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Full Length Research Paper

Investigation of genetic variability among different isolates of *Fusarium solani*

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***Fusarium solani* (Mart). Sacc. is an important wilt causing pathogenic fungi. Plant diseases have caused severe losses to human beings. In the present investigation five different isolates of *F. solani* were subjected to genetic variability analysis in terms of DNA-polymorphism using RAPD-PCR. *F. solani* isolate V_3 (*Lycopersicum esculentum* Mill.) and V_5 (*Solanum melongena* L.) showed 78.2% similarity. Sequences of isolates V_1 (*Lense esculentum* L.) and V_2 (*Acacia* sp.) were 74.2% similar. Isolates (V_1 and V_2) and (V_3 and V_5) also show 65.5% similarities among their sequences. Whereas isolate V_4 (*Gladiolus sp*.) gives 70.3% similar results. Genetic variability pattern among isolates of *F. solani* were also supported by UPGMA dendrogram and percentage similarity table.**

Key words: *Acacia* sp. *Fusarium solani*, *Gladiolus* sp. *Lens esculenta*, *Lycopersicum esculentum*, RAPD-PCR *Solanum melongena*.

INTRODUCTION

Vegetables play a significant role in human nutrition, especially as sources of vitamins, minerals, and dietary fiber (Wargovich, 2000). Egg plant is economically important vegetable crop in Asia and Africa, and although it is also grown in Europe and the United States. The global area under brinjal cultivation has been estimated at 1.85 million ha with total production of brinjal fruit of about 32 million. Brinjal is grown over 8670 hectare area throughout Pakistan with the annual production of 91260 tones, out of which the Punjab, Province has the highest share in terms of area of sowing (4890 ha) and production, 60890 tons (Anonymous, 2007). Among the many diseases that attack the brinjal crop, wilt is major damaging disease that causes the severe yield losses. The pathogen can survive in the soil for many years (Babu et al., 2008). *Fusarium* Wilt is due to the species *Fusarium solani*. Disease symptoms are often helpful in making decisions, but a definitive diagnosis requires clear identification of pathogen so in order to apply appropriate controls, it is extremely important to make an accurate and timely diagnosis of plant diseases (Frederick et al., 2000). Molecular techniques are important tools in solving the problems of species restriction and also provide alternative methods for taxonomic studies (MacLean et al., 1993). In the last years characterizations of plant genetic resources based on molecular markers have been increased. Studies using a broad range of markers applied on hundreds of plant species are the theoretical basis for understanding genetic diversity to propose both breeding and conservation strategies (Laurentin, 2009). Polymerase chain reaction can be applied to measure responses of experimental stimuli and to gain knowledge of potential changes in protein level and function (Mark et al., 2005). The development and application of molecular diagnostic methods have made it possible to study plant diseases with the help of new technologies (McCartney et al., 2003). RAPD has been used widely for the detection of genetic variability in plants because of its simplicity and lack of need for any prior information about the genetic material of plant. RAPD patterns remains constant in plant whether it is young or old (Welsh and McClelland, 1990; Micheli et al.,

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Table 1. List of decamers used in RAPD.

Serial No.	Primer designation	Primer sequence
1	RAPD 1	3′ AGGGGTCTTG 5′
2	RAPD2	3′ AATCGGGCTG 5′
3	RAPD 3	3′ CAGGCCCTTC 5′

1994). This technique has also been reported very useful for identification and genotyping of ornamental as well as of many other varieties (Temiesak et al., 1993). According to McClelland and Welsh, (1994) high quality templates should be used to assure reproducible RAPDs. RAPD markers were also used by Katherine et al. (2003) to examine the degree of genetic variation within the putatively asexual basidiomycetes fungus (lepiotaceae). Due to simplicity of this process RAPD is used as molecular markers for taxonomic and systematic analysis of plants and is used widely in plant breeding and genetic relationships (Bartish et al., 2000). RAPD fingerprinting method can be used for studying phenotypically similar *Candida* strains according to molecular era (Steffan et al., 1997) and this technique is more accurate and rapid for the identification of *Candiada* species (Rocha et al., 2008). Recently RAPD has been used widely for estimation of genetic material of many endangered plants (Zheng et al., 2008).

MATERIALS AND METHODS

Molecular characterization of *Fusarium* species

Genetic similarity among five different isolates of *F. solani* was examined. Four samples were obtained from FCBP (First Fungal Culture Bank of Pakistan IAGS University of The Punjab Lahore) and one sample was isolated from *Solanum melongena* plant. Fungal cultures were further purified as a single spore culture on Malt Extract Agar (MEA) plates by single spore isolation technique (Choi et al., 1999) and incubated at 27˚C ± 2°C. After 2 weeks fungal colony was removed from the Petri plate by scratching the surface with a sterilized needle and then placed in the (Pre-chilled at -80°C) sterilized mortar. Fungal tissues were ground with liquid nitrogen to form a fine powder with the help of a pestle. Fungal DNA was extracted by using the CTAB method described by Doyle and Doyle (1990) with some modifications.

DNA quality analysis

The target fungal genomic DNA was isolated by doing 1% agarose gel electrophoresis. To 70 ml of 0.5 × TAE buffer(10ml 50 × TAE, 990ml distilled water) 0.7 g of agarose was added and subjected to heat in a microwave oven until a clear, transparent solution was obtained. After cooling for about 5 min, 2 µL of ethidium bromide(EtBr) was added from 10 mg/ml stock solution (0.2 g EtBr in 20ml ddH2O) in the melted gel. The melted agarose was poured into a flat bed gel tray and comb was inserted. The gel was allowed to solidify completely at room temperature. Then comb was carefully removed and gel tray was placed in the electrophoresis tank containing 0.5 × TAE buffer.DNA samples and the DNA standard marker were loaded into the wells of the solidified gel submerged in 0.5 × TAE buffer. Gel electrophoresis was carried out at 100 volts for about 40 min. The DNA bands in the gel were visualized using UV transilluminatior and photographed by using gel documentation system (Wise Doc MUV-M2O).

RAPD analysis (random amplification of polymorphic DNA)

Each of the five fungal DNA extract was amplified with three different decamer primers. In order to determine genetic variability among different isolates of *Fusarium solani* RAPD technique was applied. PCR amplification involved the following steps.

Primer screening

In RAPD analysis 3 primers (decamers) were used. Table 1 show the decamers used in RAPD.

Reactions for RAPD-PCR

PCR tube contains 25 µL RAPD reaction mixture; which is composed of 0.5 µL Taq Polymerase, 2.5 µL PCR Buffer, 2.0 µL $MgCl_2$, 5.0 µL dNTPs, 5.0 µL Primer, 5.0 µL Template DNA, 8.0 µL, Double distilled deionized water. All the chemicals were placed in ice under sterile conditions.

Conditions of RAPD-PCR

Polymerase chain reaction (PCR) tubes containing the reaction mixture were placed in the PCR machine. Machine was programmed under the following conditions of temperature. The initial process of denaturation was done at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing was done at 40°C for 1 min and final extension for 10 min at 72°C. Termination of reaction was done at 22°C. Until further analysis on agarose gel the amplified products were stored at 4°C.

Amplified DNA fragment analysis

RAPD sample (25 µL) was mixed with 3 µL of loading dye and the mixture was then loaded in the wells of 1% agarose gel. Electrophoresis was carried out same as described earlier for genomic DNA. Bands were visualized through the documentation system [Wise Doc MUV-M20] and were recorded. The number of amplified DNA bands for each *Fusarium* isolate was recorded along with their sizes. According to genetic similarities and differences *F. solani* isolates were grouped in different clusters.

RESULTS

RAPD markers were used to examine the degree of genetic variation within the isolates of *Fusarium solani*. The accession numbers of fungal isolates are given in Table 2. Initially three random decamer primers were chosen in order to generate RAPD profile of the five fungal isolates, RAPD primers were selected for the further studies as it produced consistent and reproducible bands for all of the fungal isolates. The results of primer (RAPD 3 primer) are shown in Figure 1. Number of shared RAPD bands was compared between each pair of isolates to quantify the similarity between fungal isolate.

Table 2. Serial and accession numbers of Isolates obtained from FCBP.

Serial No.	Accession No	Isolation source
1	136	*Lense esculenta*
2	438	*Acacia*
3	443	*Lycopersicum esculentum*
4	277	*Gladiolus*
5	1127	*Solanum melongena*

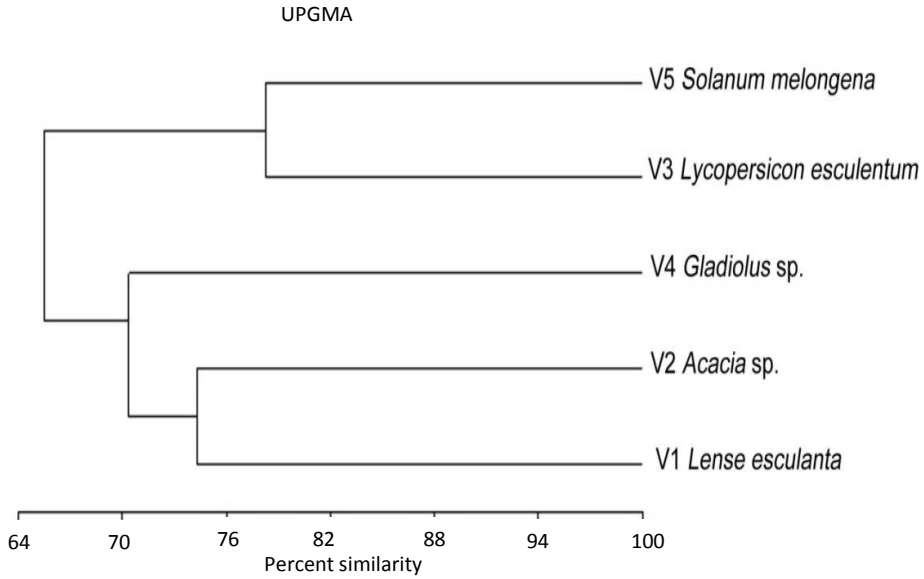

Figure 1. Dendrogram showing different isolates of *Fusarium solani*.

Table 3. Percentage similarity among different isolates of *Fusarium solani.*

Node	Group 1	Group 2	Percentage similarity (%)	Objects in group
1	*L. esculentum*	*S. melongena*	78.261	2
2	*L. esculenta*	Acacia	74.286	2
3	Node 2	Gladiolus	70.370	3
4	Node 3	Node 1	65.536	5

Data was recorded and used to generate a Phylogenetic tree using Multivariate Statistical Package, ver 3. (MVSP 3.0). The percentage similarity of *F. solani* isolates are shown in Table 3.

It is evident from the dendrogram that *F. solani* isolate V3 (*Lycopersicum esculentum*) is closer to V5 (*Solenum melongena*) isolate of *F. solani* and they have the highest percentage similarity that is 78.2% and occupy the node position 1. Similarly V1 (*Lense esculenta*) and V2 (*Acacia*) occupied second position on the node and showed the 74.2% similarity in their sequences (Table 3). Node 2 is occupied by V4 (*Gladiolus*) and shared 70.3% of its rapid bands. Some similarities were also observed in the isolates present on node 3 (V1 and V2) and node 1 (V3 and V5). Both of them share 65.5% similarity.

DISCUSSION

In this study, the suitability of RAPD techniques for molecular characterization of *F. solani* isolates, isolated from different sources, was observed. It was observed that RAPD 3 marker provide clear polymorphism and provide profiles which differed markedly between isolates of *F. solani* from different plant host and this further revealed polymorphism with reference to different isolates of *Fusarium solani* (Figure 2), and established DNA fingerprints which is useful for genetic

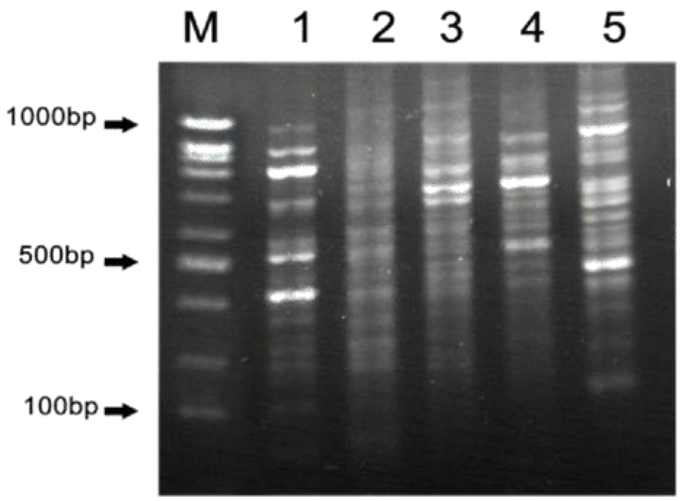

Figure 2. RAPD profile of *Fusarium solani* by using decamer RAPD 3. The size of DNA was compared with 1.0 kb DNA ladder (L).

characterization and specific identification of *F. solani* isolates from other different host plants. According to Goodwin et al. (2001) and Sunnucks (2000) the genetic diversity of some *Penicillium* species was reported by random amplified polymorphic DNA. The use of RAPD markers was also reported by Pitt (1973) for molecular characterization of 10 *Penicillium* species. It is evident from the present study that *F. solani* isolated from *L. esculentum* is closer to *F. solani* which was isolated from *S. melongena* as they share 78.2% similarity which is the highest percentage among all the other isolates. Similarly *Lense esculenta* and *Acacia* showed 74.2% similarity in their sequences. 65.5% similarity was also observed among the isolates present on node 3 (*L. esculenta* and *Acacia*) and node 1 (*L. esculentum* and *S. melongena)*. So it was concluded from the study that isolate of *F. solani* isolated from Gladiolus differs from all the four isolates of *F. solani* as it does not show similarity with any of the isolate. *Fusarium* species usually require time consuming and lengthy pathogenicity and vegetative compatibility analysis (Williams et al., 1990). Therefore RAPD analysis has been used widely among phytopathogenic fungi including *Fusarium* species for their detection and genetic characterization (Kim et al., 1993; Miller, 1996; Gulino et al., 2003). Thirty isolates of Pestalotiopsis and two isolates of *Bartalinia robillardoides* were genotypically compared by RAPD techniques and 241 reproducible polymorphic bands were obtained using 23 random primers (Tejesvi et al., 2007). By using RAPD analysis, population of *Fusarium* spp. from different plant hosts have been grouped and recommended by various workers that RAPD markers could be a quick and reliable alternative for different isolates of *Fusarium* sp. (Hyun and Clark, 1998; Ibrahim and Nirenberg, 2000; Jana et al., 2003).

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