Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (3): 278-283, 2007
ISSN 1816-4935

Microorganisms Associated with Rot of Minisetts of White Yam (*Dioscorea rotundata* Poir)

E. Asare-Bediako, F.A. Showemimo and Y. Opoku-Asiama
Department of Crop Science, School of Agriculture,
University of Cape Coast, Ghana

Abstract: Two cultivars of white yam (Pona and Dente) minisetts were used in a study to identify microorganisms causing rot in white yam. Laboratory analysis showed presence of *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *Aspergillus* sp., *A. tamari*, *Cladosporium* sp. *Corynebacterium* sp. *Fusarium* sp. *Penicillium* sp. *Rhizopus stolonifer* and *Trichoderma* sp. Pona minisetts were more heavily infected and so suffered more severe rot than Dente minisetts. Among the pathogenic isolates, *Sclerotium. rolfsii* caused the most severe rot in both Pona and Dente, followed by *A. niger* and *Fusarium* sp. while the least were *R. stolonifer*, *Trichoderma* sp. and *Corynebacterium* sp. Use of disinfectants were suggested as pre-planting treatment to control the pathogens.

Key words: Control, cultivars, growth rate, isolation, pathogenicity, severity, yam rot

INTRODUCTION

White yam (*Dioscorea rotundata* Poir) is the major and important yam species grown in Ghana (Tetteh and Saakwa, 1994). Among this yam species Pona is the most preferred cultivar by both farmers and consumers because of its early maturity, high market value and excellent organoleptic qualities. However, the production of yam in general is constrained by high cost and availability of planting materials, which constitute over 33% of the cost in yam production. As many as 30% of the previous harvest which should have been sold for income or eaten are reserved as seed yam for next cropping season (Orkwor and Asadu, 1997).

The adoption of yam minisett technique has considerably contributed to the increased supply of planting materials in Ghana. However, Pona minisetts, unlike minisetts of other white yam cultivars had not been very successful due to the high spoilage of the setts in the sprouting medium. There were little or no documented work on the identification of the microorganisms associated with rotting of Pona minisetts. Cornelius (1998) identified twelve microorganisms associated with rotting of Pona tubers in storage. These were *Aspergillus flavus*, *A. niger*, *Botryodiplodia threobromae*, *F. cumorum*, *F. oxysporium*, *Fusarium* sp. *Rhizopus stolonifer*, *Penicillium brevi-compactium*, *Penicillium* sp. *Scutellonema bradys* and *Erwinia carotovora*. It has, however, not been established as to which of these pathogens cause *Pona* minisetts to rot in the sprouting medium.

This study attempt to identify and compare the microorganisms associated with the rot of Pona and Dente yam minisetts, their pathogenicity and suggests an effective control package.

MATERIALS AND METHODS

Pona and Dente yam minisetts planted in untreated sawdust medium were used for identification of microorganisms associated with rot in minisetts. The individual fungi and bacterium were then isolated. Isolation of fungi from the minisetts of Pona and Dente were done on Water Agar (WA) and

Corresponding Author: F.A. Showemimo, Department of Crop Science, School of Agriculture, University of Cape Coast, Ghana

Potato Dextrose Agar (PDA). Pieces of tissues from the advancing margins of the rots in Pona and Dente yam minisetts were removed with flamed scalpel, surface sterilized in 1.0% sodium hypochlorite solution for 3 min, rinsed in several changes of sterile distilled water and plated on petri plates of water agar. The plates were enclosed in clean polythene bags and incubated under temperature of 28°C in the laboratory. Fungi that grew out from the plated tissues were sub-cultured on plates of Potato-dextrose Agar (PDA) to obtain pure cultures. Pure isolates on PDA slants were stored at 4°C in refrigerator until needed.

Morphological characteristics of mycelia stained in lactophenol and observed under a compound microscope were recorded. Conclusive identification of fungal isolates was based on the following culture characteristics on PDA: growth rate and colour and morphology of mycelia, conidia, conidiophores, sporangia, sporangiophores and sclerotia as described by Sampson *et al.* (1995).

Dilution plate method was used in the isolation of the bacteria. About 5 g tissues each was removed using a sterile scalpel from advanced portion of the lesion on the Pona and Dente minisetts. The tissue was surface sterilized for 3 min in a 1% sodium hypochlorite solution. After rinsing in several changes of sterile distilled water, the tissue was chopped up with a sterile scalpel in a drop of sterile distilled water on a sterile glass slide and streaked onto petri plates of Nutrient Agar (NA) with a sterile inoculation loop. After incubation colony characteristics such as pigment production was observed and recorded to aid proper identification of the isolate. Motility test was carried out to ascertain whether the bacterial cells were motile. The procedure followed was according to the method described by Goszcznska *et al.* (2000). A loopful of bacterial suspension was removed from the bottom of the slant and examined under high power microscope and the characteristics recorded.

Gram stain reaction (Bradbury, 1970) was done to reveal the shape and gram reaction of the cells of the bacterial isolates. A smear from a bacterial colony on a plate was prepared on a clean grease-free microscope slide. The smear was air-dried and underside of the slide was passed over a flame three times to fix the bacterial cells onto the slide. The smear was then stained with a 1.0% crystal violet for 2 min, washed with tap water and excess water drained off. Gram's iodine was applied to the smear for a minute and then washed off with water. It was then decolourized rapidly (25 sec) with 50% acetone-spirit and washed with water immediately. Counter-staining was done for a minute with 1.0% Neutral red, washed thoroughly with water and blotted dry. The stained film was examined under oil immersion objective of the microscope and the characteristics recorded.

Aerobic growth test was carried out by inoculating the ESP80a aerobic and anaerobic broths with 10 mL cell suspension of 18 h old bacterial culture under aseptic conditions. They were then incubated at 34°C for 24 h and then examined for growth.

Spore formation test was done by suspending cells of the bacteria growing on Nutrient Agar (NA) in a drop of water on a slide and air-dried. The slide was flooded with 5.0% (W/V) aqueous malachite green and stained for 10 min. It was washed thoroughly under running water and dried briefly. Counter-staining was done by flooding the slide with 0.5% (W/V) aqueous safranin for 50 sec, rinsed thoroughly with water and blotted dry. Cells were then observed under oil immersion objective of the microscope. Acid-fast staining was carried out to determine whether the test bacterium was acid-fast or non-acid -fast. Catalase production was tested for using nutrient agar. Conclusive identification of the bacterium isolate was based on Bergey's manual (Breed *et al.*, 1957).

Pathogenicity test was carried out by inoculating healthy yam minisetts of Pona and Dente with pure cultures of each fungal and bacteria isolates according to the method described by Okafor (1966). An isolate was confirmed pathogenic if it caused rot similar to that observed on the diseased minisetts from where it was isolated. Data obtained were computed into frequency and percentage.

RESULTS

Ten fungal and one bacterial species were isolated from 130 partially rotten minisetts of Pona and Dente yam cultivars. The fungi belong to seven genera. The fungal species were: *Aspergillus niger* van

Tieghem; *Aspergillus flavus* Link; *Aspergillus ochraceus* Wilhelm; *Aspergillus tamari* Kita; *Aspergillus* sp., *Penicillium* sp., *Rhizopus stolonifer* (Ehrenb) Lind; *Sclerotium rolfsii* Saccardo; *Fusarium* sp., *Cladosporium* sp., *Trichoderma* sp. The bacterium was *Corynebacterium* sp. *Aspergillus niger* was the most frquently encountered (52.3%) of the microorganisms on Pona minisetts. This was followed by *A. flavus* (44.6%), *Rhizopus stolonifer* (38.5%), Sclerotium rolfsii (30.8%), *Fusarium* sp. (20.0%) and *A. ochraceus* (16.9%) and *Cladosporium* sp. (16.6%). *Penicillium* sp., *A. tamari* and *Trichoderma* sp. were less frequently encountered with percentage occurrence being 9.3, 9.2 and 6.2%, respectively. However, on Dente minisetts the most encountered organisms was *Aspergillus flavus* (43.1%), followed by *A. niger* (30.8%) and the least was *Trichoderma* sp., *Penicllium* sp. was not found on Dente minisetts.

All the microorganisms identified were more frequently on Pona minisetts than on Dente minisetts, with mean frequency of occurrence being 20.1 and 13.8%, respectively. However, there were no significant difference among the isolates with respect to their frquency of occurrence on the yam minisetts (Table 1). Figure 1 show the growth rate of the isolated fungal pathogens that cause yam rot disease in yam tubers and minisetts; *Rhizopus stolonife*, *Sclerotium rolfsii*, *Trichoderma lignorum* and *Trichoderma* sp. had the highest growth rate, while, microorganisms with least growth rate are *Penicellium citrinum*, *Aspergillus* sp., *Penicellium* sp. and *Cladosporium* sp.

Yam minisetts and tubers inoculated with the isolates and incubated under humid conditions were visually assessed (Table 2). All the 10 fungi and the bacterium isolate inoculated into healthy yam minisetts and yam tubers caused rot disease. The rot colour symptoms associated with causative fungi and bacterium are also presented in Table 2. The infected yam tuber and minesett colour differed as the causative organism differ; all the *Aspegillus* sp. that caused yam rot changed the yam colour to pale yellow initially to brown or dark brown. The remaining microorganisms turn the yam colour to brown initially and then to varous shades of brown colour finaly. Morphological characteristics of the microorganisms isolated from yam minisetts and yam tubers used for the pathogenicity test as was seen under the microscope were similar to those used for the inoculation. At the end of the pathogenicity test the entire minisetts of both Pona and Dente, which were inoculated with *Sclerotium rolfsii* had rottened completely. Besides *Sclerotium rolfsii*, *Aspergillus niger* caused severe rot in both yam minisetts and yam tubers, followed by *Fusarium* sp., *Aspergillus ochraceus*, *Aspergillus flavus*, *Penicillium* sp., *Cladosporium* sp. Thus, moderately pathogenic, while, *Trichoderma* sp., *Rhizopus stolonifer* and *Aspergillus* sp. caused mild rot of both minisetts and tuber. Generally, apart from *Aspergillus ochraceus*, the test microorganisms caused more severe rot in Pona minisetts than in Dente minisetts as seen by their severity ranking.

Table 1: The frequency of occurrence of the fungi and bacteria isolated from pona and dente yam minisetts

	Frequency of occurrence (%) on indicated yam cultivar	
Pathogens	Pona	Dente
Aspergillus flavus	44.6	43.1
Aspergillus niger	52.3	30.8
Aspergillus ochraceus	16.9	13.9
Aspergillus sp.	9.3	4.6
Apergillus tamari	9.2	15.4
Cladosporium sp.	16.9	12.3
Corynebacterium sp.	7.7	1.5
Fusarium sp.	20.0	7.7
Penicillium sp.	9.3	0.0
Rhizopus stolonifer	38.5	29.2
Sclerotium rolfsii	30.8	18.5
Trichoderma sp.	6.2	3.1
Mean infection (%)	20.1	13.8

t = 1.1985, p = 0.24

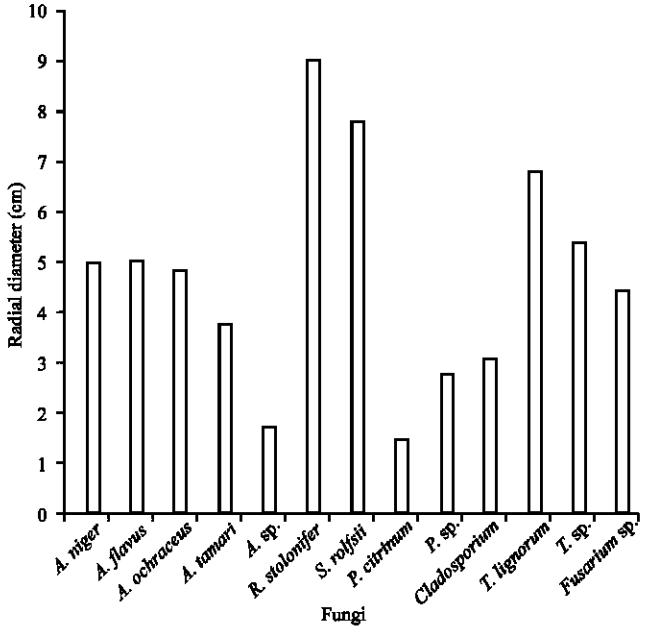

Fig. 1: Fungi isolates and their growth rate

Table 2: Symptoms and severity ranking of pathogens causing rot in yam

		Severity ranking	
Microorganism	Symptom	Pona	Dante
Aspergillus flavus	Dark brown soft rot	6*	6
Aspergillus niger	Dry purple to yellowish brown firm rot with black charcoal-like margins	8	7
Aspergillus ochraceus	Yellow to dark brown firm rot	4	6
Aspergillus tamari	Pale yellow to brown dry rot	4	4
Cladosporium sp.	Brown to radish brown dry rot	5	4
Corynebacterium sp.	Brown to dark brown dry rot	4	4
Fusarium sp.	Cream to brown dry rot	6	6
Penicillium sp.	Yellowish brown soft rot	3	-
Rhizopus stolonifer	Light brown to yellowish brown soft rot	5	5
Sclerotium rolfsii	Purple to pink soft rot	9	8
Trichoderma sp.	Brown dry rot	4	3

The rot symptoms caused by the micrroganisms were the same in both pona and dente yam minisetts. *Ranking based on 0-9 scale; 0 = least severe and 9 = most severe

DISCUSSION

Wide range of microorganisms were associated with the rot of yam minisetts in sprouting medium. This is expected because both the sprouting medium and the mother seed yam from which the minisetts were cut out are potential source of yam rot pathogens as reported by Cornelius (1998), Osai and Ikotun, (1994).

Sclerotium rolfsii and *Aspergilus niger* belonged to the group of fast growing fungi and they caused more severe rot to both yam minisetts and tubers than the other pathogenic fungi although they were less frequently encountered on the yam minisetts. This confirms their high level of pathogenicity as reported by IITA (1988). Therefore, they caused the most serious threat to yam minisetts especially Pona minisetts as they were able to rot the entire yam tubers within few days. Meanwhile,

A. niger had been found by Noon and Colhoun (1979) and Cornelius (1998) to cause severe decay in yam tubers. The fact that *A. niger* was encountered on most of Pona and Dente minisetts, is an indication that it could be a serious threat to yam minisetts especially Pona minisetts.

Fusarium sp., *Aspergillus ochraceus*, *Aspergillus flavus*, *Penicillium* sp., *Cladosporium* sp. were moderate in their severity on yam tubers and minisett, but they are more severe in Pona minisetts than Dente. Thus, Pona was more susceptible to rot pathogens than Dente. This may be attributed to differential genotypic sensitivity to spores, mycelia and phytoalexins of the pathogens. Similar findings had been reported by Okafor (1966), Cornelius (1998) and Emehute *et al*. (1998). These microorganisms are important pathogenic causative agents of rot in yam minisetts.

Penicillium species had been reported to be serious pathogens of yam storage rot (Adimora *et al*., 1990; Cornelius, 1998). However, *Penicillium* sp. identified in this study caused moderate rot in yam minisetts. *Cladosporium* sp. was found to cause mild rot of both minisetts and tubers of yam. *Rhizopus stolonifer* infected more yam minisetts but less ability to cause rot. This was due to the fact that *R. stolonifer*. is a secondary invader or saprophyte and its potential to cause rot only increases in association with other fungi such as *Fusarium solani* and *Fusarium oxysporum*. *Trichoderma* sp. and *Corynebacterium* sp. isolated in this study caused mild rot to both yam minisetts and tubers, thus, not considered as highly pathogenic. However, the mild rot caused by the bacterium; *Corynebacterium* sp. to yam ninisetts and tubers could be due to the fact that the symptoms of decay was superficial, involving the periderm and some underlying tissues of the yam tubers (Adeniji, 1970; Noon, 1978; Nwankiti and Arene, 1978; Noon and Colhoun, 1979; Hahn, 1994). Based on earlier studies (Emehute, *et al*., 1998; Asare-Bediako *et al*., 2006). These yam rot pathogens can be effectively controlled, slow their growth rate and or inhibit their activities by the use of disinfectants such as Benlate, woodash, quicklime and neem extract as pre-sprouting treatment on the minsetts and media/nurseries.

In conclusion 12 fungal pathogens and one bacterium were identified but ten fungi and one bacterium were of any economical importance. The most frequently encountered fungal pathogen in both Pona and Dente yam cultivars were *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Sclerotium rolfsii*. All the identified microorganisms were causative pathogens of yam rot (both minisetts and tubers) but *Sclerotium rolfsii* and *Aspergillus niger* were the most virulent. Pona cultivar was more susceptible than Dente, use of disinfestants to control the pathogens was suggested.

REFERENCES

Adeniji, M.O., 1970. Fungi associated with decay of yam In Nigeria. Phytopathology, 60: 590-592.

Adimora, L.O., K.A. Oduro and H.B. Damptey, 1990. Studies of causal agents of rot in *Dioscorea rotundata*, Poir var. Gboko (white yam). Ghana J. Sci., 29-30: 101-106.

Asare-Bediako, E., F.A. Showemimo, Y. Opoku-Asiama and D.H.A.K. Amewowor, 2006. *In vitro* Analysis of growth media and the control of yam minisett-rot. Afr. J. Biotecnol., (In Press).

Bradbury, J.F., 1970. Isolation and preliminary study of bacteria from plsnts. Rev. Plant Pathol., 45: 213-218.

Breed, R.S., E.D. Murray and N.R. Smith, 1957. Bergey's Manual of Determinative Bacteriology. 7th Edn., E and S. Livingstone, Ltd. London, pp: 1094.

Cornelius, E.W., 1998. Causes and control of tuber rot of white yam (*Dioscorea rotundata*, Poir *varieties Araba*, *Asana* and *Puna*). M.Phil. Thesis. Faculty of Agric. U.G. Legon., pp: 123.

Emehute, J.K.U., T. Ikotun, E.C. Nwauzor and H.N. Nwokocha, 1998. Crop Protection. In: Food Yams: Advances in Research, Orkwor, G.C., R. Asiedu and I.J. Ekanayake (Eds.). NRCRI/IITA. Ibadan, Nigeria, pp: 105-141.

Goszcznska, T., J.J. Serfontein and S. Serfontein, 2000. A Safrinet Manual for Phytobacteriology: Introduction to Practical Phytobacteriology. SAFRINET, South Africa, pp: 83.

Hahn, S.K., 1994. Management of yam diseases. IITA Research Guide 39. Training Program. IITA, Ibadan, Nigeria, pp: 16.

IITA, (International Institute of Tropical Agriculture), 1988. Nematology Subprogram. Annual Report. IITA, Ibadan, Nigeria.

Noon, R.A., 1978. Storage and market diseases of yams. Trop. Sci., 20: 177-188.

Noon, R.A. and J. Colhoun, 1979. Market and storage diseases of yams imported into the United Kingdom. Phytopath. Z., 94: 289-302.

Nwankiti, A.O. and O.B. Arene, 1978. Diseases of yam in Nigeria. Pest Articles and News summaries, 24: 486-494.

Okafor, N., 1966. Microbial rotting of stored yams (*Dioscorea* sp.) in Nigeria. Exp. Agric., 2: 179-182.

Orkwor, G.C. and C.L.A. Asadu, 1997. Agronomy. In: Food Yams: Advances in research. Orkwor, G.C., R. Asiedu and I.J. Ekanayake (Eds.). NRCRI/IITA. Ibadan, Nigeria, pp: 105-141.

Osai, E.O. and T. Ikuton, 1994. Microbial rot of yam minisetts. *Fitopatologia Brasileira* (1994) 19 (3): 408-412. In: Rev. Plant Pathol., (1995) 74: 4581-5323.

Sampson, R.A., E. S. Hoekstra, J.C. Frisvad and O. Filtenborge, 1995. Introduction to food-borne fungi 4th Edn. Centraabureau voor Schimmel cultures. Wageningen, Netherlands, pp: 313.

Tetteh, J.P. and C. Saakwa, 1994. Prospects and Constraints to yam production. In: Tropical Root Crops in a Developing Economy. Proc. of the 9th symp. of the Intern. Soc. Trop. Root Crops. Accra, Ghana, pp: 355-359.