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Characterization of *Fusarium udum* isolates by physiological race typing

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Abstract

Seven pigeonpea varieties obtained from and recommended by International Crops Research Institute for Semi-Arid Tropics were used as differentials in order to determine the possible existence of races of *F. udum* in Kenya. Twenty-one isolates were used for inoculation in a glasshouse experiment. *Fusarium* wilt incidence was significantly different (P = 0.05) among the *F. udum* isolates and pigeonpea varieties, with a mean of 24.9% wilt at six weeks after root-dip inoculation. Pathogenic variability was observed among the isolates. The 21 isolates fitted in 11 *F. udum* physiologic races. The distinct races with a frequency of two or more isolates were races 0, 16, 24, 48 and 56. Race 0 was dominant with percent frequency of 23.8%, followed by races 48 and 56 with 14.3% each. By performing a pairwise similarity among the 11 races, two distinct groups of physiologic races could be identified. Race 0 had 0% similarity with the other races. The remaining 10 races could be linked to each other at a similarity of over 65%. Therefore race 0 appeared to be independent while the other 10 races could be closely related. The present study has confirmed the possible existence of physiologic races of *F. udum*. Breeding pigeonpea varieties for resistance to *Fusarium* wilt should take into account the existence of pathotype groups of *F. udum*.

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Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most widely grown and eaten grain legume in the semi-arid tropics of the world. Kenya is the world's second largest producer after India (Omanga and Matata, 1987; Nene and Sheila, 1990). *Fusarium* wilt of pigeonpea has been reported from fifteen countries (Nene *et al*., 1989), but it is apparently more important in India and Eastern Africa. *Fusarium* wilt is the most important disease of pigeonpea in Kenya (Onim, 1981) and India (Sen Gupta, 1974). In Eastern Africa, wilt incidence has been found to be quite high in Kenya (15.9%), Malawi (36.3%) and Tanzania (20.4%) (Kannaiyan *et al*., 1984). In some fields, wilt incidence could be as high as 90%. Losses due to wilt vary from a negligible amount to 100% depending on the stage at which the crop is attacked (Kannaiyan and Nene, 1981). *Fusarium* wilt has been identified as one of the main causes of low yields in farmer's fields in Kenya (Onim, 1981). Surveys in Kenya showed that *Fusarium* wilt incidence and mean ranged from 5% to 60% and 12% to 14.3%, respectively (Onim, 1981; Songa *et al*., 1991; Kiprop, 2001).

The breeding of *Fusarium* wilt resistant varieties of pigeonpea is the best method of its control. Breeding *Fusarium* wilt resistant varieties of pigeonpea has been conducted since the turn of the last century. However, variability in virulence of *F. udum* isolates from pigeonpea has been reported, suggesting possible genetic variation within this fungus.

Gupta *et al*. (1988) reported seven strains of *F. udum* determined by virulence on different varieties of pigeonpea in Madhya Pradesh (India). Reddy and Raju (1993) reported two distinct strains of *F. udum* after virulence tests of 13 isolates collected from various parts of India. Okiror (1986) observed variation in pathogenicity of 12 isolates of *F. udum*

obtained from 3 districts in Kenya. Physiologic races in *F. udum* have been suggested to exist but not determined conclusively (Mukhurjee *et al.*, 1971; Baldev and Amin, 1974; Shit and Sen Gupta, 1978; ICRISAT, 1987). In the present study twenty- one isolates of *F. udum* were characterized using selected host differentials to determine any differences in virulence towards various pigeonpea genotypes, and to find out possible existence of physiologic races.

Materials and Methods

Experimental site, pigeonpea differentials and *F. udum* isolates.

The experiment was conducted in a glasshouse whose prevailing temperatures were 22 to 30°C with about 12 hours photoperiod (natural light) at the Field Station, Kabete Campus, University of Nairobi at 1820 m.a.s.l. The seven pigeonpea were kindly supplied by International Crops various districts, AEZs and one

genotypes (varieties) used in this experiment Research Institute for Semi-Arid Tropics (ICRISAT) Centre (Hyderabad, India) and ICRISAT Experimental Station (Kiboko, Kenya). These were: *Fusarium* wilt resistant varieties ICP 8863, ICP 9174, ICP 8858, ICPL 87105, C-11 (ICPL 138) and ICEAP00040, and the wilt susceptible variety KAT 60/8. Nineteen isolates of *F. udum* from altitudes in Kenya and one isolate each from Malawi and India were used in this study. The isolates were NY02, TK02, MK10, NB01, MR04, NB03, MAL01a, KT05, TN05, MR02, TT02, ML01, MS10, TT08, MK02, MS04, TN01, NY07, KR03, IND01b and MB05

Inoculum preparation and seed pregermination.

The inoculum was prepared from each isolate by flooding 7 day-old PDA cultures with 100 ml of sterile distilled water.

The conidial suspension was filtered through cheesecloth, and the inoculum concentration determined using a haemocytometer and then adjusted to 1.0×10^6 conidia/ml. The pigeonpea seeds were surface sterilized for one minute in 1% sodium hypochlorite and washed in two series of sterile distilled water before planting in 12 cm diameter polythene bags filled with sterilized riverbed sand. The bags were kept in the glasshouse and watered at regular intervals with sterile distilled water.

Inoculation, transplanting of seedlings and experimental design.

Seven-day-old seedlings were uprooted carefully from the sand medium and the roots washed in running sterile distilled water. The roots were trimmed to about 4 cm from the collar region, and then dipped into 1.0×10^6 conidial suspension for 30 minutes before transplanting them into 20 cm diameter polythene bags containing sterilized mixture of red soil (Vertisol) and riverbed sand (3:1 v/v) (Dhingra and Sinclair, 1985; ICRISAT, 1990). For each *F. udum* isolate five seedlings were inoculated and this was replicated four times. Control seedlings were dipped in sterile distilled water. The pots were placed in the glasshouse and watered at regular intervals with sterile distilled water. The experimental design was completely randomised design and was replicated three times. The experiment was repeated once.

***Fusarium*s wilt assessment and data analysis.**

Observations on the symptom development were carried out daily until the initial symptoms were noted. The records on per cent wilt incidence on seedlings due to *F. udum* were taken thereafter on a weekly basis from the third week after inoculation and the final records taken six weeks later. The disease scale used was a modification of the one used by Reddy and Raju (1993, 1997), where 0-10% = resistant plants, 11-20% = moderately resistant plants, 21-30% = moderately susceptible plants, 31-60% = susceptible plants, and 61-100% = highly susceptible plants. The wilt incidence data was analysed by Analysis of Variance (ANOVA) and General Linear Model (GLM) procedures using the SAS system computer package release 6.12 (SAS Institute Inc., Cary, USA). Means separation was by Least Significant Difference (LSD) and Student- Newman-Keuls (SNK). In order to determine the races of the isolates, the race typing computer package Habgood-Gilmour Spreadsheet (HaGiS) was used (Habgood, 1970; Gilmour, 1973; Herrmann *et al.*, 1999).

Results

Twenty-one isolates of *F. udum* induced significantly different (P = 0.05) wilt incidences on pigeonpea varieties (Table 1).

Table 1. Wilt incidence (%) of 7 pigeonpea varieties inoculated with 21 isolates of *F. udum* six after root-dip inoculation

Isolate	Wilt incidence (%)[1] on pigeonpea varieties							Mean[3]	CV (%)	SE	LSD (5%)
	KAT 60/8[2]	C-11	ICPL 87105	ICEAP 00040	ICP 8858	ICP 9174	ICP 8863				
TT08	100.0	53.3	40.0	26.7	20.0	0.0	0.0	34.29 a	24.46	95.24	17.09
ML01	86.7	53.3	33.3	13.3	33.3	6.7	6.7	33.33 ab	41.40	190.47	24.17
KT05	80.0	40.0	26.7	26.7	13.3	6.7	20.0	30.49 abc	51.63	247.62	27.56
NB03	86.7	33.3	20.0	26.7	6.7	26.7	6.7	29.54 abcd	51.21	228.57	26.48
NB01	86.7	40.0	33.3	13.3	6.7	0.0	13.3	27.61 abcde	35.33	95.24	17.09
MAL01a	93.3	13.3	33.3	33.3	13.3	0.0	6.7	27.60 abcde	54.74	228.57	26.48
MS04	93.3	13.3	53.3	26.7	0.0	6.7	0.0	27.61 abcde	35.33	95.24	17.09
NY07	80.0	33.3	26.7	33.3	6.7	0.0	6.7	26.67 abcde	54.28	209.52	25.35
MB05	80.0	26.7	40.0	13.3	0.0	20.0	0.0	25.71 bcdef	37.95	95.24	17.09
MK02	86.7	26.7	33.3	13.3	0.0	6.7	6.7	24.77 cdef	61.06	228.57	26.45
MK10	100.0	13.3	13.3	13.3	20.0	6.7	6.7	24.76 cdef	49.85	152.38	21.62
NY02	86.7	26.7	13.3	20.0	6.7	13.3	6.7	24.77 cdef	52.88	171.42	22.93
TK02	53.3	46.7	13.3	6.7	6.7	13.3	26.7	23.81 cdefg	48.50	133.33	20.22
MS10	73.3	20.0	13.3	0.0	20.0	13.3	20.0	22.84 cdefg	57.28	171.43	22.93
MR04	66.7	13.3	26.7	6.7	13.3	13.3	13.3	21.90 defg	63.01	190.47	24.47
MR02	66.7	20.0	26.7	13.3	13.3	13.3	0.0	21.90 defg	74.55	266.67	28.60
TN05	53.3	33.3	20.0	13.3	0.0	26.7	6.7	21.90 defg	56.35	152.38	21.62
TN01	73.3	13.3	20.0	26.7	6.7	0.0	0.0	20.00 efg	43.64	76.19	15.29
TT02	60.0	6.7	20.0	6.7	0.0	20.0	13.3	18.10 efg	72.36	171.43	22.93
IND01b	66.7	13.3	13.3	6.7	13.3	6.7	6.7	18.10 efg	63.81	133.33	20.22
KR03	60.0	20.0	13.3	0.0	6.7	0.0	13.3	16.19 g	80.87	171.43	22.93
Mean[4]	77.78 a	26.66 b	25.39 b	16.19 c	9.84 d	9.53 d	8.58 d	24.85			
CV (%)	17.45	52.61	61.96	85.24	120.09	105.83	121.21	51.98			
SE	184.13	196.83	247.62	190.48	139.68	101.59	107.94	166.89			
LSD (5%)	22.36	23.12	25.92	22.74	19.47	16.61	17.12	7.85			

[1]Disease scale: resistant (R) = 0-10% wilt, moderately resistant (MR) = 11-20% wilt, moderately susceptible (MS) = 21-30% wilt, and susceptible (S) = 31-100% wilt (Reddy and Raju, 1997).
[2]Wilt susceptible variety as control.
[3]Means with the same letter down the column are not significantly different at 5% level using LSD test.
[4]Means with the same letter across the row are not significantly different at 5% level using LSD test.

The various interactions among 7 pigeonpea varieties and 21 isolates of *F. udum* were significantly different (P = 0.05). The wilt incidence varied from 0 to 100% with a mean of 24.9%. Although variety-isolate interactions were significant (P = 0.05) for wilt incidence, their contribution to total variation was lower than that of varieties and isolates separately. The significant difference (P = 0.05) was much higher for varieties than for isolates, which meant that there was more variation among pigeonpea varieties (8.6 to 77.8% willt) than among *F. udum* isolates (16.2 to 34.3% wilt).

The pigeonpea varieties responded differentially to different *F. udum* isolates in some cases. The highest level of mean disease incidence was observed six weeks after root-dip inoculation on the susceptible control KAT 60/8 at 77.8% wilt, followed by C-11 and ICPL 87105 at 26.7% and 25.4% wilt, respectively (Table 1). Pigeonpea varieties with least mean wilt incidence were ICP 8863, ICP 9174 and ICP 8858 with 8.6%, 9.5% and 9.8% wilt, respectively. Pigeonpea varieties ICP 8863, ICP 9174 and ICP 8858 did not show consistent differences in their reaction against the 21 isolates tested. Varieties C-11 and ICPL 87105 showed a consistently different reaction (susceptibility) to the isolates followed by variety ICEAP 00040. Varieties C-11 and ICPL 87105 had resistant reaction (R or MR) to 10 isolates and had susceptible reaction (MS or S) to 11 isolates, with both having a maximum of 53.3% wilt incidence (Tables 1 and 2).

Table 2. Presence (1)[1] or absence (0) of *Fusarium* wilt among pigeonpea differential varieties and physiologic races of *F. udum*

Isolate	Pigeonpea differential varieties							Disease presence[3]	Physiologic race[4]	
	ICP 8863	ICP 9174	ICP 8858	ICEAP 00040	ICPL 87105	C-11	KAT 60/8[2]		Habgood (binary)	Gilmour (octal)
TT08	0	0	0	1	1	1	1	3	56	7
ML01	0	0	1	0	1	1	1	3	52	46
KT05	0	0	0	1	1	1	1	3	56	7
NB03	0	1	0	1	0	1	1	3	42	25
NB01	0	0	0	0	1	1	1	2	48	6
MAL01a	0	0	0	1	1	0	1	2	24	3
MS04	0	0	0	1	1	0	1	2	24	3
NY07	0	0	0	1	1	1	1	3	56	7
MB05	0	0	0	0	1	1	1	2	48	6
MK02	0	0	0	0	1	1	1	2	48	6
MK10	0	0	0	0	0	0	1	0	0	0
NY02	0	0	0	0	0	1	1	1	32	4
TK02	1	0	0	0	0	1	1	2	33	14
MS10	0	0	0	0	0	0	1	0	0	0
MR04	0	0	0	0	1	0	1	1	16	2
MR02	0	0	0	0	1	0	1	1	16	2
TN05	0	1	0	0	0	1	1	2	34	24
TN01	0	0	0	1	0	0	1	1	8	1
TT02	0	0	0	0	0	0	1	0	0	0
IND01b	0	0	0	0	0	0	1	0	0	0
KR03	0	0	0	0	0	0	1	0	0	0

[1]Presence of disease: 1 = 21-100% wilt; absence of disease: 0 = 0-20% wilt
[2]Wilt susceptible pigeonpea variety as control
[3]Number of pigeonpea differential varieties with wilt disease per *F. udum* isolate excluding the control (KAT 60/8)
[4]Physiologic races, excluding the effect of the control variety, using di-Habgood binary numbers and Gilmour-Code octal number (Habgood, 1970; Gilmour, 1973; Herrmann *et al*., 1999).

The minimum wilt incidence on these varieties was 6.7% on C-11 and 13.3% on ICPL 87105. Variety ICEAP 00040 had resistant and susceptible reaction to 14 and 7 isolates, respectively (Table 2). The maximum and minimum wilt incidence on this variety was respectively 33.3% and 0.0%. Varieties ICP 8858, ICP 9174 and ICP 8863 showed resistance to 20, 19 and 20 isolates, respectively. The maximum and minimum wilt incidence was 33.3% and 0.0%, and 26.7% and 0.0% on ICP 8858, and ICP 9174 and ICP 8863, respectively.

All *F. udum* isolates were virulent to wilt susceptible pigeonpea variety KAT 60/8 (control) but reacted differently with the varieties C-11, ICPL 87105, ICEAP 00040, ICP 8858, ICP 9174 and ICP 8863 (Tables 1 and 2). Isolates TT02, IND01b, KR03, MS10 and MK10 had low virulence (plants were resistant or moderately resistant) in 6 differential varieties. Isolates with low virulence in 5 differential varieties were MR02, MR04 and TN01, while isolates that showed high virulence to 5 differential varieties were TT08, ML01, KT05, NB03 and NY07.

Using the di-Habgood or Gilmour-Code of race typing (Habgood, 1970; Gilmour, 1973; Herrmann *et al*., 1999) the 21 isolates fitted in 11 *F. udum* physiologic races (Table 2). The distinct races with a frequency of two or more isolates were races 0, 16, 24, 48 and 56 (Habgood binary number method). Race 0 was dominant with percent frequency of 23.8%, followed by races 48 and 56 with 14.3% each, races 16 and 24 with 9.5% each, and the least dominant were races 8, 32, 33, 34 and 42 with 4.8% each. By performing a pairwise similarity (Dice coefficient) among the 11 races, two distinct groups of physiologic races could be identified. Race 0 had 0% similarity with the other races. The remaining 10 races could be linked to each other at a similarity of over 65%. Therefore race 0 appeared to be independent while the other 10 races could be closely related. Isolate IND01b from India belonged to race 0 together with MK10, MS10, TT02 and KR03 from Kenya, while isolate MAL01a from Malawi belonged to race 24 together with isolate MS04 from Kenya.

Discussion

Seven pigeonpea varieties namely KAT 60/8 (control), C-11, ICPL 87105, ICP 8863, ICP 8858, ICP 9174 and ICEAP 00040 were used to assess virulence variability of *F. udum* isolates. The differences in responses of the seven pigeonpea varieties to 19 isolates of *F. udum* from Kenya and one isolate each from Malawi and India confirmed virulence variability in virulence of 79 isolates. Okiror (1986) reported variation in virulence of 12 isolates of *F. udum* from Kenya using 6 pigeonpea lines (Munaa and 5 NPP lines). Gaur and Sharma (1989) made similar observations on variability in virulence of 7 isolates of *F. udum* from India using 18 pigeonpea varieties. Studies using other pigeonpea varieties against *F. udum* isolates have been reported by Mukherjee *et al*. (1971), Sarojini (1951), Baldev and Amin (1974), Shit and Sen Gupta (1978), and Gupta *et al*. (1988).

The pigeonpea varieties responded differentially when inoculated with 21 isolates of *F. udum*. For example, varieties C-11 and ICPL 87105 showed a consistently different reaction to the isolates, followed by variety ICEAP 00040.

Pigeonpea varieties ICP 8863, ICP 9174 and ICP 8858 did not show consistent differences in their reaction to 21 isolates of *F. udum*. In this study the pigeonpea varieties viz ICP 8863, ICP 9174 and ICP 8858 were found to be resistant to 21 isolates of *F. udum*. Pigeonpea variety ICEAP 00040 gave a moderately resistant type of reaction to the 21 isolates, while varieties C-11 and ICPL 87105 gave a moderately susceptible reaction to the same isolates. The present findings correlate with other reports from India especially resistance observed in varieties ICP 8863, ICP 8858 and ICP 9174. However, differences or similarities in the resistance of variety C-11 to *F. udum* isolates do exist among different reports. Reddy and Raju (1993) found pigeonpea varieties ICP 8863 (Maruti), ICP 9174 and C-11 to be resistant to *Fusarium* wilt. Gaur and Sharma (1989) found varieties ICP 8858 and C-11 to be resistant to wilt while variety ICP 8863 was ICPL 87105 (18.9-32.9% wilt) and ICP 2376 (21.3-95.8% wilt), while variety ICP 9145 was found to be resistant (0.0% wilt) at Katumani wilt 'sick' plot but susceptible (71.0% wilt) at Kiboko wilt 'sick' plot (Songa *et al*., 1995). The present findings showed ICP 8863 and ICPL 87105 to be resistant (8.6% wilt) and susceptible (25.4% wilt), respectively to wilt. In the present study pigeonpea varieties KAT 60/8, C-11, ICPL 87105, ICEAP 00040, ICP 9174, ICP 8863 and ICP 8858 were resistant to 0, 10, 10, 14, 19, 20 and 20 isolates of *F. udum*, respectively. Changaya-Banda *et al*. (1996) has also reported differential reactions of 7 pigeonpea differential lines namely ICP 2376, ICP 9145, ICP 8858, ICP 8859, ICP 8862, ICP 8863 and ICP 9174 to 75 isolates of *F. udum* from Malawi.

Eleven physiologic races of *F. udum* were identified when 21 isolates were used to moderately susceptible (30-70% wilt). Nene and Kannaiyan (1982) found varieties ICP 8858, ICP 8863 and C-11 (ICP 7118) to be resistant to wilt. Although Baldev and Amin (1974), Shit and Sen Gupta (1978), Gupta *et al*. (1988), and Nene and Kannaiyan (1982) found pigeonpea variety C-11 to be resistant to *Fusarium* wilt, the ranges of wilt incidence were 0-25%, 0-55%, 0-50%, and 2.2-70%, respectively. Singh and Mishra (1976) however found variety C-11 to be susceptible to *Fusarium* wilt. Reddy *et al*. (1990) have indicated that variety C-11 does not have high level of resistance but variety ICP 8863 has high and stable resistance to *Fusarium* wilt.

Pigeonpea varieties reported to be resistant to *Fusarium* wilt in Kenya include ICP 8863 (0.0% wilt), ICP 87051 (9.6% wilt) and BDN 1 (3.3% wilt) (Songa *et al*., 1995). *Fusarium* wilt susceptible varieties in Kenya include KAT 60/8 (44-63.1% wilt), inoculate 7 pigeonpea differential varieties. The distinct races were race 0, 16, 24, 48 and 56. The least distinct races were race 8, 32, 33, 34 and 42. Race 0 was most dominant and appeared to be independent from the rest while the remaining 10 races were closely related. The methods of Habgood (1970) and Gilmour (1973) have been used elsewhere to identify physiologic races of various plant pathogenic fungi. Habgood method has been used to identify races of, for example, *Puccinia striiformis* of wheat (Kema and Lange, 1992) and *Erysiphe graminis* of rye (Kast and Geiger, 1982). Physiologic races have been identified in plant pathogens such as tomato wilt fungus *F. oxysporum f.sp. lycopersici* (Alexander and Tucker, 1945) and *Colletotrichum lindemuthianum* causing anthracnose of common bean (Menezes and Dianese, 1988).

The present study has confirmed the possible existence of physiologic races of *F. udum*.

Earlier findings have suggested a possible existence of physiologic races of *F. udum* but no specific studies were undertaken to document their existence (Mukhurjee *et al*., 1971; Baldev and Amin, 1974; Shit and Sen Gupta; 1978; Okiror, 1986; ICRISAT, 1987; Changaya-Banda *et al*. 1996).

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