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Genetic Variability among *Fusarium udum* Isolates from Pigeonpea

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Thirty three isolates of *Fusarium udum* obtained from pigeonpea (*Cajanus cajan)* plant showing wilt symptoms were collected from various states in India and tested for variability in Amplified Fragment Length Polymorphism (AFLP). AFLP analysis of 33 *F. udum* isolates using four primer combinations generated a total of 72 fragments with 68 being polymorphic (94.38 % polymorphism). Cluster and Principal Component Analysis had grouped *Fusarium udum* isolates collected from different parts of India into four races. Among the races identified, race 3 had maximum number of isolates grouped into it and its presence in five pigeonpea growing states of India whereas race 1 and 2 were present only in Karnataka and Andhra Pradesh. In Karnataka state all four races were observed indicating that varieties with horizontal resistance against all four races are important to combat this disease.

Key words: Amplified Fragment Length Polymorphism, *Cajanus cajan*, *Fusarium udum.*

Fusarium wilt caused by *Fusarium udum* Butler is the most important disease of pigeonpea (*Cajanus cajan* (L.)Millsp) (Kannaiyan *et al*., 1984) and causes yield loss up to 100 per cent depending on the stage at which the crop is attacked (Kannaiyan and Nene, 1981). Control strategies for *Fusarium* wilt of pigeonpea should target population of *F. udum*. However *F. udum* shows a great deal of variation in cultural and morphological characteristics (Rai and Upadhyay, 1982 and Gaur and Sharma, 1989). The high variation in cultural and morphological characteristics of this pathogen could be due to environment conditions, the age of the isolates, subculturing, method of storage and cultural conditions. Wide variations in virulence to different genotypes of pigeonpea among *F. udum* isolates (Shit and Sengupta, 1978) could be due to environment conditions and the inoculation techniques. To overcome the problems associated with the standard methods such as cultural and morphological characterization and virulence/race typing, attempts have been made to use natural variation present in the DNA as a means for grouping fungal pathogens into race. The genetic markers that have been used to characterize phyto-pathogenic fungal species by using Amplified Fragment Length Polymorphisms (AFLPs). 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, and Vos *et al.,* 1995). AFLP has been used to detect genetic variation between and within species of fungi and to group isolates of plant pathogenic fungi into races.

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* , there is a need to investigate the existence of variability among *F. udum* isolates from different geographical locations in India. The use of molecular markers will enable extensive analysis of phyto - pathogenic fungi, survival of *F. udum* at different soil moisture and temperature.

Materials and Methods

Fungal isolates

Thirty three single spore isolates of *F. udum* were obtained from major pigeonpea growing states in India (Table 1). The stem portion of plants showing symptoms of *Fusarium* wilt were collected and 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 ^{0}C in a 12 hr light/dark cycle for 36-48 hr and colonies showing growth and morphology of *F. udum* (Booth *et al.,* 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.

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Table 1. *Fusarium udum* isolates collected from different locations in India

Sl. No	State	District	Taluk	Village	Isolate designation
1	Andhra Pradesh	Guntur	Chilakalurpet	Appanpuram	APFu-1
2	Karnataka	Bangalore	Bangalore	GKVK	KFU-1
3	Karnataka	Ramanagara	Magadi	Byraganapur	KFU-2
4	Karnataka	Ramanagara	Magadi	Dhannampalya	KFU-3
5	Andhra Pradesh	Guntur	Inkallu	Inkallu	APFu-2
6	Andhra Pradesh	Karnool	Karnool	Kichvala	APFu-5
7	Andhra Pradesh	Karnool	Karnool	Mahadevanpet	APFu-6
8	Andhra Pradesh	Mahabub Nagar	Jetcherla	Mannemkonda	APFu-8
9	Andhra Pradesh	Guntur	Narasaraopet	Kesanapalli	APFu-3
10	Andhra Pradesh	Khammam	Veira	Menavalu	APFu-7
11	Andhra Pradesh	Sangareddi	Kodangal	Regadimylapuram	APFu-9
12	Karnataka	Gulbarga	Sedam	Konkanahalli	KFU-10
13	Andhra Pradesh	Sangareddi	Thandur	Bandimidipalli	APFu-10
14	Andhra Pradesh	Sangareddi	Thandur	Thandur	APFu-11
15	Karnataka	Gulbarga	Aland	Nimbala	KFU-6
16	Bihar	Muzaffarpur	Muzaffarpur	Muzaffarpur	BFu-1
17	Karnataka	Bijapur	Hindi	Salotagi	KFU-4
18	Karnataka	Gulbarga	Gulbarga	Sirunuru	KFU-7
19	Karnataka	Gulbarga	Gulbarga	Uppalav	KFU-8
20	Karnataka	Gulbarga	Sedam	Kolluru	KFU-9
21	Maharastra	Akola	Akola	PDKV Akola	MSFu-1
22	Maharashtra	Badnapur	Badnapur	ARS Badnapur	MSFu-3
23	Karnataka	Kolar	Malur	Chokkandahalli	KFU-14
24	Karnataka	Hassan	Channaraya-pattana	Kandali	KFU-12
25	Uttar Pradesh	Kanpur	Kanpur	Kanpur	UPFu-1
26	Karnataka	Gulbarga	Aland	Aland	KFU-5
27	Karnataka	Hassan	Arasikere	Margundanahalli	KFU-11
28	Karnataka	Hassan	Hassan	Ankapur	KFU-13
29	Maharashtra	Latur	Latur	Latur	MSFu-4
30	Tamil Nadu	Coimbatore	Coimbatore	Coimbatore	TNFu-1
31	Tamil Nadu	Pudukottai	Alangudi	Thiruvarankulam	TNFu-5
32	Madhya Pradesh	Khargaon	Khargaon	JNKVV Farm	MPFu-1
33	West Bengal	Murshidabad	Murshidabad	Murshidabad	WBFu-1

Extraction of total genomic DNA

Total genomic DNA of *F. udum* was isolated from mycelia by employing the method of Raeda and Broda (1985) with minor modifications. For DNA extraction, fungal mycelia were harvested from the isolates grown in potato dextrose broth incubated at 28 ^{0}C for three to five days in orbital shaker at 200 rpm. After incubation the fungal biomass was filtered through Whatman No. 1 filter paper and 50 mg of freeze dried mycelium was ground with the help of pestle and mortar in liquid nitrogen until fine powder of mycelium was obtained. The mycelial powder was transferred to 2 ml Eppendorf tubes. To this 500 µl of extraction buffer was added and the resulting slurry was incubated at 60^{0}C for 20 minutes in a water bath. Equal volume of phenol : chloroform : isoamyl alcohol (25:24:1) was added to incubated slurry, mixed gently and centrifuged at 10,000 rpm at 4^{0}C for 60 minutes. The supernatant was transferred to new sterile Eppendorf tubes and to which 1 µl RNase solution (10mg/ml) was added and kept for incubation for 10 minutes at 37^{0}C. To this, equal volume of chloroform: Isoamyl alcohol (24:1) was added and mixed gently. The tubes were centrifuged for 10 minutes at 10,000 rpm at 4^{0}C. The supernatant was transferred to new sterile Eppendorf tubes and equal volume of Isopropyl alcohol was added. Centrifugation step was repeated twice. The supernatant was collected and to this ice cold 70 per cent ethyl alcohol and 3 M sodium acetate (pH 5) was added and kept for over night at 0 ^{0}C, followed by centrifuge at 13,000 rpm for 30 min, then the supernatant was drained out and pellet air dried. The pellet was re suspended in 20-100 µl TE buffer (pH 8). DNA was stored at -20 ^{0}C. Finally the quality and quantity of DNA was assessed in 1 per cent agarose gel.

AFLP analysis

AFLP analysis was performed as described by Vos *et al.* (1995)

Preparation of the double stranded adapter

Single stranded adapter sequences need to be annealed together. Both *Eco*RI and *Mse*I adapters were used at 5 pmol/µl and 50 pmol/µl respectively.

Double digestion

The reaction mix was done in microtubes and incubated at 37 ^{0}C for 2 to 3 hours.

Ligation of adapters

Twenty microlitres of digested reaction mix was added to get 24 µl ligation reaction mix. Ligation reaction was done at 37^{0}C for 16-18 hours overnight. The digested / ligated AFLP templates were then diluted $T_{10}E_{0.1}$ pH 8 buffer in the ratio of 1: 5 (i.e 1µl of DNA template: 4µl of $T_{10}E_{0.1}$) buffer and stored at -20 ^{0}C.

Amplification of AFLP templates

Pre-amplification

Pre-amplification is a PCR where, each primer has a one nucleotide base of selection. The nucleotide bases of selection chosen depend on the final primer combination for re-amplification. Amplification was performed in Eppendorf master thermal cycler PCR at 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s. The pre-amplified products were diluted by adding $T_{10}E_{0.1}$ pH 8 in buffer ratio of 1: 5 (i.e 1µl of DNA template: 4µl of $T_{10}E_{0.1}$).

Re-amplification

Re -amplification is a PCR where each primer has 3 nucleotide extensions. Amplification was performed in Eppendorf master thermal cycler PCR at 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s; followed by 11 cycles in which the annealing temperature was lowered by 0.7°C in each cycle; and finally 24 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 60 s.

Denaturation of AFLP products

Ten microlitres of stop loading dye was added to each sample. Denaturation was carried out by heating to 94^{0}C for 5 min and then cooling to 10^{0}C for 5 min. Finally the product was stored at -20^{0}C.

Visualizing AFLP products

AFLP products were visualized on denaturing 4.5% 19:1 (w/w) acrylamide/bisacrylamide gel followed by silver staining techniques.

Analysis of AFLP profiles

Amplification profiles of four pair of primer combinations on 33 *F. udum* isolates were scored as presence (1) or absence (0) and the 0, 1 matrix generated was used for statistical analysis.

Cluster analysis and estimating genetic relationship

Squared Euclidean Distance (SED) matrix of AFLP marker for 33 *F. udum* isolates was obtained using the Sequential Agglomerative Hierarchial Nested clustering (SAHN) feature using NTSYS pc version 2.0 (Rohlf, 1998). Principal component analysis (PCA) for molecular data was carried out using PAST software and scatter plot was developed for components 1 and 2.

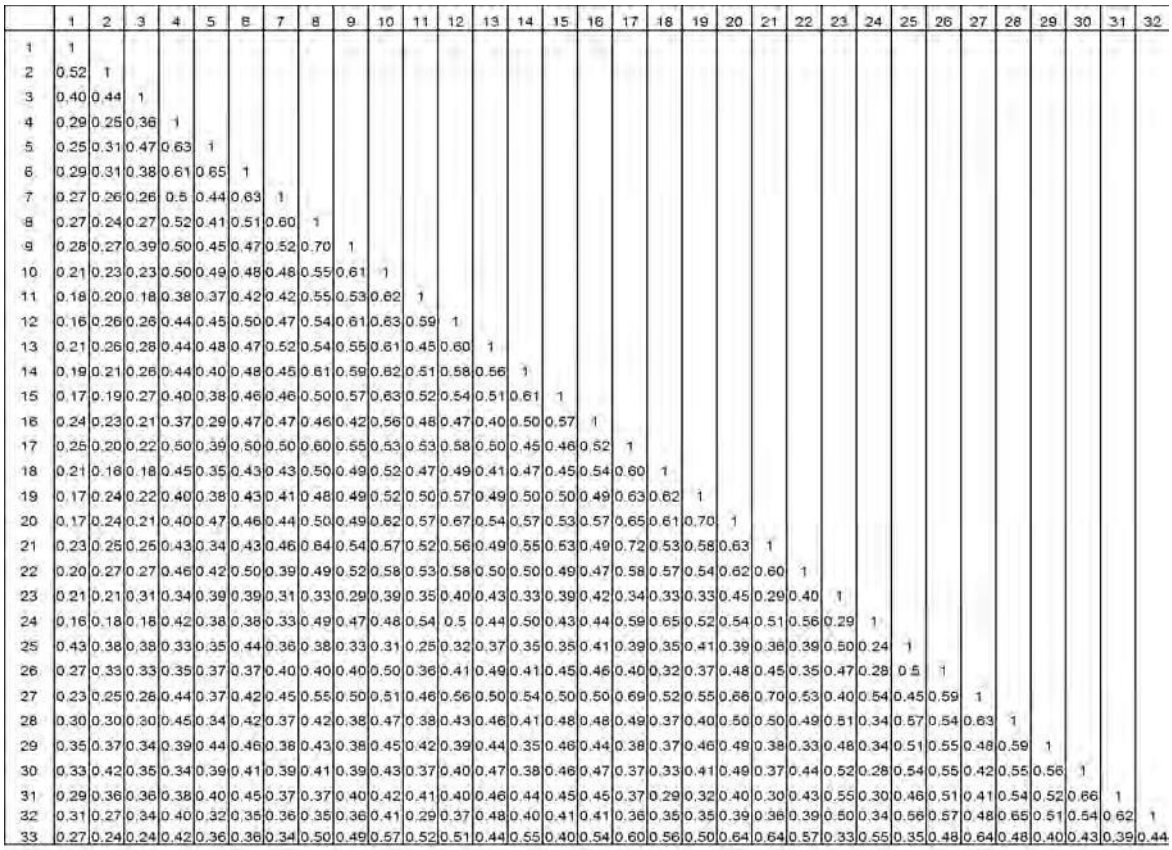
Results and Discussion

Pathogenicity test

The pathogenicity of the 33 *F. udum* isolates was carried out on susceptible pigeonpea cultivar TTB-7. *F. udum* giant culture was thoroughly hand mixed with autoclaved soils separately at 1:4 w/w ratio. The mixture was filled in 15 cm diameter polythene bag, previously surface sterilized in five per cent sodium hypochlorite solution. The polythene bags were washed and incubated for ten days under glass house condition. On eleventh day ten seeds of pigeonpea cultivar TTB-7 surface sterilized in 2.5 per cent sodium hypochlorite solution for 10 minutes were sown in each of the polythene bags. Plants grown in polythene bags were watered regularly so as to maintain 50 per cent water holding capacity of the soil. Necessary plant nutrients were supplied through Hoagland's solution. Seeds were sown in polythene bags with sterile soil served as control. Usually the typical wilting symptoms started appearing after 20-30 days of sowing. Observations on wilt incidence were recorded at fortnight intervals starting from 15 days after sowing and final observations were recorded after 120 days of sowing. The pathogen was reisolated from infected seedlings and was compared with the original culture.

Molecular variability of* Fusarium udum *isolates using AFLP

The 33 *F. udum* isolates were subjected to AFLP marker analysis for accessing the genetic variability existing among the isolates. The AFLP data generated were subjected to grouping based on UPGMA. AFLP analysis of 33 *F. udum* isolates using four primer combinations generated a total of 72 fragments with 68 being polymorphic (94.38 % polymorphism), and were grouped into 4 clusters (Table 2). The formation of large number of clusters evidenced that the isolates selected are marked by considerable high molecular diversity. Among the four clusters, cluster II was the largest with 18 isolates (KFU-4, KFU-6, KFU-7, KFU-8, KFU-9, KFU-10, KFU-11, KFU-12, APFU-3, APFU-7, APFU-8, APFU-9, APFU-10, APFU-11, MSFU-1, MSFU-3, BFU-1 and WBFU-1) followed by cluster I (KFU-5, KFU-13, KFU-14, TNFU-1, TNFU-5, MSFU-4, MPFU-1 and UPFU-1). Cluster III had four isolates *viz*., KFU-3, APFU-2, APFU-5 and APFU-6 and cluster IV KFU-1, KFU-2 and APFU-1 comprised three isolates. The results of the present study indicated that all four clusters were distantly related to each other with a similarity range between 0.25 and 0.73.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	1																															
2	0.52	1																														
3	0.40	0.44	1																													
4	0.29	0.25	0.36	1																												
5	0.25	0.31	0.47	0.63	1																											
6	0.29	0.31	0.38	0.61	0.65	1																										
7	0.27	0.26	0.26	0.5	0.44	0.63	1																									
8	0.27	0.24	0.27	0.52	0.41	0.51	0.60	1																								
9	0.28	0.27	0.39	0.50	0.45	0.47	0.52	0.70	1																							
10	0.21	0.23	0.23	0.50	0.49	0.48	0.48	0.55	0.61	1																						
11	0.18	0.20	0.18	0.38	0.37	0.42	0.42	0.55	0.53	0.62	1																					
12	0.16	0.26	0.26	0.44	0.45	0.50	0.47	0.54	0.61	0.63	0.59	1																				
13	0.21	0.26	0.28	0.44	0.48	0.47	0.52	0.54	0.55	0.61	0.45	0.60	1																			
14	0.19	0.21	0.26	0.44	0.40	0.48	0.45	0.61	0.59	0.62	0.51	0.58	0.56	1																		
15	0.17	0.19	0.27	0.40	0.38	0.46	0.46	0.50	0.57	0.63	0.52	0.54	0.51	0.61	1																	
16	0.24	0.23	0.21	0.37	0.29	0.47	0.47	0.46	0.42	0.56	0.48	0.47	0.40	0.50	0.57	1																
17	0.25	0.20	0.22	0.50	0.39	0.50	0.50	0.60	0.55	0.53	0.53	0.58	0.50	0.45	0.46	0.52	1															
18	0.21	0.18	0.18	0.45	0.35	0.43	0.43	0.50	0.49	0.52	0.47	0.49	0.41	0.47	0.45	0.54	0.60	1														
19	0.17	0.24	0.22	0.40	0.38	0.43	0.41	0.48	0.49	0.52	0.50	0.57	0.49	0.50	0.50	0.49	0.63	0.62	1													
20	0.17	0.24	0.21	0.40	0.47	0.46	0.44	0.50	0.49	0.62	0.57	0.67	0.54	0.57	0.53	0.57	0.65	0.61	0.70	1												
21	0.23	0.25	0.25	0.43	0.34	0.43	0.46	0.64	0.54	0.57	0.52	0.56	0.49	0.55	0.53	0.49	0.72	0.53	0.58	0.63	1											
22	0.20	0.27	0.27	0.46	0.42	0.50	0.39	0.49	0.52	0.58	0.53	0.58	0.50	0.50	0.49	0.47	0.58	0.57	0.54	0.62	0.60	1										
23	0.21	0.21	0.31	0.34	0.39	0.39	0.31	0.33	0.29	0.39	0.35	0.40	0.43	0.33	0.39	0.42	0.34	0.33	0.33	0.45	0.29	0.40	1									
24	0.16	0.18	0.18	0.42	0.38	0.38	0.33	0.49	0.47	0.48	0.54	0.5	0.44	0.50	0.43	0.44	0.59	0.65	0.52	0.54	0.51	0.56	0.29	1								
25	0.43	0.38	0.38	0.33	0.35	0.44	0.36	0.38	0.33	0.31	0.25	0.32	0.37	0.35	0.35	0.41	0.39	0.35	0.41	0.39	0.36	0.39	0.50	0.24	1							
26	0.27	0.33	0.33	0.35	0.37	0.37	0.40	0.40	0.40	0.50	0.36	0.41	0.49	0.41	0.45	0.46	0.40	0.32	0.37	0.48	0.45	0.35	0.47	0.28	0.5	1						
27	0.23	0.25	0.26	0.44	0.37	0.42	0.45	0.55	0.50	0.51	0.46	0.56	0.50	0.54	0.50	0.50	0.69	0.52	0.55	0.66	0.70	0.53	0.40	0.54	0.45	0.59	1					
28	0.30	0.30	0.30	0.45	0.34	0.42	0.37	0.42	0.38	0.47	0.38	0.43	0.46	0.41	0.48	0.48	0.49	0.37	0.40	0.50	0.50	0.49	0.51	0.34	0.57	0.54	0.63	1				
29	0.35	0.37	0.34	0.39	0.44	0.46	0.38	0.43	0.38	0.45	0.42	0.39	0.44	0.35	0.46	0.44	0.38	0.37	0.46	0.49	0.38	0.33	0.48	0.34	0.51	0.55	0.48	0.59	1			
30	0.33	0.42	0.35	0.34	0.39	0.41	0.39	0.41	0.39	0.43	0.37	0.40	0.47	0.38	0.46	0.47	0.37	0.33	0.41	0.49	0.37	0.44	0.52	0.28	0.54	0.55	0.42	0.55	0.56	1		
31	0.29	0.36	0.36	0.38	0.40	0.45	0.37	0.37	0.40	0.42	0.41	0.40	0.46	0.44	0.45	0.45	0.37	0.29	0.32	0.40	0.30	0.43	0.55	0.30	0.46	0.51	0.41	0.54	0.52	0.66	1	
32	0.31	0.27	0.34	0.40	0.32	0.35	0.36	0.35	0.36	0.41	0.29	0.37	0.48	0.40	0.41	0.41	0.36	0.35	0.35	0.39	0.36	0.39	0.50	0.34	0.56	0.57	0.48	0.65	0.51	0.54	0.62	1
33	0.27	0.24	0.24	0.42	0.36	0.36	0.34	0.50	0.49	0.57	0.52	0.51	0.44	0.55	0.40	0.54	0.60	0.56	0.50	0.64	0.64	0.57	0.33	0.55	0.35	0.48	0.64	0.48	0.40	0.43	0.39	0.44

Fig 1. Squared Euclidean Distance (SED) matrix of AFLP marker data of 33 *Fusarium udum* isolates of Pigeonpea

The Squared Euclidean Distance (SED) matrix revealed maximum genetic similarity of 0.70

Table 2. Number of AFLP fragments and the level of polymorphism detected in 33 *Fusarium udum* isolates of pigeonpea

Primer combination	No. of bands obtained	No. of polymorphic bands obtained	Per cent polymorphic bands
E+AAT/ M+CTG	26	25	96.15
E+AGC/ M+CTT	14	14	100.00
E+AAT/ M+CTA	21	19	90.47
E+TCG/ M+CTG	11	10	90.90
Total	72	68	94.38

between KFU-11 to MSFU-1, KFU-9 to KFU-8 and APFU-3 to APFU- 8 indicating maximum genetic similarity between the isolates studied. Whereas the minimum genetic similarity of 0.16 was observed between KFU-10 to APFU-1, KFU-1 to KFU-7 and KFU-12 to APFU-1 indicating maximum dissimilar between the isolates studied (Fig 1).

Results obtained from UPGMA cluster analysis was further corroborated by Principal component analysis (PCA) with variance-covariance matrix. Graphical plot of principal components 1 and 2 revealed grouping of isolates into four major groups. Cluster 1 had three isolates and belonged to race 1, cluster 2 belonged to race 2 with 4 isolates, cluster 3 had 16 isolates and cluster 4 had 8 isolates whereas two isolates could not be grouped (Table 3 & Fig. 2, 3). Cluster I had one isolate from Andhra Pradesh (APFu-1) and two from Karnataka (KFU-1 and 2). Cluster 2 had three isolates from Andhra Pradesh (APFU-2, 5 and 6) and one from Karnataka (KFU-3). Cluster 3 had five isolates from Andhra Pradesh (APFU-3, 7, 9, 10 and 11), seven from Karnataka (KFU-4, 7, 8, 9, 10, 11 and 12), two from Maharashtra (MSFU-1 and 3) and one each from Bihar (BFu-1) and West Bengal (WBFu-1). Cluster 4 had three isolates from Karnataka (KFU 5, 13 and 14), two from Tamil Nadu (TNFu-1 and 5) and one each from Maharashtra (MSFu-4), Madhya Pradesh (MPFu-1) and Uttar Pradesh (UPFu-1). Two isolates from Andhra Pradesh (APFu-3 and 8) did not belong to any group.

AFLP molecular marker analysis classified 33 isolates of *F. udum* into four major groups based on cluster analysis although there were some minor differences at the level of subgroups suggesting that there could be a minimum of four races in the pathogen (Table 3). Clustering of various pathogen isolates into different groups by the AFLP molecular markers however did not reflect the geographical distribution of the various pigeonpea growing areas from where these isolates were collected. From the combined analysis of PCA and cluster analysis it appears that race 3 which comprises of 18 isolates is the most predominant whereas race 1 with three isolates is rare. Maximum diversity was seen among

Table 3. Identification of *Fusarium udum* races in pigeonpea using AFLP markers

Race	Clustering	PCA	Combined	Area of spread
Race 1	1,2,3	1,2,3	1,2,3	Karnataka and Andhra Pradesh
Race 2	4,5,6,7	4,5,6,7	4,5,6,7	Karnataka and Andhra Pradesh
Race 3	8,9,10,11,12,13,14, 15,16,17,18,19,20, 21,22,24, 27,33	8,9,10,11,12,13,14, 15,16,17,18,19, 20,22,21,24, 27, 33	8,9,10,11,12,13,14, 15,16,17,18,19,20, 21,22,24,27,33	Karnataka, Andhra Pradesh, Bihar, Maharashtra and West Bengal
Race 4	23,25,26,28,29,30, 31,32	23,25,26,28,29,30, 31,32	23,25,26,28,29,30, 31,32	Tamil Nadu, Madhya Pradesh, Karnataka, Uttar Pradesh and Maharashtra

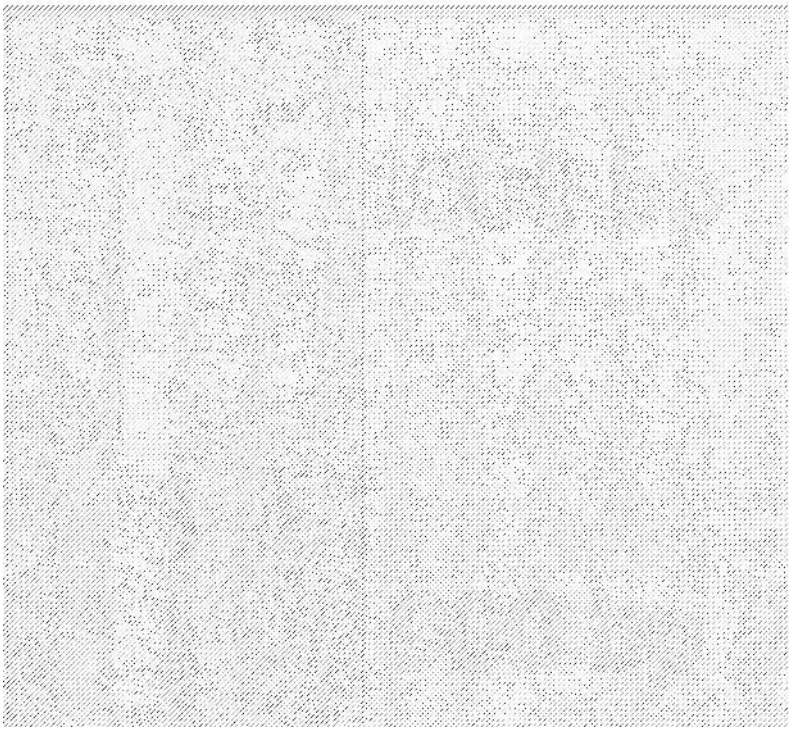


Fig 2. AFLP profile of 33 *Fusarium udum* isolates for the primer combination *Eco R1*+AGC/*Msc1*+CTT. L=100bp DNA molecular weight ladder (Lanes 1-33 represent the isolates shown in index)

1 = APFu-1, 2= KFu-1, 3= KFu-2, 4 = KFu-3, 5 = APFu-2, 6 = APFu-5, 7 = APFu-6, 8 = APFu-8, 9 = APFu-3, 10 = APFu-7, 11= APFu-9, 12 = KFu-10, 13 = APFu-10, 14 = APFu-11, 15 = KFu-6, 16 = BFu-1, 17 = KFu-4, 18 = KFu-7, 19 = KFu-8, 20 = KFu-9, 21 = MSFu-1, 22 = MSFu-3, 23 = KFu-14, 24 = KFu-12, 25 = UPFu-1, 26 = KFu-5, 27 = KFu-11, 28 = KFu-13, 29 = MSFu-4, 30 = TNFu-1, 31 = TNFu-5, 32 = MPFu-1, 33 = WBFu-1

the Karnataka isolates belonging to four different races followed by Andhra Pradesh with two predominant races. Major pigeonpea growing districts of Karnataka and Andhra Pradesh had Race 3 of *Fusarium udum*.

These results are in agreement with those obtained by Sivaramakrishnan *et al.* (2002) who suggested that, cluster analysis of similarity index data from the two DNA markers of RAPD and AFLP, classified the isolates into three major groups suggesting the existence of a minimum of three specific races of the pathogen prevailing in the pigeonpea growing areas of India. The phenetic dendrogram generated by UPGMA as well as principal coordinate analysis (PCA) by Mohmed Abdel-Satar *et al. (*2003) grouped all the *Fusarium* spp isolates into five major clusters. The results of the present study are also in conformity with earlier investigators *viz*., Zhu *et al.* (1998), Aggarwal *et al. (*2002), Bensnard *et al.* (2002), Kiprop *et al*. (2005), Kamel Abd. Elsalam *et al.,* 2004, Lakhdar Belabid

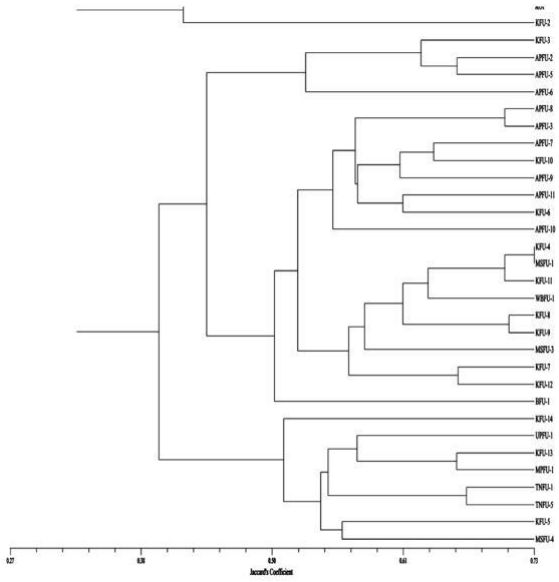


Fig 3. Genetic diversity among 33 isolates of *Fusarium udum*

1 = APFu-1, 2= KFu-1, 3= KFu-2, 4 = KFu-3, 5 = APFu-2, 6 = APFu-5, 7 = APFu-6, 8 = APFu-8, 9 = APFu-3, 10 = APFu-7, 11= APFu-9, 12 = KFu-10, 13 = APFu-10, 14 = APFu-11, 15 = KFu-6, 16 = BFu-1, 17 = KFu-4, 18 = KFu-7, 19 = KFu-8, 20 = KFu-9, 21 = MSFu-1, 22 = MSFu-3, 23 = KFu-14, 24 = KFu-12, 25 = UPFu-1, 26 = KFu-5, 27 = KFu-11, 28 = KFu-13, 29 = MSFu-4, 30 = TNFu-1, 31 = TNFu-5, 32 = MPFu-1, 33 = WBFu-1

et al. (2004), Jane Stewart *et al.* (2006) and Bogale *et al.* (2006). They also reported the use of AFLP markers to identify strains or races in *Fusarium* spp. and the use of these markers for studying inter and intraspecific variability among populations from different as well as from the same geographic regions.

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