British Biotechnology Journal
4(5): 612-621, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Molecular Identification of Biodiversity of *Fusarium* species Isolated from Wilted Oil Palm and Date Palm in Nigeria

N. I. Chidi[1*], A. A. Adekunle[2], E. I. Eziashi[1], I. B. Omamor[1], E. E. Odigie[1] and I. J. Osagie[1]

[1]Nigerian Institute for Oil Palm Research (NIFOR), Plant Pathology Division, P.M.B. 1030, Benin City, Edo State, Nigeria.
[2]Department of Botany, University of Lagos, Yaba, Lagos State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author NIC wrote the protocol and the draft of the manuscript. Author AAA designed the study. Author EIE performed the statistical analysis. Author IBO helped in the location of samples collection. Authors EEO and IJO carried out identification of pathogens. All authors read and approved the final manuscript.

Original Research Article

Received 28th February 2013
Accepted 9th March 2014
Published 13th May 2014

ABSTRACT

Biodiversity of sixteen *Fusarium* isolates, isolated from the roots of oil palm and date palm in Nigeria was studied. Ten *Fusarium* isolates out of the sixteen were obtained from the oil palm while six isolates were from date palm. Random Amplified Polymorphic DNA (RAPD) was used to detect the phylogenetic similarity between them. The unweighted Pair Group Method with Arithmetic average (UPGMA) dendrogram clearly separated these sixteen *Fusarium* isolates into five groups (clusters). The first at SC values of 100 grouped six *Fusarium* isolates (5, 2, 13, 9, 8 and 14,) of both oil and date palms. They belonged to *F. oxysporum* and *F. chlamydosporum.* The second at SC values of 100 had one *Fusarium* isolate (16) of date palm. It belonged to *F. solani.* The third at DC values of 71-100 grouped six isolates (6, 4, 3, 7, 11 and 10) of oil and date palms. They all belonged to *F. solani.* The fourth at DC value of 76 had one *Fusarium* isolate (1) of oil palm, it belonged to *F. nelsonii.* The fifth at SC value of 100 grouped two *Fusarium* isolates (15,12) of date palm. They belonged to *F. nelsonii* and *chlamydosporum*. The sequence alignment technique showed there were similarities between these sixteen

**Corresponding author: Email: eziashius@yahoo.com;*

Fusarium isolates. The analysis of RAPD showed that the sixteen *Fusarium* isolates were five groups while sequence alignment technique grouped them into five *Fusarium* isolates. The slight difference in similarity may be attributed to ecological origin and zone of the *Fusarium* isolates.

Keywords: Fusarium; biodiversity; RAPD; similarity; sequence; date palm; oil Palm.

1. INTRODUCTION

Fusarium wilt or vascular wilt is presently the most serious disease of the oil palm in West and Central Africa. The disease which attacks oil palm seedlings in the nursery as well as young and adult palms in plantations and in the groves is caused by soil-borne fungus, *Fusarium oxysporum f. sp. elaeidis* [1,2]. The external symptoms of the disease in the adult palms include stunted growth, yellowing of the leaves, desiccation and fracture of the fronds, followed by death of the palms within 6 months (acute type) or a few years (chronic type). The internal symptoms include blackening and necrosis of the cortex and plugging of the vascular system. In the nursery seedlings, the symptoms commence with stunted growth, followed by yellowing of leaves, desiccation and death.

Fusarium wilt disease was first discovered in 1946 at Zaïre [3] and is now widespread throughout several African countries (Ivory Coast, Benin, Ghana, Nigeria, Cameroon, Sao-Tome). It recently spread to Brazil [4] and Ecuador [5]. On the other hand, this disease has never been reported from south-east Asian countries such as Malaysia which is now the world's leading exporter of palm oil. Identification of *Fusarium* isolates requires laborious pathogenicity tests for 6–8 months. Therefore, a sampler method is necessary to identify suspected isolates.

Strains of *F. oxysporum* cause vascular wilt disease in many agricultural crops and have been classified into special forms based on their host specificity [6]. *F. oxysporum* f. sp. *albedinis* is the causal agent of Bayoud disease, a devastating disease of date palm (*Phoenix dactylifera* L.) [7]. The pathogen is easily spread by exchange of contaminated material; at present, strict phytosanitary rules are applied at borders of date palm-growing countries that are free of Bayoud disease. Detection and identification of *F. oxysporum* sp. *albedinis* remain difficult, mainly because time-consuming inoculation tests are required to assess pathogenicity and the fungus lacks a known sexual stage.

Nigerian Institute for Oil Palm Research (NIFOR) breeding programmed developed high yielding, early maturing, disease resistant (*Fusarium* tolerant) hybrid oil palm variety (the *tenera* hybrid) which yields 15 - 25tones fresh fruit bunch (FFB) and 3-5tones of palm oil as against 3 - 5tones FFB or 0.5tones palm oil per hectare by the unimproved palms in the natural groves, representing five-fold increase. Breeding for oil palm resistance to the devastating *Fusarium* wilt disease has been high on NIFOR's research agenda [8]. As a result of the breeding programmes and the effective procedures for screening materials against the devastating *Fusarium* wilt, it has become possible to undertake re-planting of oil palm in locations where high incidence of the disease previously occurred [9]. Cochard, et al. [9] noted that as a result of breeding for resistance to *Fusarium* wilt, it has become possible to grow and sustain the oil palm in several locations in West Africa where the disease is a problem.

Random Amplified Polymorphic DNA (RAPD) markers generated with single primers of arbitrary nucleotide sequence have been used in detecting intraspecific polymorphisms among fungi [10]. This technique can generate specific DNA fragments that can be used for the identification of isolates, and in molecular ecology [11]. For plant pathogenic fungi, RAPD analysis provided markers to differentiate races A, 3 and 4 of *F. oxysporum* f. sp. *vasinfectum* [10], races 0, 2 and 1,2y of *F. oxysporum* f. sp. *melonis* [12] and races 0, 1B/C, 5, and 6 of *F. oxysporum* f. sp. *ciceris* [13]. Cramer et al. [14] reported specific RAPD banding which distinguish among races *F. oxysporum* f. sp. *phaseoli* and *F. oxysporum* f. sp. *betae*. Identification of pathogenic races 0, 1B/C, 5 and 6 of *Fusarium oxysporum* f. sp. *ciceri* has been reported using 40 RAPD primers [15].

The diversity of *Fusarium* isolates in the environment is vast. However the use of morphological and microscopic methods of identifying these fungal isolates sometimes lacks accuracy and this is a major problem in disease management. Little information is available regarding the variability in *Fusarium* isolates from oil palm and date palm. The objective of this study was the identification and analysis of genetic diversity/similarity among sixteen *Fusarium* isolates from oil palm and date palm

3. MATERIALS AND METHODS

3.1 Source of Samples

The root samples were collected from wilted date palms showing bayoud disease from Jigawa state and from oil palms showing vascular wilt disease in NIFOR main station, Benin City, Edo State of Nigeria. Root samples were also collected from oil palm NIFOR sub station, Abak in Akwa Ibom State, Nigeria

3.2 Isolation and Identification of Fungi

One gramme of soil sample was collected into each McCartney bottle containing 9ml of sterile water. The bottles were agitated using an orbit shaker for 15minutes. 1ml of the solution was serially transferred from 10^{-1} to 10^{-5} using sterile syringes. 0.1ml from each of the serially diluted samples was dispensed into sterile Petri dishes and 9ml of potato dextrose agar (PDA) medium (45ºC) was poured into each of the Petri dishes. The plates were incubated at ambient temperature for 48hours. The emerging *Fusarium* colonies were sub cultured aseptically into solidified PDA plates until pure cultures were obtained. Twenty seven *Fusarium* isolates were isolated from the roots of wilted date and oil palms. The isolates were selected based on their cultural, morphological and pathogenic characters. The pathogenic isolates were defined as those isolates which after inoculation of the roots of susceptible plant lines of date and oil palm seedlings produced internal symptoms of blackening and necrosis of the cortex. The inoculum was prepared as described by Belabid and Fortas [16] and spore suspensions were adjusted to $3.5x10^6$ microconidia/ml. Ten mL of spore suspension was inoculated on each plant and 10mL of sterile water was used for the control. Growth of the fungi was viewed under light microscope using Motic Camera 2000 connected to a computer. The *Fusarium* isolates identified were confirmed by comparing their morphology using the descriptions of Talbot [17], Deacon [18] and Bryce [19].

3.3 Mycelium Production and DNA Extraction

Fungal isolates were grown in a 125ml Erlenmeyer flasks containing 75ml liquid culture of Lentil-dextrose. Each flask was inoculated with one mycelial plug (5mm diameter) taken from the edge of a 5-day old PDA culture. The cultures were incubated at 22°C for 10 days in the dark. The mycelium was washed in sterile deionized water, dried with filter paper, freeze-dried for 48h, and stored at - 45ºC until ready for use. DNA was extracted following the procedure described by Saghai-Maroof et al. [20], using 0.5ml TE buffer, treated with RNAase to degrade the RNA and stored at 4ºC. DNA was quantified using a spectrophotometer (Beckman DU-65). The quality of the extracted DNA was visually checked on 1.6% agarose gels.

3.4 Random Amplification of Polymorphic DNA (RAPD) Analysis

This was carried out using 11 operon primers (G-16; 5'AGCGTCCTCC 3', J-17; 5'ACGCCAGTTC 3', A-O1; 5' CAGGCCCTTC 3', AG02; 5' CTGAGGTCCT 3', AG03; 5' GGCGGGAGTG 3', AG07; 5' CACAGACCTG 3', AG08; 5' AAGAGCCCTC 3', AG09; 5' CCGAGGGGTT 3' AG 14; 5' CTCTCCCCGA 3', AG 17; 5' AGCCGAAGTG 3', X-20; 5' CCCAGCTAGA 3'). The 10.5mL cocktail mix for RAPD reaction consisted of 10X buffer (Biorad) 1.25mL, $MgCl_2$ (Bioline) 1.0mL, 5% Tween 20 at 1.25mL, 2.5mM dNTPs (Sigma) 0.5mL, Taq polymerase (Biorad) 0.2mL, RAPD primers (Operon) 0.5mL, Distilled water 5.25mL and Template DNA 2.5mL. The application conditions were; an initial denaturation step of 3mins at 94ºC followed by 45 cycles each consisting of denaturation step of 1min at 94ºC annealing step of 1min at 37ºC and extension step of 1min at 72ºC. The last cycle was followed by 5min extension at 72ºC. The amplified DNA fragments were electrophoresed on 1.5% Agarose gel in TAE buffer and visualized by staining with ethidium bromide under UV light".

3.4.1 Sequencing analysis

The DNA was purified before subject to sequencing. The PCR mix used consisted of 0.5µl of Big Dye Terminator Mix, 1µl of 5X sequencing buffer, 1µl of Operon J-17 primer with 6.5µl distilled water and 1µl of the PCR product making a total of 10µl. The initial Rapid thermal ramp to 96ºC was for 1 min followed by 25 cycles of 96ºC for 10 seconds, 50ºC for 5 seconds and 60ºC for 4 minutes. It was finally followed by Rapid thermal ramp to 4ºC and kept on hold.

3.4.2 Gene sequencer (ABI machine)

The Cocktail mix was a combination of 9µl of Hi di formamide with 1µl of the purified product making a total of 10µl. The product was directly sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing. All sequencing products were precipitated by ethanol according to the manufacturer's protocol and were analyzed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA).

3.4.3 Data analysis

Data were subjected to cluster analysis using the unweighted Pair Group Method with Arithmetic average (UPGMA) The program generated dendrogram, which grouped the test lines on the basis of Nei genetic distance [21]. DNA sequences were edited with SeqMan II

4.03 (Lasergene Sequence Analysis Software, DNASTAR, USA). It was then used as a query to search for similarities using the BLAST network services at the National Centre for Biotechnology Information (NCBI) [22]. The experiment was repeated twice.

4. RESULTS AND DISCUSSION

Out of twenty seven isolates of *Fusarium* isolated from diseased oil palms and date palms, only sixteen of them were selected for further study based on their pathogenic effects on susceptible plant lines of oil and date palm seedlings. Ten isolates out of the sixteen were obtained from oil palm while six isolates were isolated from date palm.

Out of the 11 operon primers used for the RAPD analysis only operon J-17 was selected. The electrophoresed DNA samples using primer J-17 indicated high purity which the other 10 primers lacked. The selection was also based on the highest polymorphic bands (8 bands) obtained when compared with others (Table 1). The relationship between the sixteen *Fusarium* isolates was expressed according to their DNA banding patterns (Fig. 1). Genetic similarity and distance coefficient were based on RAPD markers. The unweighted Pair Group Method with Arithmetic average (UPGMA) dendrogram clearly separated these sixteen *Fusarium* isolates into five groups (clusters). Similarity coefficient (SC) and distance coefficient (DC) between them ranged from 71 to 100. The first at SC values of 100 grouped six *Fusarium* isolates (5, 2, 13, 9, 8, and 14) of both oil and date palms together. They belonged to *F. oxysporium*, *F. solani* and *F. chlamydosporum.* The second at SC values of 100 had *F. solani* (16). The third grouped six *Fusarium* isolates (6, 4, 3, 7, 11 and 10) of the same oil palm. They belonged to *F. solani* of oil and date palms. The fourth at DC value of 76 had *F. solani* (1) of oil palm. The fifth also at SC value of 100 grouped *F. nelsonii* and *F. chlamydosporium* (15 and 12) of date palm (Fig. 2).

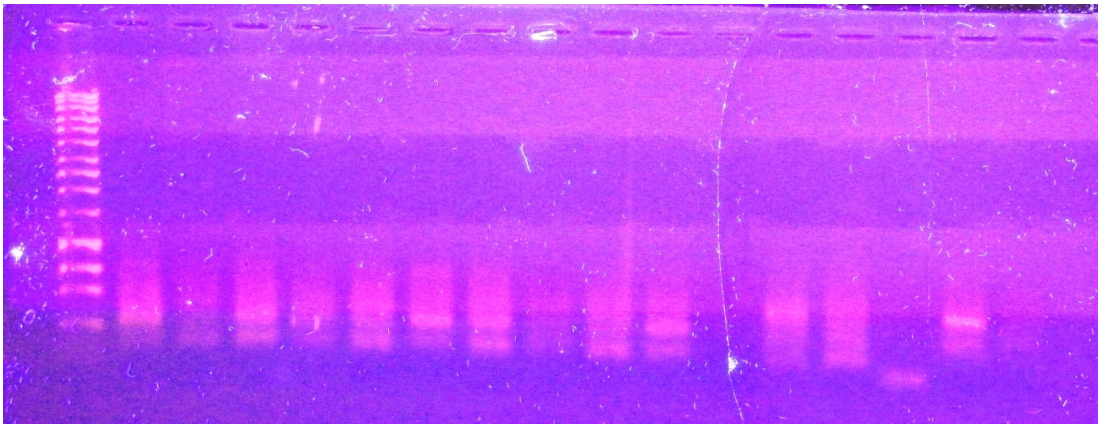

Fig. 1. Shows the polymorphic bands of *Fusarium* isolates using the OP-J17

M-Marker; 1-F.solani; 2-F-oxysporum; 3-F.solani; 4-F.solani; 5-F.oxysporum;6-F.solani;7-F.solani;8-F.oxysporum;9-F.oxysporum;10-F.solani; (oil palm)11-F.solani;12-F.chlamydosporum;13-F.oxysporum;14-F.chlamydosporum;15-F.nelsonii;16-F.solani (date palm)

Table 1. Number of polymorphic bands obtained from eleven primers among sixteen *Fusarium* isolates

Primer name	Sequence (5'-3')	PCR product length	Polymorphic bands	Monomorphic bands	% polymorphism
OPERON G-16	AGCGTCCTCC	600 bp	7	-	100
OPERON J-17	ACGCCAGTTC	800bp	8	-	100
OPERON A-01	CAGGCCCTTC	500 bp	5	-	100
OPERON AG02	CTGAGGTCCT	800 bp	7	-	100
OPERON AG03	GGCGGGAGTG	800 bp	6	-	100
OPERON AG07	CACAGACCTG	800 bp	7	-	100
OPERON AG08	AAGAGCCCTC	750 bp	7	-	100
Operon AG 09	CCGAGGGGTT	700 bp	6	-	100
Operon AG 14	CTCTCCCCGA	700 bp	7	-	100
Operon AG 17	AGCCGAAGTG	600bp	6	-	100
Operon X-20	CCCAGCTAGA	700bp	5	-	100

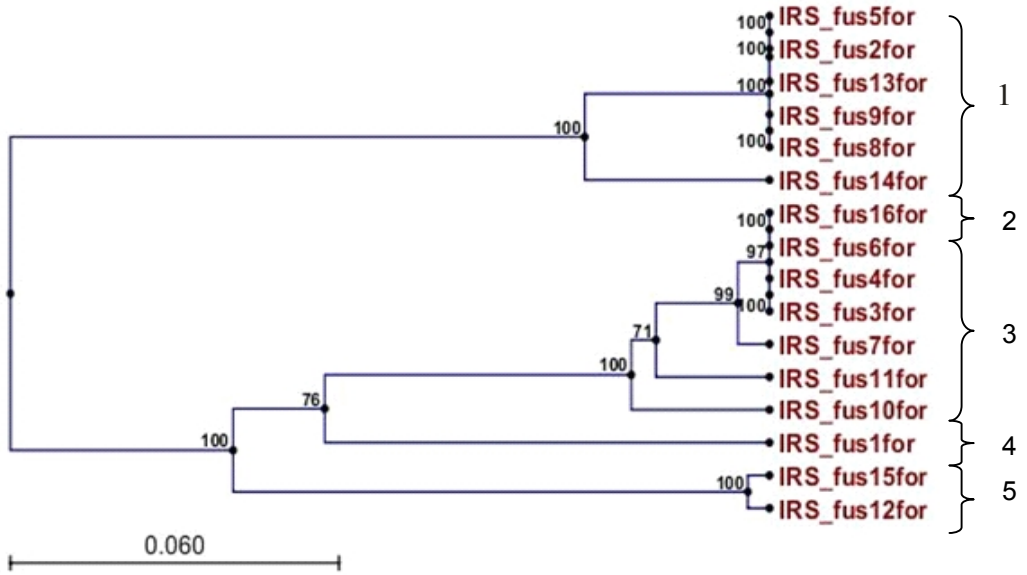

Fig. 2. Shows the phylogenetic relationship between Fusarium isolates

1-F.solani; 2-F-oxysporum; 3-F.solani; 4-F.solani; 5-F.oxysporum;6-F.solani;7-F.solani;8-F.oxysporum;9-F.oxysporum;10-F.solani; (oil palm)11-F.solani;12-F.chlamydosporum;13-F.oxysporum;14-F.chlamydosporum;15-F.nelsonii;16-F.solani (date palm)

The operon J-17 primer was used for the sequence alignment of the sixteen *Fusarium* isolates (Fig. 3). The first regions of alignment were isolates 3, 4, 6, 16, 10, 7, 11 and 15. They belonged to *F. solani* and *F. nelsonii* of oil and date palms, the second region of alignment was isolate 1, which belonged to *F. solani* of oil palm, the third region of alignment was isolate 12. It belonged to *F. chlamydosporum* of date palm, the fourth region of alignment were isolates 2, 5, 8, 9, and 13. They belonged to *F. oxysporum* of both oil and date palms and the fifth region of alignment was isolate 14 which belonged to *F. chlamydosporum* of date palm.

The results were compared to each other doing a BLAST alignment search for similarity in the NCBI database of FUSARIUM-ID database. The identification result of the sixteen isolates was determined by the partial DNA sequence (Table 2). Out of the sixteen isolates analysed eight were identified as *F. solani,* five *F. oxysporum*, two *F. chlamydosporum* and one *F. nelsonii* when matched with sequence based match on the *Fusarium* data base with primer region length between 27 – 30 (Fig. 3).

The present study showed biodiversity among *Fusarium* isolates obtained from wilted/infected oil and date palms from different ecological zones. Out of the 10 isolates from wilted oil palms, 6 isolates were *F. solani* and 4 were *F. oxysporum.* Also, out of the 6 isolates from date palms, 2 isolates were *F. solani*, 2 isolates were *F. chlamydosporum*, one *F. oxysporum* and one *F. nelsonii.* This agrees with Irma et al. [23] who reported that *Fusarium proliferatum, F. subglutinans*, and *F. verticillioides* from different zones are known causes of ear and kernel rot in maize worldwide. *Fusarium* is a known pathogen that cause wilt disease in oil palms and date palms. This agrees with Goertz et al. [24] report that *F. graminearum* which is as an important pathogen of *Fusarium* head blight in small-grain cereals also infects maize. Distinguishing species using morphological characters is difficult even for specialists [25,26], so molecular technique is usually needed to accurately identify species within the genus.

Table 2. Identification based on DNA sequencing

Isolate	Source	Location	Morphological identification	Sequence based identification	Identity (%)
1	Oil Palm	OPC	*F. solani*	*F. solani*	86
2	Oil Palm	NIFOR	*F. oxysporum*	*F. oxysporum*	100
3	Oil Palm	NIFOR	*F. solani*	*F. solani*	98
4	Oil Palm	NIFOR	*F. solani*	*F. solani*	98
5	Oil Palm	NIFOR	*F. oxysporum*	*F. oxysporum*	100
6	Oil Palm	ABAK	*F. solani*	*F. solani*	100
7	Oil Palm	ABAK	*F. solani*	*F. solani*	98
8	Oil Palm	ABAK	*F. oxysporum*	*F. oxysporum*	100
9	Oil Palm	ABAK	*F. oxysporum*	*F. oxysporum*	100
10	Oil Palm	ABAK	*F. solani*	*F. solani*	100
11	Date Palm	Genepool 3b (Jigawa)	*F. solani*	*F. solani*	99
12	Date Palm	Gruduba(Jigawa)	*F. chlamydosporum*	*F.chlamydosporum*	100
13	Date Palm	Limawa (Jigawa)	*F. oxysporum*	*F. oxysporum*	100
14	Date Palm	Genepool 1 (Jigawa)	*F. chlamydosporum*	*F. chlamydosporum*	100
15	Date Palm	Maai (Jigawa)	*F. nelsonii*	*F. nelsonii*	100
16	Date Palm	Genepool 3b (Jigawa)	*F. solani*	*F. solani*	100

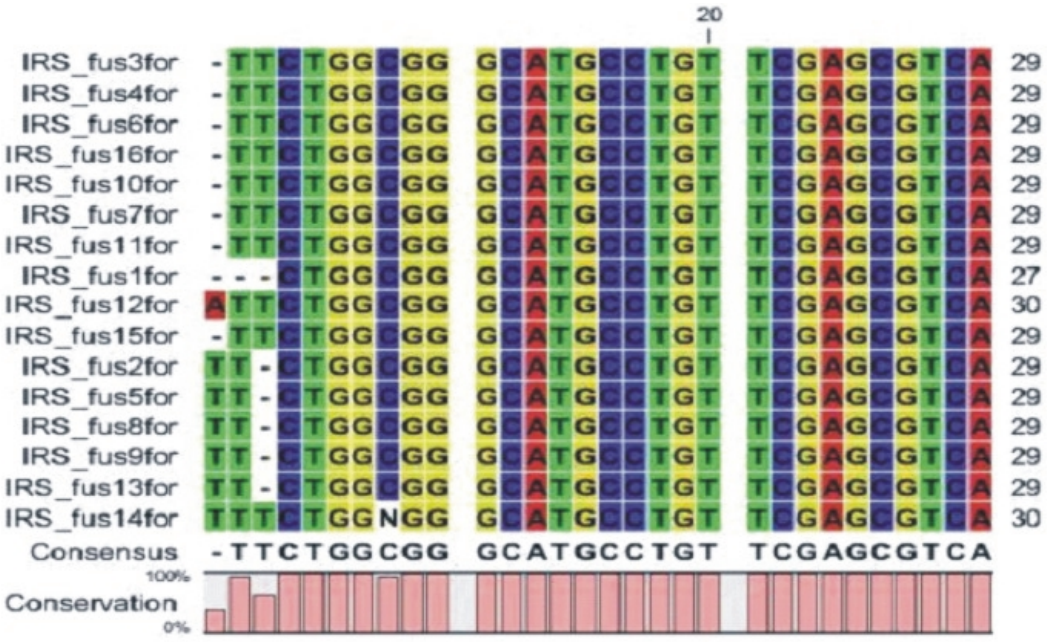

Fig. 3. Shows the sequence alignment of 16 *Fusarium* isolates

1-F.solani; 2-F-oxysporum; 3-F.solani; 4-F.solani; 5-F.oxysporum;6-F.solani;7-F.solani;8-F.oxysporum;9-F.oxysporum;10-F.solani; oil palm)11-F.solani;12-F.chlamydosporum;13-F.oxysporum;14-F.chlamydosporum;15-F.nelsonii;16-F.solani (date palm)

However, in this study DNA sequence-based identifications and RAPD PCR assays were used to confirm morphological identifications. All sequences were compared with sequences in the NCBI GenBank database and in FUSARIUM-ID database for species determination [27]. In this way the sixteen isolates were identified as *F. solani, F. oxysporum, F. chlamydosporum and F. nelsonii.* The use of dendrogram in establishing biodiversity among *Fusarium* species was reported by Irma et al. [23] when phylogenetic analysis was used to show the genetic changes in *F. napiforme* and *F. psedonygamai*. Also the role of dendrogram in establishing biodiversity was also proven when El-kazzaz et al. [28] used the phylogenetic tree of *F. oxysporum* f. sp. *lycopersici* isolate in expressing gene patterns which differed from that of the rest tested isolates. Sequence alignment grouped the sixteen *Fusarium* isolates into five regions even though few of the aligned were from different species. The slight differences were not significant. The use of sequence alignment supported the RAPD technique showing genetic relationship among *Fusarium* isolates from both oil and date palms from different ecological zones.

5. CONCLUSION

This study demonstrated that RAPD and sequence alignment techniques were efficient and faster method of evaluating the biodiversity of *Fusarium* isolates derived from different ecological zones. The sequence alignment technique is a useful tool in gene alignment grouping of different isolates and their genera. The cluster analysis of RAPD grouped the sixteen *Fusarium* isolates into five groups while sequence alignment technique also grouped them into five *Fusarium* isolates. The slight difference in similarity may be attributed to ecological origin and zone of the *Fusarium* isolates.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fraselle JV. *Fusarium oxysporium* Schl. f. as the cause of vascular wilt of the oil palm (*Elaeis guineensis* Jacq.). Transactions of the Brit. Mycol. Soc. 1951;34:492-96.
2. Aderungboye FO. A wilt disorder of nursery seedlings. NIFOR Twelfth Annual Report. 1976;51.
3. Wardlaw CW. *Fusarium oxysporum* on the oil palm. Nature. 1946;158:56.
4. Van de Lande HL. Vascular wilt of oil palm (*Elaeis guineensis*) in Brazil. Oil Palm Newslett. 1986;27.
5. Renard JL, Gascon JP, Bachy A. Research on vascular wilt of the oil palm. Oleagineux. 1972;27:581–91.
6. Armstrong GM, Armstrong JK. Formae speciales and races of *Fusarium oxysporum* causing wilt diseases. In Fusarium: diseases, biology and taxonomy. eds Nelson PE, Toussoun TA, Cook RJ. (Pennsylvania State University Press, University Park). 1981;391-99.
7. Louvet J, Toutain G. Bayoud, Fusarium wilt of date palm. in Fusarium: diseases, biology and taxonomy. eds Nelson P. E., Toussoun T. A., Cook R. J. (Pennsylvania State University Press, University Park). 1981;13–20.
8. Okwuagwu CO, Ataga CD, Okolo EC, Ikuenobe CE, Ugbah MM. The production of NIFOR elite tenera hybrid planting material. The NIFOR EWS. Technical Report, Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria; 2005.
9. Cochard B, Amblard P, Durand-Gasselin T. Oil palm genetic improvement and sustainable development. OCL. 2005;12(2):141–47.
10. Assigbetse KB, Fernandez D, Dubois MP, Geiger JP. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. Phytopathol. 1994;84:622-26.
11. Hadrys H, Balick M, Schierwater B. Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. 1: 55-63. Hall BG (1990). Spontaneous point mutations that occur more often when advantageous than when neutral. Genetics. 1992;126:5-16.
12. Namiki F, Shiomi T, Nishi K, Kayamura T, Tsuge T. Pathogenic and genetic variation in the Japanese strains of *Fusarium oxysporum* f. sp. *melonis*. Phytopathol. 1998;88:804-10.
13. Jiménez-Gasco M, Pérez-Artés W, Jiménez-Diaz RM. Identification of pathogenic races 0, 1B/C, 5, and 6 of *Fusarium oxysporum* f. sp. *ciceris* with random amplified polymorphic DNA (RAPD). Eur J. Plant Pathol. 2001;107(2):237-48.
14. Cramer RA, Byrne PF, Brick MA, Panalla L, Wicklife E, Schwartz HF. Characterization of *Fusarium oxysporum* isolates from common bean and sugar beet using pathogenicity assay and random-amplified polymorphic DNA markers. Phytopathol. 2003;151:352-60.
15. Jimenez-Gasco MM, Navas-Cortes JA, Jimenez-Diaz RM. The *Fusarium oxysporum* f. sp. *ciceris*/*Cicer arietinum* pathosystem: A case study of the evolution of plant pathogenic fungi into races and pathotypes. Int'l Microbiol. 2004;7:95-04.
16. Belabid L, Fortas Z. Virulence and vegetative compatibility of Algerian isolates of *Fusarium oxysporum* f. sp. *lentis*. Phytopathol. Mediterr. 2002;41:179-87.

17. Talbot OHK. An Introduction to Seed Technology. Leonard Hill Publishers, London; 1971.
18. Deacon JW. Introduction to modern mycology. Blackwell Scientific Publications, London; 1980.
19. Bryce K. The Fifth kingdom. Mycologue Publications, Ontario; 1992.
20. Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci, USA. 1984;81:8014-18.
21. Nei M. Genetic distance between populations. Am. Nat. 1972;106:283-92.
22. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 1997;25:3389-402.
23. Irma MR, Maria de JYM, Hilda V, Gabino GS, Doralinda AGP. Biodiversity of *Fusarium* species in Mexico associated with ear rot in maize, and their identification using a phylogenetic approach. Mycopathol. 2007;163:31-9.
24. Goertz A, Oerke EC, Steiner U, Wallwijk C, de Vriees I, Dehne HW. Biodiversity of *Fusarium* species causing ear rot in Germany. Cereal Res Commun. 2008;36:617-22.
25. Summerell BA, Salleh B, Leslie JF. A utilitarian approach to *Fusarium* identification. Plant Disease. 2003;87:117-28.
26. Leslie JF, Summerell BA. The Fusarium Laboratory Manual. Blackwell Publishing Professional, Ames, IA, USA; 2006.
27. Geiser DM, Jimenz Gasco MM, Kang S, Kuldau GA, O' Donnell K. Fusarium-IDv.1.0: A DNA sequence date base for identifying *Fusarium.* Eur J of Plant Pathol. 2004;110:473-79.
28. El-Kazzaz MK, El-Fadly GB, Hassan MAA, El-Kot GAN. Identification of some *Fusarium* spp. using Molecular Biology Techniques. Egypt J Pytopathol. 2008;36:57-69.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=494&id=11&aid=4544