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Seed Survival and Transmission of Cassava Anthracnose Disease, and Seed Treatment Effect on Seedling Growth

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Abstract

Open pollinated seeds from cassava genotypes were used to investigate the survival and transmission of cassava anthracnose disease fungus. Seeds from each genotype were surface- sterilized, cultured on potato dextrose agar (PDA), and incubated for 8 days, at 25 ± 2°C. Microscopic examination indicated that *Colletorichum gloeosporioides* was one of the seed-borne fungi, with up to 40 per cent incidence in some genotypes. Seeds from five genotypes with high incidence of *C. gloeosporioides*, were selected for transmission studies. They were planted in steam-pasteurized soils in jiffy pots in the greenhouse. The pots were placed closed to each other to increase canopy relative humidity needed for anthracnose development at temperatures of 25-32 °C. After six weeks, some plants exhibited symptoms that resembled those of cassava anthracnose disease. The stems, leaves and roots of these plants were washed, surface sterilized, plated on PDA and incubated for 5-7 days. Microscopic examination of the fungus identified conidia of *C. gloeosporioides*. The rest of the plants were monitored for 3 months under vector-free conditions for typical anthracnose symptoms. Mean maximum wilt and defoliation of 35-38per cent was recorded on some genotypes. *C. gloeosporioides* f. sp. *manihotis* was confirmed by pathogenicity tests on young healthy cassava seedlings with stem puncture inoculations. Treatment of infected seeds with three synthetic fungicides; benlate, captan, thiram, two anti-microbial plant products from *Azadirachta indica* A. Juss and *Ocimum gratissimum* Linn at full strength concentration, and hot water treatment, significantly reduced fungal incidence, increased seedling emergence and plant vigour index of the cassava genotypes.

Introduction

Cassava (*Manihot esculenta* Crantz) constitutes the principal carbohydrate source for more than 800 million people in developing countries (FAO, 1993). The crop has been constantly attacked in the past three decades by some forty different bacterial, fungal and viral diseases (Lozano, 1989). Cassava anthracnose disease (CAD) caused by *Colletotrichum gloeosporioides* f.sp. *manihotis* Henn (Penz) Sacc, is an epidemic disease in most cassava-growing areas of the tropics. It is characterised by cankers on stems and branches, leaf spots and tip die-back (Muimba *et al.*, 1983; IITA, 1987). Leaf infection could lead to a reduction in photosynthesis, thereby decreasing the production of the much-needed carbohydrates (Lozano and Booth, 1974). Infection could also lead to a significant loss in planting materials and total crop failure when the infected cuttings are used (Makambila and Bakala-Koumouno, 1982). Available information on the epidemiology of the disease and how it can be effectively managed in areas of sudden outbreaks is limited. From available literature, CAD is a limiting factor in the cultivation of cassava in many regions in the tropics (Terry and Oyekan, 1976; IITA, 1987). Limited surveys carried out in the Congo and the Democratic Republic of Congo (former Zaire), and investigations at the International Institute of Tropical Agriculture (IITA), indicate that CAD is of economic importance for cassava farmers in tropical Africa.

Cassava seed is an important material for crop improvement studies. It is a useful tool for studies in breeding for resistance to major diseases, germplasm conservation and stability. Apart from a report by Persley (1979) of the seed-borne nature of CBB with a low percentage seed transmission under favourable conditions, little attention has been focused on cassava seed pathology.

Post harvest treatment of cassava seeds before storage has been shown to increase seed viability and planting quality (Lozana *et al.*, 1984). In some developing countries where the use of resistant genotypes as the best control option has not been attained, anti-microbial products such as neem (*Azadirachta indica* A. Juss) and *Ocimum gratissimum* Linn, have been used as promising alternative control agents (Amuah, 1989; Akpa *et al.*, 1991).

With the increasing seed production technology, there is need to focus research on cassava seed-borne diseases, possible transmission mechanisms and also to develop control strategies under the integrated control management principle.

Materials and Methods

Experimental site: The study was conducted in the greenhouse and Advance Plant Pathology Laboratory of the Root and Tuber Crop Improvement Programme (TRIP), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Survival of *C. gloeosporioides* f.sp. *manihotis* and other associated fungi in cassava seeds: Seeds from 13 open pollinated cassava genotypes were stored at 5-10°C for ten

months after harvest from the IITA cassava breeding field at Ibadan, Nigeria. The seeds were used to investigate the presence of CAD fungus, and other fungi present in the seeds. Two hundred seeds from each genotype were surfaced-sterilized for approximately 3-5 minutes in 10 per cent sodium hypochlorite solution and rinsed with five successive changes of sterile distilled water. Solidified Potato Dextrose Agar (PDA) in 9cm pyrex petri dishes were cut with a sterile 6cm corkborer, and aseptically transferred with a sterile forceps into empty sterile plates. The sterile seeds were dried on sterilized filter papers and placed on the PDA containing 100mg/dm^3 sodium novobiocin (PDA + N) added to suppress bacterial growth. The seeds were incubated at 25 ± 2 C for 8 days under intermittent mixed irradiation for 12h/day from near ultra-violet and daylight type of fluorescent tubes suspended 50cm above the plates. Each seed lot contained 10 seeds per petri plate, in 10 replications arranged in a randomized complete block design. The plates were examined for fungal growth and identification using a stereo binocular compound microscope. Records on number of infected seeds, the type of fungi identified and the percentage infection were taken.

Seed transmission of *C. gloeosporioides* f.sp *manihotis*: Cassava seeds from five genotypes (TMS 91/00059, 91/01188, 91/00052, 91/00165 and 91/00313) determined to have higher incidence of *C. gloeosporioides* after the seed fungal survival and identification study were selected. Seedling nurseries were established in wooden boxes in the greenhouse. Two hundred seeds were used from each seed lot. The seeds were planted in fine steam-sterilized soils in jiffy pots and watered daily until seedling emergence. At the height of 10-15cm, the seedlings were transferred to plastic pots (10.5cm diameter) filled with a sterilized mixture of soil and sand (2:2v/v). Pots were placed close to each other on benches, to obtain the thick canopy needed for anthracnose disease development. Seedling pots were arranged in a randomized complete block design with 4 replications per treatment, and each treatment consisted of 5 plants per cassava genotype. The plants were watered daily throughout the growth period. Temperature and relative humidity in the greenhouse were monitored with a hygrothermograph. A temperature range of 26-32 C in the day and 22-26 C at night and relative humidity range of 80-98 per cent was maintained.

Plants showing symptoms of infection were sampled to determine the causal agent. To do this, stems, leaves and roots of infected plants were washed, air-dried and surfaced-sterilized with 10per cent sodium hypochlorite for 3-5mins and rinsed in five successive changes of sterile distilled water. The plant segments were dried on sterilized filter paper and plated on PDA + N in petri dishes. The petri dishes were incubated for 5-7 days at 25 ± 2 C, to allow for growth of any micro-organisms associated with the infection. The rest of the plants were monitored for typical anthracnose symptoms, and symptomatic plants were recorded to determine the percent infection, disease severity, leaf infection, defoliation and wilting at two weeks interval for three months.

Effect of different seed treatment methods on *C. gloeosporioides* f.sp *manihotis* survival and the effect on seedling growth: Seed samples determined to have a high incidence of CAD pathogen were selected from six cassava genotypes (TMS 85/00136, 91/01188, 30572, 91/00684, 91/00300 and 91/00344), stored for 12 months at 5 C and 60 per cent relative humidity, and subjected to the following treatments:

Surface-sterilization: The seeds from each cassava genotype were soaked for 3-5 minutes in 10 per cent sodium hypochlorite. They were rinsed in 3 successive changes of sterile distilled water, dried in sterilized filter paper and plated on PDA + N medium and incubated at 25 ± 2 C for 7 days under 12h/12h alternate cycle of near ultraviolet light and darkness. The other treatments followed the same incubation conditions.

Hot air: Seed lots were incubated in an oven with temperature maintained at 60 C for 24 hrs (Perley, 1979) and transferred aseptically with forceps to PDA + N medium in 9cm petri dishes. They were incubated under the same conditions as above.

Hot water: Seed samples were put in canvas bags and immersed in a water bath maintained at 60 C for 2 minutes. They were dried on sterilized filter papers and plated on PDA + N medium.

Fungicides: Three synthetic fungicides, 0.3 per cent thiram (tetramethyl thiurum disulphide), 0.3 per cent captan (trichloromethyl-tio-4 cyclohexane-2, 2-dicarboximide), 0.3 per cent benlate Imethyl-N-(-butyl-carbomo-2-benzimadazole carbamate)], and 2 antifungal plant products at full strength concentration from *A. indica* and *O. gratissimum* were used. Uniform seed dressing was achieved by adding the required amount of fungicide into each 500ml flask containing the test seed samples and vigorously shaking on a mechanical shaker for 30 mins (Nisar *et al.*, 1990). The treated seeds were plated on PDA + N medium and placed in the same incubation conditions as described above. Untreated seed samples from each seed lot served as controls. After 7 days of incubation, seed samples were observed under a stereo binocular microscope and the incidence of *C. gloeosporioides* f.sp *manihotis* recorded.

Effect of different seed treatment methods on C. incidence and seedling growth: Seed genotypes determined microscopically to have high CAD infection were subjected to the different seed treatment methods, and the control treated with sterile distilled water. The seed lots were planted in fine steam-sterilized soils in jiffy pots per treatment, and placed in wooden boxes in the greenhouse

Each treatment contained 200 seeds per genotype arranged in a randomized complete block design with 4 replications. Watering was done daily to obtain a soil moisture level of 90-100 per cent field capacity before seed planting. Seedling emergence was recorded in each treatment 7 days after planting, and at 2 days interval thereafter, for 14 days. Seedlings were considered emerged when the cotyledons were opened and epicotyl exposed. After attaining a height of 15-20cm, the seedlings were transplanted into plastic pots (10.5cm diameter) filled with steam-sterilized soil. Watering was done daily until the end of the study. Data for mean shoot length (MSL), mean root length (MRL), and seedling vigour index (SVI) were taken. Seedling vigour index was calculated as follows: **SVI = MSL + MRL x % seedling emergence.**

Statistical analysis: The data were subjected to analysis of variance (ANOVA) procedure using SAS statistical package (SAS Institute, 1989). Fischer's protected least significant difference (LSD) and Duncan's multiple range tests of mean separation were performed only when the ANOVA showed significance.

Results

Survival of *C. gloeosporioides* f.sp *manihotis* and associated fungi on cassava seed: The incidence of *C. gloeosporioides* f. sp. *manihotis* and other associated fungi (*Cuvularia spp*, *Rhizopus spp*, *Macrophoma spp*, *Pennicilium spp*, and *Aspergillus spp)* identified, was determined 8 days after incubation at 25 ± 2 C (Table 1). A maximum mean percentage incidence of 40 per cent for *C. gloeosporioides* f.sp *manihotis* was recorded on TMS 91/00059, with a minimum mean survival of 12 per cent recorded on seed of genotype 90/00333. There were significant differences (P 0.05) in fungal incidence among the genotypes. Other associated fungi that survived on the seed had mean maximum values of 66 per cent for *Curvularia spp.* on genotype 90/00333, 41 per cent for *Rhizopus spp* on 91/00344, 47 per cent for *Macrophoma spp* on TMS 90/00333, 51 per cent for *Pennicilium spp* on TMS 89/01084 and 53 per cent for *Aspergillus spp* on genotype TMS 91/00313. The lowest mean fungal incidence of 8 per cent for *Pennicilium spp* was on genotype TMS 91/00052, and 16 per cent for *Aspergillus spp* on TMS 91/00344.

Cassava anthracnose disease expression on cassava seedlings: CAD was observed on all the genotypes at 10 months after planting (MAP), with significant differences (P 0.05) in the level of symptoms expressed (severity, wilt, defoliation and leaf infection) (Table 2). The genotype 91/00313 recorded the highest mean CAD incidences of 63 and 83 per cent at 10 and 12 months after planting respectively, while the least incidences were recorded on the genotype 91/00059 with mean values of 36 and 64 per cent. There was an increase in CAD severity at 12 MAP, with a mean maximum score of 3.2 for genotype 91/00313 and least score of 2.2 for genotype 91/00059. At 10 MAP, the highest mean leaf infection of 30.6 per cent was recorded for 91/00052 and the least of 11 per cent for genotype 91/00059. At 12 MAP, the highest mean leaf infection of 42 per cent was for genotype 91/00165 and the least value of 16 per cent for genotype 91/00059. Wilt symptoms were less than 30 per cent at 10 MAP in all the test genotypes, while at 12 MAP, the highest wilt incidence of 38 per cent was on genotype 91/00313 and the least of 21.4 per cent on 91/00059. There were significant differences (P 0.05) in defoliation among the genotypes, with a mean maximum defoliation of 38 per cent on genotype 91/00313 at 12 MAP and the least value of 21.2 per cent on genotype 91/00052.

Seed treatment effect on CAD incidence, and seedling growth: Seed treatment with the three synthetic fungicides (benlate, captan, thiram), and two antifungal plant products from *A. indica* and *O. gratissimum* crude extracts significantly reduced CAD incidence, and increased seedling emergence (Table 3). Seed treatment with benlate showed the best performance, with the lowest CAD incidence of 1.13 per cent. Although surface sterilization and hot air treatments were significantly different from the control, the other treatments showed better efficacy, with an increase in seedling emergence and a mean maximum emergence of 90 per cent for benlate, 87 per cent for thiram, 86 per cent for captan and 86 per cent for *A. indica*. There were significant differences (P 05) in MSL, MRL and SVI among the treatments. Benlate, captan and *A. indica* treatments showed significant increase in MSL of 66.2, 65.4 and 64.4cm respectively. Maximum MRL of 15.4cm was recorded with benlate treatment, followed by 15cm for captan. A seedling vigour index of 7416 was highest for benlate treatment, and the least of 1862 was recorded for the control (Table 3).

The overall response of genotypes to the different treatment methods showed significant variation in CAD incidence, seedling emergence, MSL, MRL and SVI (Table 4). The genotype 85/00136 had the highest CAD incidence of 17.8 per cent, while the least of 7.9 per cent was on genotype 91/00684. Genotype 91/01188 had the highest MSL of 48.8cm, while the least MSL value of 46.8cm was on genotype 85/00136. There were variations in SVI among the genotypes with mean maximum value of 60661 on genotype 91/00684 and mean minimum of 4607 on genotype 85/00136. Seedling emergence was highest on 91/00684 with a mean value of 83.1 per cent, which was not significantly different from 91/01188 with a value of 83.0 per cent. The least seedling emergence of 68.8 per cent was on genotype 85/00136.

Discussion

This study shows that *C. gloeosporioides* f.sp *manihotis* can survive in cassava seeds. A survival incidence of 40 per cent was recorded on the genotype 91/00059. The variation in survival incidence among the genotypes could be attributed to their different levels of reaction to the fungi. An earlier report by Van der Bruggen and Maraite

Table 1: Incidence of seed-borne fungi associated with cassava seed lines

Seed Lines	Mean percentage incidence of identified fungi					
	Colletotrichum spp	*Curvularia* spp	*Rhizopus* spp	*Macrophoma* spp	Penicillium spp	*Aspergillus* spp
TMS[a] 91/00059	40.8a[b]	10.5e	13.8de	13.2de	11.0ef	47.9ab
TMS 91/01188	37.9b	24.3d	28.5b	24.0c	37.9cd	46.1ab
TMS 91/00052	35.5c	20.2de	17.6d	11.7de	8.2f	35.8bc
TMS 91/00165	32.8d	30.8c	34.2b	16.1d	26.6d	35.24bc
TMS 91/00313	32.5d	28.5c	29.5b	15.3d	13.6e	53.0a
TMS 89/00011	31.6d	25.0cd	26.7bc	15.4d	22.5de	34.5bcd
TMS 91/00300	23.1e	24.1d	23.1cd	14.2de	15.2e	30.2d
TMS 88/01084	23.9e	8.9e	27.8bc	33.7bc	51.7a	33.3cd
TMS 91/00344	22.0ef	38.8b	41.0a	8.0e	22.1de	16.7d
TMS 00684	22.3f	19.0de	18.2d	10.2e	17.5e	34.3bcd
TMS 88/00136	18.8fg	10.8e	25.8c	39.9b	21.9de	31.9cd
TMS 91/00072	13.9g	7.9e	14.7de	8.2e	35.0c	50.7a
TMS 90/00333	12.9g	66.0a	18.1d	47.9a	47.2b	39.0b
Mean	25.92	23.70	24.0	19.46	25.00	37.15
CV	15.84	23.60	13.55	20.82	15.07	14.93

[a]**TMS** = Tropical Manihot Specie. Mean of 200 seeds per seed lines. [b]Means in the same column followed by the same letter(s) are not significantly different at 0.05 by Duncan's multiple range test.

Table 2: Seed transmission of cassava anthracnose disease on cassava seedlings at 10 and 12 months after planting.

Seedling	Disease symptoms rating at 10 weeks after planting					Disease symptoms rating at 12 weeks after planting				
	%CAD incidence	CAD seveirty	%Leaf infection	%Wilt	%Defoliation	%CAD Incidence	CAD severity	%Leaf infection	%Wilt	%Defoliation
91/00313	63.0a[a]	2.8a	29.0a	26.0a	29.8a	83.0ab	3.2a	38.0a	35.0a	38.0a
91/01188	52.0ab	2.6a	19.4b	8.0b	20.4b	76.0ab	2.8b	22.0b	22.5b	26.0bc
91/00052	50.0ab	2.0b	30.6a	8.0b	7.0d	90.0a	2.3cd	38.0a	23.0cd	21.2c
91/00165	43.0ab	2.0b	29.0a	21.0a	13.4c	72.0ab	2.6bc	42.0a	32.0a	27.0b
91/00059	36.0b	2.0b	11.0c	12.0b	14.5c	64.0b	2.2d	16.0c	21.4b	22.5c
CV[b]	22.15	17.81	24.60	19.33	23.49	20.15	26.72	19.58	22.11	23.02

[a]Means in the same column followed by the same letter(s) are not significantly different at 0.05 by Duncan's Multiple Range Test.
[b]CV = Coefficient of Variation.

Table 3: Seed treatment effect on seedling emergence, disease incidence and other growth parameters.

Seed treatment	% Seedling emergence	Shoot length (cm)	Root length (cm)	Seedling vigour index[a]	% CAD incidence
Benlate	91.00a[b]	66.16a	15.38a	7416.10a	1.13h
Thiram	87.44b	65.36ab	14.04bc	6952.44b	3.58g
Captan	86.28b	63.92bc	15.00a	6821.52bc	3.21g
Neem	86.04b	64.36bc	14.24b	6756.40c	3.59f
Ocimum	83.60c	63.44c	14.12bc	6475.24d	8.54e
Hot water	82.04c	60.72d	13.52c	6110.96e	13.80d
Hot air	72.40c	42.72e	11.44d	3897.18f	19.63c
Surface sterilization	68.84e	35.08f	9.26e	2968.41g	32.21b
Control	51.20f	27.72g	8.48ef	1862.40h	38.67a

[a]Determined by: mean shoot length + mean root length x % seedling emergence.
[b]Means in the same column followed by the same letter(s) are not significantly different at 0.05 by Duncan's multiple range test.

Table 4: Overall response of seed lines after treatment to disease incidence and other growth parameters.

Seed lines	CAD incidence	Shoot length (cm)	Root length (cm)	Seedling vigour index	% seedling emergence
85/00136	17.8a[a]	46.8c	11.9d	4607.75e	68.8c
91/00304	16.0ab	50.4b	12.3cd	5406.60d	78.4b
91/00300	15.3b	50.2c	12.5bc	5036.25de	78.2b
30572	13.9c	54.6b	12.9b	5579.53c	78.4b
91/01188	12.3d	58.7a	13.0b	5711.29b	83.0a
91/00684	7.9e	57.5a	13.9a	60661.87a	83.1a
CV	24.15	20.22	19.85	22.46	19.01

CAD = Cassava anthracnose disease; [a]Means in the same column followed by the same letter(s) are not significantly different at P

(1987) showed that differences in cultivar resistance to the CAD pathogen were especially marked by the number of stem fragments invaded by the fungus. The factor that best differentiates between the resistant levels of cultivars seems to be their resistance to invasion, which corresponds to the symptoms of necrosis in the stems as a result of natural infection.

Information on seed-borne fungi of cassava is limited since cassava propagation is mostly done through stem cuttings. Persley (1979) conducted studies on the survival and transmission of *X. c.* pv. *manihotis* on cassava seeds and reported that a low percentage of successful seed transmission of *X. c.* pv. *manihoits* can occur under favourable environmental conditions. The information on CAD on seed in association with other cassava seed-borne micro-organisms is also very important in implementing post-harvest seed treatment before storage and in distribution to national research programs and to farmers for planting. The infection of cassava seeds by *C. gloeosporioides* f.sp *manihotis* reduces seed quality and viability. This can lead to an economic loss which in most cases can be high under high inoculum pressure and could also cause significant crop failure.

Investigation of CAD transmission in cassava seed genotypes showed that CAD symptoms were recorded in most of the seed genotypes at 10 months after planting in the greenhouse under vector-free conditions. This supports the fact that transmission of CAD can take place through infected seed without the presence of the transmission vector *Pseudotheraptus devastans* (Boher *et al.*, 1983). There were however variations in the level of CAD symptom expression (incidence, severity and wilt) in the test genotypes. This study suggests that pre-treatment of harvested seed or investigation of the seed health status of cassava, should be an important measure before any seed is released or stored, to reduce disease dissemination.

The effect of seed treatment methods on CAD incidence and seedling emergence showed significant variation among the seed genotypes. Seed treatment with synthetic fungicides (benlate, captan, thiram), antimicrobial plant products from *A. indica* and *O. gratissimum,* significantly reduced CAD incidence; increased seedling emergence and plant vigour index. This confirms the reactions of these fungicides in suppressing seedling diseases observed in other pathosystems. Evaluation of fungicides for seed treatment and foliar application in the management of damping-off of seedlings and blight of rapeseed caused by *Alternaria brassicae*, for example, have shown that benlate and dithane M-45 are effective as seed dressing fungicides for controlling seedling diseases (Nisar *et al.*, 1990). Fungicidal control studies have also confirmed that agrosan, benlate, captan, maneb and thiram, all at 0.3 per cent concentration, can effectively control seed-borne fungi of jute *in vitro* and *in vivo* (Amitava and Chattopadhyay, 1975).

The threat of many synthetic compounds in agricultural use due to their highly toxic, broad-spectrum and persistent nature, represents a potential risk to non-target organisms and the environment. This has stimulated the search for more acceptable alternatives in the management of farmers' disease problems. There has been a focus in the use of anti-microbial plant products for pre-treatment of planting materials. These natural plant products are not only readily available to the poor subsistent farmers who cannot afford the high cost of synthetic fungicides, but are also less toxic, biodegradable, non-phytotoxic, environmentally friendly and are very promising within the frame work of integrated pest management systems (Akpa *et al.* 1991). Natural plant products from *A. indica* and *O. gratissimum* significantly reduced CAD incidence and increased seedling emergence, in the same strength as the synthetic fungicides. These are readily available in large quantities in the tropical environments and can be used instead of the synthetic compounds which are either not available or too costly for resource-poor subsistence farmers who depend on cassava for their daily dietary needs.

This study also shows that *C.gloeosporioides* f.sp. *manihotis* is seed -borne and seed transmitted. The level of infectivity of cassava genotypes depends on the differences in their resistance to the fungus, which has been shown to be linked to the physio-chemical and genetic constitutions of the cultivars. The study has also shown the need for routine post-harvest seed health testing of cassava genotypes and pre-treatment of harvested seeds before storage or distribution to other research programmes and/or farmers. Apart from the synthetic fungicides shown to be efficient disease control agents, anti-microbial plant products are also promising control alternatives that are low cost, non-phytotoxic, environmentally friendly and should be explored and used.

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