**BIODIVERSITAS**
Volume 14, Number 2, October 2013
Pages: 55-60

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d140201

# Genetic diversity among fourteen different *Fusarium* species using RAPD marker

**SHITAL R. BONDE♥, ANIKET K. GADE, MAHENDRA K. RAI**
Department of Biotechnology, S.G.B. Amravati University, Amravati-444602, Maharashtra, India. Tel: +91-721-2662208-9; Ext 267, Fax: +91-721-2662135, 2660949, ♥email: shitalbonde@gmail.com

Manuscript received: 4 February 2013. Revision accepted: 22 May 2013.

## ABSTRACT

*Bonde SR, Gade AK, Rai MK. 2012. Genetic diversity among fourteen different* Fusarium *species using RAPD marker. Biodiversitas 14: 55-60.* We report genetic diversity of total fourteen different *Fusarium* species by RAPD-PCR analysis using 25 random primers. The genus *Fusarium* is food borne pathogen responsible for T-2 toxin production which affects human and animal health. In the present study, total 14-different species of *Fusarium* were analyzed on the basis of genetic diversity using RAPD method. A dendrogram was developed by UPGMA method. RAPD analysis was carried out by using 25 different universal primers each of them consisted of 10 bases. Genetic similarity coefficients between pair wise varied from 0.00 to 0.9 based on an unweighted paired group method of arithmetic average (UPGMA) cluster analysis. RAPD-PCR technique can be used as an important tool for the genetic differentiation *Fusarium* species.

**Key words:** *Fusarium*, genetic diversity, RAPD, UPGMA.

## INTRODUCTION

The word 'mycotoxin' is used for the toxic chemical products produced by fungi that readily colonize crops in the field or after harvest (Richard et al. 2007; Turner et al. 2009). Mycotoxins are secondary metabolites produced by certain filamentous fungi, which can be produced in food and food-products as a result of fungal growth. They cause a toxic response, termed as mycotoxicosis, when ingested by higher vertebrates and other animals (Menaka et al. 2011).

T-2 toxin is a Type A chemical class of non-macrocyclic trichothecenes. The principle fungus responsible for the production of T-2 toxinis is *Fusarium sporotrichioides* (CAST 2003). T-2 toxin is produced by various species of *Fusarium,* which are widespread on a variety of plants and in soil throughout the cold temperate regions (Omurtag et al. 2001). T-2 toxin is generally found in various cereal crops such as wheat, corn, barley, rye, oats and processed grains (malt, beer and bread) (SCF 2001). Symptoms of T-2 toxin include nausea, emesis, dizziness, chills, abdominal pain, diarrhea, dermal necrosis, irreversible damage to the bone marrow, reduction in white blood cells (aleukia), inhibition of protein synthesis, and is toxic for the hematological and lymphatic systems (Omurtag et al. 2001).T-2-contaminated products can cause severe effects in humans/animals which can result in death (Moss and Long 2002). T-2 toxin also alters the level of dopamine, tryptophan, serotonine and serotonine metabolites in the brain of rodents and pigs (Pestka and Smolinski 2005). T-2 toxin is quite known for inhibition of DNA, RNA and protein synthesis, mitochondrial function as well as other subcellular processes, and to cause death of eukaryotic cells (Gyongyossy-Issa et al. 1985). The genus *Fusarium* is commonly associated with many economically important crop diseases, however, distribution and diversity of this species is very important (Latiffah et al. 2007). Wang et al. (Wang et al. 1993) reported human toxicosis caused by mouldy rice contaminated with *Fusarium* and T-2 toxin. Mycotoxin ingestion by humans, which occurs mainly through plant-based foods and the residues and metabolites present in animal-derived foods can lead to deterioration of liver or kidney function The mycotoxigenic fungi involved with the human food chain belong mainly to three genera *Aspergillus, Fusarium* and *Penicillium.* While *Fusarium* species are destructive plant pathogens producing mycotoxins before, or immediately alters harvesting. The use of molecular markers based on the polymerase chain reaction for species identification and as diagnostic tool has become very popular during the last decade (Sabir 2006).

RAPD assays have been used extensively to define fungal populations at species, intraspecific, race and strain levels (Miller 1996; Ingle et al. 2009) and RAPD-PCR is technique for detecting genetic variability (Edwards et al. 2002; Sabir 2006). Different molecular markers are available for the differentiation of fungal taxa (Steinkellner et al. 2008). Mostly used Random amplified polymorphic DNA (RAPD) (Gupta et al. 2009) and amplified fragment length polymorphisms (AFLP) (Niessen 2007) are specific PCR-based molecular markers. These markers demonstrated remarkable genetic variation (Skaria et al. 2011). RAPD technique has been used since a long time for phylogenetic studies (Niessen 2007; Gupta et al. 2009).

There are several reports on differentiation of *Fusarium* species using RAPD markers. Gupta et al. (2009) reported the genetic polymorphism among six isolates of *F. solani* causing wilt disease in guava, isolated from different places in India. Ingle and Rai (2011) reported genetic diversity of *F. semitectum*, *Fusarium* associated with mango malformation were identified and analysed for their genetic diversity among *Fusarium* isolates (Smith et al. 2001; Arif et al. 2011). Similarly, a genetic variation in *F. oxysporum* f. sp. *fragariae* causing wilt disease in strawberry was characterized by Nagarajan et al. (2004). RAPD markers are easy, rapid for an evaluation of genetic variation (Niessen 2007). The study of RAPD analysis has been used widely in phylogenetic analysis of bacteria, fungi and plants (Singh et al. 2011).

The aim of the present study was to estimate the genetic diversity of 14-different secreting *Fusarium* species studied by RAPD-PCR.

## MATERIALS AND METHODS

### Fungal species

Different *Fusarium* were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India (Table 1).

**Table 1.** Fourteen different *Fusarium* cultures were used for RAPD analysis

| Cultures | *Fusarium* species |
|---|---|
| MTCC-3325 | *F. avenaceum* |
| MTCC-3731 | *F. equiseti* |
| MTCC-350 | *F. solani* |
| MTCC-7375 | *F. sporotrichioides* |
| MTCC-349 | *F. culmorum* |
| MTCC-3730 | *F. tricinctum* |
| MTCC-636 | *F. lateritium* |
| MTCC-156 | *F. moniliforme* |
| MTCC-1755 | *F. oxysporum* |
| MTCC-6580 | *F. nivale* |
| MTCC-2086 | *F. poae* |
| MTCC-1983 | *F. acuminatum* |
| DBT-18 | *F. graminearum* |
| DBT-21 | *F. semitectum* |

### DNA Isolation

Different *Fusarium species* were grown on Potato Dextrose Agar (PDA) at 25 ± 2$^0$C for 3 days. The mycelia grown were harvested and total DNA was extracted using fungal genomic DNA isolation kit from Chromous Biotech Pvt. Ltd, Bangalore, India according to manufacturer's instructions.

### RAPD analysis

Twenty five fungal primers from Random Fungal Primer Kit (RFu 'D') Genie Pvt. Ltd, Bangalore, India, were evaluated for PCR amplification of 14 *Fusarium* species. In the preliminary experiments, 12 out of the 25 primers tested produced distinct and reproducible band profile, and polymorphisms produced by ten primers. Four of 12 primers were used for comparative analysis of the forteen *Fusarium* species. The primers, including Rfu-9 (5'-CCTGGGTGCA-3'), Rfu-10 (5'-CCTGGGTGAC-3'), Rfu-23 (5'-CCGGCCATAC-3') and Rfu-25 (5'-CCGGCTGGAA-3') (Table 2).

**Table 2.** Primer and their sequences tested in RAPD analysis.

| Sequences 5'-3' | Primer |
|---|---|
| CCTGGGCCAG | RFu 1 |
| CCTGGGCGAG | RFu 2** |
| CCTGGGCTGG | RFu 3 |
| CCTGGGCTAT | RFu 4** |
| CCTGGGCTTG | RFu 5 |
| CCTGGGCTAC | RFu 6 |
| CCTGGGCTTA | RFu 7** |
| CCTGGGTCGA | RFu 8 |
| **CCTGGGTGCA** | **RFu 9*** |
| **CCTGGGTGAC** | **RFu 10*** |
| CCTGGCTTAC | RFu 11 |
| CCTGGGTTAC | RFu 12** |
| CGGGGGATGG | RFu 13 |
| CTCCCTGACC | RFu 14 |
| GAGCACCTGT | RFu 15** |
| GAGCACGTCA | RFu 16 |
| GAGCACGGCA | RFu 17 |
| GAGCACGGAG | RFu 18** |
| GAGCTCGCAT | RFu 19 |
| GAGGGCATGT | RFu 20 |
| CCGGCCCCAA | RFu 21 |
| CCGGCCTTAA | RFu 22** |
| **CCGGCCATAC** | **RFu 23*** |
| CCGGCCTTCC | RFu 24** |
| **CCGGCTGGAA** | **RFu 25*** |

Note: *) Primers used in this study; **) Primers produced distinct and reproducible band, but not used in this study.

Preliminary amplifications determined the optimal concentration of the component in the PCR reaction mixture and amplification conditions. Amplifications were performed in a total volume of 25 µL containing 12.5 µL PCR master mix (2X) (Fermentas Life Sciences, Canada) 5 µL of template DNA (20 ng), 1.5 µL $MgCl_2$ (25 mM), 0.3 µL *Taq* DNA polymerase (Genexy, 5U/µL), 1 µL each primer and 4.7 µL nuclease free distilled water (supplied with Fermentas PCR master mix).

PCR was carried out on gradient PCR machine (Palm-Cycler from Corbett Research, Australia). The program included an initial denaturation at 94$^0$C for 2 min, 35 cycles with denaturation at 94$^0$C for 30 sec, annealing 40 $^0$C for 1 min, extension at 72$^0$C for 2 min and final extension at 72$^0$C for 5 min with holding temperature at 4$^0$C for 10 min. All experiments were repeated for three times. PCR products were electrophorezed on 1.5% agarose by using 1X TAE buffer (Fermentas Life Sciences, Canada), stained with ethidium bromide, visualized in a UV-transilluminator and the gel were photographed using Gel Doc (AlphaImager, Gel documentation system, USA), system.

### Data analyses

Statistical analyses of all 14-different *Fusarium* were carried out using software PAST PAleontological STatistics (Version 2.07). While, Unweighted Pair Group

Method with Arithmetic Mean Analysis (UPGMA) was used to construct phylogenetic dendrogram. This method is one of the oldest techniques to be used in phylogenetic analysis, is an offshoot of the linkage methods that were popular in numerical taxonomy studies. Its simplicity and ease of interpretation has made its survival in phylogenetic studies. It works on the assumption that the rates of evolution in all lineages are same and gives output clustering in increasing order of distance (Sahoo et al. 2010).

## RESULTS AND DISCUSSION

### RAPD analysis

Genomic DNA isolated from 14 different species was subjected to RAPD-PCR analysis with 25 random decamer primers of Fungal RAPD Primer (RFu 'D') kit (Table 2). In the preliminary experiments, 12 out of the 25 primers tested produced distinct and reproducible band profile, and polymorphisms produced by ten primers. Four of 12 primers were used for comparative analysis of the fourteen *Fusarium* species. The primers, including RFu 9, RFu 10, RFu 23 and 25 generated polymorphic bands in all 14 different species (Figure 1).

All the amplified fragments were ranged 1 kb to 3.0 kb. RAPD assays of all 14 species with four above mentioned primers yielded 180 bands which were found to be polymorphic. Above data showed that RAPD is a convenient method for distinguishing the different species of *Fusarium* and also reveal a significant genetic variation among these species. There was other most studied *Fusarium* species viz. *F. oxysporum* also showed the genetic variation (Ingle and Rai 2011). Assigbetse et al. (1994) differentiated races of *F. oxysporum* f. sp. *vasinfectum* on cotton by using RAPD as molecular tool and Bonde et al. (2013) studied genetic variation of *F. equiseti* isolated from fruits and vegetables. In another study carried out by Edel et al. (2001) it was observed that the isolates of *F. oxysporum* isolated from soil samples in France showed genetic diversity. While, Nagarajan and

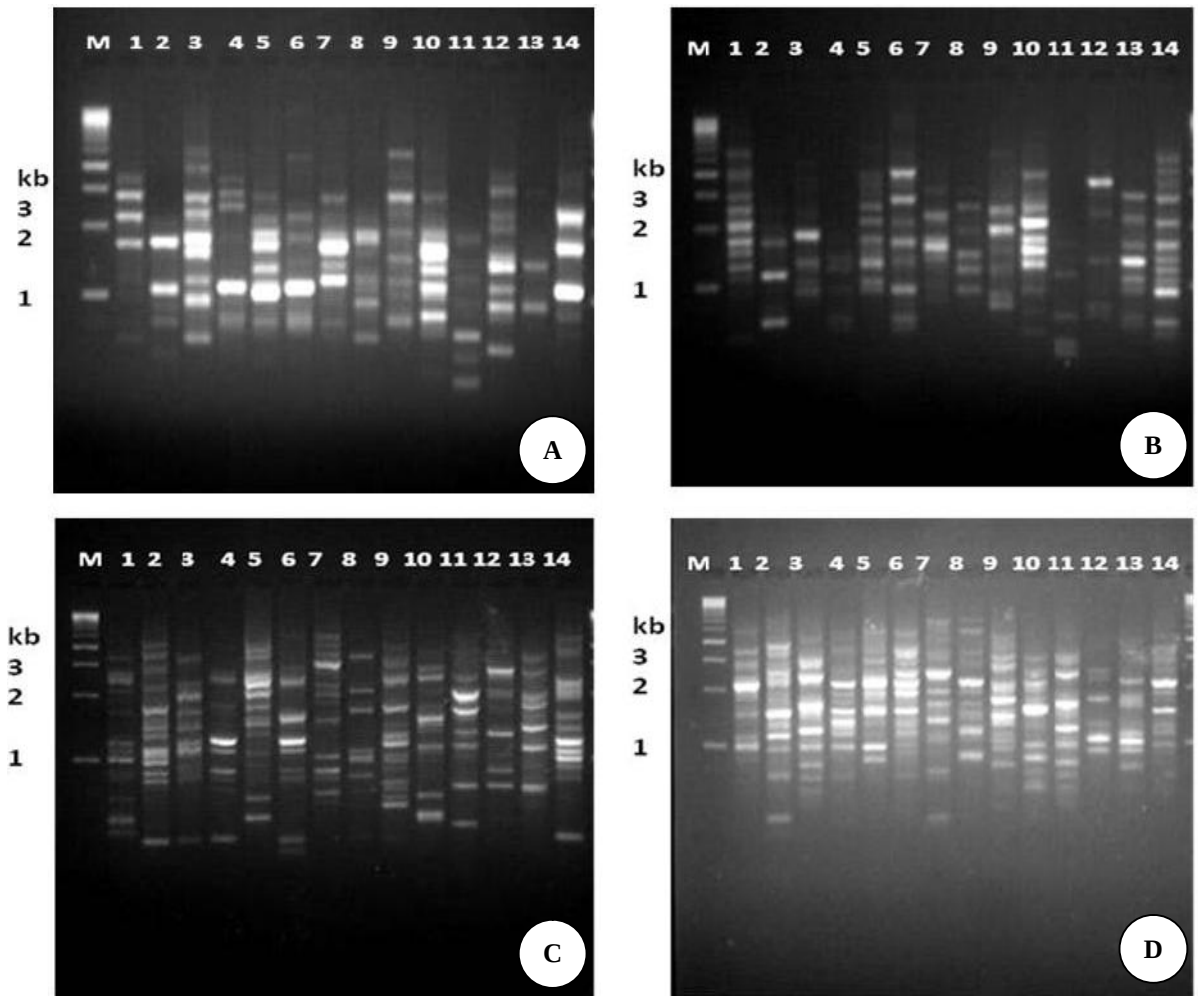


**Figure 1.** RAPD patterns on 1.5% agarose gel of amplified fragments generated from different *Fusarium sp*. with primers RFu-9 (B) RFu-10 (C) RFu-23 (D) RFu-25. Lane M, DNA marker (1 kb), lane 1. *F. avenaceum*, lane 2. *F. equiseti*, lane 3. *F. solani*, lane 4. *F. sporotrichioides*, lane 5. *F. culmorum*, lane 6. *F. tricinctum*, lane 7. *F. lateritium*, lane 8. *F. moniliforme*, lane 9. *F.oxysporum*, lane 10. *F. nivale*, lane 11. *F. poae*, lane 12. *F. acuminatum*, lane 13. *F. graminearum*, lane 14. *F. semitectum*

group (2004) studied the genetic variation in *F. oxysporum* f. sp. *fragariae* population causing wilt in strawberry using RAPD and RFLP analysis. The work carried out by Leslie et al. (2006) supports the findings of present study. They observed inter-and intra specific genetic variation in different *Fusarium* species. RAPD-PCR technique as suitable method was used to rapid identification and differentiation of *Fusarium* species (Pujo et al. 1997; El-Fadly et al. 2008). Gupta et al. (2009) reported the genetic polymorphism and diversity in isolates of *F. solani* isolated from wilt disease of Guava in India. Ingle et al. (2009) suggested RAPD marker is important, reliable tool for genetic variation among ten phytopathogenic isolates of *F. semitectum* from India.

**UPGMA**

RAPD markers along with appropriate statistical procedures are suitable for genetic variation analyses at both intra and inter-population levels (Leon et al. 2011). *Fusarium* species secreting T-2 toxin were analyzed with several UPGMA dendrograms with bootstrap analysis. In addition, bootstrap values of UPGMA dendogram obtained with the utilization of RAPDs were slightly higher. Genetic relationship calculated in the form of similarity coefficient from dendrogram showed high level of genetic similarity among all different *Fusarium*, which ranges from 0 to 0.9. Clustering was performed by UPGMA method. UPGMA analysis of the RAPD data separated the *Fusarium* species in two clusters (Figure 2).

**Table 3.** Distance matrix

| | *F. avenaceum* | *F. equiseti* | *F. solani* | *F. sporotrichioides* | *F. culmorum* | *F. tricinctum* | *F. lateritium* | *F. moniliforme* | *F. oxysporum* | *F. nivale* | *F. poae* | *F. acuminatum* | *F. graminearum* | *F. semitectum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***F. avenaceum*** | 0 | | | | | | | | | | | | | |
| ***F. equiseti*** | 1.00 | 0 | | | | | | | | | | | | |
| ***F. solani*** | 1.10 | 1.11 | 0 | | | | | | | | | | | |
| ***F. sporotrichioides*** | 1.00 | 1.01 | 1.00 | 0 | | | | | | | | | | |
| ***F. culmorum*** | 1.11 | 1.00 | 1.10 | 1.00 | 0 | | | | | | | | | |
| ***F. tricinctum*** | 1.10 | 1.10 | 9.89 | 9.99 | 1.10 | 0 | | | | | | | | |
| ***F. lateritium*** | 1.00 | 1.00 | 8.90 | 9.00 | 1.00 | 9.91 | 0 | | | | | | | |
| ***F. moniliforme*** | 1.10 | 9.89 | 1.11 | 1.01 | 1.10 | 1.10 | 1.00 | 0 | | | | | | |
| ***F. oxysporum*** | 1.00 | 1.01 | 1.00 | 1.11 | 1.00 | 9.99 | 9.00 | 1.01 | 0 | | | | | |
| ***F. nivale*** | 1.11 | 1.00 | 1.10 | 1.00 | 9.99 | 1.10 | 1.00 | 1.10 | 1.00 | 0 | | | | |
| ***F. poae*** | 1.00 | 1.00 | 1.11 | 1.01 | 1.00 | 1.10 | 1.00 | 9.90 | 1.01 | 1.00 | 0 | | | |
| ***F. acuminatum*** | 1.01 | 1.00 | 1.10 | 1.00 | 9.99 | 1.10 | 1.00 | 1.10 | 1.00 | 9.99 | 1.00 | 0 | | |
| ***F. graminearum*** | 1.09 | 8.91 | 1.10 | 1.00 | 1.10 | 1.10 | 1.00 | 9.90 | 1.00 | 1.10 | 8.91 | 1.10 | 0 | |
| ***F. semitectum*** | 1.11 | 1.00 | 1.10 | 1.00 | 1.00 | 1.10 | 1.00 | 1.10 | 1.00 | 9.00 | 1.00 | 9.99 | 1.10 | 0 |

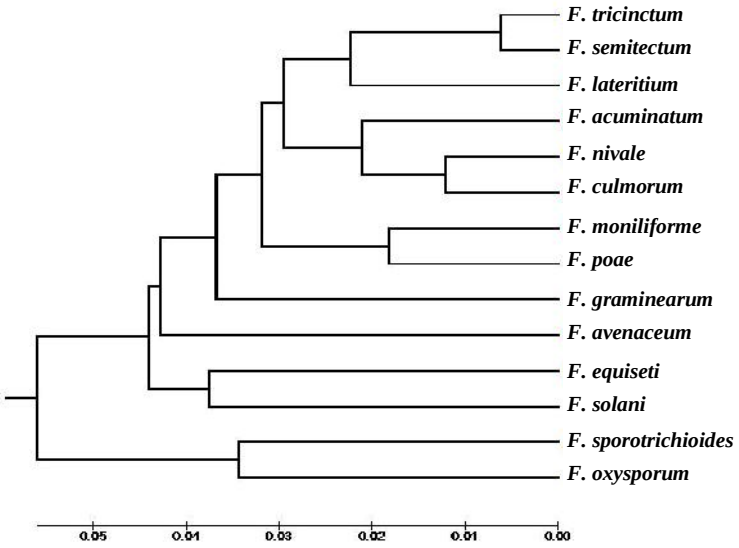


**Figure 2**. Phylogenetic analysis using UPGMA method

UPGMA dendrogram showed the *F. sporotrichioides* and *F. oxysporum* in one clade and other species in another clade. In upper clade *F. equiseti* and *F. solani* showed greater similarity than other *F. tricinctum, F. semitectum, F. lateritium, F. acuminatum, F. nivale, F. culmorum, F. moniliforme, F. poae, F. graminearum* and *F. avenaceum.* UPGMA analysis thus carried out in the present study showed the genetic variation in these 14 different *Fusarium* species. A distance matrix on simple matching coefficients was calculated from the data based on the RAPD of all 14 *Fusarium* species. The matrix was used to construct a dendrogram using distance tool with UPGMA method of PHYLIP for establishing to analyze the level of relatedness among the ten isolates. The dendrogram obtained from the data showed that hierarchical clustering separated the isolates into three groups according to their similarity coefficients. The similarity coefficients among the all isolates ranged from 0 to 0.9. Distance matrix of different 14 *Fusarium* species was obtained (Table 3).

UPGMA is a simple agglomerative or hierarchical clustering method used in bioinformatics for the phylogenetic analysis. The results obtained in the present study are noteworthy and showed the similarity with the observations of Ingle and Rai 2009, Bonde et al. (2012), Gupta et al. (2009) and Nagarajan et al. (2004). In their studies on isolates of *F. semitectum, F. equiseti, F. solani* and *F. oxysporum* respectively, they used data generated from RAPD banding pattern for the UPGMA analysis and found that there was genetic variations in different isolates of same *Fusarium* (Abd-Elsalam et al. 2003). Statistical analysis were carried out of all 14 *Fusarium* species using PAST software in which diversity graph (Figure 3) which computes a number of similarity or distance measures between all pairs of rows.

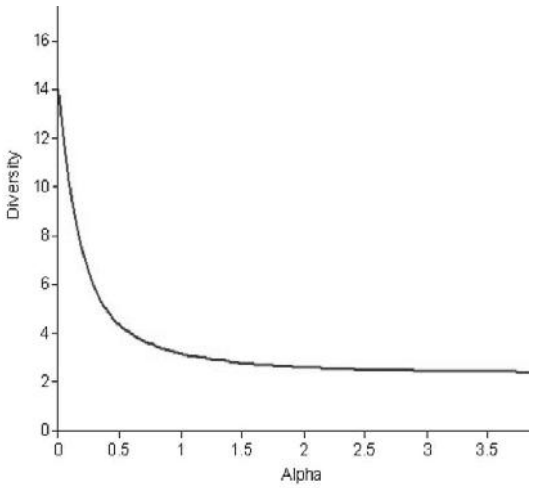


**Figure 3.** Diversity profile of 14 different *Fusarium* species

The diversity indices are applied in statistics of association data, where numbers of individuals are tabulated in rows (taxa) and possibly several columns (associations) and test for normal distribution (Table 4) asymptotically normal distribution with mean 0 and variance 1 under the null hypothesis of zero correlation, which is the basis for the calculation of *p* 0.002596 in which three statistical tests for normal distribution of one or several samples of univariate data, given in columns. Diversity indices statistics apply to association data, where numbers of individuals are tabulated in rows (taxa) and possibly several columns (associations). The available statistics are as follows, for each association:

Number of taxa (*S*), total number of individuals (*n*), Dominance = 1-Simpson index. Ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely), Simpson index 1-*D*. Measures 'evenness' of the community from 0 to 1. Diversity indexes, taking into account the number of individuals as well as number of taxa. Varies from 0 for communities with only a single taxon to high values for communities with many taxa, each with few individuals. Buzas and Gibson's evenness, Brillouin's index, Menhinick's richness index, Margalef's richness index, Equitability. Shannon diversity divided by the logarithm of number of taxa. This measures the evenness with which individuals are divided among the taxa present. Fisher's alpha-a diversity index, defined implicitly by the formula $S=a*\ln(1+n/a)$ where *S* is number of taxa, *n* is number of individuals and *a* is the Fisher's alpha. Berger-Parker dominance is simply the number of individuals in the dominant taxon relative to *n.*

The data below were generated by a random number generator with uniform distribution and tests such as Shapiro-Wilk test, Jarque-Bera test and Chi-square tests (Table 5) were studied to check the univariate normal distribution of data.

**Table 4.** Diversity indices

| | | |
|---|---|---|
| 0 | 59 | |
| Taxa_S | 14 | |
| Individuals | 2.4212309315201E58 | |
| Dominance_ | D | 0.383 |
| Shannon_H | 1.152 | |
| Simpson | 1-D | 0.617 |
| Evenness_e^H/S | 0.2261 | |
| Menhinick | 8.997E-29 | |
| Margalef | 0.0967 | |
| Equitability_J | 0.4365 | |
| Fisher_alpha | 0.1024 | |
| Berger-Parker | 0.4544 | |

**Table 5.** Shapiro-Wilk test, Jarque-Bera test and Chi-square tests as it is other statistical analysis

| **Statistical parameter** | **Values** |
|---|---|
| Min | 1E30 |
| Max | 1.10011E58 |
| Sum | 2.42123E58 |
| Mean | 1.72945E57 |
| Std. error | 1.00174E57 |
| Variance | 1.40487E115 |
| Stand. dev | 3.74815E57 |
| Median | 1.11111E55 |
| Geom. mean | 4.01722E55 |

Our results suggest existence of significant genetic variation among these *Fusarium* species secreting mycotoxin, on the basis of RAPD analysis. *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.

## CONCLUSION

RAPD marker was found to be powerful tool to analyze the genetic variation among the *Fusarium* species. These *Fusarium* species are responsible for producing mycotoxin, which is hazardous to animals and human beings. The results of the present study provide evidence that RAPD technique can be used for identification and differentiation of different *Fusarium* species. Study of mycotoxin secreting *Fusarium* is necessary to avoid T-2 toxin contamination in food and feed. We suggest that RAPD marker may be used as one of reliable alternative for the determination of genetic variation among the different *Fusarium* species.

## ACKNOWLEDGEMENTS

The authors are grateful to Defense Research and Development Organization (DRDO), New Delhi for providing financial assistance for the present research.

## REFERENCES

Abd-Elsalam KA, Schnieder F, Asran-Amal A, Khalil MS, Verreet JA. 2003. Intra-species genomic groups in *Fusarium semitectum* and their correlation with origin and cultural characteristics. J Plant Dis Prot 10: 409-418.

Arif M, Pani DR, Zaidi NW, Singh US. 2011. PCR-based identification and characterization of *Fusarium* sp. associated with mango malformation. Biotech Res Int. Article ID 141649, 6 pages, 2011. doi:10.4061/2011/141649

Assigbetse KB, Fernandez D, Dubois MP, Geiger JP. 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by Random amplified polymorphic DNA (RAPD) analysis. Phytopathol 84: 622-626.

Bonde SR, Gade AK, Rai MK. 2013. Genetic diversity among different isolates of *Fusarium equiseti* (Corda Saccardo) isolated from fruits and vegetables. Ind J Biotech (In press).

CAST [Council for Agricultural Science and Technology]. 2003. Mycotoxins: risks in plant, animal and human systems. Task Force Report no. 139. Council for Agricultural Science and Technology, Ames IA 1-191.

Edel V, Steinberg C, Gautheron N, Recorbet G, Alabouvette C. 2001. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. FEMS Microb Ecol 36: 61-71.

Edwards SG, Callaghan J, Dobson AW. 2002. PCR-based detection and quantification of mycotoxigenic fungi. Mycol Res 106: 1005-1025.

El-Fadly GB, El-Kazzaz M, Hassan MA, El-Kot GA. 2008. Identification of some *Fusarium* spp. using RAPD-PCR technique. Egyp J Phytopathol 36: 71-80.

Gupta VK, Misra AK, Gaur R, Pandey R, Chauhan UK. 2009. Studies of genetic polymorphism in the isolates of *Fusarium solani*. Austr J Crop Sci 3:101-106.

Gyongyossy-Issa MI, Khanna V, Khachatourians GC. 1985. Characterization of hemolysis induced by T-2 toxin. Biochim Biophys Acta 838: 252-256.

Ingle AP, Karwa A, Rai MK, Gherbawy Y. 2009. *Fusarium*: Molecular detection, mycotoxins and biocontrol. In: Gherbawy Y, Mach R, Rai M (eds.). Curent Advance in Molecular Mycology. Science Publishers Inc., Enfield, New Hampshire.

Ingle AP, Rai MK. 2011. Genetic Diversity among Indian phytopathogenic isolates of *Fusarium semitectum* Berkeley and Ravenel. Adv Biosci Biotech 2: 142-148.

Latiffah Z, Zariman M, Baharuddin S. 2007. Diversity of *Fusarium* species in cultivated soils in Penang. Malay J Microb 3: 27-30.

Leon T, Raj S, Britto JD, Benjamin P, Kumar JR. 2011. RAPD-PCR fingerprint analysis of *Cassia angustifolia* Vahl. in Tirunelveli District of Tamil Nadu. Int J BioTechnol 2: 17-20.

Leslie JF, Summerell BA. 2006. The *Fusarium* laboratory manual, 3rd ed. Blackwell publishing professional, Ames IA, USA.

Menaka M, Phanni M, Malathy NS . 2011. Isolation and identification of microcystin and mycotoxin from food borne fngus and to study about its antibacterial activity. EJLS 1:5-13.

Miller S. 1996. Detecting Propagules of plant pathogenic fungi. Adv Bot Res 23: 73-102.

Moss MO, Long MT. 2002. Fate of patulin in the presence of yeast *Saccharomyces cerevisiae.* Food Addit Contam 19: 387-399.

Nagarajan G, Nam MH, Song JY, Yoo SJ, Kim HG. 2004. Genetic variation in *Fusarium oxysporum* f. sp. *fragariae* populations based on RAPD and rDNA RFLP analyses. Pl Pathol J 20: 264-270.

Niessen L. 2007. PCR based diagnosis and quantification of mycotoxin producing fungi. Int J Food Microb 119:38-46.

Omurtag GZ, Yazıcıoglu Z. 2001. Occurrence of T-2 toxin in processed cereals and pulses in Turkey determined by HPLC and TLC. Food Addit Contam 18: 844-849.

Pestka JJ, Smolinski AT. 2005. Deoxynivalenol: Toxicology and potential effects on humans. J Environ Sci Health B 8: 39-69.

Pujo I, Guarro J, Gene J, Sala JP. 1997. *In vitro* antifungal susceptibility of clinical and environmental *Fusarium* spp. strains. J Antimicrob Chemoth 39: 163-167.

Richard JL. 2007. Some major mycotoxins and their mycotoxicoses-an overview. Int J Food Microbiol 119 (2) 3-10.

Sabir SM. 2006. Genotypic identification for some *Fusarium sambucinum* strains isolated from Wheat in Upper Egypt. World J Agri Sci 2 (1) 6-10.

Sahoo L, Das BK, Parhi K, Mukherjee SC. 2010.DNA fingerprinting of *Flavobacterium columnare* using RAPD-PCR. Ind J Microbiol 50: S10-S16.

SCF [Scientific Committee on Food]. 2001. Opinion on *Fusarium* Toxins. Part 5: T-2toxinandHT-2toxin. www.europa.eu.int/comm/food/fs/sc/scf/out88en.pdf._3.

Singh M, Chaudhuri I, Mandal SK, Chaudhuri RK. 2011. Development of RAPD Markers linked to *Fusarium* Wilt Resistance Gene in Castor Bean (*Ricinus communis* L). Genet Eng Biotech J (GEBJ-28): 1-8.

Skaria R, Sen S, Muneer PM. 2011. Analysis of genetic variability in rice varieties (*Oryza sativa* L) of Kerala using RAPD markers. Genet Eng Biotech J (GEBJ-24): 1-9.

Smith SN, Decay JE, Hsui HW, Jen LH, Hsieh WH, Lee HL. 2001. Soil-borne populations of *Fusarium oxysporum* f. sp. *vasinfectum*, cotton wilt fungus in California fields. Mycologia 93:737-743.

Steinkellner S, Mammerler R, Vierheilig H. 2008. Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum* strains. Eur J Plant Path 122: 395-401.

Turner NW, Subrahmanyam S, Piletsky SA. 2009. Analytical methods for determination of mycotoxins: a review. Anal Chim Acta 632 : 168-180.

Wang ZG, Feng JN, Tonq Z. 1993. Human toxicosis caused by moldy rice contaminated with *Fusarium* and T-2 toxin. Biomed Environ Sci 6: 65-70.