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BIOTECHNOLOGY

Molecular detection and genetic characterisation of fumonisin producing *fusarium* isolates from rice cultivars

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Abstract

Fusarium species mainly produce fumonisins group of mycotoxins which are classified as Group 2B human carcinogen by International Agency for Research on Cancer (IARC). In poor storage conditions, Fusarium species producing fumonisins can infect rice or paddy (Oryza sativa L.) which is the highest produced and consumed staple food in India. A rapid molecular method using primer Fum5F and Fum6R detected 85% fumonisin producers among 28 Fusarium isolates from Indian rice cultivars. Genetic variability of the isolates was studied by PCR based RAPD assay using 13 random primers. A total of 169 polymorphic bands were obtained by 13 markers with an average polymorphism information content (PIC) of 0.665 and overall polymorphism of 88%. Primer 3B showed a polymorphism of 96% with PIC value of 0.66 and it amplified 26 scorable fragments hence may be useful for the analysis of genetic variation among Fusarium isolates. Four strains (F47, F90, F92 and F96) in which fum gene wasn't amplified by Fum5F and Fum6R and supposed to be non producer of fumonisin have been consistently placed in one separate group by RAPD primers. Genetic variation of toxic Fusarium in rice from India is less studied. RAPD proved to be a suitable tool for depicting Polymorphism among the isolates. The high genetic variability among the Fusarium isolates used in the current study is a matter of concern considering the importance of Rice in India.

Highlights

- Fumonisins are Group 2B human carcinogen produced by *Fusarium* species.
- Fum5F and Fum6R primer pair detected fumonisin producers.
- RAPD primers deciphered 88% polymorphism among 26 *Fusarium* isolates.

Keywords: *Oryza sativa* L., cultivar, PIC, mycotoxins, polymorphism, PCR-RAPD

India is the second largest producer and consumer of rice in the world. Paddy or rice (*Oryza sativa* L.) is the staple food for 65% of the Indian population and is a high calorific cereal grain (DAC India, 2013). Due to unfavourable conditions during harvesting and processing, this elementary important cereal remains susceptible to infection by many mycotoxigenic fungi including species of *Fusarium* which cause subsequent deterioration during storage and produce Fumonisins (Makun *et al*., 2007; Maheshwar *et al*., 2009).

Fumonisins are group of mycotoxins produced mainly by members of the Gibberella fujikuroi complex e.g. *Fusarium verticillioides* (a synonym of *Fusarium moniliforme*) which mainly colonizes cereal grains. Fumonisin B1 (FB1) which is derived from F. verticillioides has been classified as Group 2B human carcinogen by the International Agency for Research

on Cancer (IARC). Fumonisins have been associated with leukoencephalomalacia in equine species, pulmonary edema in pigs, oesophageal cancer in humans and neural tube defects (neurotoxin) in human babies (Arino *et al.*,2007; Kushiro *et al.*,2008). Therefore the rapid detection of fumonisin producing Fusarium species is very important for its control and to prevent toxins entering the food chain.

Traditional methods for identification of *Fusarium* spp. producing fumonisins require considerable expertise, systematic sampling (as they form latent or asymptomatic infections), specialized growth media and require one or two weeks of growth before identification can be established (Nelson *et al*., 1993). Molecular genetic studies on mycotoxin production have been followed by the development of PCR-based detection of mycotoxins related fungal genes. In this regard the primer set Fum5F and Fum6R has been demonstrated to be very useful (Baired *et al*., 2008).

Fumonisin producing *Fusarium* isolates have been detected in rice from many countries across the world (Tonon *et al.,* 1997; Desjardins *et al*., 2000; Pacin *et al.,* 2002; Makun *et al*., 2007; Ebadi *et al*., 2013; Cruz *et al*., 2013;). Despite the importance of rice as a staple food and the reported occurrence of mycoflora and fumonisins, very few reports are available from India on the incidence of *Fusarium* isolates producing fumonisin and their genetic spectrum. (Maheshwar *et al*., 2009; Maheshwar and Janardhana, 2010).

A good knowledge of genetic variability in plant pathogens is required for strategies regarding plant disease management based on host resistance. Many workers have extensively studied PCR Based genetic variability or polymorphism. The Randomly Amplified Polymorphic DNA (RAPD) assay in which DNA segments are amplified by arbitrary primers, has been used for genetic characterisation of *Fusarium* species and other mycotoxigenic fungi (Nelson *et al*., 1997; Mostafa *et al*., 2002; Belabid *et al*., 2004; Sharma *et al*., 2006; Lourenço *et al*., 2007; Gupta *et al*., 2009; Mishra *et al*., 2010; Mohammadian *et al*., 2011; Gupta, 2012; Bahmani *et al*., 2012; Bonde *et al*., 2013; Ebadi *et al*., 2013). RAPD-genetic characterisation of *Fusarium* species from animal feed have already been carried out based on fumonisin production. (Ono *et al*., 2010; Yazeed *et al*., 2011; Daie-Ghazvini *et al*., 2011). Considering the importance of rice regarding food security in India genetic diversity of *Fusarium* isolates need to be explored from the view point of fumonisin production for setting up management strategies.

In view of the above mentioned facts the purpose of the present study was the quick detection of fumonisin producing *Fusarium* spp present in some popular rice (*Oryza sativa* L.) varieties of India by using the primer set Fum5F and Fum6R and depicting the extent of polymorphism among these strains of *Fusarium* by using RAPD assay.

Materials and Methods

Collection of Fusarium isolates

Twenty eight *Fusarium* cultures were studied during present investigation. These *Fusarium* cultures were collected from Plant pathology culture collection of Central Rice Research Institute, Cuttack. The source of isolation of these cultures was rice seeds.

Genomic DNA extraction and Detection of Fumonisin producers by PCR amplification

Genomic DNA of *Fusarium* cultures, grown on Potato Dextrose broth, was extracted according to Liu *et al*. (2000) with modifications.

Fumonisin producing *Fusarium* isolates were detected by Polymerase Chain Reaction (PCR) according to Baird *et al*. (2008). PCR was performed in 50 µl volumes which contained 20 ng of fungal DNA for each reaction, 1X Taq buffer (Fermentas), 1.5 mM of $MgCl_2$, 0.2 mM of each dATP, dCTP, dGTP, and dTTP, 10 picomoles of each forward and reverse primer (Fum5F and Fum6R) and 1unit of Taq DNA polymerase. The PCR was carried out in PTC-100 (MJ Research) thermal cycler which included the following temperature cycles: Initial denaturation at 95°C for 3 min. Then 32 cycles consisted of

denaturation at 95°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 3 min, and the final extension was at 72°C for 5 min.

Genetic variability of *Fusarium* isolates by RAPD PCR

Genomic DNA of *Fusarium* isolates was amplified by thirteen RAPD primers (Table 2). PCR conditions were optimized and amplification reactions were performed in thermocycler (MJ Research, PTC 100) in volumes of 25µl reaction mixture containing 1X PCR buffer, 2mM $MgCl_2$, 15 pico moles of primer, 0.3mM dNTP, 60ng of DNA and 1U of Taq DNA polymerase. Thirty five cycles of amplification was performed after initial denaturation of DNA at 93°C for 3min. Each cycle consisted of a denaturation step at 93°C for 60 seconds, annealing temperature as per Table 1 for 60 seconds and an extension step at 72°C for 2 min, with a final extension at 72°C for 5 min following the last cycle. The primers were retested in RAPD assays to assess the reproducibility of banding pattern. Only reproducible RAPD profiles were analyzed.

Table 1. Details of *Fusarium* Isolates

*Isolate ID	Source (Rice cultivar)	Fumonisin producing ability
F32	Sarala	+
F36	Sarala	+
F37	Sarala	+
F47	Sarala	-
F49	Sarala	+
F94	Sarala	+
F98	Sarala	+
F110	KMJ-1-17-2	+
F114	Akutphou	+
F115	Akutphou	+
F116	KMJ-1-17-2	+
F117	KMJ-1-17-2	+
F45	Sarala	+
F122	Meher	+
F40	Sarala	+
F113	Meher	+
F124	Meher	+
F55	Sarala	+
F111	NDR-8002	+
F44	Sarala	+
F119	Rambha	+
F90	Sarala	-
F91	Lunisree	+
F92	Sarala	-
F93	Lunisree	+
F96	Sarala	-
F97	Sarala	+
F121	Rambha	+

*CRRI, Plant Pathology culture collection

Scoring and data analysis

Data were compiled as a binary 0/1 matrix by the presence (1) or absence (0) of a band at particular position. All calculations were conducted by using the computer program NTSYS-PC version 2.02 (Rohlf, 1990). Genetic similarity matrix was calculated based on the method of simple matching coefficient and the values were used to generate a similarity matrix using SIMQUAL programme. The resulting matrix was analyzed for hierarchical clustering of the isolates by the Unweighted Pair Group Method with Arithmetic mean (UPGMA) by SAHN programme. The matrix was used to construct a dendrogram with TREE programme using the UPGMA for establishing to analyze the level of relatedness among the 28 isolates.

Results and Discussion

Detection of Fumonisin producing isolates

Among the 28 *Fusarium* isolates 86% were found to be Fumonisin producer as they consistently amplified at approx 419bp by FUM5F and FUM6R primer pairs. Those isolates were F32, F36, F37, F49, F94, F98, F110, F114, F115, F116, F117, F45, F122, F40, F113, F124, F55, F111, F44, F119, F91, F93, F97 and F121. Rest of the isolates i.e. F47, F90, F92 and F96 (14%) were non producers of fumonisin (Figure 1a and 1b).

Table 2. Details of 13 RAPD primers, amplified products and polymorphism explored by them.

Primers	Primer sequence (5' to 3')	GC %	Annealing Temp. (°C)	Amplified Fragment size range (bp)	No. of bands	No. of Polymorphic Bands	Poly-morphism %	PIC
R3	ACGATCGCGG	70	36	300-1600	11	6	54.5	0.492
R-1	CGGCCACCCT	80	38	165-2570	21	16	76.2	0.588
R-2	CGCGTGCCAG	80	38	300-2570	14	12	85.7	0.752
P-160	CATGGCCAGC	70	36	330-2750	15	13	86.7	0.639
PU-1	AGATGCAGCC	60	36	400-2860	16	14	87.5	0.599
ap12h	CGGCCCCTGT	80	36	350-2500	18	16	88.9	0.615
R-108	GTATTGCCCT	50	27	260-2875	18	16	88.9	0.653
P-117	TGGCGTCTCCA	63	40	585-2710	10	9	90	0.714
PU-2	ACGGATCCTG	60	36	355-3000	12	11	91.7	0.750
OPB-10	CTGCTGGGAC	70	36	465-3000	14	13	92.9	0.662
3B	GAGCGCCTTG	70	36	330-3800	26	25	96.1	0.66
P-54	GGCGATTTTTGCCG	57	50	820-1600	7	7	100	0.70
PU-3	ACTGGGACTC	60	35	465-4000	11	11	100	0.827

In order to investigate the genetic polymorphism within 28 *Fusarium* isolates, random amplified polymorphic DNA (RAPD) analysis was performed with thirteen random primers (Table 2). The GC content of primers was 50% (R-108) to 80% (R-1, R-2 and ap12h). Differences in banding patterns between isolates were assessed visually by observing amplified bands on agarose gel. All the thirteen primers were found to be polymorphic, amplified a total of 193 bands (averaging 14.84 bands per primer) of which 169 were polymorphic indicating a polymorphism of about 88% among 28 *Fusarium* isolates. Polymorphism by individual primers was shown in Table 2. This showed a high genetic variation among isolates. Primer wise maximum number of fragments (26 numbers) were obtained by primer 3B (Figures 2a and 2b) and lowest number by primer P-54 (7 fragments). The size of amplified bands ranged from 165bp (by primer R-1) to 4000bp (by primer PU-3; Table 2). Isolate wise from the combination of 13 primers, highest number of fragments was amplified in F115 (107) followed by F40 with 106 bands. All other isolates were having fragments ranging from 81 to 98 numbers except that of F47 which had a total of 74 fragments.

Polymorphism Information Content (PIC) of primers was found to be highest by PU-3 (0.827) and lowest for R3 (0.492) with an average of 0.665 for 13 primers. When percentage of polymorphism was taken into consideration, all the fragments amplified by primers P-54 and PU-3 were found to be polymorphic but only 7 and 11 number of amplified bands were obtained by these two respectively. Primer 3B which has GC content of 70%, showed a polymorphism of 96% with PIC value of 0.66 and it amplified 26 scorable fragments hence may be useful for the analysis of genetic variation among *Fusarium* isolates (Table 2; Figures 2a and 2b).

The similarity index depicted a minimum of 36% to maximum 98% similarity between 28 *Fusarium* isolates (Table 3). The dendrogram constructed from cluster analysis separated 28 *Fusarium* isolates in to two major groups 'A' and 'B' (Figure 3) at a similarity co efficient of 0.52. One branch in group 'A' included F47, F90, F92 and F96 at a similarity level more than 87%. These four isolates weren't amplified by Fum5F and Fum6R, indicating non producers of fumonisin and have been consistently placed in one separate group by RAPD primers ap12h, R-1, R-2, PU-1, PU-

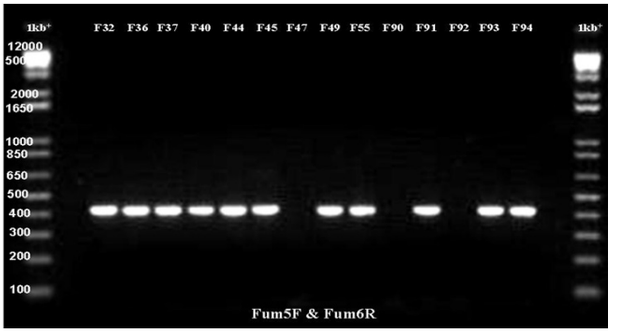

Figure 1a. PCR amplification of Fusarium isolates (F32 to F94) by primer pair Fum5F and Fum6R. F47, F90 and F92 show no amplification

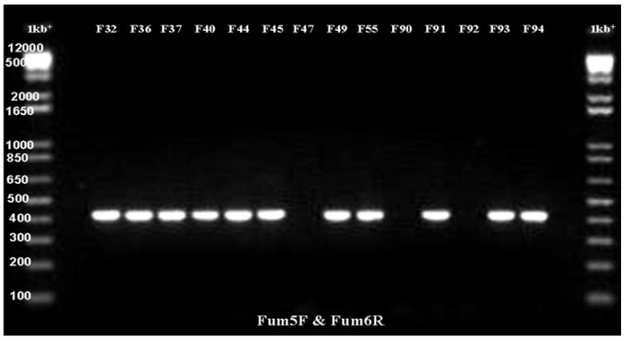

Figure 1b. PCR amplification of Fusarium isolates (F96 to F124) by primer pair Fum5F and Fum6R. F96 showing no amplification

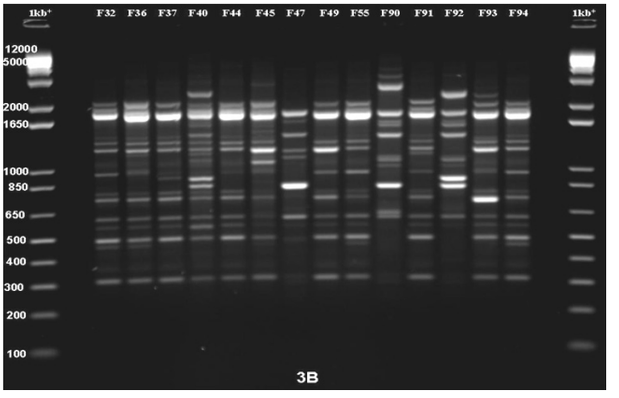

Figure 2a. RAPD patterns on 1.5% agarose gel of amplified fragments generated from Fusarium isolates with primer 3B. F32 to F94

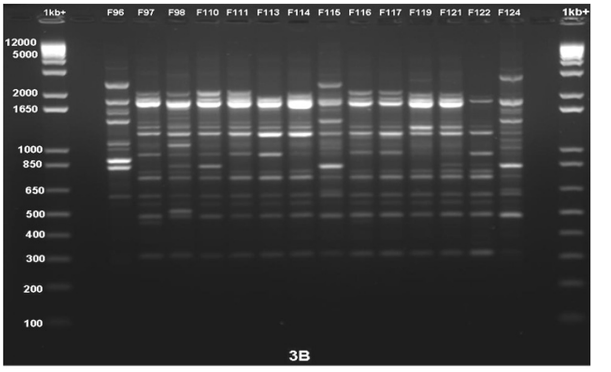

Figure 2b. RAPD patterns on 1.5% agarose gel of amplified fragments generated from Fusarium isolates with primer 3B. F96 to F124.

Genetic variability of *Fusarium* isolates

3, OPB-10 and 3B. These four isolates were grouped together more by the absence of bands at a particular position as depicted in case of primer ap12h where absence of unique bands was marked at 600bp and 480bp.

Relatively more similarity with the fumonisin non producer group was shown by F40, F115 and F124 as some primers such as P-160, R-3, R-108, R-2, PU-2, P-117 and P-54 placed them closely to the group of fumonisin non producers. The rest of isolates F32, F36, F37, F44, F45, F49, F55, F91, F93, F94, F97, F98, F110, F111, F113, F114, F116, F117, F119, F121 and F122 were distinguished from each other and placed in 14 separate branches in group B.

Detection of fumonisin producers

Amplification of fumonisin producing isolates by primer Fum5F and Fum6R confirms the findings of Baired *et al.* (2005) where 96% of *Fusarium verticillioides* have been amplified at 419bp region. Similarly Ivic *et al*. (2011) amplified 29 *F. verticillioides* isolated from barley grain in Bosnia and Herzegovina,

Table 3. Similarity matrix of 28 *Fusarium* isolates depicted by 13 RAPD primers

	F32	F36	F37	F40	F44	F45	F47	F49	F55	F90	F91	F92	F93	F94	F96	F97	F98	F110	F111	F113	F114	F115	F116	F117	F119	F121	F122	F124
F32	1																											
F36	0.96	1																										
F37	0.88	0.87	1																									
F40	0.65	0.65	0.69	1																								
F44	0.89	0.88	0.93	0.67	1																							
F45	0.86	0.86	0.84	0.63	0.82	1																						
F47	0.47	0.45	0.47	0.66	0.46	0.48	1																					
F49	0.84	0.82	0.82	0.6	0.81	0.79	0.45	1																				
F55	0.94	0.91	0.85	0.62	0.85	0.81	0.46	0.85	1																			
F90	0.39	0.36	0.39	0.61	0.39	0.41	0.86	0.35	0.36	1																		
F91	0.91	0.93	0.89	0.64	0.89	0.85	0.45	0.84	0.89	0.38	1																	
F92	0.42	0.39	0.43	0.69	0.43	0.44	0.89	0.39	0.4	0.88	0.4	1																
F93	0.88	0.9	0.91	0.64	0.91	0.82	0.44	0.84	0.87	0.36	0.89	0.4	1															
F94	0.97	0.95	0.86	0.62	0.88	0.84	0.45	0.82	0.95	0.36	0.91	0.39	0.9	1														
F96	0.41	0.38	0.41	0.68	0.41	0.42	0.88	0.38	0.4	0.87	0.39	0.98	0.39	0.38	1													
F97	0.97	0.94	0.87	0.63	0.88	0.86	0.47	0.82	0.93	0.39	0.88	0.42	0.87	0.95	0.41	1												
F98	0.83	0.81	0.88	0.66	0.84	0.84	0.51	0.77	0.79	0.45	0.82	0.47	0.84	0.8	0.45	0.82	1											
F110	0.93	0.93	0.87	0.61	0.87	0.85	0.46	0.82	0.88	0.39	0.91	0.41	0.88	0.92	0.39	0.93	0.84	1										
F111	0.9	0.91	0.9	0.62	0.87	0.86	0.47	0.81	0.86	0.39	0.91	0.41	0.87	0.89	0.39	0.9	0.86	0.95	1									
F113	0.87	0.86	0.84	0.63	0.84	0.78	0.46	0.78	0.82	0.41	0.82	0.43	0.82	0.85	0.43	0.88	0.8	0.87	0.88	1								
F114	0.87	0.86	0.93	0.66	0.92	0.81	0.48	0.82	0.83	0.42	0.87	0.44	0.89	0.85	0.42	0.88	0.86	0.91	0.91	0.86	1							
F115	0.64	0.62	0.67	0.81	0.66	0.65	0.7	0.59	0.63	0.65	0.64	0.7	0.64	0.63	0.68	0.64	0.68	0.64	0.65	0.64	0.68	1						
F116	0.91	0.92	0.86	0.65	0.85	0.85	0.47	0.82	0.86	0.42	0.89	0.42	0.86	0.88	0.4	0.91	0.82	0.93	0.91	0.85	0.88	0.66	1					
F117	0.92	0.93	0.89	0.66	0.87	0.86	0.49	0.81	0.87	0.41	0.9	0.43	0.88	0.9	0.41	0.92	0.85	0.94	0.93	0.86	0.9	0.68	0.97	1				
F119	0.88	0.88	0.92	0.69	0.9	0.83	0.52	0.84	0.86	0.42	0.87	0.46	0.91	0.88	0.44	0.9	0.88	0.89	0.9	0.87	0.93	0.69	0.87	0.9	1			
F121	0.86	0.87	0.92	0.68	0.91	0.84	0.47	0.81	0.83	0.39	0.87	0.45	0.89	0.85	0.43	0.87	0.87	0.88	0.88	0.84	0.91	0.68	0.86	0.88	0.94	1		
F122	0.85	0.82	0.84	0.64	0.81	0.78	0.51	0.78	0.82	0.43	0.81	0.47	0.8	0.82	0.47	0.86	0.82	0.86	0.85	0.92	0.84	0.68	0.85	0.86	0.85	0.84	1	
F124	0.65	0.62	0.65	0.72	0.64	0.65	0.69	0.58	0.63	0.66	0.62	0.65	0.61	0.64	0.65	0.64	0.68	0.63	0.64	0.64	0.65	0.79	0.63	0.65	0.67	0.65	0.63	1

with Fum5F and Fum6R primer pairs and 419 bp amplification were recorded for all. Hence unlike conventional culture methods, PCR amplification of FUM genes could become rapid and reliable method for detecting potential fumonisin producing fungi as this polyketide synthase gene may have unique primer binding sites (Ivic *et al*., 2011; Karthikeyan *et al*., 2011).

Genetic variability of *Fusarium* isolates

The mould contamination, particularly with mycotoxin-producing fungi is a worldwide problem. It has been estimated that 25% of the world's crop product is contaminated with mycotoxins. *Fusarium* mycotoxin such as fumonisin has adverse health effects on animals and human hence RAPD-PCR based genetic polymorphism assay was carried out among the 28 *Fusarium* isolates which were isolated from popular rice varieties of India.

Bonde *et al*. (2013) obtained 180 polymorphic RAPD loci (amplified fragments were ranged 1 kb to 3.0 kb) in 14 mycotoxic *Fusarium* species with 25 primers and suggested RAPD marker to be a powerful tool to analyze the genetic variation among the *Fusarium* species. Similarly Sharma *et al*. (2006) and Belabid *et al*. (2004) amplified 134 and 8 polymorphic markers with 10 and 6 primers respectively. A set of seven random primers used by Bahmani *et al*. (2012) revealed a total of 36 alleles in 24 fumonisic *F. verticillioides.* Gupta (2012) and Mishra *et al*. (2010) tested 30 and 25 RAPD primers respectively with the genome of *Fusarium* spp. causing wilt disease and found the DNA band size ranged from 200 bp to 5090 bp and 300bp to 2kb respectively. Mishra *et al*. (2010) also reported up to 20% genetic diversity by RAPD profiles of ten *Fusarium oxysporum f. sp. lycopersici strains*. Yazeed *et al*. (2011) observed 88% polymorphism among 21 fumonisin producing *Fusarium* species from feed samples using 5 primers. Daie-Ghazvini, *et al*. (2011) also observed high degree of genetic variation among 20 fumonisin producers using 4 RAPD primers.

In clone or genotype identification, the resolution power is decided by the number of polymorphic loci used and the allele frequency. Ono *et al*. (2010) discriminated 16 fumonisin producing *Fusarium* isolates with 60 RAPD polymorphic loci but no clear association between RAPD makers and fumonisin production was observed. In the current study, the 169 RAPD polymorphic loci were sufficient to discriminate all the *Fusarium* isolates, including the isolates from the same seed sample, e.g. the fumonisin non producers F47, F90, F92, F96 and the fumonisin producers F32, F36, F37, F49, F94, F98, F45, F40, F55, F44, F97 isolated from the same variety 'Sarala' were discriminated extensively, though the previous group (fumonisin non-producer) was placed as a separate node in the UPGMA dendrogram (Figure 3). Isolate F114 and F115 showed only 68% relatedness even if both are fumonisin producers and isolated from same rice variety 'Akutphou'. Similar genetic variability was also observed in the other isolates from seeds of same rice variety (Table 1 and Figure3).

Wang *et al*. (1997) described that even in the same plant many unrelated isolates could be found, suggesting multiple infections by independent spores. In corn plant infection with distinct biotypes of *Fusarium* genera has been reported by Kedera *et al*. (1994). Such genetic variation among *Fusarium* isolates producing fumonisin has been rarely studied in seeds of Indian rice cultivars. All the *Fusarium* isolates used in this study have been collected from Indian popular rice cultivars and the high degree of genetic diversity is a matter of concern. These variations might arise due to mutation and recombination within the *Fusarium* population. As is common with most organisms, plant-pathogenic fungi rely on the processes of mutation and recombination as the ultimate source of genetically based variation. Within a species, gene flow between populations supplements these processes as propagules spread from one epidemiological area to another and from one deme to the next (Burdon and Silk, 1997). The knowledge of the genetic structure of pathogen populations has direct agricultural applications. For instance, the genetic variation maintained within a population indicates the speed at which a pathogen evolves. This information might eventually be used to predict the efficacy of control measures, e.g. the

use of resistant cultivars or fungicides application. (Mohammadian *et al*., 2011). Most strategies for plant disease management based on host resistance require a good knowledge of variability in plant pathogens. The RAPD-PCR assay conducted in the present study revealed high genetic variation. It indicates that management strategies aiming destruction of primary source of inoculum may be more useful for management of the destructive toxigenic strains of this pathogen. *Fusarium* species secrets mycotoxin and to reduce mycotoxin contamination in food and feed by these species as well as to search for remedy for infected food and feed, the present study of genetic diversity of *Fusarium* species will be useful.

The results of the present study revealed that PCR based techniques which use multi locus markers could be used not only to differentiate the *Fusarium* from each other but also to identify fumonisin producing *Fusarium* species. DNA polymorphism amplified by random primers has been useful genetic markers in a variety of species (Williams *et al.,* 1990). Universal primers theoretically anneal to the intergenic target sites which are randomly dispersed in genomes and provide the amplifications of different lengths of fragments (Ebadi *et al*., 2013). Molecular diversity study of *Fusarium* species characterized by the use of random primers has a great rate of success. The primers used in our study; R-1, R-2, R-3, PU-1, PU-2,

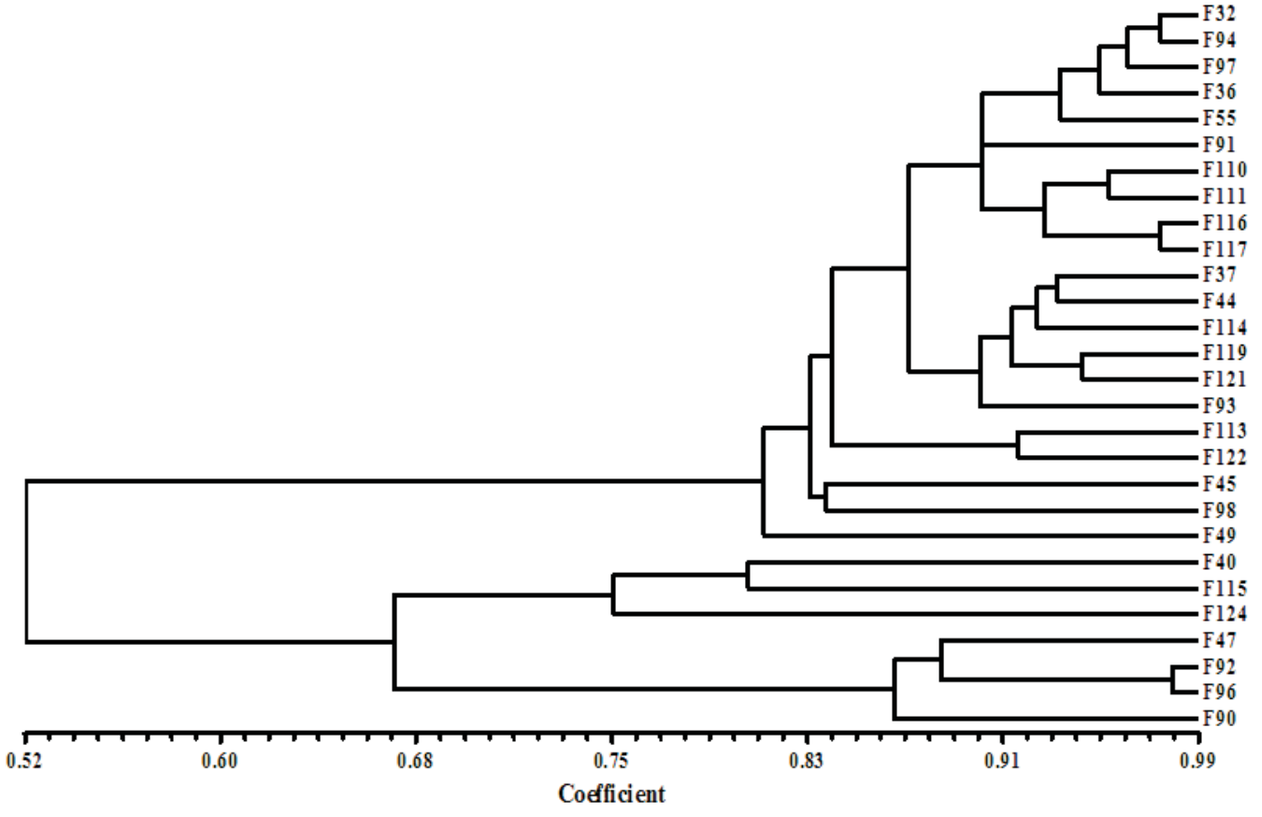

Figure 3. UPGMA cluster analysis based dendrogram depicting genetic variability among 28 Fusarium Calculated from 193 bands generated by 13 RAPD primers

PU-3, P-160, P-117, P-54, ap12h, R-108, OPB-10 and 3B has been used to differentiate *Fusarium* as well as other toxigenic fungi. (Diaz-Guerra *et al.*, 2000; Mostafa *et al.*, 2002; Lourenco *et al.*, 2007). Previously reported data indicated that, molecular techniques based on the polymerase chain reaction (PCR) have been used as a tool for genetic mapping, molecular taxonomy, evolutionary studies, and diagnosis of several fungal species. RAPD analysis also offers several advantages that may be useful in studying races of *Fusarium* (Nagarajan *et al.*, 2004; Bhim *et al.*, 2006), and also to characterize strains of many *Fusarium* spp. (El-Fadly *et al.*, 2008). The analysis of DNA products generated through random amplified polymorphic DNA (RAPD) has provided information on variation within and between *Fusarium* species which are in agreement with Belabid *et al.* (2004). RAPD is simpler to perform and the sensitivity can be increased by analysing more no of primers enabling a more detailed population genetic analysis of this fungus which is important from fumonisin production as well as pathogenic point of view.

Conclusion

As Rice is a very important staple food of India, the detection of fumonisin producing *Fusarium* isolates and the high genetic variability among them certainly indicates more study using more isolates and markers. This information may help to design suitable strategy for the management of this devastating pathogen.

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