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GENETIC VARIABILITY AMONG *Fusarium udum* ISOLATES FROM PIGEONPEA

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ABSTRACT

Thirty-eight isolates of *Fusarium udum* obtained from pigeonpea *(Cajanus cajan)* plants showing wilt symptoms were collected from various districts in Kenya and tested for variability in vegetative compatibility groups (VCG) and amplified fragment length polymorphism (AFLP). Nitrate non-utilising mutants were generated from *F. udum* isolates by selecting chlorate-resistant sectors on minimal medium amended with 15 g l^{-1} potassium chlorate. All the isolates of *F. udum* were grouped into a single VCG (VCG 1) with two subgroups VCG 1 I and VCG 1 II. The DNA of the fungal isolates was extracted using CTAB method. The AFLP analysis of 38 isolates using seven primer combinations generated a total of 318 fragments with 102 being polymorphic (32% polymorphism). The isolates could be grouped into one AFLP group with more than ten subgroups based on the analysis of the banding patterns, although most of these subgroups were not significantly distant (<50% confidence interval) genetically. Based on VCG and AFLP, the isolates could have originated from a single lineage. The VCG and AFLP of *F. udum* were independent of geographical origin of the isolates.

Key Words: Amplified fragment length polymorphism, *Cajanus cajan, Fusarium udum*

RÉSUMÉ

Trente huit variétés de *Fusarium udum* obtenu de pois cajan *(Cajanus cajan)* montra wilt symptômes étaient collectées dans plusieurs districts du Kenya et tester pour la variabilité dans le groupe de végétations compatibles (GVC) et fragment des longueur polymorphisme amplifies (FLPA). Le nitrate n'utilisant pas des mutants étaient générés de variétés de *F. udum* par sélection de secteurs résistants au chlorate sur des milieux avec minimums amendements de 15 g/l de chlorure de potassium. Toutes les variétés de *F. udum* étaient groupées dans un seul GVC (GVC1) avec deux sous-groupes GVC1I et GVC1 II. Les AND des isoles de fungi étaient extraits en utilisant la méthode cétyldiméthyléthylammonium bromure modifiée (méthode CTAB). Les analyses FLPA des 38 isoles utilisant 7 combinaisons primer a généré 318 fragments dont 102 étaient polymorphique (32% polymorphisme). Les isoles pourraient être groupes dans 1 FLPA groupe avec plus de 10 sous groupes bases sur les analyses de la tendance de bandes, même si presque tous ces groupes n'étaient pas très significativement distants génétiquement (<50% intervalle de confidence). En se basant sur GVC et les FLPA, les isoles pourraient provenir d'une seule lignée. Les GVC et les FPLA de *F. udum* étaient indépendant de l'origine géographique des isolés.

Mots Clés: Fragment de longueur polymorphique amplifié, *Cajanus cajan, Fusarium udum*

INTRODUCTION

Fusarium wilt caused by *F. udum* Butler is the most important disease of pigeonpea (*Cajanus cajan* (L.) Millsp.), and one of the major causes of low yields (Kannaiyan *et al.*, 1984). Losses due to wilt vary from negligible proportions to absolute (100%) depending on the stage at which the crop is attacked (Kannaiyan and Nene, 1981). Control strategies for *Fusarium* wilt of pigeonpea should target populations of *F. udum*. However, *F. udum* shows a great deal of variation in cultural and morphology characteristics (Booth, 1978; Rai and Upadhyay, 1982; Gaur and Sharma, 1989; Kiprop, 2001). The high variation in cultural and morphology characteristics of this pathogen could be due to environment conditions, the age of the isolates, subculturing, method of storage and culturing conditions. Wide variations in virulence (pathogenicity) to different genotypes of pigeonpea among *F. udum* isolates (Baldev and Amin, 1974; Shit and Sen Gupta, 1978; Gaur and Sharma, 1989; Kiprop, 2001) could be due to environment conditions and the inoculation techniques.

To overcome the problems associated with the standard methods such as cultural and morphological characterisation, and virulence/ race typing, attempts have been made to use the natural variation present in the DNA as a means for grouping fungal pathogens into species, forma specialis, races or pathotypes. The genetic markers that have been used to characterize phytopathogenic fungal species include vegetative compatibility groups (VCG), isozymes, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs). Among these markers, only isozyme technique has been used to determine genetic variation within *F. udum* (Shit and Sen Gupta, 1980; Okiror, 1986).

Vegetative compatibility groups (VCG) have been used frequently as a means of identifying isolates of a fungus that are closely related (Leslie, 1993). This trend has been accelerated by the development of faster methods of identifying compatible isolates using nitrate non-utilising *(nit)* mutants (Cove, 1976; Puhalla, 1985; Correll *et al.*, 1987). To be compatible isolates must show a common allele at each of the loci. Determination VCG may reflect genetic similarities, although not the degree of genetic differences, among isolates of the species (Kistler, 1997).

The AFLP technique is a recently developed molecular marker that makes use of the reliability of RFLP technique combined with the power of polymerase chain reaction (PCR) technique (Lin and Kuo, 1995; Vos *et al.*, 1995). It is a DNA fingerprinting technique that detects genomic restriction fragments and resembles in that respect to RFLP, with the major difference that PCR amplification instead of Southern hybridization is used for detecting fragments. It has been used to detect genetic variation between and within species of fungi (Majer *et al.*, 1996), and to group isolates of plant pathogenic fungi into haplotypes/ pathotypes such as *Colletotrichum lindemuthianum* (Gonzalez *et al.*, 1998) and *Pyrenopeziza brassicae* (Majer *et al.*, 1998).

In view of the economic importance of wilt as a constraint to increased pigeonpea yields and the fact that little has been reported on genetic variability of *F. udum*, the objectives of this study were to group single-spore isolates from Kenya by VCG and AFLP analysis, and to identify any relationship among these markers.

MATERIALS AND METHODS

Fungal isolates. Thirty-eight single-spore isolates of *F. udum* were obtained from the major pigeonpea growing districts in Kenya during a *Fusarium* wilt survey in 1997 (Table 1). The stem portions of plants showing symptoms of *Fusarium* wilt were collected from a total of 30 pigeonpea farms (sites) in 10 districts. Small pieces (0.5 cm^2) of vascular tissue were cut and placed aseptically onto plates having potato dextrose agar (PDA) medium. The plates were incubated at 25°C in a 12 hr light/dark cycle for 36-48 hr, and colonies showing growth and morphology typical of *F. udum* (Booth, 1978; Gerlach and Nirenberg, 1982) were transferred onto fresh PDA and incubated until conidia were produced. Conidial suspension from the cultures were prepared and streaked onto plates with tap water agar, and single germinating conidia were transferred to PDA after 24-36 hr and maintained as single-spore isolates.

Vegetative compatibility groups. Nitrate non-utilising mutants from each of the 38 single-spore isolates of *F. udum* were generated by a mycelial transfer from the edge of the colony of a single block of 5 mm^2 of 5 day-old cultures on PDA onto the centre of 9 cm (diameter) Petri plate having minimal agar medium with chlorate (MMC). This medium was based on minimal medium (MM) amended with 15 g l^{-1} $KClO_3$ and 1.6 g l^{-1} L-asparagine (Puhalla, 1985; Correl *et al.*, 1987). Ten MMC plates with single mycelial blocks were used per isolate. The plates were incubated at 25°C in a 12 hr dark/ light cycle and examined periodically for the appearance of fast-growing

TABLE 1. Sectors on MMC medium and *nit* mutants of 38 isolates of *F. udum*

Isolate	Site[1]	Sectors per colony	Total *nit* mutants	*Nit* mutant phenotypes			% VC[2]
				*nit*1	*nit*3	*Nit*M	
MK02	Makueni 2	2.1	8	6	2	0	100.0
MK03	Makueni 3	1.8	7	7	0	0	100.0
MK05	Makueni 5	2.4	10	6	4	0	75.0
MK07	Makueni 7	1.4	7	4	3	0	83.3
MS01	Machakos 13	2.0	5	5	0	0	100.0
MS03	Machakos 14	2.6	11	8	3	0	87.5
MS04	Machakos 14	2.6	11	4	7	0	50.0
MS05	Machakos 15	1.4	5	4	0	1	83.3
MS06	Machakos 16	1.3	3	3	0	0	100.0
MS07	Machakos 17	2.0	6	3	2	1	58.3
MS09	Machakos 17	1.4	2	2	0	0	100.0
MB01	Mbeere 24	2.1	6	3	0	3	75.0
MB03	Mbeere 26	1.9	5	3	2	0	100.0
MB04	Mbeere 27	1.7	4	4	0	0	100.0
MB05	Mbeere 27	1.9	4	4	0	0	100.0
MR02	Meru 34	1.4	3	3	0	0	100.0
MR03	Meru 35	2.7	6	6	0	0	100.0
MR04	Meru 37	2.1	9	8	1	0	62.5
NY01	Nyambene 40	1.3	5	5	0	0	100.0
NY02	Nyambene 41	1.4	3	0	3	0	75.0
NY03	Nyambene 41	1.4	2	2	0	0	100.0
NY04	Nyambene 42	2.3	5	3	2	0	87.5
NY07	Nyambene 44	1.3	7	5	2	0	100.0
NY08	Nyambene 44	1.4	2	2	0	0	100.0
TN04	Tharaka-Nithi 50	2.5	9	7	2	0	100.0
TN05	Tharaka-Nithi 51	2.9	15	12	3	0	75.0
TT05	Taita-Taveta 58	2.0	10	7	3	0	100.0
TT06	Taita-Taveta 59	1.4	4	4	0	0	100.0
TT08	Taita-Taveta 63	1.4	4	4	0	0	75.0
KR01	Kirinyaga 75	1.8	5	5	0	0	100.0
KR03	Kirinyaga 76	2.6	12	6	5	1	75.0
TK02	Thika 77	1.7	3	3	0	0	75.0
TK04	Thika 79	2.1	9	6	3	0	100.0
TK05	Thika 79	2.1	5	5	0	0	87.5
TK06	Thika 79	3.0	14	9	5	0	87.5
NB01	Nairobi 83	1.4	2	2	0	0	25.0
NB02	Nairobi 83	2.1	8	8	0	0	75.0
NB04	Nairobi 84	1.4	4	4	0	0	87.5
Total			240	182	52	6	
Mean		1.9	6.3	4.8	1.4	0.2	86.8
%			37.4	75.8	21.7	2.5	

[1]District with a field number

[2]Vegetative compatibility (%VC) among selected nit mutants of the isolate and four NitM testers

sectors (chlorate-resistant) from the initial colony. Transfers were made from the leading margin of any fast-growing sectors onto MM, each sector on a separate plate and examined after 3-7 days of incubation. Colonies that were having a thin expansive growth with no aerial mycelium on MM were considered *nit* mutants (Correll *et al.*, 1987). The *nit* mutants were identified as *nit*1, *nit*3 and NitM depending on their growth on nitrate, nitrite and hypoxanthine medium (Correll *et al.*, 1987).

To test for complementation or heterokaryon formation between *nit* mutants, a 5 mm^2 block of mycelium was transferred from MM to a fresh 9 cm Petri plate having MM with a NitM mutant in the centre of a daisy configuration and four *nit*1 and/or *nit*3 mutants from different isolates on the outer circle at 3 cm apart. One to three *nit*1 and/ or *nit*3 obtained from each isolate were used for complementation reaction. The plates were incubated as described above for up to 20 days. NitM mutants obtained from isolates MS05, MB01, KR03 and MS07 were used as testers for vegetative compatibility. Four types of scoring were made: wild-type growth/strong reaction (++) if a continuous line of robust aerial mycelia developed within 4-7 days where the thin expansive growth of the *nit* mutants converge; moderate or weak wild-type growth/moderate to weak reaction (+) if a continuous or broken line of aerial mycelia developed within 8-20 days; no wild-type growth/no reaction (-) after 20 days of incubation; and uncertain reaction (+-). If a line of robust growth, continuous or broken line, appeared when complementary *nit* mutants (NitM and *nit*1 or *nit*3) from different isolates were paired on minimal medium, then the isolates were of the same VCG. If a thin growth appeared at the intersection of colonies, then the isolates were of different VCG. Complementary and similar *nit* mutants from the same isolate were also paired. Complementation reaction between NitM testers and *nit* mutants of different isolates were repeated at least two times.

DNA extraction. The DNA was extracted from 38 single-spore isolates of *F. udum*. Czapek Dox salts liquid medium was used to culture the isolates and mycelia harvested after 7 days of incubation as described by Coddington and Gould (1992). Mycelial samples were frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. DNA extraction was done using 3-D-cetyltrimethylammonium bromide (CTAB) (Doyle and Doyle, 1990). After the digestion of RNA with 10 µl of 20 µg/µl RNase A (Roche Diagnostics Gmbh, Mannheim, Germany), the final DNA pellets were dissolved in 100 µl TE buffer and stored at -20°C. The DNA was quantified visually by running 3 µl of each sample on a 1% agarose gel along with undigested λ *Hind* III DNA marker (Roche Diagnostics Gmbh, Mannheim, Germany) of a known concentration (20 ng/µl) and making comparisons of their relative fluorescence in the presence of UV light and 0.1 µl/ml ethidium bromide solution.

Amplified fragment length polymorphism. AFLP assays were performed with AFLP Analysis System II of GibcoBRL (Life Technologies Inc., Merelbeke, Belgium) following the manufacturer's instructions and as described by Lin and Kuo (1995) and Vos *et al.* (1995). Genomic DNA was digested by restriction endonucleases, ligated to *Eco*R I and *Mse* I adapters, and amplified by PCR, using primers that contain the common sequences of the adapters and *Eco*R I + 2 and *Mse* I + 3 nucleotides as selective sequences.

The preamplification reaction, by 20 PCR cycles, was performed in a GeneAmp PCR system 9600 (Perkin-Elmer, Cetus, CT, USA). The *Eco*R I + 2 primers used in the selective AFLP amplification was radioactively labelled with [γ-^{32}P]ATP (Amersham International plc, Buckinghamshire, UK) by using T4 kinase. The primer combinations used were *Eco*R I + TA/*Mse* I + CAT, *Eco*R I + AA/*Mse* I + CAG, *Eco*R I + AG/*Mse* I + CAA, *Eco*R I + AG/*Mse* I + CAG, *Eco*R I + AC/*Mse* I + CAG, *Eco*R I + TG/*Mse* I + CAC, and *Eco*R I + AG/*Mse* I + CAC. The PCR amplification temperature profile was one cycle at 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s and then the annealing temperature was lowered during each cycle by 0.7°C for 12 cycles to give a touch down phase of 13 cycles. The remainder of the amplification was 23 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s.

The amplified fragments were added equal volume (20 µl) of formamide dye [98% (w/v) formamide, 10 mM EDTA, 0.1% (w/v)

bromophenol blue, 0.1% (w/v) xylene cyanol] and analysed on 6% denaturing polyacrylamide sequencing gel using Model SQ sequencing gel unit (Life Technologies Inc., Merelbeke, Belgium). A 100 bp molecular weight marker λ *Hind* III DNA labelled with [_γ^{32}P]ATP was loaded (3 µl) in the first and the last wells of the gel. Gels were run for about 150 minutes until the xylene cyanol (slower dye) was two-thirds down the length of the gel.

The gel was removed from the sequencing unit and fixed in 10% acetic acid for 20 min, with regular shaking at 5 min interval. Placing it in an oven at 80°C for 30 to 60 min dried the gel. Autoradiographs were obtained by exposing Kodak Biomax MR film (Kodak Scientific Imaging Film, Eastman Kodak Co., USA) to the dried gel in an exposure cassette for 15 to 17 hr at room temperature.

Data analyses. Bands observed by AFLP analysis were assigned a number in relation to their migration within the gel. Band with the highest molecular weight were assigned number 1 and so on in ascending order until the band of the lowest molecular weight was assigned. It was assumed that bands of the same molecular weight in different individuals were identical. For each individual, the presence or absence of each band was determined and designated 1, present or 0, absent in order to obtain binary banding data. Binary banding data from seven primer combinations were combined in order to determine the relationships of the genetic distances among the *F. udum* isolates. A pairwise distance matrix was generated with the PHYLIP (Phylogeny Inference Package) version 3.5c computer software (Felsenstein, University of Washington, Washington DC, USA) using Nei and Li distance index (Nei and Li, 1979): Dx = 1-2Nxy/(Nx + Ny), where Nxy is the number of bands shared between a pair of isolates, and Nx and Ny are the number of bands in isolate x and y, respectively. The genetic distance between the isolates in the combined matrix was subjected to cluster analysis by UPGMA method using NEIGHBOR programme (Vos *et al.*, 1995). A consensus tree was generated using the Majority-rule and Strict Consensus tree program. To determine the statistical significance of the dendrogram branches, the data were bootstrapped with 1,000 replications (Nei and Li, 1979).

RESULTS

Vegetative compatibility groups. The sectors of *F. udum* isolates that were produced on MMC per colony ranged from 1.2 to 3.0 with an overall mean of 1.9 (Table 1). The chlorate-resistant sectors that were unable to utilise nitrate as the sole source of nitrogen on MMC and consequently grew as thin expansive colonies with no aerial mycelium on MM were recovered at a mean frequency of between 0.14 and 0.52 sectors per colony with a mean of 0.33 sectors. These sectors were designated *nit* mutants and were 37.4% of the total chlorate-resistant sectors. The *nit* mutants that did not utilise nitrate but utilized nitrite and hypoxanthine on the respective media as the sole source of nitrogen were designated *nit*1, those that did not utilize nitrite designated *nit*3, and those that did not utilise hypoxanthine designated NitM. Two hundred and forty sectors were *nit* mutants, majority of which were *nit*1 with 182 *nit* mutants (75.8%) and a mean of 4.8 per isolate. Fifty-two *nit* mutants (21.7%) were *nit*3 with a mean of 1.4 per isolate while 6 *nit* mutants (2.5%) were NitM with a mean of 0.2 per isolate.

A total of 292 vegetative compatibility reactions between four NitM tester mutants and *nit*1 and/or *nit*3 mutants of different *F. udum* isolates were performed (Table 1). Vegetative compatibility reaction was a more robust wild-type with dense aerial mycelial growth between *nit*1 and NitM phenotypes of different isolates than between *nit*3 and NitM. Compatibility reaction between *nit*1 and *nit*3 mutants of different isolates was moderate to weak. All the isolates formed heterokaryons with at least one tester and all could be linked to the other testers. Thirty-one isolates (81.6%) formed heterokaryons with all the four NitM testers while 7 isolates (18.4%) formed heterokaryons with one, two or three NitM testers. Isolates MK05, MB01, MR04, TN05 and TK02, for example, did not develop heterokaryon with NitM tester from isolate MS07 but formed wild-type mycelial growth with the remaining three NitM testers. The above five isolates could be

linked to NitM tester of MS07 by 31 isolates that formed heterokaryons with all the testers. It was with this criterion that 38 single-spore isolates of *F. udum* from Kenya were grouped into a single vegetative compatibility group, VCG 1. However, 36 (94.7%) isolates formed strong reactions with at least one NitM while two (5.3%) isolates namely NB01 and NB02 formed either moderate/weak, uncertain or no reaction with NitMs. This resulted in the subdivision of VCG 1 into two subgroups, namely VCG 1 I with 36 isolates and VCG 1 II with two isolates. There was no correlation between VCG 1 subgroups with geographical origin of the isolates.

Amplified fragment length polymorphism. A total of 318 bands were amplified from seven primer combinations with 102 being polymorphic (Table 2). Polymorphism was 32% with an average of 15 polymorphic bands per primer combination. Polymorphism was highest among isolates with primer combination *Eco*R I (E)+TA/*Mse* I (M)+CAT, followed by E+TG/M+CAC and E+AG/M+CAA.

Analysis of the binary banding data from all the seven primer combinations showed that the 38 isolates of *F. udum* from Kenya were genetically related and could belong to one AFLP group (Fig. 1). This is due to the presence of a boostrap value of >50% confidence interval at the second (53%) and third (54%) forks of the dendrogram. However, more than 10 subgroups exist within the single AFLP group, although majority are not genetically distinct (<50% confidence interval). Exceptions for these subgroups are groups of isolates MR03, TK06, MB05, MS05 and MR02, and isolates MB03 and NY07.

Correlation between the geographical origin (district) of the isolates and AFLP subgroups was not observed. The single AFLP group comprised of isolates of *F. udum* from a single VCG. There was no clear relationship between AFLP subgroups and VCG subgroups. The isolates that belong to VCG 1 II (NB01 and NB02) were at different AFLP subgroups.

TABLE 2. Primers, amplified bands and polymorphic bands obtained by AFLP analysis of 38 isolates of *F. udum*

Primer combination (*Eco*R I/*Mse* I)	Amplified bands	Polymorphic bands	Polymorphism (%)
TA/CAT	30	19	63
AA/CAG	69	17	25
AG/CAA	46	17	37
AG/CAG	44	8	18
AC/CAG	52	12	23
TG/CAC	50	22	44
AG/CAC	27	7	26
Total	318	102	32

DISCUSSION

The chlorate-resistant sectors recovered from MMC that were *nit* mutants represented 37% of the total sectors. The recovery of these sectors differed considerably between the isolates with a mean frequency of 0.14 and 0.52 sectors per colony as have been observed on other *Fusarium* species elsewhere (Clark *et al.*, 1995; Correll *et al.*, 1987; Sunder and Satyavir, 1998). These findings indicate that the sectoring of individual isolates of a particular fungal species differs and hence the number of chlorate-resistant sectors recovered, which may be due to the type of fungal species, type of medium and culturing conditions. All the *nit*mutants recovered from *F. udum* could be divided into three distinct phenotypic classes: *nit*1, *nit*3 and NitM. The majority of the *nit* mutants recovered on MMC were *nit*1 mutants (75.8%), followed by *nit*3 mutants (21.7%), and the least were NitM mutants (2.5%). This observation correlates with the findings of Correll *et al.* (1987) when generating *nit* mutants from *F. oxysporum*. However, Clark *et al.* (1995) generated more NitM than *nit*3 from *F. lateritium* after increasing percentage of $KClO_3$ in the media to 3.0, 4.5 and 6.0%. Most other findings on the generation of chlorate-resistant sectors have used 1.5% $KClO_3$ as an amendment to potato dextrose agar or minimum medium (Sunder and Satyavir, 1998; Katan *et al.*, 1991; Puhalla, 1985). Varying the amount of chlorate in the medium therefore could determine the percentage of NitM and *nit*3 mutants generated.

Thus, the *F. udum* isolates from Kenya were grouped into a single vegetative compatibility group, VCG 1, but with two subgroups due to differential reactions of mutants. Subgroup VCG 1 I comprised 36 isolates while VCG 1 II comprised

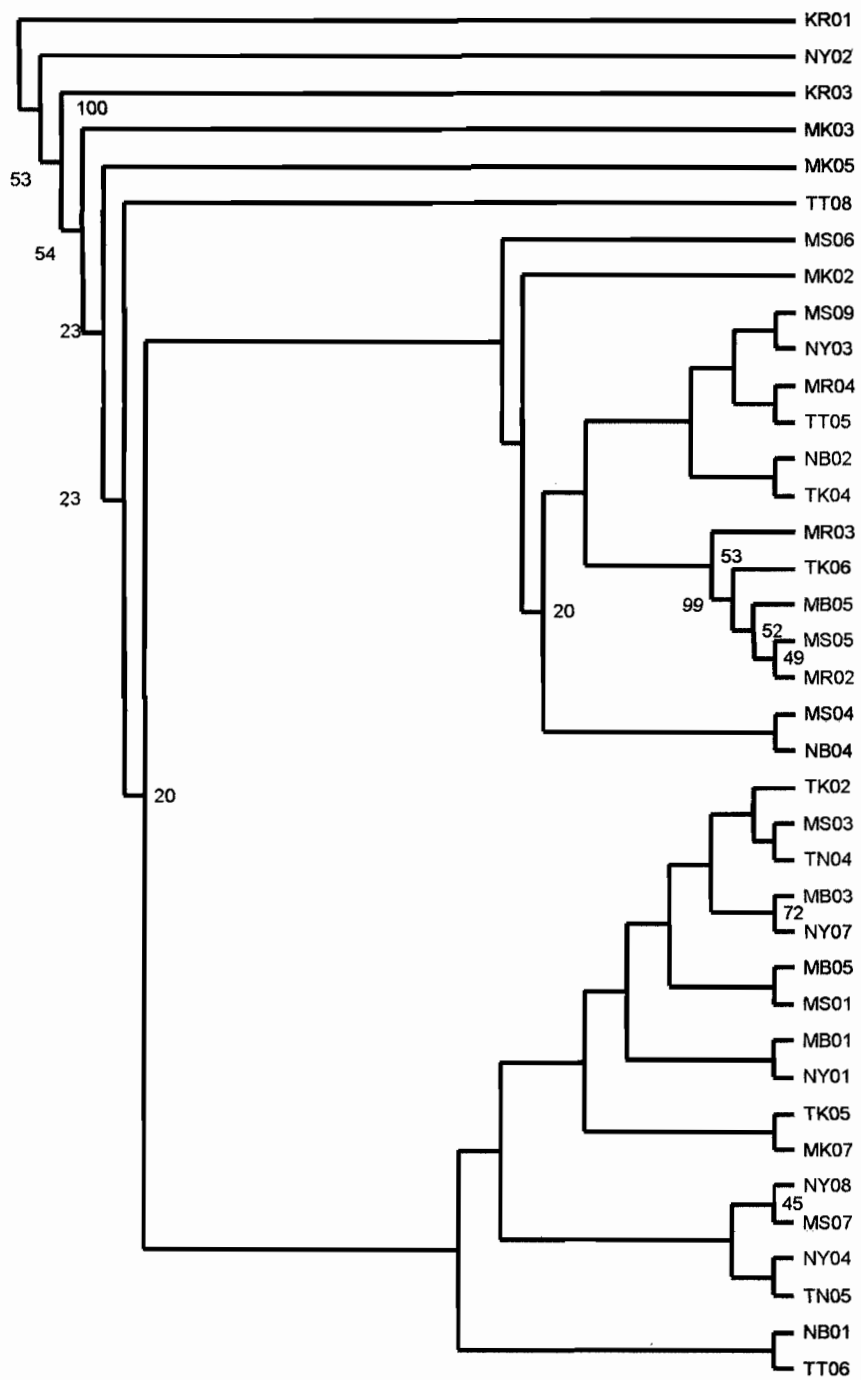

Figure 1. Dendrogram generated from seven primer combinations in AFLP analysis showing genetic relationships among 38 isolates of *F. udum* obtained by the strict consensus tree using UPGMA method in the PHYLIP 3.5c program. The numbers on the branches represent the confidence intervals generated by bootstrapping with 1,000 replications.

2 isolates. Populations of other *Fusaria* with a single VCG include *F. oxysporum* f.sp. *dianthi* (Katan *et al.*, 1989) and *F. oxysporum* f.sp. *canariensis* (Plyler *et al.*, 2000), while those with more than one VCG include *F. oxysporum* f.sp. *radicis-lycopersici* (Katan and Katan, 1999) and *F. moniliforme* (Sunder and Satyavir, 1998). More than one subgroup has been observed in three VCGs of *F. oxysporum* f.sp. *radicis-lycopersici* (Katan and Katan, 1999). On the basis of vegetative compatibility, pathogenic isolates of *F. udum* from Kenya could be derived from a single lineage.

Using seven primer combinations with *Eco*R I (E) + 2 and *Mse* I (M) + 3 selective nucleotides at the 3'-end of the primers on 38 isolates, a total of 318 bands were amplified with 102 polymorphic bands. However, the number of amplified bands and polymorphism obtained from individual primer combinations varied, with primers having more Ts and As showing high polymorphism and generally more bands amplified than primers having more Gs and Cs. An exception was observed with primer combination E + TA/M + CAT which showed the highest polymorphism (63%) but with second lowest number of amplified bands (30 bands). Janssen *et al.* (1996) has showed that the choice of the restriction enzymes (endonucleases), and the length and composition of the selective nucleotide determines the complexity of the final AFLP fingerprint. The present findings are consistent with the work of Majer *et al.* (1996) who also showed that the number of polymorphisms varied with primer combination in AFLP analysis of pathogenic fungi *Cladosporium fulvum* and *Pyrenopeziza brassicae* isolates. They however, used E + 2 and M + 2 nucleotides. Gonzalez *et al.* (1998) have also used 2 instead of 3 selective nucleotides (E + 2/M + 2) in order to generate adequate number of bands for AFLP analysis of *Colletotricum lindemuthianum* isolates. Primer selectivity is good for primers with 1 or 2 selective nucleotides in simple genomes such as fungi, bacteria and some plants, although selectivity is still acceptable with primers having 3 selective nucleotides, but it is lost with the addition of the fourth nucleotide (Vos *et al.*, 1995).

Statistical analysis of AFLP data indicates that *F. udum* isolates from Kenya are genetically related and could consist of one AFLP group. However, the isolates comprising MR03, TK06, MB05, MS05 and MR02, and MB03 and NY03 appeared as significantly distant subgroups. The fungus *F. udum* is a deuteromycete and therefore natural populations of the pathogen may consist of clonal lineages produced by asexual reproduction. The correlation between AFLP of the isolates and their geographical origin could not be determined, although Gonzalez *et al.* (1998) has identified smaller subgroups of two major AFLP groups that were associated with the geographical location from which isolates of *C. lindemuthianum* were obtained in Mexico. The single AFLP group consisted of one VCG, although there was no correlation among their subgroups.

Based on VCG and AFLP, pathogenic isolates of *F. udum* from Kenya appear to originate from a single lineage. There is genetic variability in *F. udum*, as indicated by the presence of VCG and AFLP subgroups. The genetic variability that exists in *F. udum* should be taken into consideration in pigeonpea improvement programmes aimed at breeding for resistance against *Fusarium* wilt disease. However, further investigations should utilize more isolates of *F. udum*, pathogenic and non-pathogenic, from diverse geographical origin (within and between countries) in VCG and AFLP analysis in order to fully understand the genetic variability within this fungus.

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