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Full Length Research Paper

Fungal contaminants of the oil palm tissue culture in Nigerian institute for oil palm research (NIFOR)

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Twenty-five species of fungi belonging to 14 genera were identified as fungal contaminants of the oil palm tissue culture materials (explant, callus/embroid and plantlets). Of these genera *Penicillium* sp. occurred most frequently (40.8%), followed by *Curvularia* sp. (14.5%) *Cladosporium* sp. (13.4%), *Aspergillus* sp. (10.1%), *Acremonium, Fusarium* and *Alternaria* spp. (4.5%) respectively. *Rhizopus* (3.4%), *Trichoderma, Pestalotia* and *Helminthosporium* spp. (1.1%) respectively. *Paecilomyces, Dreschlera* and *Pythium* spp. were the least frequents (0.6%) respectively. These fungal species were found to cause death of the culture material. Some probable sources of contaminations such as handling of plant materials, culture vessels and the laboratory were discussed.

Key words: Fungal contaminants, oil palm tissue culture.

INTRODUCTION

Plant tissue culture is the *in vitro* technique of growing "sterile" plant cells, tissue or organs separate from the mother plant on artificial/synthetic medium. Among various uses (George and Sherrington, 1984), it is an important technique for rapid multiplication of plant materials from tissue and cells of desirable plants. Although aseptic conditions are usually employed but many plant cultures do not stay aseptic *in vitro* as they get contaminated. Contamination with micro-organism is considered to be the single most important reason for losses during *in vitro* culture of plants, such micro-organisms include viruses, bacteria, yeast, fungi, mites and trips have been shown to be harmless to the plant though they introduce fungi, yeast and bacteria into sterile plant culture (Cassels, 1996), which are considered harmful to the plant cultures.

The nutrient media in which the plant tissue is cultivated is a good source of nutrient for microbial growth. These microbes compete adversely with plant tissue culture for nutrient. The presence of these microbes in these plant cultures usually results in increased culture mortality, the presence of latent infections can also result in variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting (Kane, 2003). Odutayo et al. (2004) reported that *Pseudomonas syringae* pv phaseolicoli, *Bacillus licheniformis, B. subtilis, Cornebacterium* sp. and *Erwinia* sp. with the contamination of *Hibiscus canabinus* and *Telfaria occidentalis* in Nigeria.

While contamination with bacteria was said to be the most serious and has been described extensively in the literature (Horsch and King, 1983). Fewer publications described yeast and fungal contaminants and their effects on plantlets grown *in vitro* (Enjalric et al., 1988). In the Tropics, however, fungal contaminants are very common and cause serious harm to plant tissue culture.

The aim of this study was to investigate and identify the common contaminants of the oil palm tissue cultures with the hope of devising means of preventing contamination of the cultures

MATERIALS AND METHODS

The culture materials used were leaf explants, callus/embroid and plantlet. The plant tissue culture medium used was Murashige and Skoop (1962). The medium was sterilized by autoclaving at 121°C for 15 min pressure. The explants were excised and surfaced sterilized with Sodium hypochlorite (Sigma-Aldrich Chenire) for three minutes (Yedidia et al., 1999). The excised explants were then aseptically transferred to the culture medium, labeled and incubated at 24°C ± 2 for three weeks. Contaminated tissue culture tubes were removed from the Tissue Culture Unit of Nigerian Institute for Oil Palm Research (NIFOR) and visually examined. The contaminants were isolated from explant, callus/embroid and plantlets by planting the infected tissues on solid PDAC (Potato Dextrose Agar

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with 0.05 g chloramphenicol per litre) and incubated at 28°C ±2 for 3 days. The isolated micro-organisms (fungi) were maintained on solid PDAC and 0.1% PDAC liquid medium at 28°C. Some of the isolated fungi were identified in this laboratory, while some others were sent to the International Mycological Institute, Surrey, United Kingdom for identification.

RESULTS

Fungal, yeast and some bacterial contaminants produced visible growth in plant tissue culture media and were detected during visual assessment of cultures. The fungi were identified on the bases of morphological characteristic such as colony form and color, type of mycelium, fruiting bodies and spores (Commonwealth Mycological Institute Descriptions of Pathogenic Fungi and Bacteria, 1996). Twenty-five fungal species belonging to fourteen genera have so far been identified as oil palm tissue culture contaminants (Table 1). Of these 14 fungal genera identified, the genus *Pencillium* occurred most frequently (40.8%), followed by *Curvularia* (14.5%), *Cladosporium* (13.4%), *Aspergillus* (10%), *Acremonium, Fusarium and Alternairia* (4.5%) respectively; *Rhizopus* (3.4%). *Trichoderma, Pestalotia and Helminthosporium* (1.1%) respectively, *Paecilomyces, Dreshchlera and Pythium* were the least frequent (0.6%) respectively. Three non sporulating saprophyte and six sporulating but unidentified fungi were insolated. Of these 25 species only *Pencillium pinophylium* and *P. purpurogenum* which in most cases occurred together. Mites and trips were however, not observed in any of the culture bottles.

The fungi identified in this study were observed to utilize the nutrients of the plant tissue culture medium for their metabolism and usually outgrown the culture materials. The genera *Aspergillus* and *Penicillium* associated more with the leaf explants while *Cladosporium* associated more with callus/embroid material. *Curvularia* was found in association with callus and plantlet materials. However, the plantlet material did not host a lot of the nutrient degraders when compared with leaf explants and callus materials. As a result, some of the plant tissues were completely covered within a short time. The culture materials appeared reduced in growth and were subsequently killed by the fungal contaminants.

DISCUSSION

Apart from *Fusarium oxysporum* f. *sp elaeidis, Fusarium* sp. and *Pythium splender* which inhibited the Rhizosphere of plants, all other inhibited the aerial plant surfaces. Thus members of the genera *Cladosporium* and *Curvularia* (Magie, 1948), *Alternaria and Helminthosprium* (Atkins, 1950) are known to cause various leaf spots and other disease of plants. *F. oxysporum* f. sp. *elaeidis* has been reported to cause *Fusarium* wilt of the oil palm while *P. splender* in combination with *Rhizctonia lamellifera* causes blast of the oil palm seedlings (Robertson et al., 1968). Thus nearly all species isolated belonged to genera that are known as common plant pathogens and their introduction into the culture bottles might have been due to inadequate sterilization of the explants. Sterilization of leaf explant might have been inefficient. Some fungal genera such as *Aspergillus, Fusarium, Microsporium, Nieurospora, Cladosporium and Philalophora* have also been identified as fungal contaminants of plant tissue cultures (Blake, 1988). Fungal contaminants of plant tissue culture have been reported to increase culture mortality (Kane, 2003).

It is probably that complete coverage of the culture materials could have caused death by suffocation while it is unlikely that they died of starvation as the culture medium was far from depletion at the time of their death. It is not unlikely that some fungal contaminants exuded substances that were harmful to culture materials. Such fungi as *Aspergillus niger* and *Asperigillus flavus* are known to produce oxalate and aflatoxin poisoning respectively and *F. oxysporium* f.s. *elaeidis* has also been shown to produce metabolites (Obuekwe and Osagie, 1989) which could have adverse effects on the culture materials thus causing death. Eziashi et al (2006) reported that the growth of *Ceratocystis paradoxa* was inhibited *in vitro* by *Trichoderma* species during volatile, non-volatile and direct-diffusible metabolite tests. Similar observations have been made on plant tissue culture materials contaminated by bacteria (Lynch, 1977; Baker and Schippers, 1987).

Ex-plant taken from *in vivo* plants can be a source of contamination. In the tropics, high numbers of fungal populations abound on the aerial surfaces and the rhizosphere of plants. Such ex-plants taken from *in-vivo* plants are sometimes very difficult to complete surface sterilization. In the present study, the abundance of *Pencillium* sp. in the culture could be attributed to inefficient surface sterilization as it was the only genus isolated from explants meant for the initiation of tissue culture after surface sterilization with 0.35% Sodium hypochlorite for 3 min. Increase in the concentration/or exposure time will probably reduce fungal contaminants and consequently death of the culture materials. The appearance of *F. oxysporum* f.sp *elaeidis* at later *in vitro* stage only suggested that it must have been introduced with the initial plant materials. Leaf explant from *F. oxysporum* f.sp *elaeidis* recently infected oil palm will pass as healthy and surface sterilization will not destroy the pathogen as the microconida are carried from root to the shoot system through the xylem. Consequently, the pathogen is likely to contaminate the plant tissue culture after many subcultures or transfer. Similar findings have been reported of latent bacterial, which are not detected by visual examination or destroyed by surface sterilization (Bastinaens, 1973).

Handling of plant materials, cultures vessels and media preparation could be a source of large proportions of contaminants found in this study. This might have been introduced due to sub-standard condition under which the

Table 1. Fungi isolated from oil palm tissue culture materials and their m frequencies.

Culture materials of the fungi isolated				
Fungal contaminants	**Leaf explants in culture**	**callus/Embroid**	**Plantlet in culture**	**% frequently**
Acremonium sp.	5	3	-	4.5
Alternaria sp.	-	8	-	4.5
Aspergillus flavus Link	2	1	-	1.7
Aspergillus niger Tieghem	2	1	3	3.4
Aspergillus tamarii Rita	7	2	-	5.0
Cladosporium cladosporides (Fres) de Vries	-	2	-	1.1
Cladosporium oxysporium Berk & Curtis	1	9	2	6.7
Cladosporium sphaerosphernum Penzig	-	2	-	1.1
Cladosporium sp.	2	4	2	4.5
Curvularia verriclosa Tandon & Bildrami	-	7	2	5.0
Curvularia sp.	1	4	12	9.5
Dreschlera sp.	-	1	-	0.6
Fusarium oxysporium f.sp. elaedis Schlecht	-	-	5	2.8
Fusarium sp.	-	-	3	1.7
Helminthosporium sp	-	2	-	1.1
Paecilomyces liacinu (thom) Samso n	1	-	-	0.6
Penicillium pinophylum	21	3	-	13.4
Penicillium purpourogenum Stoll	18	1	-	10.6
Penicillium sp.	4	-	-	2.2
Pestalotia sp.	1	1	-	1.1
Pythium splender	-	-	1	0.6
Rhizopus oryzae Went & Prinseon Greetings	-	-	1	0.6
Rhizopus sp.	5	-	-	2.8
Trichoderma harzianum Rifai	1	-	-	0.6
Trichoderma s	1	-	-	0.6

operator worked. Nearly all-fungal contaminants, species of *Cladosporium, Curvularia, Aspergillus, Penicillium* and *Alternaria* (Table 1) are likely to have originated from source within the laboratory, probably during handling and cleaning of plant cultures in not so aseptic environments. Incomplete sterilization of culture vessels and media could also have increased the incidence of fungal contamination. Introduction of fungal yeast, and bacterial contaminants during handling of clean plant cultures have been reported by various authors (Kunneman and Faaij-Groene, 1988). Introduction of contaminants at a rate of between 5 and 15% per sub-culture has also been reported (Leifert, 1990). Consequently, adequately training of operators and high standards laboratory cleanliness is a vital pre-requisite to successful plant tissue culture. The micro-organisms involved in the contamination of oil palm tissue cultures are either aerial or soil micro-organisms depending on the source of the ex-plants.

The following precautions have considerably helped in minimizing the contaminations and often obtaining contaminant free cultures: 1. Selection of clean, healthy part, which were free of soil. 2. Adequate surface sterilization of explants for tissue culture:- i. 0.35% Sodium hypochlorite for 3 min for leaf explants; ii. Washing the roots under the tap to free them of any adhering soil; iii. Then immersing the root explants in 70% alcohol for 3 min followed by immersion in 0.1% mercuric chloride for 3 min. 3. All operations were performed in a sterile bench with other aseptic practices. With the above protocol, contamination of tissue culture materials has been reduced to manageable level.

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REFERENCES

Atkins JC Jr. (1950). *Heminthosporium victoriae* as a leaf spotting pathogens. Phytopathol. 40:p. 785.

Baker AW, Schippers B (1987). Microbial cyanide production I the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth stimulation. Soil biology and biochemistry 19: 451-457

Bastinaens L (1973).Endogenous bacterial plant and their implications in tissue culture a review Mededelingen van de faculteit Landbouwettenschappen. Rij ksuniversitelt Gent 48: 1-11

Blake J (1988). Mites and Trips as bacterial and fungal vectors between plant tissue culture. Acta Horticultural 225: 163-166.

Cassels AC (1996). Production of healthy plants. In proceedings of the Institute of Horticultural Symposium: Micropropagation in culture. Alerson PG, Dullforce, WM (edition). Nothingham. University of Nottingham Trent Print Unit. pp. 53-71

Commonwealth Mycological Institute, (1996). Description of Pathogenic Fungi and Bacteria, Nos. 91& 94.

Enjalric F, Carron MP, Lardet L (1988). Contamination of primary culture in tropical areas. The case of Hevea brasiensis. Acta Horticulture 225: 25-65.

Eziashi EI, Uma NU, Adekunle AA, Airede CE (2006). Effects of metabolites produced by *Trichoderma* species against *Ceratocystis paradoxa* in culture medium. Afr. J. of Biotech. 5 (9): 703-706.

George EF, Sherrigton PD (1984). Plant Propagation by tissue culture, Eversley, Bassingstoke; Exegetices Ltd. pp. 88-93.

Horsch RB, King JM (1983). A convert contamination of cultured plant cells, elimination of a Hyphomicrobium spp. from cultures of *Datura innoxia* (Mill). Plant cell Tissue Culture 2: 21-28.

Kane M (2003). Bacterial and fungal indexing of tissue cultures http://www.hos.ufl.edu/moreweb/TissueCulture/class1/Bacterial%20and%20fungal%20indexing%20of20tissue%20cultures.doc.

Kunneman BOAM, Faaij-Groenen GPM (1988). Elimination of bacteria contaminants :a matter of detection and transplanting Procedures. Acta Horticultural 225: 183-189.

Leifert C (1990). Contaminants of plant tissue cultures, Ph. D. Thesis, Nottingham University, School of Agriculture.

Lynch JM (1977). Phytotoxicity of acetic acid production in the anaerobic decomposition of wheat straw. Journal of applied Bacteriol. 42: 81-87.

Magie RO (1948). Curvularia spot, a new disease of gladiolus. Plant Disease Reporter 32: 11-23.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarium 15: 475-497.

Obuekwe CO, Osagie IJ (1989). Morphological changes in infected wilt-resistant and wilt-susceptible oil palm progenies and hydrolytic enzyme activities associated with *Fursarium oxysporum* f sp elaeidis pathogens, Oeagureux, 44 (11): 8-9.

Odutayo OI, Oso RT, Akinyemi BO Amusa NA (2004). Microbial contaminants of cultured *Hibiscus cannabalis* and *Telfaria occident-talis* cultured tissue. Afr. J. Biotechnol. 3: 301- 307.

Robertson JS, Prendergast AG, Sly AG (1968). Disease and Disorder of the Oil Palm J. of the Nigerian Inst. For Oil Palm Res. 16: 389-400.

Yedidia I, Benhamou N, Chet I (1999). Induction of defence responses in cucumber by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol. 64: 1061-1070.