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Cultural characteristics, pathogenicity and vegetative compatibility of *Fusarium udum* isolates from pigeonpea (*Cajanus cajan* (L.) Millsp.) in Kenya

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Abstract

Seventy-nine single-spore isolates of *Fusarium udum*, the causal agent of wilt disease of pigeonpea, from Kenya, India and Malawi were characterized according to their cultural characteristics, pathogenicity and vegetative compatibility group (VCG). The isolates exhibited high variation in pathogenicity on a wilt-susceptible pigeonpea variety, and in mycelial growth and sporulation on potato dextrose agar medium. The 79 isolates were categorized into two virulence groups, two groups of radial mycelial growth and four groups of sporulation. Radial mycelial growth showed a moderate negative correlation ($r = -0.40$; $P = 0.01$) with sporulation. However, mycelial growth and sporulation had no correlation with virulence. Pairings between complementary nitrate non-utilizing (*nit*) mutants of *F. udum* generated on chlorate containing minimal medium revealed that all the isolates belonged to a single VCG (VCG 1) with two subgroups, VCG 1 I and VCG 1 II. Vegetative compatibility was independent of cultural characteristics and pathogenicity. This is the first report of vegetative compatibility in *F. udum*.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most widely-grown and eaten grain legumes in the semi-arid tropics of the world. Wilt caused by *Fusarium udum* Butler is the most important disease of this crop (Kannaiyan et al., 1984). The disease is widely distributed in pigeonpea growing districts in Kenya. Comparisons of single-spore isolates of *F. udum* from the same or different geographical origin have shown that the fungus is highly variable in cultural characteristics (Reddy and Chaudhary, 1985; Gaur and Sharma, 1989) and pathogenicity (Shit and Sen Gupta, 1978; Gupta et al., 1988). However, studies on genetic diversity using isozyme markers revealed low variation in *F. udum* (Shit and Sen Gupta, 1980; Okiror, 1986). Vegetative compatibility grouping (VCG) has been used frequently as a means of identifying isolates of a fungus that are closely related (Leslie, 1993). This methodology has been applied to a long and growing list of Fusaria, but not to *F. udum*. In some cases, VCG has a correlation with pathogenicity, while in others no relationship was observed (Leslie, 1993; Clark et al., 1995). Thus, vegetative compatibility studies on *F. udum* help in understanding the genetic variability within this fungus resulting in an improvement in wilt resistance pigeonpea breeding programmes. In view of the economic importance of wilt and the fact that little has been reported on genetic variability of *F. udum*, the objectives of this study were to group the isolates from Kenya by VCGs, cultural characteristics and pathogenicity, and to determine if a relationship exists between these parameters.

Materials and methods

Fungal isolates

Seventy-five isolates of *F. udum* were obtained from wilt-infected pigeonpea plants collected from 55 sites in 12 districts of Kenya in 1997. All the isolates were purified on tap water agar and established as single-spore isolates. Two single-spore isolates were obtained from each strain of *F. udum* from Malawi (IMI number 275452) and India (IMI number 205514), both strains received from the International Mycological Institute, UK. All these isolates were maintained on Spezieller Nahrastofarmer agar (Nirenberg, 1976) at −20 °C. The medium consisted of 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose and 20 g agar per litre.

Pathogenicity tests

The conidial suspensions of the 79 single-spore isolates of *F. udum* were prepared in 100 ml sterile distilled water (SDW) from 7-day-old cultures on PDA. The conidial suspension was filtered through two layers of cheesecloth and the concentration was adjusted to 10^6 conidia ml^{-1}. Seven-day-old pigeonpea seedlings of wilt susceptible variety KAT 60/8, grown on sterile riverbed sand, were uprooted carefully, the roots washed with SDW and trimmed to about 4 cm from the collar region using a sterile scalpel. The seedlings were dipped in inoculum suspension for 30 min and transplanted in 20 cm diam plastic pots having a sterile mixture of red soil (Vertisol) and riverbed sand (3 : 1 v/v). Five inoculated seedlings were planted per pot with four replications for each isolate. Control seedlings were dipped in SDW. The plants were kept in a greenhouse having a maximum temperature between 23 and 30 °C with 12 h natural light per day at the University of Nairobi (Kenya). The plants were checked daily for the appearance of wilt symptoms. The records on percentage of wilted seedlings were taken 6 weeks after inoculation (Reddy and Raju, 1997). The data were analysed by ANOVA using SAS system software (SAS Institute Inc., Cary, NC, USA). The experiment was repeated once.

Cultural characteristics

All the isolates were grown on PDA plates in triplicate for 8 days at 25 °C in a 12 h light/dark cycle to determine the radial mycelial growth (colony diameter) and sporulation (conidia ml^{-1}). The colony diameter of each culture in the plate was measured using a vernier calliper by taking an average of four radial measurements at random. To estimate sporulation, conidial suspension was prepared from each culture plate using a modification of Calpouzos and Stallknecht (1965). Two discs of 6 mm diam each were cut from the opposite sides of the centre of the colony, but 1.5 cm from the middle point, and suspended in a universal bottle with 10 ml of SDW. This was put on a rotary shaker at 250 rpm for 20 min, the spore suspension then filtered through two layers of sterile cheesecloth and the conidial concentration determined by using a haemocytometer. A mean of four counts was obtained per colony. The data were analysed by ANOVA using SAS system software (SAS Institute Inc., Cary, NC, USA). The experiment was repeated once.

Vegetative compatibility

To generate nitrate non-utilizing mutants a 5 mm^2 mycelial block of each isolate from 5-day-old cultures on PDA was transferred to the centre of a 9 cm diam Petri dish containing nitrate minimal medium with chlorate (MMC). This medium consisted of minimum medium (MM) (per litre: 30 g sucrose, 2 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.2 ml trace element solution (5 g citric acid, 5 g $ZnSO_4{\cdot}7H_2O$, 1 g $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, 0.25 g $CuSO_4{\cdot}5H_2O$, 0.05 g $MnSO_4{\cdot}H_2O$, 0.05 g H_3BO_4, 0.05 g $NaMoO_4{\cdot}2H_2O$, and 95 ml distilled water) and 20 g agar) amended with 15 g $KClO_3$ and 1.6 g L-asparagine. Ten MMC plates with a single mycelial block were used per isolate. The plates were incubated at 25 °C in a 12 h dark/light cycle and examined periodically for the appearance of fast-growing sectors (chlorate-resistant) from the initial colony. Transfers were made from the leading margin of fast-growing sectors onto MM. Colonies having a thin expansive growth with no aerial mycelium on MM were considered *nit* mutants. 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 m^2 block of mycelium was transferred from MM to a fresh 9 cm Petri dish containing MM with a NitM mutant in the centre of a daisy configuration and four *nit*1and/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 7 days but mutants that exhibited moderate to weak reactions were incubated for up to 20 days (Katan et al., 1991; Katan and Katan, 1999). NitM mutants obtained from isolates MS05, IND01a, MB01, KR03, TT04, MR06, MS07 and TK03 were used as testers in pairings with *nit*1 and/or *nit*3 mutants of all isolates for vegetative compatibility tests. Four types of scoring were made: strong reaction (++) if wild-type growth developed within 4–7 days where the thin expansive growth of the *nit* mutants converge, moderate or weak reaction (+) if wild-type growth developed within 8–20 days, no reaction (−), and uncertain reaction (+−) (Katan et al., 1991; Katan and Katan, 1999). If a wild-type growth appeared at the intersection of colonies on pairing of complementary *nit* mutants (NitM and *nit*1 or *nit*3) from different isolates on minimal medium, then the isolates were of the same VCG and if it remained thin, then the isolates were of different VCG. Complementary and similar *nit* mutants from the same isolate were also paired. Complementation reactions between NitM and *nit*1 and/or *nit*3 mutants of different isolates were repeated at least two times.

Results

Pathogenicity tests

All 79 isolates of *F. udum* were pathogenic to the wilt-susceptible pigeonpea variety KAT 60/8, although they showed high significant ($P = 0.01$) variation in virulence (Table 1). Fusarium wilt symptoms on pigeonpea plants were observed from the eighth day of inoculation, and the fungus re-isolated from the stems of infected plants on the 14th day. The wilt incidence on infected plants ranged from 35% to 100%. The isolates were classified into two groups; those causing up to 60% wilt were moderately virulent and those causing more than 60% wilt were highly virulent. The number of isolates in the former group was 17 (21.5%) and in the latter group was 62 (78.5%). The highly virulent group was widely distributed in all the districts. The districts with a high proportion of the moderately virulent group were Meru with 57% and Thika with 50% of isolates in this group. Some isolates from the same group collected from the same site showed significant variation in pathogenicity.

Cultural characteristics

The *F. udum* isolates exhibited high variation in radial mycelial growth and sporulation on PDA. There were significant differences ($P = 0.01$) among the isolates in terms of radial mycelial growth and sporulation (Table 1). Based on inter-quartile ranges and one-sample *t*-tests, the 79 single-spore isolates were classified into different groups using radial mycelial growth and sporulation. There were 61 (77.2%) isolates with fast radial mycelial growth and 18 (22.8%) isolates with slow radial mycelial growth (over 79.0 mm and below 79.0 mm colony diameter, respectively). Eight (10.1%) isolates had very high sporulation, 23 (29.1%) isolates high sporulation, 28 (35.4%) isolates moderate sporulation and 20 (25.3%) isolates low sporulation, with over 15.9×10^5, 8.0–15.9×10^5, 5.0–7.9×10^5 and below 5.0×10^5 conidia ml^{-1}, respectively. The radial mycelial growth of isolates was highly variable in most districts with the exception of Thika, Meru and Kirinyaga districts, and Malawi having only isolates with fast growth. The districts that had predominantly high to very high sporulation groups were Machakos and Mbeere, while those with predominantly low to moderate sporulation groups were Meru, Tharaka Nithi, Thika and Nairobi. Some isolates collected from the same site were significantly different ($P = 0.01$) with respect to radial mycelial growth and/or sporulation. For example, isolates that showed significant differences in radial mycelial growth and sporulation from the same site were TN01 and TN02, NY07 and NY08, MB02 and MB03, and TT04 and TT05.

Generally, isolates with fast radial mycelial growth had low to high sporulation while those with slow radial mycelial growth had moderate to very high sporulation. The correlation coefficient between radial mycelial growth and sporulation was negative and moderate ($r = -0.40$; $P = 0.01$). Pathogenicity was not correlated with mycelial growth ($r = -0.05$, $P = 0.67$) and sporulation ($r = 0.11$; $P = 0.33$) and was statistically non-significant ($P = 0.05$).

Vegetative compatibility

1465 chlorate-resistant sectors were produced on MMC from 79 single-spore isolates of *F. udum* with a mean of 18.5 sectors per isolate (Table 1). The isolates differed considerably in their sectoring frequency with a range of 1.2 (IND01a) to 3.0 (TK06) sectors per colony. The majority of the sectors appeared in the

Table 1. Pathogenicity, radial mycelial growth and sporulation of *Fusarium udum* isolates from pigeonpea, and characterization of their chlorate-resistant mutants

Isolate	Site[a]	Wilt[b] (%)	Colony diameter (mm)	Spores (conidia $ml^{-1} \times 10^5$)	Chlorate-resistant sectors	*nit* mutants	*nit* mutant phenotypes		
							*nit*1	*nit*3	NitM
MK01	Makueni 1	75	81.3	9.4	18	7	7	0	0
MK02	Makueni 2	90	81.9	13.4	21	8	6	2	0
MK03	Makueni 3	75	81.2	9.6	18	7	7	0	0
MK04	Makueni 4	85	78.8	7.1	25	7	6	1	0
MK05	Makueni 5	80	80.7	11.0	24	10	6	4	0
MK06	Makueni 6	55	80.4	11.5	18	7	7	0	0
MK07	Makueni 7	75	80.2	5.8	14	7	4	3	0
MK08	Makueni 11	40	77.3	17.9	13	2	2	0	0
MK09	Makueni 11	60	77.8	11.2	18	7	6	1	0
MK10	Makueni 12	80	84.2	6.5	21	7	5	2	0
MS01	Machakos 13	90	80.8	11.2	20	5	5	0	0
MS02	Machakos 13	65	82.0	3.8	18	5	5	0	0
MS03	Machakos 14	75	80.8	7.3	26	11	8	3	0
MS04	Machakos 14	85	80.8	15.3	26	11	4	7	0
MS05	Machakos 15	85	77.3	5.7	14	5	4	0	1
MS06	Machakos 16	65	79.8	8.9	13	3	3	0	0
MS07	Machakos 17	65	77.5	17.7	20	6	3	2	1
MS08	Machakos 17	55	79.5	9.2	19	8	4	4	0
MS09	Machakos 17	55	79.5	2.7	14	2	2	0	0
MS10	Machakos 18	85	81.4	3.6	18	4	4	0	0
KT01	Kitui 19	95	80.2	6.4	27	18	12	6	0
KT02	Kitui 19	90	78.7	24.6	23	10	10	0	0
KT03	Kitui 20	65	82.9	6.2	18	6	6	0	0
KT04	Kitui 21	70	76.7	8.9	24	10	8	2	0
KT05	Kitui 23	80	83.3	3.8	14	3	2	1	0
MB01	Mbeere 24	55	82.6	5.0	21	6	3	0	3
MB02	Mbeere 25a	80	84.5	8.6	18	4	4	0	0
MB03	Mbeere 26b	90	78.9	5.6	19	5	3	2	0
MB04	Mbeere 27	85	78.3	11.1	17	4	4	0	0
MB05	Mbeere 27	95	72.7	12.8	19	4	4	0	0
MB06	Mbeere 28	70	81.9	11.0	19	13	9	4	0
MB07	Mbeere 28	85	80.9	11.1	17	7	7	0	0
MR01	Meru 33	100	84.1	7.1	21	7	4	3	0
MR02	Meru 34	55	82.6	6.7	14	3	3	0	0
MR03	Meru 35	70	83.9	4.8	27	6	6	0	0
MR04	Meru 37	65	79.5	6.9	21	9	8	1	0
MR05	Meru 38	55	83.0	5.2	13	2	2	0	0
MR06	Meru 38	55	84.3	6.5	17	8	4	2	2
MR07	Meru 39	55	84.9	9.8	18	5	5	0	0
NY01	Nyambene 40	85	84.2	4.0	13	5	5	0	0
NY02	Nyambene 41	95	84.3	4.8	14	3	0	3	0
NY03	Nyambene 41	75	85.7	6.3	14	2	2	0	0
NY04	Nyambene 42	70	82.7	11.9	23	5	3	2	0
NY05	Nyambene 43	70	79.6	6.1	17	6	6	0	0
NY06	Nyambene 43	70	81.5	7.8	18	9	7	2	0
NY07	Nyambene 44	100	78.4	21.1	13	7	5	2	0
NY08	Nyambene 44	100	81.6	5.8	14	2	2	0	0
TN01	T-Nithi 47	65	80.2	9.4	17	3	3	0	0
TN02	T-Nithi 47	70	75.1	5.8	13	3	3	0	0
TN03	T-Nithi 48	90	81.5	3.6	26	10	7	3	0
TN04	T-Nithi 50	95	85.4	4.0	25	9	7	2	0
TN05	T-Nithi 51	55	76.6	3.5	29	15	12	3	0

Table 1. Continued

Isolate	Site[a]	Wilt[b] (%)	Colony diameter (mm)	Spores (conidia $ml^{-1} \times 10^5$)	Chlorate-resistant sectors	*nit* mutants	*nit* mutant phenotypes		
							*nit*1	*nit*3	NitM
TT01	T-Taveta 55	100	78.6	16.9	17	6	5	1	0
TT02	T-Taveta 56	60	81.5	6.0	18	9	7	2	0
TT03	T-Taveta 57	75	86.3	9.0	18	4	4	0	0
TT04	T-Taveta 58	90	80.3	8.2	18	5	4	0	1
TT05	T-Taveta 58	100	81.8	4.4	20	10	7	3	0
TT06	T-Taveta 59	100	82.5	17.5	14	4	4	0	0
TT07	T-Taveta 62	85	81.2	6.2	17	5	5	0	0
TT08	T-Taveta 63	80	71.4	21.4	14	4	4	0	0
TT09	T-Taveta 64	85	82.0	4.0	19	6	6	0	0
ML01	Malindi 70	75	83.5	4.8	12	4	4	0	0
KR01	Kirinyaga 75	60	83.5	17.1	18	5	5	0	0
KR02	Kirinyaga 76	65	84.6	1.4	14	5	5	0	0
KR03	Kirinyaga 76	55	81.9	3.8	26	12	6	5	1
TK01	Thika 77	80	88.8	5.0	18	4	4	0	0
TK02	Thika 77	65	87.7	4.6	17	3	3	0	0
TK03	Thika 78	90	88.5	3.1	21	9	6	2	1
TK04	Thika 79	45	85.4	3.8	21	9	6	3	0
TK05	Thika 79	35	84.3	8.8	21	5	5	0	0
TK06	Thika 79	55	85.7	6.1	30	14	9	5	0
NB01	Nairobi 83	75	83.9	4.8	14	2	2	0	0
NB02	Nairobi 83	90	82.6	4.4	21	8	8	0	0
NB03	Nairobi 84	80	81.2	6.8	17	4	4	0	0
NB04	Nairobi 84	75	73.6	7.6	14	4	4	0	0
MAL01a	Malawi	80	80.1	5.1	17	5	5	0	0
MAL01b	Malawi	90	80.4	5.4	23	5	2	3	0
IND01a	India	70	73.6	7.5	12	8	6	0	2
IND01b	India	65	73.3	8.8	13	8	5	1	2
Total					1465	505	399	92	14
Mean		74.9	81.2	8.2	18.5	6.4	5.1	1.2	0.2
L.S.D. ($P = 0.05$)		16.4	1.0	0.3					

[a]District in Kenya or country with the field number. T-Nithi = Tharaka Nithi, T-Taveta = Taita. Strain from India was collected at Hyderabad, and Malawi strain from unknown site.
[b]Pathogenicity on Fusarium wilt susceptible pigeonpea variety (KAT 60/8).

first and second week of incubation, and a few in the third week (Figure 1). The chlorate-resistant sectors that were unable to utilize nitrate as the sole source of nitrogen and consequently grew as thin expansive colonies with no aerial mycelium on MM were recovered at a mean frequency of between 0.14 and 0.68 sectors per colony. These sectors were designated *nit* mutants and were 34.5% of the total chlorate-resistant sectors. The *nit* mutants that did not utilize 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 utilize hypoxanthine designated NitM. All *nit* mutants produced wild-type growth on PDA medium (Figure 2). Five hundred and five sectors were *nit* mutants, of which 79.0%, 18.2% and 2.8% were *nit*1, *nit*3 and NitM, respectively.

Vegetative compatibility reaction was more robust in the 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. A total of 1248 vegetative compatibility reactions between eight NitM mutants and *nit*1 and/or *nit*3 mutants of different *F. udum* isolates were performed with varying degrees of heterokaryon formation. All the isolates formed strong or moderate/weak heterokaryons with at least one NitM and could be linked to other NitMs (Figure 3). Forty-nine (62%) isolates formed strong or moderate/weak

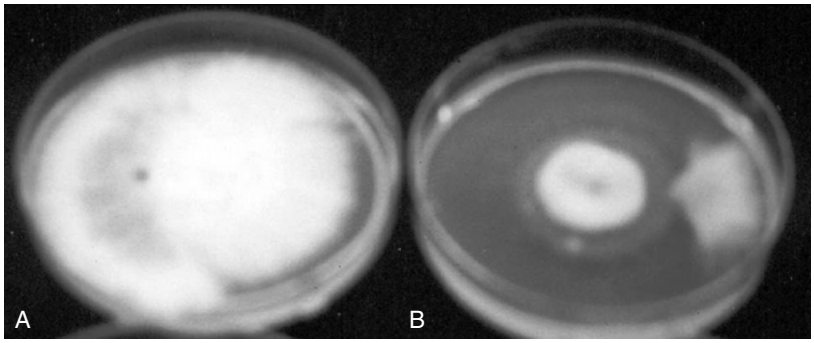

Figure 1. Formation of chlorate-resistant sectors by isolates MK10 (plate A) and MK07 (plate B) of *F. udum* three weeks after incubation on nitrate minimal medium amended with chlorate.

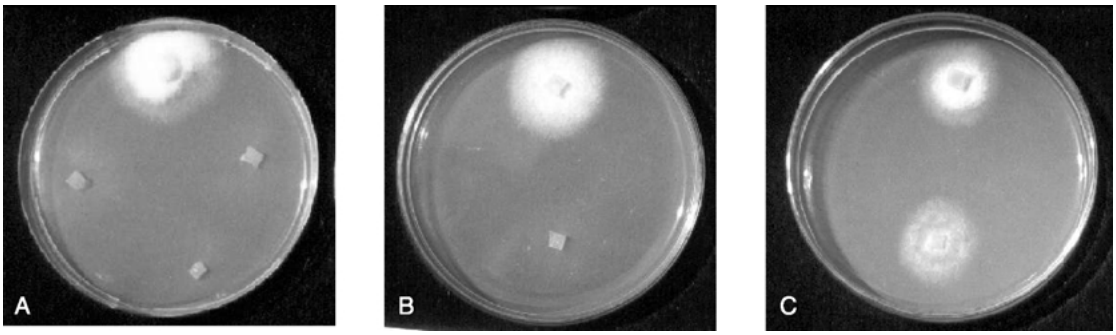

Figure 2. Plate A shows wild-type growth of parental strain (top block), and thin growths of mutants NitM (right block), *nit*1 (bottom block) and *nit*3 (left block) of isolate TK03 on nitrate minimal medium; plate B shows wild-type growth of *nit*1 (top block) and thin growth of NitM (bottom block) of isolate MS05 on hypoxanthine medium; and plate C shows wild-type growths of *nit*1 (top block) and NitM (bottom block) of isolate MS05 5 days after incubation on nitrite medium.

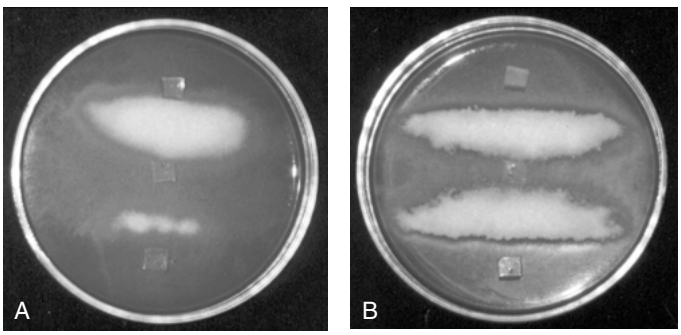

Figure 3. Formation of complementary heterokaryons in pairings between *nit* mutants of *F. udum* isolates 12 days after inoculation on nitrate minimal medium: Plate A shows strong reaction of NitM from isolate MS05 (centre block) with *nit*1 from isolate MB06 (upper block) and a moderate/weak reaction with *nit*1 from isolate TN01 (lower block), and plate B shows strong reactions of NitM from isolate IND01a (centre block) with *nit*1 from isolate KT02 (upper block) and *nit*1 from isolate MAL01b (lower block).

heterokaryons with all the eight NitM. Isolate MS04 did not develop heterokaryons with NitMs from isolates KR03, TT04 and TK03 but it formed wild-type mycelial growth with the remaining five NitM. Isolates MS05, MS10, MR02, TT05, MAL01a, for example, developed wild-type mycelial growth with the eight NitM. Therefore, isolate MS04 could be linked to NitM of KR03, TT04 and TK03 by the above isolates. Isolate MR05 formed moderate/weak heterokaryons with only NitM of IND01a and TK03 but could be linked to the other six NitM through isolates MR06 or NY07. Using a similar criterion, the 30 isolates that did not develop heterokaryons with all NitM were linked to 49 isolates that showed wild-type mycelial growth with all NitM. It was using this criterion that the *F. udum* single-spore isolates from Kenya, and one strain each from Malawi and India were grouped into one vegetative compatibility group, VCG 1. However, 73 (92.4%) isolates formed strong reactions with at least one NitM while six (7.6%) isolates namely MR05, TN01, TN02, TN03, NB01 and NB02 formed either moderate/weak, uncertain or no reactions with NitMs. This enabled the subdivision of VCG 1 into two subgroups, namely VCG 1 I with 73 isolates and VCG 1 II with six isolates. The isolates of *F. udum* in subgroups VCG 1 I and II were highly variable with respect to cultural characteristics (radial mycelial growth and sporulation) and pathogenicity.

Discussion

Variations in pathogenicity of *F. udum* isolates enabled their classification into two virulence groups. Both virulence groups were found in eight districts while only the highly virulent group was found in five districts indicating that virulence may vary depending on the geographical origin of the isolates. Isolates with moderate virulence were dominant in Meru and Thika districts. Variability in pathogenicity of *F. udum* isolates from different geographical origins has been observed elsewhere (Shit and Sen Gupta, 1978; Okiror, 1986; Gupta et al., 1988; Gaur and Sharma, 1989).

Single-spore isolates of *F. udum* showed variation in their cultural characteristics. In the present study, *F. udum* was classified into two groups by radial mycelial growth and four groups by sporulation on PDA medium. Other workers have also indicated considerable variability in *F. udum* with respect to these characteristics (Shit and Sen Gupta, 1978; Okiror, 1986; Gupta et al., 1988; Gaur and Sharma, 1989). It was found in this study that, on average, isolates with slow growth had higher sporulation while those with fast growth had lower sporulation agreeing with earlier findings (Gupta et al., 1988). Pathogenicity was not correlated with sporulation and radial mycelial growth, which also supports earlier observations (Okiror, 1986; Gaur and Sharma, 1989).

Isolates of *F. udum* differed considerably in their sectoring frequency on chlorate medium with a mean of 0.14 and 0.68 sectors per colony. Earlier reports have also revealed a wide variation in sectoring frequency in isolates of other species of *Fusarium* (Correll et al., 1987; Clark et al., 1995; Sunder and Satyavir, 1998). These findings indicate that the sectoring of individual isolates of a particular fungal species differs and hence also the number of chlorate-resistant sectors recovered. Although the recovery frequency of chlorate-resistant sectors in the present study was lower than that recovered from other *Fusarium* species, the differences could be due to the type of fungal species, type of medium used for sectoring 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 (79%), followed by *nit*3 mutants (18%), and NitM mutants (3%). Correll et al. (1987) recovered *nit* mutants of *F. oxysporum* at frequencies of 59–66% for *nit*1, 10–28% for *nit*3 and 10–25% for NitM on MMC. Clark et al. (1995) recovered *nit* mutants of *F. lateritium* at frequencies of 77.2% for *nit*1, 7.4% for *nit*3 and 15.4% for NitM on KPS, and 77.7% for *nit*1, 9.7% for *nit*3 and 12.6% for NitM on KMM. These findings indicate that the majority of *nit* mutants recovered in fungal populations are usually *nit*1 while *nit*3 and NitM are in the minority.

In the present study, isolates of *F. udum* from Kenya, Malawi and India comprised one vegetative compatibility group, VCG 1. However, VCG 1 could be divided into two subgroups due to differential reactions of mutants. Subgroup VCG 1 I constituted 73 isolates with at least one strong reaction with NitM while subgroup VCG 1 II constituted six isolates with either moderate/weak, uncertain or no reaction with NitM. Populations of other fusaria like *F. oxysporum* f.sp. *canariensis* (Plyler et al., 2000), *F. oxysporum* f.sp. *vasinfectum* (Katan and Katan, 1988), *F. oxysporum* f.sp. *dianthi* (Katan et al., 1989) have also been reported to be comprised single VCG, while those of *F. oxysporum* f.sp. *radicis-lycopersici* (Katan and Katan, 1999) and *F. moniliforme* (Sunder

and Satyavir, 1998) represented eight and 10 VCGs, respectively. More than one subgroup in VCG 0090, VCG 0091 and VCG 0094 of *F. oxysporum* f.sp. *radicis-lycopersici* have been observed (Katan and Katan, 1999). In the present study, the isolates of *F. udum* in the two subgroups of VCG 1 were found to have isolates with significant differences in cultural characteristics and pathogenicity. Sunder and Satyavir (1998) observed that isolates of *F. moniliforme* from different VCGs and also within the same VCG varied considerably in virulence and gibberrelic acid (GA_3) production. Correll et al. (1986) have reported a correlation of VCG and colony size, with virulence of *F. oxysporum* f.sp. *apii* isolates; however in the present study, vegetative compatibility showed no definite relationship with cultural characteristics and pathogenicity. On the basis of vegetative compatibility as a genetic marker, the data presented in this study indicate that *F. udum* could be derived from a single lineage. However, to draw more definite conclusions, it will be necessary to study more isolates from different geographical origins. Further investigations using molecular markers and race typing are necessary to fully understand the genetic variation within *F. udum*.

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References

Calpouzos L and Stallknecht GF (1965) Sporulation of *Cercospora beticola* affected by an interaction between light and temperature. Phytopathology 55: 1370–1371

Clark CA, Hoy MW and Nelson PE (1995) Variation among isolates of *Fusarium lateritium* from sweetpotato for pathogenicity and vegetative compatibility. Phytopathology 85: 624–629

Correll JC, Klittich CJR and Leslie JF (1987) Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77: 1640–1646

Correll JC, Puhalla JE and Schneider JF (1986) Identification of *Fusarium oxysporum* f.sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76: 396–400

Gaur VK and Sharma LC (1989) Variability in single spore isolates of *Fusarium udum* Butler. Mycopathologia 107: 9–15

Gupta O, Kotasthane SR and Khare MN (1988) Strain variation in *Fusarium udum* in Madhya Pradesh, India. International Pigeonpea Newsletter 7: 22–25

Kannaiyan J, Nene YL, Reddy MV, Ryan JG and Raju TN (1984) Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and America. Tropical Pest Management 30: 62–71

Katan T and Katan J (1988) Vegetative-compatibility grouping of *Fusarium oxysporum* f.sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. Phytopathology 78: 852–855

Katan T and Katan J (1999) Vegetative compatibility grouping in *Fusarium oxysporum* f.sp. *radicis-lycopersici* from UK, the Netherlands, Belgium and France. Plant Pathology 48: 541–549

Katan T, Hadar E and Katan J (1989) Vegetative compatibility of *Fusarium oxysporum* f.sp. *dianthi* from carnation in Israel. Plant Pathology 38: 376–381

Katan T, Zamir D, Sarfatti M and Katan J (1991) Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Phytopathology 81: 255–262

Leslie JF (1993) Fungal vegetative compatibility. Annual Review Phytopathology 31: 127–151

Nirenberg H (1976) Untersuchungen uber die morphologische und bioligische Differenzierung in der *Fusarium*-section *Liseola*. Mitteilungen aus der Biologischen Bundesanstalt fur Land-und forstwirtschaft, Berlin-Bahlem 169: 1–117

Okiror MA (1986) Breeding for resistance to *Fusarium* wilt of pigeonpea (*Cajanus cajan* (L.) Millsp.) in Kenya. PhD Thesis, University of Nairobi, 202 pp

Plyler TR, Simone GW, Fernandez D and Kistler HC (2000) Genetic diversity among isolates of *Fusarium oxysporum* f.sp. *canariensis*. Plant Pathology 49: 155–164

Reddy NPE and Chaudhary KCB (1985) Variation in *Fusarium udum*. Indian Phytopathology 38: 172–173

Reddy MV and Raju TN (1997) Evaluation of pigeonpea (*Cajanus cajan*) varieties for resistance to wilt caused by *Fusarium udum* and sterility mosaic disease in a perennial system. Indian Journal of Agricultural Sciences 67: 437–439

Shit SK and Sen Gupta PK (1978) Possible existence of physiological races of *Fusarium oxysporum* f.sp. *udum*, the incitant of the wilt of pigeonpea. Indian Journal of Agricultural Sciences 48: 629–632

Shit SK and Sen Gupta PK (1980) Pathogenic and enzymatic variation in *Fusarium oxysporum* f.s.p. *udum*. Indian Journal of Microbiology 20: 46

Sunder S and Satyavir (1998) Vegetative compatibility, biosynthesis of GA_3 and virulence of *Fusarium moniliforme* isolates from bakanae disease of rice. Plant Pathology 47: 767–772