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Full Length Research Paper

Evaluation of pathogenic potential and genetic characterization of *Fusarium solani*: A cause of *Fusarium* wilt in potato

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***Fusarium* wilt, caused by *Fusarium solani,* is economically important disease of potato in Pakistan. Presently, pathogenic potential of *F. solani* was studied by inoculating potato plants with three *F. solani,* strains to screen the most virulent isolate among *F. solani* FCBP-016, *F. solani* FCBP-434 and *F. solani* FCBP-470. Pathogenicity test depicted that *F. solani* FCBP-434 was the most pathogenic isolate with variation in genetic level that was determined by RAPD-PCR. *F. solani* FCBP-434 was 55.66% different with both isolates. This disparity in genetic constitution might be cause of high pathogenicity.**

Key words: *Fusarium* wilt, potato, pathogenesity test, RAPD-PCR.

INTRODUCTION

Potato (*Solanum tuberosum*) is the most widely distributed crop in the world, cultivated in about 140 countries, more than 100 of which are located in tropical and sub tropical zones (Beukema, 1993). Potato ranks third among food crops after wheat and rice and fifth in total production in Pakistan. It produces high energy and nutritional value per unit area than wheat, rice and maize.

Although potato production in Pakistan has increased many folds but it's per acre yield is far less than in other parts of the world (Malik, 1995). Among the various factors responsible for its low per acre production, potato diseases are considered to be the most important. More than 18 potato diseases are reported in the country, of which 13 are of common occurrence. Their importance, however, varies considerably in different potato growing areas (Ahmad et al., 1991). Most commonly occurring potato diseases in Pakistan are early blight, powdery and common scab, black scurf, stem rot, soft rot, brown rot, wilts, potato cyst nematode and root knot nematode (Ahmad, 1998).

Fusarium wilt is a fungal disease which can be caused by several species of *Fusarium*, including *F. eumartii*, *F. oxysporum*, *F. avenaceum*, and particularly *F. solani*. Roberts et al. (2005) found that most of root infecting fungi including *Fusarium* spp. are known to attack many cultivated plants and parasitize 36 hosts in Pakistan. It may become established in many types of soil, but it is likely to cause most damage on light sandy soils.

The present study is, therefore, designed to screen out the most pathogenic isolate of *F. solani,* among the test isolates of potato wilt disease in Pakistan, on the basis of genetic characterization.

METHODOLOGY

Procurement and maintenance of cultures

The pure cultures of *F. solani* FCBP-016, *F. solani* FCBP-434 and *F. solani* FCBP-470 were obtained from first Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Science, University of the Punjab, Lahore. They were maintained and subcultured monthly on Malt extract agar medium at 4°C.

Pathogenicity test

For pathogenecity test the protocol of Grogan et al. (1975) was

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Table 1. Reagents and their working concentration.

Reagents	Working concentration	Reaction mixture (µL)
PCR buffer (10X)	(1.0 X)	5.0
MgCl2 (25mM)	(1.5-3.0 mM)	5.0
dNTPs (2.0mM)	(0.2 mM)	5.0
RAPD primers	(100 pMol/ µL)	2.0
Template DNA	(1-1.5 µg)	5.0
Taq Polymerase (2.5U/ µL)	1 U	0.5
Double distilled Deionized water		27.5
Total volume		50

adopted. Two kilograms of soil were sterilized at 70°C for 24 h in hot oven. The conidial suspension containing 2 x 10^5 conidia/ml was prepared. Pathogenicity test was performed by inoculating the conidial suspension @ 10-15 ml/plant on to the 1-month-old potted potato plants and soil with three selected isolates of the fungus. Inoculation of the fungus was made by applying spores and mycelium, scraped from colony surfaces, to petiole stubs left after clipping off leaves by spraying. The plants were kept covered with polythene bags for 48 h to maintain sufficient moisture for spore germination and development of disease. Disease rating was based on a scale of 0-5. Disease severity was calculated with the help of following formula.

$$\text{Disease severity} = \frac{\text{Affected area of a plant}}{\text{Total area of the plant}} \times 100$$

Screening of the most pathogenic isolate was carried out on the basis of results of pathogenicity test. The most pathogenic species was isolated and subjected to further RAPD-PCR assays to evaluate their genetic diversity.

Molecular analysis of fungal isolates

The genomic DNA of three different isolates of *F. solani* was extracted by CTAB method (Saghai-Maroof et al*.,* 1984) with some modifications. Thirteen primers of A and B series were used in RAPD analysis (A-01 GGGTAACGCC, A-02 GTTGCGATCC, A-06 GGTCCCTGAC, A-08 GTGACGTAGG, A-11 CAATCGCCGT, A-12 TCGGCGATAG, A-13 CAGCACCCAC, B-05 TGCGCCCTTC, B-13 TTCCCCCGCT, B-14 TCCGCTCTGG, B-16 TTTGCCCGGA, B-18 CCACAGCAGT, B-19 ACCCCCGAAG). RAPD amplifications were carried out in Master cycler gradient PCR (TECHNE TC-412) with pre heat lid temperature of 105°C and initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation 94°C for 1 min, annealing at 25°C for 1 min, and primer extension at 72°C for 1 min. Final extension was set for 5 minutes at 72°C. The reaction was terminated at 4.0°C hold. The bands were examined under UV Transilluminator (WiseDoc MUV-M20) and photographed on gel documentation system (Table 1).

Statistical analysis

Minitab (2004) software was used to calculate genetic distances and similarities between isolates and to draw a dendrogram based on the genetic distances using the unweighted pair group method and arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

Pathogenicity test

The pathogenicity of three isolates of *F. solani* (FCBP-016, FCBP-434, FCBP-470) was assessed which enabled the reproduction of typical symptoms of the disease over the time-scale after 15 days of incubation at 25°C. Five levels of pathogenicity were detected on potato plants (Figure 1). The less and the least pathogenicity was noticed by FCBP-470 and FCBP-016 isolates*,* respectively. However, *F. solani* FCBP-434 was proved to be the most pathogenic. It is clearly depicted from the results that typical symptoms appeared on the leaves of potato as wilts. Wilted leaves gradually turned yellowish to brown and severely infected leaves defoliated. Rapid disease progress resulted in blight of stems and eventual death of the entire plant. Dark brown dieback lesions appeared on the stems of potato plants 25 days after artificial inoculation but no symptom developed on control plants. Leaves on the infected plants were defoliated 25 days after artificial inoculation, resulting in complete blight of stems and eventual death of the entire plant as are observed in naturally infected plants. Leaves on the infected plants were defoliated 15 days after artificial inoculation, resulting in complete blight of stems and eventual death of the entire plant as are observed in naturally infected plants. Similar results were reported from various host plant - *F. oxysporum* combinations (Kim et al., 2005).

Scrutinization of genetic variability through RAPD analysis

Rapid results were obtained with only two primers, that is, A-02 and B-05. The results of these primers are shown in Figure 2 in which one DNA fragment was amplified with decamer primer A-02 and its size is 1500-2000 bp while two DNA fragments were amplified with B-05 primer, the size of one band is almost 2000 bp and second is 2500 bp. The dendrogram generated from each primer and cluster of primers by MINITAB are presented in Figure 3. The RAPD data obtained with 13 primers was evaluated to analyze the genetic parity or disparity among different genotypes. A dendrogram was constructed on the basis of genetic distances by UPGMA method and two main groups of cluster were identified in the homology tree: *F. solani* FCBP-016 and *F. solani* FCBP-470 on one side and *F. solani* FCBP-434 on the other side (Figure 3). The findings indicate that *F. solani* FCBP-016 and *F. solani* FCBP-470 are more similar to each other than *F. solani* FCBP-434. *F. solani* FCBP-016 and *F. solani* FCBP-470

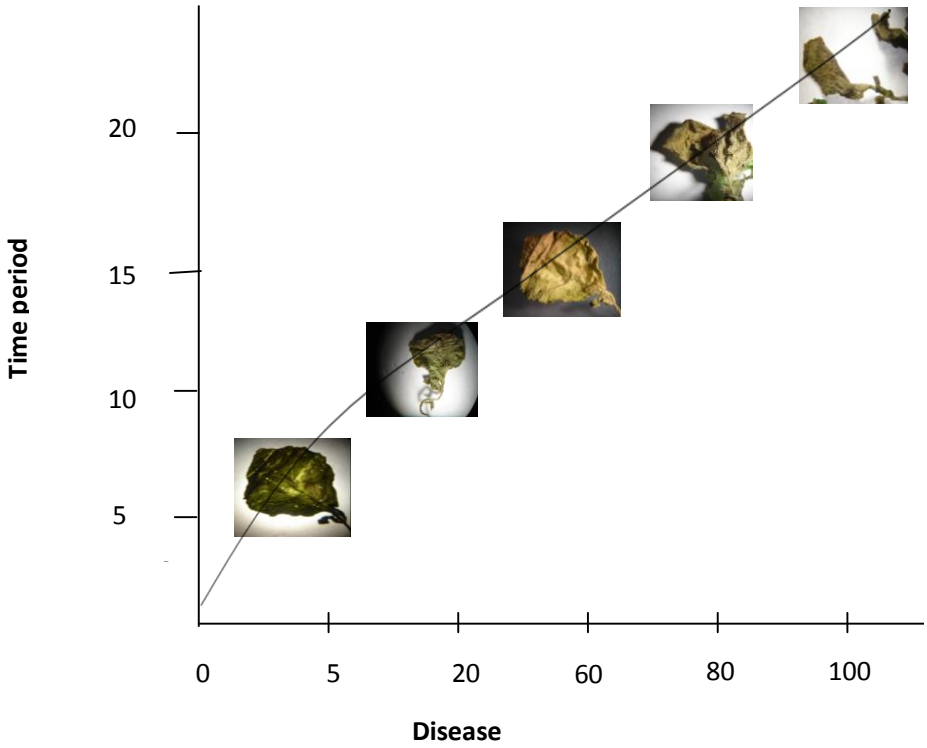

Figure 1. Periodic progression of *Fusarium* wilt of potato.

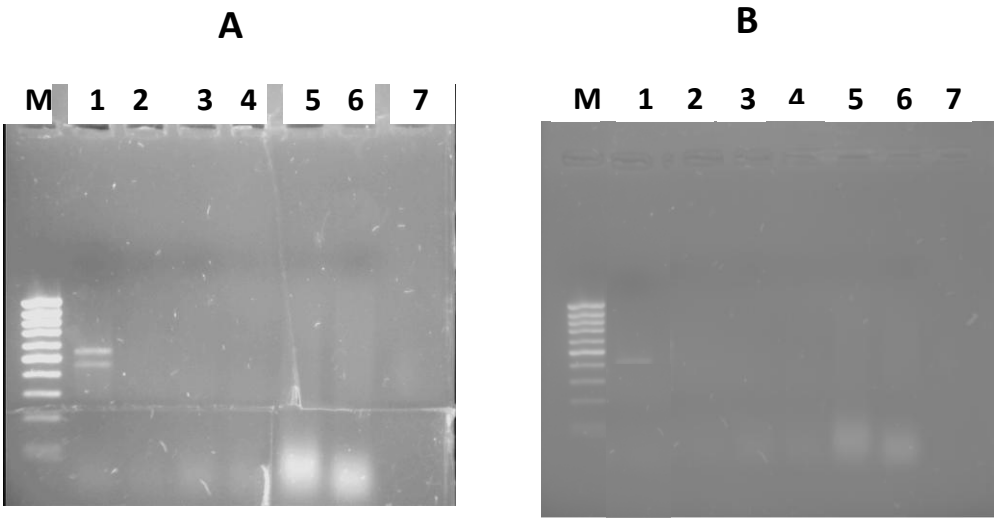

Figure 2. RAPD DNA fragments amplified with decamer primers B-05, B-18, A-02 (A) and A-02, A-06 and A-08 (B). Lane M indicates DNA marker and lane 1(A) indicates amplification of *F. solani* FCBP-434 while lane 1(B) indicates amplification of *F. solani* FCBP-470.

are 100% similar to each other but they show only 44.34% similarity with *F. solani* FCBP-434. In several studies RAPD fingerprinting technique has been employed to detect mutation, genetic relatedness and genetic variation within and between natural bacterial and human DNA and fungal populations (Keinath et al., 1995; Jones and Kortenkamp, 2000).

Due to the different genetic make up of *F. solani* FCBP-434 from others, it may be a reason for its trend towards high pathogenicity than *F. solani* FCBP-016 and *F. solani*

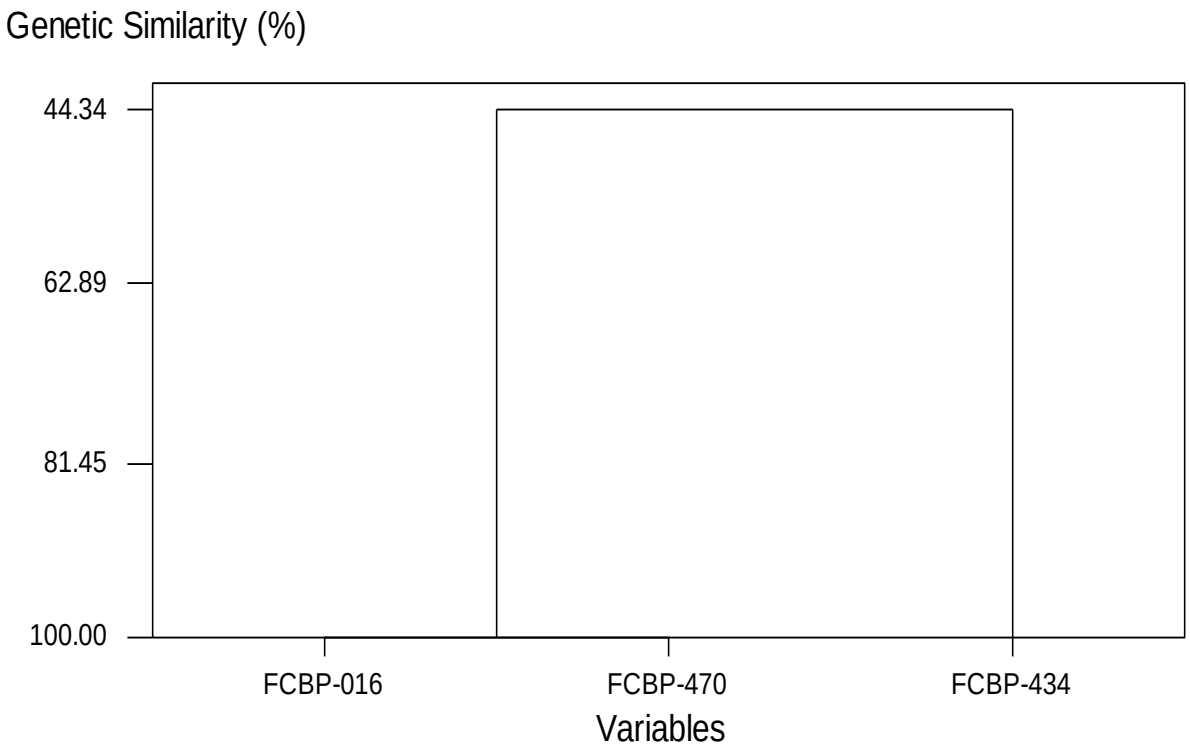

Figure 3. Homology tree constructed by an un-weighted pair group with arithmetic averages clustering algorithm from the pair wise matrix of genetic similarity amongst genotypes of *F. solani* FCBP-016, 434 and 470.

FCBP-470. The findings of pathogenicity test and molecular analysis showed that *F. solani* FCBP-434 have high pathogenic potential to cause severe infection in crop plants.

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