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# Genetic diversity of *Fusarium* wilt pathogen of Tobacco from Karnataka, India

**K. Sumana[1], K.R. Kini[2], Venkataramana[3] and N.S. Devaki[1*]**

[1]Department of Molecular Biology, Yuvaraja's College, University of Mysore, Mysore, Karnataka, India, [2]Department of Studies in Biotechnology, University of Mysore, Mysore, Karnataka, India, [3]Defence Food Research Laboratory, Karnataka, India.

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**Abstract** Genetic diversity of 14 isolates of *Fusarium oxysporum* f. sp. *nicotianae* (FON) the causal organism of tobacco wilt disease was studied using characteristics such as cultural, molecular and pathogenicity. All isolates differed in cultural characteristics. They were subjected to RAPD and were found to be varying. Further, these isolates were also subjected to RFLP of 740bp amplified product which when digested *with Taq1*enzyme showed six restriction patterns indicating a high genetic diversity. UPGMA analysis showed 5 distinct clusters based on the banding patterns obtained from RAPD and 5 clusters based on the RFLP analysis. All 14 isolates were subjected to pathogenicity test. The isolate 5 FON showed lowest pathogenicity and the isolate 13 FON showed highest pathogenicity. Comparison was made between the results of pathogenicity and UPGMA analysis. The least and highest pathogenic isolates belonged to different clusters only in RFLP.

**Key words:** Genetic diversity, *Fusarium oxysporum* f. sp. *nicotianae*, RAPD, RFLP, Pathogenicity, ITS

## Introduction

*Fusarium oxysporum* Schlecht. is a devastating plant pathogen on many commercially important plants including cereals, peanut, soybean, lima bean, cucumber, papaya, eggplant, corn, cotton, tomato, cucumber, alfalfa, date palm, tobacco and many more various formae specialis based on host differentials (Nelson *et al.,* 1997, Nagasaka *et al.,* 2003). Tobacco is selected as the host plant for the present study. *F. oxysporum* f. sp. *nicotianae* (FON) causes wilt of tobacco mainly in transplanted crop (Gopalachari, 1984; Shenoi and Nagarajan, 2000). All caltivars are susceptible to diseases. This is due to the presence of variations in pathogen. The fungal taxonomists have used macroconidia,

[*] **Corresponding author**: N.S. Devaki; **e-mail:** devakins@yahoo.co.in

microconidia, chlamydospore, host range and secondary metabolites as important criteria for identifying as well as understanding the variability in filamentous fungi (Duggal *et al.,* 1997). Molecular methods such as the PCR have been described to resolve genetic variation among isolates within or between formae specialis of *F. oxysporum* (McDonald, 1997). Various molecular techniques such as Random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphisms (RFLPs) of rDNA have been widely used for assessing genetic diversity in case of many fungal species (Annamalai *et al.,* 1995; Manulis *et al.,* 1994). Another strategy to detect genetic variation is the use of defined PCR amplified fragments as substrate for RFLP analysis. The spacer regions including the internal transcribed spacer (ITS) and the intergenic spacer (IGS) have been used to examine relationships among more closely related taxa (Appel and Gordon, 1995; Edel *et al.,* 1995). For these reasons, the molecular method has been recently introduced in Fusarium systematics and the molecular variation at the DNA level has been studied by many workers. The knowledge of this will help in evolving resistant cultivars (Singh *et al.,* 2006).

Molecular techniques for identifying species of the genus Fusarium and variability within the species, have become important tool in recent years (Singh *et al.,* 2006; Kim *et al.,* 1992; Mes *et al.,* 1994). These studies showed that nucleotide sequences of ITS regions were useful for identifying the Fusarium species. Thus the ITS region has been used in classifying many other fungal species because of its systematic and taxonomic usefulness (Chillali *et al.,* 1998; Schilling *et al.,* 1994; Dusabenyagasani *et al.,* 1999; Carter *et al.,* 2000). PCR-RFLP analysis is useful in detecting DNA polymorphism in conserved sequences (Nakamura *et al.,* 1998). This was found out using RAPD profiles of fungal pathogen *F. oxysporum w*here variations in different isolates were seen. Since *F. oxysporum* is a highly variable species, RAPD alone may not be sufficient to study genetic variability (Sharma *et al.,* 2005, Rozlianah and Saraiah, 2006). Thus this technique has to be supplemented by more reliable techniques where polymorphism in ribosomal DNA is taken as criteria. The conserved regions such as internal transcribed spacer (ITS) are targeted to identify genetic variability. Here the polymorphism can be more stable and reliable than RAPD. Hence this technique is adapted in the present study. Further the grouping obtained is compared with variations in the pathogenicity. Much work is not available with regard to the correlation among morphological, cultural, pathogenicity, RAPD profile and ITS -RFLP variations. An attempt is made for the first time during the present study to identify the existence of such relationship if any.

## Material and methods

### *Isolation and establishment of the pathogen culture*

Standard cultures of *Fusarium oxysporum* was obtained from MTCC, India and ITCC, New Delhi. Standard cultures were maintained on Potato dextrose agar medium. *Fusarium oxysporum* strains in this study were isolated by transferring mycelium growing from infected root and stem bits of tobacco plants collected from southern parts of Karnataka, India (Johnston and Booth, 1968) to the petriplate containing potato dextrose agar (Himedia). After 7 days of incubation at room temperature, a spore suspension was made and spread on to fresh PDA plate and incubated for 18 h at room temperature. A single germinating conidium was then removed from the PDA plate, transferred to PSA (Potato Sucrose Agar) plate and after 7 days of growth, culture plates were stored in plastic bag at $4^{o}$C. Identification was made as per the monographs of Booth (1975). The identity of each strain was verified by morphological characteristics and by comparing the ribosomal DNA (rDNA) sequences with data at Gene Bank (NCBI, Bethesda, MD, USA, www.ncbi.nlm.nih.gov). Cultures were also sent to Fusarium Research Centre, Penn State University, University Park, PA, USA for confirmation of identification. Standard cultures of *F. oxysporum* used as positive control for molecular work in the experiment were obtained from MTCC, India and *F. graminearum* and *F. cerealis* used as negative control were obtained from ITCC, New Delhi. All cultures were maintained on Potato dextrose agar medium. Cultures obtained by single spore isolation technique (Bayaa *et al.*, 1994) grown on PSA were further subjected to DNA extraction, RAPD and RFLP studies.

### *Cultural studies*

Fourteen isolates obtained by single spore isolation were maintained in PSA. These isolates were inoculated to PDA and incubated at $23\pm2^{0}$C for 7 days. Observations were done at regular intervals to study the cultural characteristics. 7-day-old PDA cultures were used for quantifying and studying the characteristics of microconidia, macroconidia and chlamydospores. 5mm diameter PDA culture plugs (two in number) of actively growing mycelia were suspended in 1ml of physiological saline and subjected to vigorous agitation for the release of the spores.

### *DNA extraction and molecular identification of the fungal pathogen*

FON isolates were inoculated to Potato Sucrose Broth (PSB) and was grown for 20 days at 30$^{o}$C with constant agitation. The mycelia were harvested, patdried and kept at -20$^{o}$C until further use. The frozen mycelia were ground into fine powder in liquid nitrogen using sterile pestle and mortar and DNA was extracted from the mycelial powder of each isolate following the method of Raeder and Broda (1985). The DNA extracted from 14 isolates, standard *F. oxysporum* cultures (Positive control), *F. graminearum* and *F. cerealis* (Negative control) were subjected to Agarose gel electrophoresis. The bands obtained in all the lanes were compared.

### *RAPD analysis*

RAPD-PCR was performed as described by Kini *et al.,* (2002). RAPD primers were obtained from Eurofins Biotechnology, India. Genomic DNA samples isolated from 14 isolates of FON were subjected to RAPD PCR analysis with 45 decamer primers. 11 primers belonging to OPA, OPC, OPD, OPF, OPH and OPN series produced distinct and reproducible band patterns. 2 out of 11 primers namely OPA2 and OPC7 resulted in the formation of polymorphic bands.

PCR reaction was performed in 25µl reaction volumes containing 1-1.5 U Taq. DNA polymerase, DNTP mix (0.2mM each of dCTP, dGTP, dATP, dTTP), 1X PCR buffer (10mM Tris HCl, pH 8.5, 50mM $MgCl_2$, Bangalore Genei, Pvt. Ltd., Bangalore, India), 0.5µM primer and 25-50ng of genomic DNA. The reaction mixture was vortexed and centrifuged briefly. Amplification was performed in a thermal cycler (JH Bios, USA). Initial denaturation for 3min at 97℃ followed by 45 cycles of 1min at 94℃, 1min at 36℃ and 2min at 72℃ and final extension step of 10min at 72℃. All reactions were repeated thrice to verify reproducibility. The amplified products were separated by electrophoresis in 1.5% agarose gel in 0.5X TAE buffer at 80volts, in Tris EDTA (90mM Tris, 90mM Boric acid, 2mM EDTA). Gels were stained with ethidium bromide and visualized with UV light and photographed by using gel documentation system. In the control treatment PCR mixture was supplemented with sterile distilled water instead of genomic DNA.

RAPD data from all amplifications were recorded by scoring all DNA bands (obtained from OPA2 and OPC7) and were compiled in a binary matrix. The data were converted to distance matrices based on Nei (1978) unbiased minimum distance. The distance matrices were then used to construct a dendrogram by the unweighted pair-group method with arithmetic averages

(UPGMA) using tools for population genetic analysis (TFPGA ver.1.3) (Miller, 1997).

### *ITS-RFLP- analysis*

ITS-RFLP was performed by targeting the ITS+5.8S region rDNA of FON isolates. ITS region was synthesized by using ITS1 and ITS4 primers reported by White *et al.,* (1990). The amplified PCR products were subjected to restriction digestion by using *TaqI, EcoRI* and *MboII* enzymes by taking the reaction mixtures (buffer 1.5µl, enzyme 1.0µl, amplicon 10µl and water 2.5µl). The reaction mixture was subjected to PCR set at 37℃ for one hour. PCR products were subjected to 1% agarose gel electrophoresis. The analysis of restriction digestion after electrophoresis was the same as described for RAPD product analysis. The sequence of forward and reverse primer was ITS1 – 5' TCC GTA GGT GAA CCT GCG G-3'; ITS4 –5' TCC TCC GCT TAT TGA TAT GC-3'.

### *Pathogenicity studies*

50 g of sorghum seeds were sterilized in 100 ml of distilled water for two consecutive times. 5mm plugs of 7-day old cultures (two in number) were inoculated aseptically and the flasks were incubated at 28 ±2℃ for 15 days. Sorghum cultures were transferred to 10cm diameter plastic pots and the sterile potting mix of 2kg and the pots were kept for inoculum multiplication for a week at room temperature. 60-day-old healthy tobacco seedlings were transplanted into plastic pots filled with sterile potting mix and the sorghum culture. Seedlings were punctured by sterile needle to the main axis of the root system by Pin Prick method (Jahagirdhar *et al.,* 2001). For each isolate sixty seedlings were planted and three replicates were maintained and the experiment was repeated thrice.

## Results

### *Isolation and establishment of the pathogen culture*

A total of 14 isolates of *F. oxysporum* were brought into pure culture from root and stem bits of infected tobacco plants (Table. 1). Based on morphological characteristics the isolates were identified as *F. oxysporum*. Identification was confirmed by Fusarium Research Centre, Penn State University, University Park, PA, USA. Our morphological identifications were further confirmed by molecular method (PCR) (Fig.1). A single band of 700 bp

was obtained in all the 14 isolates and that was on par with standard culture of *F. oxysporum.*

**Table 1.** The areas of collection of the isolates of *Fusarium oxysporum* f. sp. *nicotianae* used in the present study

| Sl. No | Isolate No. | Tobacco cultivar | Tobacco growing belts | Areas of collection | Year |
|---|---|---|---|---|---|
| 1 | 1 FON | Kanchan | Piriyapatna | kogilavadi | 2005 |
| 2 | 2 FON | Kanchan | Piriyapatna | Mylapura | 2005 |
| 3 | 3 FON | Kanchan | Piriyapatna | Nandinathapura | 2005 |
| 4 | 4 FON | Kanchan | Hunsur | Kothegala | 2005 |
| 5 | 5 FON | Kanchan | Piriyapatna | Beguru | 2005 |
| 6 | 6 FON | Kanchan | Piriyapatna | Konasooru | 2006 |
| 7 | 7 FON | Kanchan | Piriyapatna | Aaranahalli | 2006 |
| 8 | 8 FON | Kanchan | Piriyapatna | Choranahalli | 2006 |
| 9 | 9 FON | Kanchan | Hunsur | Hosahalli | 2006 |
| 10 | 10 FON | Kanchan | Piriyapatna | Boonahalli | 2006 |
| 11 | 11 FON | Kanchan | Hunsur | Badaga | 2007 |
| 12 | 12 FON | Kanchan | Piriyapatna | Pudaganahalli | 2007 |
| 13 | 13 FON | Kanchan | Piriyapatna | Pudaganahalli | 2007 |
| 14 | 14 FON | Kanchan | Piriyapatna | Pudaganahalli | 2007 |

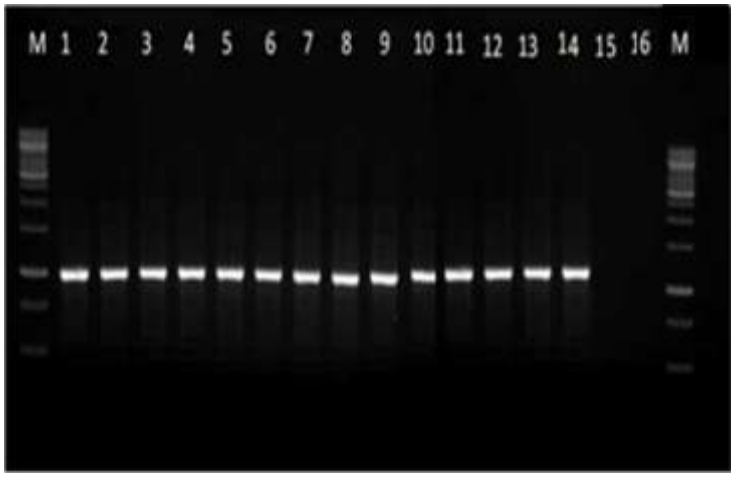


**Fig. 1.** Molecular detection of *Fusarium oxysporum* isolates
Lane M- 1 kb DNA lader, Lane 1- standard strain of *F. oxysporum,* lane 2-14, isolates of *F. oxysporum,* Lane-15, *F. graminearum,* Lane 16- *F. cerealis,* Lane 17-marker

### *Cultural studies*

Isolates showed significant variations in cultural characteristics, production of microconidia, macroconidia and chlamydospores (Table. 2).

Colony diameter was found to be significantly more in 3 FON, 4 FON, 6 FON and 9 FON and lesser in 1 FON, 5 FON, 8 FON and 14 FON. Maximum sector formation was observed in 3 FON, 4 FON, 6 FON, 9 FON and 13 FON and minimum sector formation was seen in 1 FON, 5 FON, 8 FON and 14 FON. Pigmentation varied from pale yellow to dark brown. Among the isolates 13 FON was found to be with dark brown pigmentation in the medium. Microconidia and chlamydospore formation was less in the isolates *viz*., 1 FON, 6 FON and 8 FON; macroconidia production was also recorded to be lesser in 1 FON, 5 FON, 6 FON, 7 FON, 11 FON and 12 FON isolates; 2 FON and 10 FON isolates recorded maximum number of all types of spores.

**Table 2.** Cultural characteristics of 7-day-old *Fusarium oxysporum* grown on Potato Dextrose Agar

| Isolates | Colony diameter in mm | Sector formation * | Pigmentation | Number of spores quantified from 2 culture plugs of 5mm diameter | | |
|---|---|---|---|---|---|---|
| | | | | Microconidia | Macroconidia | chlamydospores |
| **1 FON** | 20 | + | Dark pink | 0.7143 ±0.184 a | 0.000 ± 0.000 a | 0.7143 ±0.184 a |
| **2 FON** | 45 | ++ | Pale pink | 30.714 ± 1.898 ef | 1.000 ±0.000 abc | 30.714 ± 1.898 ef |
| **3 FON** | 60 | +++ | Dark brown | 9.4286 ±1.394 bc | 1.142 ±0.142 ab | 9.4286 ±1.394 bc |
| **4 FON** | 60 | +++ | Dark brown | 9.4286 ±1.394 bc | 1.142 ±0.142 ab | 9.4286 ±1.394 bc |
| **5 FON** | 20 | + | Pale yellow | 7.000 ±1.112 abc | 0.000 ±0.000 a | 7.000 ±1.112 abc |
| **6 FON** | 60 | +++ | Dark brown | 0.000 ± 0.000 a | 0.000 ± 0.000 a | 0.7143 ±0.184 a |
| **7 FON** | 30 | ++ | Pinkish white | 25.285 ±2.965 b | 0.000 ±0.000 a | 25.285 ±2.965 b |
| **8 FON** | 20 | + | Dark pink | 0.7143 ±0.184 a | 0.000 ± 0.000 a | 0.7143 ±0.184 a |
| **9 FON** | 60 | +++ | Dark brown | 9.4286 ±1.394 bc | 1.142 ±0.142 ab | 9.4286 ±1.394 bc |
| **10 FON** | 45 | ++ | Pale pink | 30.714 ± 1.898 ef | 1.000 ±0.000 abc | 30.714 ± 1.898 ef |
| **11 FON** | 30 | ++ | Pinkish white | 25.285 ±2.965 b | 0.000 ±0.000 a | 25.285 ±2.965 b |
| **12 FON** | 30 | ++ | Pinkish white | 25.285 ±2.965 b | 0.000 ±0.000 a | 25.285 ±2.965 b |
| **13 FON** | 25 | +++ | Dark brown mycelium and media also turned brown | 9.4286 ±1.394 bc | 1.142 ±0.142 ab | 9.4286 ±1.394 bc |
| **14 FON** | 20 | + | Pinkish white | 0.000 ± 0.000 a | 0.000 ± 0.000 a | 0.7143 ±0.184 a |

*+ minimum, ++ moderate , +++ maximum sector formation

### *RAPD DNA Finger printing analysis*

The primers OPA2 and OPC7 generated a total of 124 scorable bands which were polymorphic. Amplified fragments ranged from 100bp to 1Kb. The number of DNA fragments amplified and scored per isolate for individual primer ranged from 2 to 5. Cluster analysis with UPGMA using genetic distances showed that the isolates are divided into 5 main groups (Fig. 2).

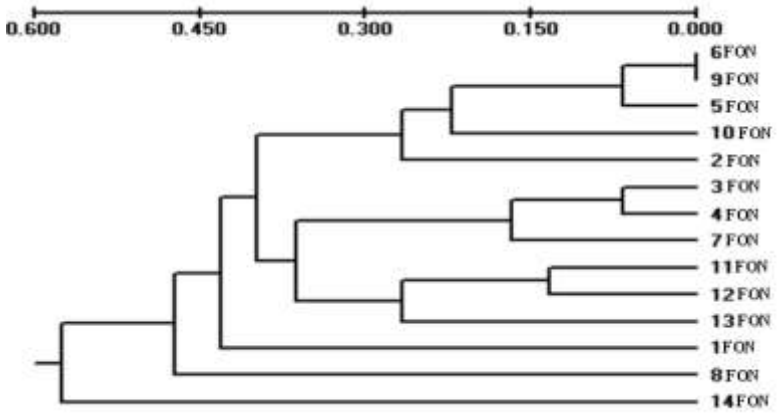


**Fig. 2.** Dendrogram of 14 isolates of *Fusarium oxysporum* revealed by UPGMA cluster analysis of genetic similarities based on RAPD data.

### *rDNA RFLP analysis*

DNA fragments of approximately 740 bp were amplified using ITS1 and ITS4 primer pair for all FON isolates. The ITS +5.8S region of all the FON isolates was digested with *TaqI, EcoRI* and *MboII* enzymes. *EcoRI* and *MboII* did not show any variation but produced the same restriction patterns for all the isolates. *TaqI* produced 6 restriction patterns (Fig.3; Table. 3). Cluster analysis based on UPGMA separated the FON isolates into 5 main clusters (Fig. 4). All the isolates were grouped into 2 main clusters, A and B. Within cluster A, the isolates were further divided into sub-clusters A1and A2. The isolates in sub-cluster A1 (8 FON, 14 FON, 1 FON, and 13 FON) and isolates in sub-cluster A2 (2 FON and 10 FON) were found to be almost similar with respect to the restriction pattern. 7 out of 14FON isolates were clustered in sub-cluster B1. Within sub-cluster B1 the isolates were sub divided into I and II clusters. The isolates of I cluster (7 FON, 11 FON, 12 FON) and cluster II (6 FON, 9 FON, 3 FON, 4 FON) were found to be highly variable in their restriction patterns. The isolate 5 FON formed sub-cluster B2.

**Table 3.** Estimated sizes of restriction fragments of *Fusarium oxysporum* isolates

| Sl.No | Isolate No | Estimated size of restriction fragments Of FON isolates digested by *TaqI* enzyme |
|---|---|---|
| 1 | 9 FON and 6 FON | 620 bp |
| 2 | 3 FON and 4 FON | 520 bp |
| 3 | 7 FON, 11 FON and 12 FON | 550, 340 bp |
| 4 | 5 FON | 420, 320 bp |
| 5 | 1 FON, 8 FON, 13 FON and 14 FON | 480, 320 bp |
| 6 | 10 FON and 2 FON | 320, 370 bp |

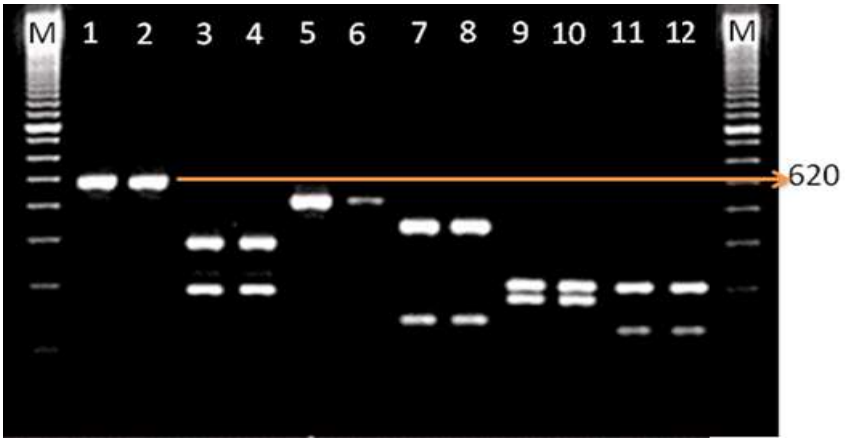


**Fig. 3.** ITS-RFLP- analysis of Fusarium oxysporum f. sp. nicotianae. Restriction band patterns of ITS amplicon digested with Taq 1 enzyme.
Lane M, DNA Marker (DNA digested with TaqI), lane1and2, 9 FON; 3and 4, 5 FON; 5and 6, 3 FON; 7and 8, 11 FON and; 9and 10, 1 FON; and 11and 12, 10 FON.

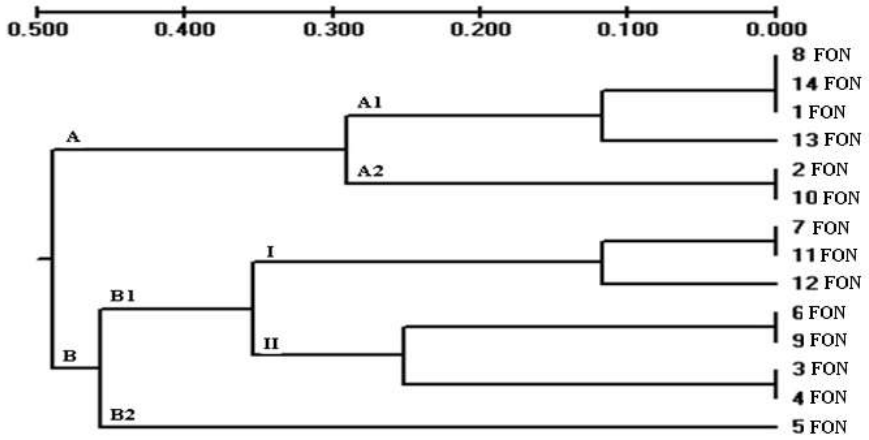


**Fig. 4.** Dendrogram generated from UPGMA cluster analysis based on PCR-RFLP of ITS+5.8S regions of r DNA of *Fusarium oxysporum*

### *Pathogenicity studies*

Isolates showed variation in the number of days required for initiation of the disease. Isolates 1 FON, 2 FON, 8 FON, 10 FON and 13 FON showed initiation of the disease on 2nd day itself. 3 FON, 4 FON, 6 FON, 7 FON, 9 FON, 11 FON, 12 FON and 14 FON initiated the disease on 3rd day. Isolate 5 FON took 4 days to initiate the disease. On the seventh day of inoculation all the isolates except 5 FON resulted in 100% infection. 5FON showed 80% infection on the seventh day (Table. 4).

**Table 4.** Pathogenicity studies of 14 isolates of *Fusarium oxysporum*

| isolates | Day1 | Day2 | Day3 | Day4 | Day5 | Day6 | Day7 |
|---|---|---|---|---|---|---|---|
| **control** | 0.00±0.00 a | 0.00±0.00 a | 0.00±0.00 a | 0.00±0.00 a | 0.00±0.00 a | 0.00±0.00 a | 0.00±0.00 a |
| **1 FON** | 0.00±0.00 a | 1.40±0.50 b | 1.47±0.51b c | 2.00±0.53 cd | 2.27±0.45 bcd | 3.20±0.56 c | 3.93±0.25 c |
| **2 FON** | 0.00±0.00 a | 1.13±0.64 b | 1.53±0.51 bc | 2.00±0.53 cd | 2.47±0.51 cd | 2.73±0.45 bc | 3.87±0.35 c |
| **3 FON** | 0.00±0.00 a | 0.27±0.45 a | 1.27±0.45 bc | 1.53±0.51 bc | 2.07±0.70 bcd | 2.60±0.50 bc | 3.93±0.25 c |
| **4 FON** | 0.00±0.00 a | 0.40±0.50 a | 1.27±0.45 bc | 1.93±0.45 c | 2.60±0.50 de | 2.87±0.51 bc | 4.00±0.00 c |
| **5 FON** | 0.00±0.00 a | 0.00±0.00 a | 0.13±0.35 a | 1.27±0.45 b | 1.60±0.50 b | 2.87±0.51 bc | 3.13±0.64 b |
| **6 FON** | 0.00±0.00 a | 0.40±0.50 a | 1.20±0.41 b | 1.47±0.51 bc | 2.00±0.53 bcd | 2.67±0.48 bc | 4.00±0.00 c |
| **7 FON** | 0.00±0.00 a | 0.27±0.45 a | 1.40±0.50 bc | 1.60±0.50 bc | 2.20±0.41 bcd | 2.60±0.50 bc | 3.93±0.25 c |
| **8 FON** | 0.00±0.00 a | 1.13±0.64 b | 1.47±0.51 bc | 1.87±0.64 bc | 2.47±0.51 cd | 2.60±0.50 bc | 4.00±0.00 c |
| **9 FON** | 0.00±0.00 a | 0.40±0.50 a | 1.40±0.50 bc | 1.87±0.64 bc | 2.33±0.72 cd | 2.93±0.59 bc | 4.00±0.00 c |
| **10 FON** | 0.00±0.00 a | 1.20±0.41 b | 1.40±0.50 bc | 1.80±0.56 bc | 2.33±0.61 cd | 2.87±0.64 bc | 3.87±0.35 c |
| **11 FON** | 0.00±0.00 a | 0.00±0.00 a | 1.13±0.35 b | 1.53±0.51 bc | 1.80±0.41 bc | 2.60±0.50 bc | 3.67±0.48 c |
| **12 FON** | 0.00±0.00 a | 0.00±0.00 a | 1.33±0.48 bc | 1.67±0.48 bc | 2.20±0.77 bcd | 2.67±0.48 bc | 3.80±0.41 c |
| **13 FON** | 0.00±0.00 a | 1.13±0.35 b | 1.80±0.67 c | 2.60±0.73 d | 3.20±0.41 e | 4.00±0.00 d | 4.00±0.00 c |
| **14 FON** | 0.00±0.00 a | 0.00±0.00 a | 1.33±0.48 bc | 1.67±0.48 bc | 1.93±0.59 bcd | 2.40±0.63 b | 3.87±0.35 c |
| **F value** | 0.00 | 24.394 | 16.148 | 16.367 | 24.326 | 40.772 | 168.390 |

The values given above are three replicates ± standard deviation. The values followed by different alphabets
differ significantly as indicated by ANOVA ($\alpha = 0.05$). * F values significant at 0.000 level.

## Discussion

During the present study, isolates of FON were collected from different locations of Hunsur tract of Karnataka within 30,000 hectares of tobacco growing area. Variability in cultural characteristics was observed among all the 14 isolates of FON. Similar observations were done in different formae specialis of *F. oxysporum* (Sharma *et al.,* 2005; Rozlianah and Saraiah, 2006). This is the first report on the variations of FON for KLS. The study was done to investigate the potential of the RAPD and RFLP techniques to detect polymorphisms among the different isolates of *Fusarium* infecting tobacco. Distinct variation in the isolates with respect to RAPD was observed within the above mentioned geographical area itself. In previous studies, RAPD analysis has been used successfully to delineate groups within formae specialis of *F. oxysporum*. One group was delineated with *F. oxysporum* f. sp. *albedinis* (Tantaoui *et al.,* 1996). 2 groups within f. sp. *dianthi* (Manulis *et al.,* 1994), f. sp. *ciceris* (Honnareddy and Dubey, 2006), f. sp. *erythroxyli* (Nelson *et al.,* 1997) and 2 to 3 groups within f. sp. *pisi* (Grajal- Martin *et al.,* 1993). In the present study genetic variations was detected among the isolates of FON using RAPD marker where five clusters were found. OPA series were better than all the other series of primers viz., OPD, OPF, OPH and OPN for amplification. Similar results were recorded by Honnareddy and Dubey (2006) with OPB and OPN series primers. In general these primers showed potential for differentiating various isolates of FON.

In our study, the RFLP analysis showed variation among the internal transcribed spacer (ITS) region where six distinct restriction patterns were observed. This is a clear indication for the presence of high genetic diversity. Such genetic diversity based on RFLP analysis is also reported by Leong *et al.,* (2009) in *F. oxysporum* f. sp. *cubense*. This is the first report on the diversity of FON based on ITS +5.8S rDNA.

The isolates of FON were found to be varying in their pathogenicity which was identified through the virulence i.e., the number of days taken to initiate the disease and the number of plants completely wilted. All isolates were found to be pathogenic. Based on the virulence, the isolates can be grouped into two distinct groups. The results of the grouping of RAPD did not show any relationship with the pathogenicity grouping. Such lack of relationship between RAPD profile and pathogenicity was reported in *Fusarium oxysporum* f. sp. *lycopersici* (Rozlianah and Saraiah, 2006). The highly virulent strain 13FON which took only 3 days to initiate significant amount of disease in host plant and the less virulent strain 5FON belonged to separate subclusters of RFLP but not RAPD. Such lack of relationship between RAPD profile and pathogenicity was reported in *Fusarium oxysporum* f. sp.

*lycopersici* (Rozlianah and Saraiah, 2006). However, in case of RFLP study the two different clusters included separately the virulent and the less virulent strains of the pathogen. This indicates the usefulness of RFLP as a tool for understanding the genetic diversity which may include the significant difference in the virulence level of pathogens also.

In conclusion, isolates of FON are different from each other and have the high level of genetic variation within a small geographical area like Hunsur tract of Karnataka, which is revealed by comparing cultural, molecular and pathogenicity studies. This is the first report of such study for *F. oxysporum* infecting tobacco. These observations will be helpful for screening the germplasm for large number of FON strains isolated from a small geographical area itself and which in turn will help in breeding for disease resistance.

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