained. A collection of 20 isolates was constituted. Microscopic observation of each isolate was done with an optical microscope (model Olympus BH-2) at a magnification of 400x, and fungal identification was done with reference to the key of Barnett and Hunter (1972).

RESULTS

Description of root rot symptoms

At the first site, a swelling of the tuber and browning of the bark close to the stem were noted on the roots collected from all localities. Splits of these roots showed light brown coloration which is characteristic of CRR disease. A pungent smell was also noted. It was observed that the rotting was different for samples collected from the different localities (Table 1). In fact, rotting of some roots was humid and pasty which is characteristic of soft root rot while on others it was friable, characteristic of dry rot. It has been reported that relative humidity plays a major role in cassava fungal disease development (Makambila, 1994).

Isolation and identification of pathogenic fungi

Fungal isolations realized on rotten cassava samples from the eight localities revealed a great diversity of species associated with cassava root rot. In all, 20 fungal isolates were grouped into seven genera. Figure 1 presents

Table 1. Typological characterization of cassava root rot.

Nature of infection	Infected part		Region	Ecosystem
	Aerial part	Root		
Soft (wet) rot	Wilting, leaves fall-off	Brown necrotic lesions, pasty rotting	Littoral	Humid forest zone with monomodal rainfall
Soft (wet) rot	Wilting, no falling of leaves	Brown necrotic lesions, pasty rotting	Southwest	Humid forest zone with monomodal rainfall
Dry rot	Dehydration of plant, dark brown coloration	Friable rotting	West	Upper plateaux

**Figure 1.** Diversity of fungal species associated with CRR as observed in PDA after 7 days.

presents the mycelial growth and coloration of colonies of each of these genera.

The morpho-cultural and microscopic characteristics of CRR causal agents isolated in this study are presented in Table 2.

With respect to localization, *Fusarium* sp. was the most common genus with a 50% relative prevalence, followed by *Botryodiplodia theobromae* (15%), *Colletotrichum* sp. (10%) and *Trichoderma viride* (10%) (Table 3).

DISCUSSION

Determination of infectious routes of plant pathogens and their mechanisms of infection are of great importance in any disease control program (Twumasi et al., 2014). Host-plant resistance and biological control are the cornerstones of crop protection measures against biotic stress on cassava (Herren, 1994). In fact, knowledge on the identity of the pathogen and disease infection

mechanisms is of prime importance. This could be achieved by isolation and identification of disease causal agents on appropriate culture media. The choice of PDA medium for the isolation of the pathogenic fungal species in this study was based on its successful use in previous studies (Maheshwari et al., 1999; Attrassi et al., 2005), which have been mentioned as an appropriate medium for isolation of a wide range of fungi. Isolation and identification of the pathogenic fungi in this study help to show the presence of seven fungi, namely *Colletotrichum* sp., *Fusarium* sp., *Pestalotia* sp., *Geotrichum* sp., *Sphaerostilbe repens*, *Trichoderma viride* and *Botryodiplodia theobromae*. With a relative prevalence in terms of isolation frequency equal to 50%, fungi of the genus *Fusarium* sp. were the most encountered. They were respectively followed by *B. theobromae* (15%), *T. viride* and *Colletotrichum* sp., respectively 10%, *S. repens*, *Geotrichum* sp. and *Pestalotia* sp., respectively 5%. With reference to the symptoms observed on the roots/tubers of cassava, the genera *Botryodiplodia* sp. and

Table 2. Morpho-cultural and microscopic characteristics of fungi isolated in this study.

Fungi	Morpho-cultural characteristics on PDA	Microscopic characteristics
<i>Botryodiplodia theobromae</i>	Very rapid growth, abundant cotton-like mycelium, colorless when young and becomes deep gray or black with age	Bicellular and ovoid conidia
<i>Colletotrichum</i> sp.	White cotton-like mycelium which turns to gray with time forming acervuli	Abundant unicellular, fusiform conidia
<i>Fusarium</i> sp.	White mycelium which turns to ochre-yellow on old cultures	Spores with crescent form. Septate hypha.
<i>Pestalotia</i> sp.	White cotton-like mycelium-forming black acervuli	Hyphae are septate. Spores are fusiform.
<i>Sphaerostilbe repens</i>	Localized growth; colonies with twisted outlines, whitish when young and turning progressively red with age	They bear stilbosporous. The mycelium is undifferentiated.
<i>Trichoderma viride</i>	Rapid growth, sparse mycelium initially colorless, but rapidly turns green with profused green conidia	Pyramidal branched conidiophores.
<i>Geotrichum</i> sp.	Less abundant milky white cotton-like mycelium	Hyaline Conidia. Septate hyphae with dichotomous branches.

Table 3. Relative prevalence of each pathogenic fungal species with respect to localization.

Fungi	Localization	Relative prevalence (%)
<i>B. theobromae</i>	Douala, Batoke, Dschang	15
<i>Colletotrichum</i> sp.	Batoke, Kumba, Dibombari, Dschang	10
<i>Fusarium</i> sp.	Mondoni, Kumba, Souza, Douala	50
<i>Pestalotia</i> sp.	Douala	5
<i>Geotrichum</i> sp.	Kumba	5
<i>S. repens</i>	Souza	5
<i>Trichoderma viride</i>	Douala, Ekona, Kumba, Souza, Dschang	10

Sphaerostilbe sp. seem to be associated with dry root rot while the genus *Fusarium* sp. is associated with wet (soft) cassava root rot (Theberge, 1985). These symptoms correspond to a particular climatic condition. For instance, the high prevalence of *Fusarium* sp. among our isolates could be due to the conditions of high rainfall, particularly common in the Littoral and Southwest regions, rendering the soils humid and favoring soft or wet rot.

Damages caused by *Colletotrichum* sp. on cassava have been signaled elsewhere. A special form of this genus, *Colletotrichum gloeosporioides* f. sp. *Manihotis* is known to cause anthracnose diseases on cassava (Amusa, 1998; Magdalena et al., 2012) which is an important disease of cassava in tropical Africa, transmitted through breeder seeds and post-harvest debris in the field (Fokunang et al., 1997, 2001). The disease has been reported to cause total crop failure where infected propagation materials are used as seed sources (Ikotun and Hahn, 1991; Magdalena et al., 2012).

The fungi of the genus *Geotrichum* sp. probably play a role in the process of fermentation and post-harvest

deterioration of tuberized roots of cassava (Noon and Booth, 1977; Raimbault et al., 1985; Oyewole and Odunfa, 1988). However, despite the fact that it contributes to crop devastation, a study revealed that *Geotrichum* sp. possibly produces dihydroisocoumarins which could be capable of inhibiting the action of *Plasmodium falciparum* (Palangpon et al., 2003).

Fungi of the genus *Trichoderma* sp. are saprophytes found in the soil. Their capacity to inhibit mycelial growth of other fungi such as *B. theobromae* and *Fusarium* sp. has been put to evidence (Manjula et al., 2005). The species *viride* though with very slow mycelial growth is seemingly capable of inhibiting the development of fungi at a distance (Cherif and Benhamou, 1990). In fact, trichodermine (an antibiotic) has been derived from *T. viride* (Dennis and Webster, 1971). The presence of *Colletotrichum* sp. and *Pestalotia* sp. at the level of cassava tuberized roots need further investigation given that Makambila (1994) highlighted the responsibility of *C. gloeosporioides* in anthracnose of cassava stem. Their specific identification would permit establishment of the

relationship between rhizospheric and aerial isolates.

The *B. theobromae* identified in this study has also been reported to cross infect other crops like cocoa, mango, banana and yam with significant tissue damage and economic losses. The cross-infectivity of this fungus on several crops calls for a review of biocontrol strategies that recommend adoption of mixed- or inter-crop systems to control fungal rot in farms (Twumasiet al., 2014).

CONCLUSION AND PERSPECTIVES

From this study, seven pathogenic fungi were revealed to be associated with cassava root rot disease namely: *Colletotrichum* sp., *Fusarium* sp., *Pestalotia* sp., *Geotrichum* sp., *S. repens*, *T. viride* and *B. theobromae*. Results also show a variability of types of infection with respect to locality or origin of cassava samples revealing an influence of ecological conditions on relative abundance of the pathogenic fungi. This study will help to improve cassava production; thereby contributing to poverty alleviation and food security. Given the nutritional and economic importance of cassava as well as the seriousness of the phytosanitary problems associated with the cassava sector, it will be appropriate in perspective to realize a more elaborate study to determine the prevalence of these pathogens in relation to pertinent factors. An aggressivity/pathogenicity assessment of the fungi as well as their biomolecular characterization is also imperative to confirm their identity and pathogenic importance for their possible use in the screening of cassava clones for resistance.

Conflict of Interests

The authors have not declared any conflict of interest.

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