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ASSESSMENT OF CASSAVA ROOT AND STEM ROTS IN ECOZONES OF TOGO AND EVALUATION OF THE PATHOGEN VIRULENCE

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Abstract

Among the diseases of cassava (*Manihot esculenta* Crantz), root and stem rots are important in different ecozones of West Africa. This study on the prevalence of cassava root and stem rot diseases was carried out in forest and wet savanna ecozones of Togo and the causing pathogens were isolated, identified and pathologically characterized. Cassava rots were found in both the ecozones, but were more frequently observed in the forest than in the wet savanna zone. A total of 39 fungal strains were isolated from diseased root and stem samples collected from cassava fields. *Botryodiplodia theobromae*, *Fusarium* sp., *Sclerotium rolfsii* and *Pythium* sp. were the fungi isolated from the rotted cassava roots and stems. *B. theobromae* was the most frequently isolated fungus (51.3% of the isolated root rot pathogens), followed by *Fusarium* sp. (33.3% of the isolates), while *S. rolfsii* and *Pythium* sp., were less frequently found. Virulence tests on cassava stem cuttings and on cassava roots of field plants revealed only *B. theobromae* strains extremely virulent when inoculated into stem cuttings, whereas most of the fungi were highly virulent when inoculated into both cassava stem cuttings and roots of field plants, except *S. rolfsii* strains, which were less virulent when inoculated into stem cuttings. A pathogenic specialization of strains for roots or stems seemed to occur.

Introduction

Cassava, (*Manihot esculenta* Crantz) of the family Euphorbiaceae, is a major food crop in sub-Saharan Africa and is the basic staple food for more than 500 million people in tropical and sub-tropical parts of the world (FAO and FIAD, 2000). It has mainly been a subsistence crop in Africa. In Togo, cassava was the first crop in terms of production among the major staple crops in 1999, followed by yam and maize (Anon., 1999). However, cassava has become an industrial crop due to the identification of several industrial processes in which cassava can be a raw material. For example, cassava has been found very useful in the production of ethanol and starch for various industrial uses (IITA, 1990; Hillocks & Waller, 1997). These properties and increased food demand of the rapidly growing population has led to an expansion of cassava cultivation in many African countries.

Unfortunately, cassava production is greatly reduced due to the attack by pests and diseases (Hahn *et al.*, 1989). The yield was constantly below the African average yield and the production generally stagnated over the last years in Togo (Anon., 1999). Cassava is attacked by more than 30 pathogens (Nilmanee, 1986; IITA, 1990), causing various degree of losses (Lozano *et al.*, 1981). Among cassava diseases, cassava root and stem rots are

most important in different ecozones of West Africa (Wydra & Msikita, 1998; Hillocks & Wydra, 2002). One major constraint to this in-ground storage of cassava is root rot disease. Cassava roof rots are caused by a complex of soilborne pathogens which induce damages that eventually reduce the yield (Ambe, 1994). Cassava yield losses of up to 80% due to rot diseases have been reported (Theberge, 1985). In some areas, total crop losses have been attributed to rot diseases (Hillocks & Waller, 1997).

Among the organisms commonly reported are *Phytophthora drechsleri* and *Rosellinia necatrix* (Booth, 1978; Theberge, 1985; Hillocks & Waller, 1997); *Armillaria mellea* and *Rigidoporus lignosus* (Booth, 1978; IITA, 1990); *Botryodiplodia theobromae* (Sivaprakasam *et al*., 1977; Aderiye & Ogundana, 1984; Akinyele & Ikotun, 1988; Lo & Clark, 1988; Boher *et al*., 1997); *Sclerotium rolfsii* (IITA, 1990); *Nattrassia mangiferae* (Msikita *et al*., 1997) and *Colletotrichom gloeosporioides* f.sp. *manihotis* (Fokunang *et al*., 2002). The cassava diseases surveys were recently carried out in ecozones of several African countries (DPV, 1978; Tchabana, 1992; Boher *et al*., 1997; Wydra & Msikita, 1998; Msikita *et al*., 2000; Wydra and Verdier, 2002), which revealed cassava rots as one of the most important diseases found in the production areas. In Togo, since only primary studies on cassava rot diseases were conducted only in few localities in the forest zone (DPV, 1978; Tchabana, 1992; Boher *et al*., 1997), and no suitable control measures have been taken in the country.

There is a need of the present status of cassava rots and the pathogen characteristics which are the prerequisite of any further study in controlling a disease. Our studies aimed to determine the present status of cassava root and stem rots in two ecozones of Togo and to identify and characterize pathologically the causing pathogens.

Materials and Methods

Survey area: The study was conducted in the forest and the wet savanna zones, which are among the main cassava producing agroecological zones in Togo. The forest zone in the South-West with a sub-equatorial climate having one long rainy season (March to June), one short dry season (July to August), one short rainy season (September to October) and one long dry season (November to March) is characterized by a rainforest vegetation, whereas the wet savanna zone in the Center with a tropical climate with one long rainy season (April to September) and one long dry season (October to March) is characterized by more shrubby vegetation, (Lamouroux, 1979). The average annual rainfall is about 1,400 mm in both of the ecozones with the average temperature of 24°C and in the forest zone and 27°C in the wet savanna zone. However, a maximum annual rainfall of to 2,027 mm in the forest zone and 1,810 mm in the wet savanna zones were recorded (Anon., 2001).

Collection of the specimens: During the cassava disease survey plants were checked for obvious symptoms of root and stem rots. Fungal mycelium or fruiting bodies on the stem base of the plant as well as wilting indicated infection. The suspected plants with rot symptoms were uprooted and partly or completely rotten root and/or stem samples were collected for pathogen isolation.

Isolation and identification of root and stem rot pathogens: From samples with pathogen signs, parts of the diseased tissue area were directly cultivated on potato dextrose streptomycin agar, and the isolated fungi were then transferred to potato dextrose agar (PDA). In the case of root and stem samples without pathogen signs on the

surface, parts were first washed under running water to remove soil and debris, gently dried and surface-disinfected in 1% Sodium hypochloride for 3 minutes. With a sterile scalpel the outer surface of the diseased part was removed up to the starchy part. Pieces about 5 mm were cut between infected and healthy tissue and were laid on Potato Dextrose Agar-streptomycin medium in a Petri dish. Petri dishes were incubated at room temperature (25 - 30°C) until the sporulation of the fungus. During this incubation, Petri dishes were often checked and the fungi purified in case of mixed cultures. Fungal isolates were identified by culture appearance and microscopical observation using taxonomic keys (Barnett & Barry, 1972; Gams *et al.*, 1998).

Virulence test of fungal strains: The isolated and identified fungi from the collected samples were tested for their virulence on stem cuttings in the laboratory and on root tubers of living plants in the field at the experimental site of the "Institut Togolais de Recherche Agronomique'' station in Lome (Togo).

a. Laboratory test for virulence on cassava stem cuttings: The virulence test was conducted on stem cuttings of 12 month-old plants of the susceptible variety Ben86052 (Wydra, unpublished data). Cuttings of 40 cm length were surface-disinfected in 1% Sodium hypochloride (NaOCl) solution for 3 minutes, rinsed twice in sterile distilled water and gently dried. In the center of each cutting, a hole of about 1 cm depth was bored with a sterile cork borer of 6 mm diameter. The pure fungal culture along with the agar medium cut with the same sterile cork borer was laid into the hole. Each strain was inoculated into 8 stem cuttings. The control cuttings were treated the same way, but received only sterile agar medium without fungal inoculum. After inoculation, cuttings were incubated for five days on a grid in a closed box with 5 mm depth of water at room temperature of 25°C to 30°C and approximately 80 to 85% relative humidity as reported by Firdous *et al*., (2009). Cuttings were evaluated by measuring the surface of necrotic lesions. The bark of the cuttings was removed with a scalpel to approach the woody surface on which lesions were clearly observable. The surface of lesions was reproduced on a transparent paper using a fine pencil, and was measured using a planimeter.

b. Field test for virulence on cassava roots of living plants: The virulence test was conducted in the field on root tubers of cassava field plants of the susceptible variety Ben86052 (Wydra, unpublished data). Root tubers of ten month-old plants were used. In the field, the soil was carefully removed to find the roots without damaging them. The middle part of each root of 14 to 40 cm length was gently cleaned and a hole of about 1 cm depth was made with a sterile cork borer of 6 mm diameter. The fungal culture on an agar plug previously cut with the same sterile cork borer was laid into the hole. Each strain was inoculated into three roots of the same plant. After inoculation, roots were fully covered with dry weeds. Evaluation followed six days after inoculation by measuring the necrotic lesion length caused by the fungus as adopted also by Shah *et al*., (2009) to evaluate the bacterial lesion on the rice leaf. Therefore, the cortex of the roots was removed using a scalpel to approach the starch part.

Statistical analysis: For analysis, the values for surface and length of the necrotic lesions of stems and roots, respectively, of each replication were log-transformed. Analysis of variance (ANOVA) of the variable log-transformed values was performed using the General Linear Model procedure in the SAS system (Anon., 1990; 1997). The Student-Newman-Keuls test was used to compare means of necrotic lesion values (Danielie, 1975).

Table 1. Cassava root and stem rot fungi isolated from cassava root and stem samples in the forest and in the wet savanna zones of Togo.

Strain code	Strain name	Ecozone
CRI01	*Fusarium* sp.	Forest
CRI02	*Fusarium* sp.	Forest
CRI03	*Fusarium* sp.	Forest
CRI04	*B. theobromae*	Forest
CRI05	*Fusarium* sp.	Forest
CRI06	*B. theobromae*	Forest
CRI07	*B. theobromae*	Forest
CRI08	*S. rolfsii*	Forest
CRI09	*B. theobromae*	Forest
CRI10	*B. theobromae*	Forest
CRI11	*B. theobromae*	Forest
CRI12	*S. rolfsii*	Forest
CRI13	*B. theobromae*	Forest
CRI14	*B. theobromae*	Forest
CRI15	*B. theobromae*	Forest
CRI16	*B. theobromae*	Forest
CRI17	*B. theobromae*	Forest
CRI18	*Fusarium* sp.	Forest
CRI19	*Fusarium* sp.	Forest
CRI20	*B. theobromae*	Forest
CRI21	*B. theobromae*	Forest
CRI22	*B. theobromae*	Forest
CRI23	*Fusarium* sp.	Forest
CRI24	*Fusarium* sp.	Forest
CRI25	*Pythium* sp.	Forest
CRI26	*B. theobromae*	Forest
CRI27	*B. theobromae*	Forest
CRI28	*Pythium* sp.	Forest
CRI29	*S. rolfsii*	Wet Savanna
CRI30	*S. rolfsii*	Wet Savanna
CRI31	*Fusarium* sp.	Wet Savanna
CRI32	*Fusarium* sp.	Wet Savanna
CRI33	*Fusarium* sp.	Wet Savanna
CRI34	*B. theobromae*	Wet Savanna
CRI35	*B. theobromae*	Wet Savanna
CRI36	*Fusarium* sp.	Wet Savanna
CRI37	*B. theobromae*	Wet Savanna
CRI38	*Fusarium* sp.	Wet Savanna
CRI39	*B. theobromae*	Wet Savanna

Results and Discussion

Prevalence of the disease and identification of the pathogens: Cassava root and stem rots were found more frequently in the forest zone than in the wet savanna zone (Table 1). In the forest zone, the disease occurred in the highland in the region of Danyi as well as in the lowland in the region of Adeta. Cassava rots were also found in the wet savanna zone, but were less frequently observed than in the forest zone and occurred in the region of Sotouboua. Compared to the previous work which reported cassava rots in the plateau of

Dayi (Anon., 1975; Tchabana, 1992), the diseases were widely found in the forest zone, and also in the wet savanna zone where cassava rots have never been reported before.

A total of 39 fungal strains were isolated from diseased root and stem samples collected from cassava fields in the forest zone and in the wet savanna zone (Table 1). Most of the strains (72%) were isolated from samples collected from the fields in the forest zone and 11 strains (28%) from the wet savanna ecozone. Four genera of fungi viz., *Botryodiplodia*, *Fusarium*, *Sclerotium* and *Pythium* were isolated from all samples collected. Results showed that *B. theobromae* was the most frequently isolated fungus (51.3%), followed by *Fusarium* sp. (33.3%), while *S. rolfsii* and *Pythium* sp., were less frequently found, with 10.3 % and 5.1 %, respectively.

Cassava root and stem rots were not restricted to the forest zone although they were more frequently found in this zone. The occurrence of cassava root and stem rots was observed in the wet savanna zone which is one of the main cassava producing regions in Togo. High frequency of the rot disease found in the forest zone could be due to the environment conditions which may be more favorable for the disease occurrence in this zone than in the wet savanna ecozone. The forest zone with its high rainfall up to 2,000 mm or more and mean temperature of about 24°C could offer better growth conditions of the rot pathogens than in the wet savanna zone, where the maximum rainfall did not exceed 1,800 mm, and the temperature was about 27°C or more. Cassava rots due to *P. drechsleri* were reported to be more severe in the humid areas of South America (Oliveros *et al*., 1974; Lozano & Booth, 1976; Booth, 1978).

If the isolated fungi *B. theobromae*, *Fusarium* sp., *S. rolfsii* and *Pythium* sp., were also among the rot pathogens found in the previous studies conducted in the forest zone - especially in the plateau of Danyi - of Togo (Ptcholo, 1991; Tchabana, 1992). The later author found cassava root and stem rots only in the plateau, while the present studies revealed the disease in the lowlands of the forest zone, as well as in the wet savanna zone. The isolated fungi were also among the most important cassava root and stem rot pathogens found in other countries in West Africa such as Nigeria and Benin, and in Central Africa in Cameroon (Afouda *et al*., 1995; Afouda & Wydra, 1996). *B. theobromae*, *Fusarium* spp., *S. rolfsii* and *S. repens* were also reported to be associated with cassava root and stem rots by Akinyele & Ikotun (1989) and by Lozano (1989) in Latin America. The species *B. theobromae* and *S. rolfsii* were among the most damaging fungal pathogens and frequently associated with peanut rots in Côte d'Ivoire (Savary *et al*., 1988).

Virulence of root and stem rot fungi on cassava stem cuttings: A total of 28 strains of root rot fungi were tested for virulence on stem cuttings of the susceptible cassava variety Ben86052. Necrotic lesions on the surface of the cuttings were measured in mm^2 at five days after inoculation (dpi). Values were log-transformed and analysis of variance was performed (Table 2). The analyses allowed to form the following virulence classes: "a", "ab", "bc", "bcd", "bcde", "cde", "cdef", "def", "ef", "f" and "g". Highly significant ($p<0.01$) differences of the surface of necrotic lesions were observed. Five strains of *B. theobromae* - group "a" with the lesion surfaces ranging from 490.3 to 420.4 mm^2 - were defined as "extremely virulent" with significantly different necrotic lesion surfaces compared to those of all other strains. Strains of *Fusarium* sp., and *Pythium* sp., and four *B. theobromae* strains ranging in the groups from "b" to "f" - with necrotic lesion surfaces ranging from 280.1 to 67.1 mm^2 - were classified as highly virulent. The group "g" significantly different from the above groups contains the *S. rolfsii* strains - with surface lesions of 20.3 and 20.4 mm^2 - and is classified as lowly virulent group. All the *B. theobromae* strains, except one, caused higher necrotic lesion surfaces (>120 mm^2) than the other fungi.

Table 2. Virulence of root and stem rot fungi on cassava stem cuttings, measured as surface necrotic lesions (mm^2) 5 days after inoculation.

Strain code	Lesion surface[3] (mm^2)	Rank[2]	Strain name
CRI01	67.1	f	*Fusarium* sp.
CRI02	93.0	ef	*Fusarium* sp.
CRI03	81.1	ef	*Fusarium* sp.
CRI04	450.3	a	*B. theobromae*
CRI05	110.1	def	*Fusarium* sp.
CRI06	170.3	bcde	*B. theobromae*
CRI07	280.1	ab	*B. theobromae*
CRI08	20.3	g	*S. rolfsii*
CRI09	110.4	def	*B. theobromae*
CRI10	130.1	cdef	*B. theobromae*
CRI11	150.4	cde	*B. theobromae*
CRI12	20.4	g	*S. rolfsii*
CRI13	480.1	a	*B. theobromae*
CRI14	490.3	a	*B. theobromae*
CRI15	430.3	a	*B. theobromae*
CRI16	420.4	a	*B. theobromae*
CRI17	190.4	bcd	*B. theobromae*
CRI18	88.4	ef	*Fusarium* sp.
CRI19	120.3	cdef	*Fusarium* sp.
CRI20	190.3	bcde	*B. theobromae*
CRI21	120.1	def	*B. theobromae*
CRI22	230.1	bc	*B. theobromae*
CRI23	95.1	ef	*Fusarium* sp.
CRI24	112.1	def	*Fusarium* sp.
CRI25	68.1	f	*Pythium* sp.
CRI26	158.4	cde	*B. theobromae*
CRI27	123.3	def	*B. theobromae*
CRI28	68.1	f	*Pythium* sp.
Control	0.0	h	

[1]Means of eight replications;
[2]Discrimination of the means using the Student-Newman-Keuls (SNK) test
[3]Values followed by the same rank letter do not differ significantly at $p \leq 0.01$ according to SNK test.

Virulence of root and stem rot fungi on cassava roots of field plants: Thirty-one fungal strains were inoculated into roots of cassava variety Ben86052 in the field. The length of the necrotic lesions was measured in cm six days after inoculation and values were transformed and analyzed by discrimination analysis (Table 3). The necrotic lesion length on roots was representative for the virulence of the pathogen, and mostly high length of lesions on the surface corresponded to a big part of rotten tissue inside the root. The analyses allowed to form the virulence classes, “a”, “b”, “bc”, “bcd”, “bcde” and “de”. All the tested strains caused symptoms significantly different ($p<0.01$) from the control. *B. theobromae* strain CRI26 (group “a”, 25.0 cm) was extremely virulent with the highest necrotic lesion length of 25.0 cm. The other strains tested ranged between 8.5 and 17.3 cm necrotic lesion without significant difference between them and were classified as virulent strains. However, differences between fungal strains in causing necrotic lesions were observed. Seven *B. theobromae* strains (four from the forest zone and three from the wet savanna zone two *Fusarium* sp. strains, one from each of the

ecozones, three *S. rolfsii* strains one from the forest zone and two from the wet savanna zone) and one *Pythium* sp., strain from the forest zone caused severe necrotic lesions of > 14 cm length and were classified as highly virulent, while two *B. theobromae* strains and one *Fusarium* sp. strain caused smaller necrotic lesions of < 10 cm length on cassava roots and were classified as virulent. Some necrotic lesions were also observed on the control roots due to the action of soil microorganisms since the experiment was conducted under field conditions.

Table 3. Virulence test of root and stem rot fungi on roots of cassava plants in the field by measuring length (cm) of necrotic root tissue 6 days after inoculation with fungal pathogens.

Strain code	Lesion length[1] (cm)	Rank[2]	Species
CRI01	11.33	bcde	*Fusarium* sp.
CRI02	15.83	bcd	*Fusarium* sp.
CRI03	9.17	cde	*Fusarium* sp.
CRI10	10.83	bcde	*B. theobromae*
CRI11	15.83	bcde	*B. theobromae*
CRI12	14.17	bcde	*S. rolfsii*
CRI13	12.50	bcde	*B. theobromae*
CRI14	12.17	bcde	*B. theobromae*
CRI15	14.33	bcde	*B. theobromae*
CRI16	10.83	bcde	*B. theobromae*
CRI17	8.50	de	*B. theobromae*
CRI18	13.00	bcde	*Fusarium* sp.
CRI21	9.67	bcde	*B. theobromae*
CRI22	13.00	bcde	*B. theobromae*
CRI23	10.67	bcde	*Fusarium* sp.
CRI24	12.67	bcde	*Fusarium* sp.
CRI25	12.83	bcde	*Pythium* sp.
CRI26	25.00	a	*B. theobromae*
CRI27	15.33	bcde	*B. theobromae*
CRI28	16.17	bcd	*Pythium* sp.
CRI29	15.67	bcd	*S. rolfsii*
CRI30	15.00	bcde	*S. rolfsii*
CRI31	10.33	bcde	*Fusarium* sp.
CRI32	17.33	bc	*Fusarium* sp.
CRI33	12.50	bcde	*Fusarium* sp.
CRI34	17.67	b	*B. theobromae*
CRI35	11.33	bcde	*B. theobromae*
CRI36	11.33	bcde	*Fusarium* sp.
CRI37	14.67	bcde	*B. theobromae*
CRI38	10.33	bcde	*Fusarium* sp.
CRI39	14.33	bcde	*B. theobromae*
Control	5.33	f	

[1]Means of three replications

[2]Discrimination of the means using the Student-Newman-Keuls (SNK) test

[3]Values followed by the same rank letter do not differ significantly at $p \leq 0.01$ according to SNK test.

Comparison of stem and root inoculation: A high diversity in the expression of the virulence was observed among the root and stem rot pathogen strains with both methods. A certain pathogenic specialization on stems or roots occurred. The *B. theobromae* strain CRI14 caused the highest necrotic area on the cassava stem cuttings, while its effect on roots was moderate. The situation was reversed with strain CRI33 which showed a moderate effect on stem cuttings, but caused the highest necrotic lesion when inoculated into roots. *B. theobromae* strains CRI16, CRI17 and CRI21 expressed higher effects on stem cuttings than on roots. *Fusarium* sp., strain CRI02, *S. rolfsii* strain CRI12 and *Pythium* sp., strain CRI28 were more virulent on cassava roots than on stems. *B. theobromae* strains CRI11 and CRI27 and *Fusarium* sp., strain CRI24 were highly virulent on stem cuttings as well as on roots, while *Fusarium* sp., strains CRI03 and CRI23 showed low effects on both cassava stem cuttings and roots.

Conclusion

The present studies were to elucidate the present status of cassava rots in two major production zones of Togo, the forest and the wet savanna zones, and to identify and characterize pathologically the causing pathogens. The survey revealed the presence of cassava root and stem rots in both of the ecozones. The disease was more frequently found in the forest zone than in the wet savanna ecozone. *B. theobromae*, *Fusarium* sp., *S. rolfsii* and *Pythium* sp. were the cassava root and stem rot fungi isolated and identified from the samples collected from different localities. Among them, only *B. theobromae* strains were extremely virulent when inoculated into stem cuttings whereas most of the fungi were highly virulent when inoculated into cassava roots of living plants in the field.

The disease is becoming more and more serious compared to the situation found a decade age. Therefore, measures should be taken to limit their spread and to reduce or avoid possible epidemics. The results of the present studies are of high importance, being the first wide survey on cassava rots never done in Togo earlein and also pronick a database on cassava rots useful for further investigations. Recommendations resulting from the studies on root and stem rots are, to select resistant varieties, and determine suitable cultural practices that could significantly reduce or avoid the disease occurrence.

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