INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 8 | ISSUE 1 | APRIL, 2015 | 81-85

● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/8.1/81-85

RAPD based molecular diversity analysis of different *Fusarium udum* Butler isolates of pigeonpea wilt

■ AMOL S. SHINDE*, S.R. KALASKAR, A.H. RATHOD, W.A. SHEIKH **AND** S. ACHARYA

Centre of Excellence for Research on Pulses, Sardarkrushinagar Dantiwada Agricultural University, SARDARKRUSHINAGAR (GUJARAT) INDIA

ARITCLE INFO

Received : 10.10.2014
Revised : 01.02.2015
Accepted : 17.02.2015

KEY WORDS :
RAPD, Butler, PCR, Primer, Molecular marker, Pigeonpea wilt, Molecular diversity

***Corresponding author:**
Email: amolshinde0110@gmail.com

ABSTRACT

Genetic diversity in pigeonpea wilt pathogen (*Fusarium udum* butler) was analyzed using 10 isolates collected from major pulse growing regions of Gujarat. The genomic DNA extracted from each isolate of *Fusarium udum* was subjected to polymerase chain reaction using 20 random decamer primers from OPC series. Only 10 of the 20 RAPD primers were selected on the basis of polymorphism for pooled analysis. The 10 earmarked RAPD primers selected from OPC series amplified 67 DNA fragments with size ranging from 157 to 2068bp. Out of these, 47 were polymorphic giving 70 per cent polymorphism. The total number of amplified fragments varied from 3 in OPC-8 to 11 in OPC-4. The average polymorphic bands per primer were 4.7 and per cent polymorphism ranged from 40 in OPC-15 to 100 in OPC-5. The PIC value varied from 0.64 in OPC-8 to 0.88 in OPC-4.

How to view point the article : Shinde, Amol S., Kalaskar, S.R., Rathod, A.H., Sheikh, W.A. and Acharya, S. (2015). RAPD based molecular diversity analysis of different *Fusarium udum* Butler isolates of pigeonpea wilt. *Internat. J. Plant Protec.*, **8**(1) : 81-85.

INTRODUCTION

Pigeonpea wilt caused by *Fusarium udum* Butler is one of the most serious soil borne diseases of pigeonpea in India and in other parts of the world (Nene *et al.*, 1989). Damage is further compounded when the vascular wilt fungus, *Fusarium udum*, is associated with the nematode. The percentage of wilted plants in such cases may go as high as 93.6 and can reach 100. Although much progress has been made in developing pigeonpea lines with resistance to biotic constraints and tolerance to abiotic stresses, yield loss in these crops is very high due to the high incidence of diseases and insectpests (Nene and Sheila, 1990). *Heterodera cajani* enhances the aggressiveness of *F. udum* in wilt-susceptible pigeonpea genotypes but not in wilt-tolerant or wilt-resistant genotypes. Losses due to wilt vary from negligible proportions to absolute (100 %) depending on the stage at which the crop is attacked (Datta and Lal, 2013). Control strategies for *Fusarium* wilt should target populations of *Fusarium udum*. However, *Fusarium udum* shows the great deal of variation in cultural and morphology characteristics. The high variation of cultural and morphology characteristics of this pathogen could be due to environment conditions, the age of the isolates, sub culturing, method of storage and culturing conditions. Wide variation in virulence to different genotypes of pigeonpea among Fusarium wilt isolates could be due to environmental conditions and inoculation techniques (Kiprop *et al.*, 2005).

The wilt hamstrung yields and sickens the soil of *Fusarium udum* that makes cultivation of crops rather impossible. Further, there are enormous variations in cultural and morphology characteristics of *Fusarium udum* among which Kanpur race, Bangalore race, Coimbatore race and

Hyderabad race have been widely reported (Mahesh *et al*., 2010). A lot of research has been conducted on Fusarium wilt since the 1930s, especially in India, yet the genetics of this destructive disease is still ambiguous (Odeny *et al*., 2009). Pal (1934) reported that resistance to wilt in pigeonpea was controlled by multiple factors while Shaw (1936) and later Pathak (1970) reported two complementary genes for resistance to Fusarium wilt. Pawar and Mayee (1986) reported that the resistance to Fusarium wilt is governed by a single dominant gene. In recent years, numerous DNA based methods have been increasingly used to study variability in pathogenic Fusarium population (Kiprop *et al*., 2002; Sivaramakrishnan *et al*., 2002). Considering wilt being very complex character and highly dependent upon physical selection conditions, environment, inoculums and favorable conditions, the present study carried out to ascertain molecular polymorphism in different isolates of *Fusarium udum* Butler.

MATERIAL AND METHODS

The present investigation entitled RAPD based molecular diversity analysis of different *Fusarium udum* butler isolates of pigeonpea wilt was carried out at the Department of Plant Molecular Biology and Biotechnology, C.P. College of Agriculture, Sardarkrushinagar and the Center of Excellence for Research on Pulses, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

***Fussarium udum* isolates :**

The experimental material comprising ten *Fussarium udum* isolates *viz.*, Dantiwada 1 (DNT1), Dantiwada 2 (DNT2), Navsari 1 (NVS1), Navsari 2 (NVS2), Banaskantha1 (BK1), Banaskantha 2 (BK2), Sabarkantha 1 (SB1), Sabarkantha 2 (SB2), Baroda 1 (BD1), Baroda 2 (BD2) were collected from different pigeonpea growing area of Gujarat.

Isolation of *Fusarium udum* :

Total 10 isolates of *Fusarium udum* were collected from different part of Gujarat. Small pieces (0.5cm^2) of vascular tissue was cut and placed aseptically onto plates having potato dextrose agar (PDA) medium. The plates were incubated at 25ºC in 12 hrs light/dark cycle for 36 to 48 hrs and colonies showing growth and morphology typical of *Fusarium udum* were transfer onto fresh PDA and incubated until conidia were produced. Conidial suspension from the culture was prepared and transferred in to PDA after 24 to 36 hrs and maintained as single spore isolates.

DNA isolation :

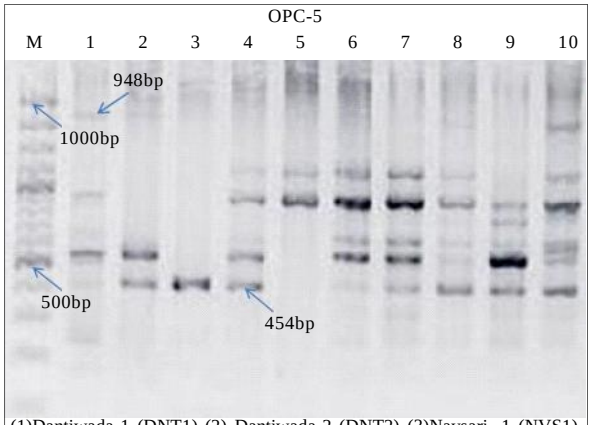
The DNA was extracted from 10 isolates of *Fuarium udum*. Czapek Dox medium was used to culture the isolates and mycelia were harvested after 7 days of incubation as described by Coddington and Gould (1992). Mycelial samples were frozen in liquid nitrogen and grounded to a fine powder with mortar and pestle. DNA extraction was done using Cetyl trymethyl ammonium bromide (CTAB) as proposed by Doyle and Doyle (1990). After the digestion of RNA with 10µl of 20µg/µl RNase A, the final DNA pellets were dissolved in 100 µl TE buffer and stored at 20ºC. The DNA was quantified by nanodrop and the quality was checked by running 3 µl of each sample on 0.8 per cent agarose gel.

RAPD-PCR analysis :

RAPD-PCR analysis was undertaken by using 10-mer primers. RAPD analysis was performed in 25µl reaction volumes containing 1×taq buffer (2.5 µl), 0.2 mMol dNTPs (0.5 µl), 1 unit of Taq Polymerase (1 µl), 4.0 mMol $MgCl_2$ (1.5 µl), 30 pmol primer (1 µl), and 10–20 ng genomic DNA. PCR reactions were carried out in a thermal cycler (Eppendorf) using the following profile: denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 1 min for 30 cycles, and a final extension at 72°C for 5 min. Following amplification, the samples were separated by electrophoresis in 1.4 per cent agarose gel and viewed in gel documentation unit.

RESULTS AND DISCUSSION

The 10 earmarked RAPD primers selected from OPC series amplified 67 DNA fragments with size ranging from 157 to 2068bp. Out of these, 47 were polymorphic giving 70 per cent polymorphism. The total number of amplified fragments varied from 3 in OPC-8 to 11 in OPC-4. The average polymorphic bands per primer were 4.7 and per cent polymorphism ranged from 40 in OPC-15 to 100 in OPC-5 (Plate 1). The PIC value varied from 0.64 in OPC-8 to 0.88 in OPC-4 (Table 1). In consonance to the present findings Gupta *et al*. (2001) have also reported enormous polymorphism in 16



(1)Dantiwada-1 (DNT1) (2) Dantiwada-2 (DNT2) (3)Navsari -1 (NVS1) (4)Navsari -2 (NVS2) (5)Banaskantha-1(BK1) (6)Banaskantha- 2 (BK2) (7)Sabarkantha- 1 (SB1) (8)Sabarkantha -2 (SB2) (9)Baroda- 1 (BD1) (10)Baroda 2 (BD2)

Plate 1 : RAPD pattern of different *Fusarium udum* isolates produced by primer OPC-05

isolates of *Xanthomonas oryzae* pv. *oryzae* (*X.o.* pv. *oryzae*) representing different geographical regions of India and Philippines. Similarly in cotton too, Elsalam *et al.* (2004) characterized 40 *Fusarium oxysporum* f.sp. vasinfectum isolates obtained from cotton growing provinces in Egypt and 5 reference strains representing physiological races 1, 2, 3, 4 and 5 through random RAPD and AFLP and reported high degree of polymorphism. Gherbawy *et al.* (2002) used RAPD technique for identifying *Fusarium subglutinans*, *F. proliferatum* and *F. verticillioides* strains isolated from maize in Austria. Pasquali *et al.* (2003) characterised isolates of *Fusarium oxysporum* pathogenic on *Argyranthemum*

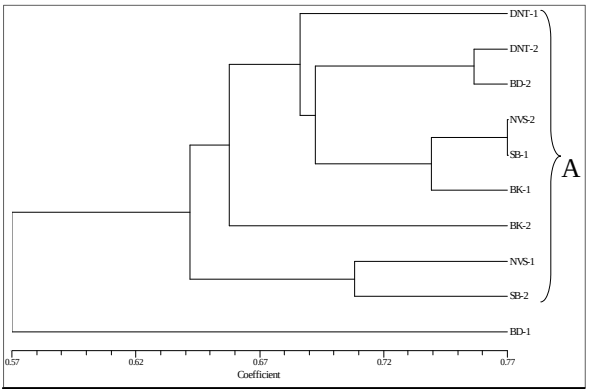


Fig. 1 : Dendrogram showing clustering of ten isolates of *Fusarium udum* constructed using UPGMA based on Jaccard's similarity coefficient obtained from OPC primer series

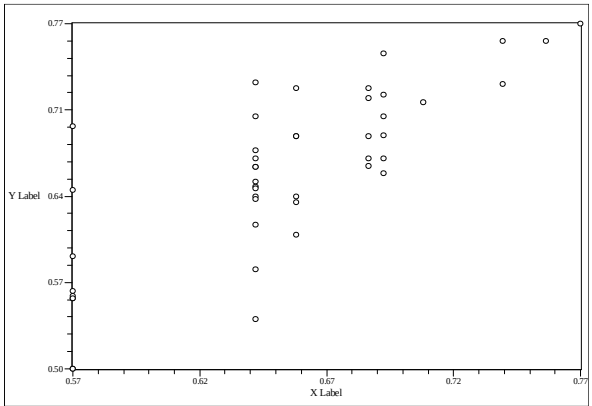


Fig. 2 : Scatter plot showing cophenetic correlation of ten *Fusarium udum* isolates using OPC primer series

Table 1 : Results of RAPD analysis in ten isolates of *Fusarium udum*

Sr. No.	Primers	Primer sequence (5'-3')	Molecular weight range (bp)	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Per cent polymorphism	Pic value
1.	OPC-02	GTGAGGCGTC	272-840	8	7	1	87	0.84
2.	OPC-04	CCGCATCTAC	345-2068	11	7	4	63	0.88
3.	OPC-05	GATGACCGCC	454-948	7	7	0	100	0.85
4.	OPC-07	GTCCCGACGA	310-1547	7	5	2	71	0.74
5.	OPC-08	TGGACCGGTG	515-641	3	2	1	66	0.64
6.	OPC-10	TGTCTGGGTG	234-1336	7	5	2	71	0.84
7.	OPC-13	AAGCCTCGTC	669-1745	7	3	4	42	0.84
8.	OPC-15	GACGGATCAG	256-864	5	2	3	40	0.73
9.	OPC-17	TTCCCCCCAG	174-1174	7	6	1	85	0.83
10.	OPC-19	GTTGCCAGCC	157-535	5	3	2	60	0.76

Table 2 : Jaccard's similarity co-efficient for different *Fusarium udum* isolates based on RAPD data analysis

R/C	DNT-1	DNT-2	NVS-1	NVS-2	BK-1	BK-2	SB-1	SB-2	BD-1	BD-2
DNT-1	1.0000									
DNT-2	0.7222	1.0000								
NVS-1	0.6363	0.7000	1.0000							
NVS-2	0.6842	0.6545	0.6603	1.0000						
BK-1	0.6666	0.6666	0.6734	0.7254	1.0000					
BK-2	0.6065	0.6315	0.5789	0.6842	0.6363	1.0000				
SB-1	0.7142	0.6851	0.6603	0.7735	0.7600	0.6842	1.0000			
SB-2	0.6440	0.6428	0.7115	0.6666	0.6481	0.5396	0.7272	1.0000		
BD-1	0.5892	0.5555	0.5576	0.5000	0.5000	0.5614	0.6415	0.6923	1.0000	
BD-2	0.6607	0.7600	0.6346	0.7169	0.7000	0.7222	0.7500	0.6140	0.5555	1.0000

frutescens L. using RAPD technique.

The dendrogram based on UPGMA analysis grouped all the *Fusarium udum* isolates except BD-1 into one main cluster designated as Cluster A. Among the nine isolates included in Cluster A, NVS-2 and SB-1 were found to be close to each other (Fig. 1).

Jaccard's pair-wise similarity co-efficient values for 10 isolates were calculated and are presented in Table 2. The genetic similarities ranged from 0.50 to 0.77 with average genetic similarity among them as 0.71. The highest similarity index value of 0.77 was observed between NVS-2 and SB-1; and the lowest value of 0.50 was exhibited between BD-1 and NVS-2; and BD-1 and BK-1 (Table 2).

Goodness of fit of clustering of OPC primers data was accomplished by estimating matrix of cophenetic values using the COPH program followed by comparing the estimated cophenetic matrices to the original matrices produced by SIMQUAL. The two matrices were plotted against each other and the association statistics were calculated by MXCOMP function in the NTSYSpc version 2.0. The scattered diagram of the *Fusarium udum* isolates is presented in Fig. 2. The matrix correlation was found to be r = 0.78 and the degree of goodness were appropriate for OPC primer series (Pawar *et al.*, 2012).

Data analysis :

Data was scored for computer analysis on the basis of the presence or absence of the PCR products. If a product was present in an isolate, it was designated as '1' and if absent; it was designated as '0'.The data generated by OPC primers were analyzed with the software NTSYSpc version 2.02 (Rohlf, 1994). The PIC values were calculated with formula :

$$PIC = 1 - \sum pi^2$$

(where, *pi* is the frequency of the *i th* allele) given by Smith *et al.*, 1997.

Conclusion :

The OPC primer series exhibited enormous polymorphism amongst the 10 isolates of *Fusarium* ranging from 40 per cent in OPC-15 to 100 per cent in OPC-5. The RAPD analysis grouped all the 10 isolates, except BD-1 in one group. This was despite 70 per cent polymorphism with PIC value varying from 0.64 to 0.88 so this data could be utilize to identify some new races of *Fusarium udum* butler. The genetic similarities among 10 isolates ranged from 0.50 between BD-1 with NVS-2 and BK-1 to 0.77 between NVS-2 and SB-1. In cluster analysis grouped all the *Fusarium udum* isolates except BD-1 into one main cluster designated as Cluster A, so there is need to more molecular study on BD-1 isolates towards identification of new race of *Fusarium udum* butler.

REFERENCES

Coddington, A. and Gould, D.S. (1992). *Use of RFLPs to identify races of fungal pathogen. In: techniques for rapid detection of plant pathogens.* Duncan, J.M. and Torrance, L. (Eds). Blackwell Scientific Publications, Oxford. 162-176pp.

Datta, J. and Lal, N. (2013). Application of molecular markers for genetic discrimination of *Fusarium* wilt pathogen races affecting chickpea and pigeonpea in major regions of India. *Cell. Mol. Biol.* **58**(1) : 55-65.

Doyle, J.J. and Doyle, J.L. (1990). A rapid total DNA preparation procedure for fresh plant tissue. *Focus,* **12** : 13-15.

Elsalam, K.A.A., Moawad, R.O., Migheli, Q. and Nirenberg, H.I. (2004). Genetic characterization of *Fusarium oxysporum* f. sp. Vasinfectum isolates by random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), *J. Plant Dis. & Protec.,* **111**(6) : 534-544.

Gherbawy, Y.A.M.H., Adler, A. and Prillinger, H. (2002). Genotypic identification of *Fusarium subglutinans, F. proliferatum* and *F. verticillioides* strains isolated from maize in Austria. *Mycobiol.,* **30** (3) : 139-145.

Gupta, V.S., Rajebhosale, M.D., Sodhi, M., Singh, Sukhwinder, Gnanamanickam, S.S., Dhaliwal, H.S. and Ranjekar, P.K. (2001). Assessment of genetic variability and strain identification of *Xanthomonas oryzae* pv. *oryzae* using RAPD-PCR and IS1112-based PCR. *Curr. Sci.*, **80** (8) : 1043-1048.

Kiprop, E.K., Baudoin, J.P., Mwang'ombe, A.W., Kimani, P.M., Mergeai, G. and Maquet, A. (2002). Characterization of Kenyan Isolates of *Fusarium udum* from pigeonpea [*Cajanus cajan* (L.) Millsp.] by Cultural Characteristics, Aggressiveness and AFLP Analysis. *J. Phytopathol.,* **150** (10) : 517-525.

Kiprop, E.K., Ombe, A.W.M., Baudoin, J.P., Kimani, P.M. and Mergeai, G. (2005). Genetic variability among *Fusarium udum* isolates from pigeonpea. *African Crop Sci. J.,* **13** (3) : 163-172.

Mahesh, M., Muhammad, S., Prasad, P.S. and Sreenivasa, S. (2010). Studied on cultural variability of *fusarium udum* isolates in India. *Internat. J. Sci. & Nature,* **1**(2) : 219-225.

Nene, Y.L. and Sheila, V.K. (1990). Pigeonpea: Geography and importance. In: *The Pigeonpea*. Nene, Y.L., Hall, S.D. and Sheila, V.K. (Eds.). CAB International, UNITED KINGDOM. 1-10pp.

Nene, Y.L., Sheila, V.K. and Sharroa, S.B. (1989). A world list of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L. Millsp.) pathogens. Legumes Pathology Progress Report-7, Patancheru, A.P. (India): International Crops Research Institute for the Semi-Arid Tropics.

Odeny, D.A., Jayashree, B., Gebhardt, C. and Crouch, J. (2009). New microsatellite markers for pigeonpea [*Cajanus cajan* (L.) millsp.]. *BMC Res. Notes,* **2** : 35. DOI: 10.1186/1756-0500-2-35.

Pal, B.P (1934). Recent progress in plant breeding at pusa. *Agr. Livest. India,* **4** : 505–515.

Pasquali, M., Acquadro, A., Balmas, V., Migheli, Q., Garibaldi, A. and Gullino, M.L. (2003). RAPD characterization of *Fusarium oxysporum* isolates pathogenic on *Argyranthemum frutescens* L. *J. Phytopathol.*, **151** (1) : 30-35.

Pathak, G.N. (1970). Red Gram. In: Pulse crops of Indian Council of Agricultural Research. New Delhi, India. 14–53pp.

Pawar, N.B and Mayee, C.D. (1986). Reaction of pigeonpea genotypes and their crosses to *Fusarium* wilt. *Indian Phytopathol.*, **39** : 70–74.

Pawar, S.V., Deshpande, G.D. and Dey, Utpal (2012). Survey and survillance of pigeonpea wilt in Marathwada region. *Internat. J. Pl. Protec.*, **5**(2) : 394-396.

Rohlf, F.J. (1994). NTSYS-PC. Numerical taxonomy and multivarate analysis system verson 2.02. Stat Unversity of New York, Stonybrook, New York.

Shaw, F.L.F. (1936). Studies in Indian pulses: the inheritance of morphological characters and of wilt resistance in Arhar (*Cajanus indicus* Spreng). *Indian J. Agric. Sci.*, **6** : 139–187.

Sivaramakrishnan, S., Kannan, S. and Singh, S.D. (2002). Detection of genetic variability in *Fusarium udum* using DNA markers. *Indian Phytopathol.*, **55** (3) : 258-263.

Smith, J.S.C., Chin, E.C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L., Mitchel, S.E., Kresorich, S. and Tiegle, J. (1997). An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. *Theoretical & Appl. Genet.*, **95** (1-2) : 163–173.