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Basal Susceptibility of Tomato Varieties against Different Isolates of *Fusarium oxysporum* f. sp. *lycopersici*

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Abstract

Basal susceptibility of tomato varieties was studied against Fusarium wilt by using ten different isolates of *Fusarium oxysporum* f. sp. *lycopersici* (Fol). These isolates were collected from infected tomato plants from different tomato fields. A total of 230 combinations of Fol isolates and tomato varieties were evaluated and disease index was calculated. Mean disease index against all Fol isolates was used to govern susceptibility of single tomato variety against Fusarium wilt disease. Based on this mean disease index, varieties were classified into five groups viz., immune, resistant, moderately resistant, susceptible and very susceptible. None of the variety was completely resistant or immune against Fol. Three varieties viz., Pride Burn, Red Power, Sun Grape were moderately resistant. All other varieties were either susceptible or very susceptible against Fol infection. Varying levels of susceptibility of tomato varieties was observed against different isolates of Fol. Clusters analysis based on disease index values placed all tomato varieties in three different groups. Genetic finger printing of all Fol isolates was performed by using ISSR markers. Dendrogram based on the ISSR analysis divided all Fol isolates in two major groups. This is first study carried out in Pakistan by using multiple strains of Fol to declare basal susceptibility of tomato germplasm against Fusarium wilt.

Keywords: *Fusarium oxysporum* f. sp. *Lycopersici*; Tomato; Mean disease index; ISSR markers

Introduction

Tomato (*Lycopersicon esculentum* Miller) is the second major vegetable product of Pakistan (Mirza, 2007). Tomato farming covered 63 thousand hectares during 2009-2010, with an average yield of 10522 kg/ha (Anonymous, 2011). This yield is very low as compared to that of the developed countries, where it can reach up to an average of 1562 kg/hectare (Sajjad *et al.*, 2011). Several fungal, bacterial and some viral diseases of tomato contribute in severe yield loss of tomato under field conditions.

Fusarium wilt disease has ever been the most destructive plant diseases in history (Halila and Strange, 1996). All members of *F. oxysporum* are successful saprophytes and capable to survive for long periods of time under most of the edaphic conditions. Some isolates induce root-rot and vascular diseases on specific hosts (Olivain *et al.*, 1981; Olivain and Alabouvette, 1997; 1999; Olivain *et al.*, 2003) and are classified into approx. 120 *formae speciales* and races, based on the plant species and cultivars they infect (Armstrong and Armstrong, 1981; Tello and Lacasa, 1988; Gordon and Okamoto, 1992; Alabouvette *et al.*, 2001). Pathogenic isolates of *F. oxysporum* often display a high degree of host specificity (Sakai, 1998). Infection occurs when the pathogen penetrates in roots of the plant. *Fusarium oxysporum* f. sp. *lycopersici* (Fol) is responsible for important crop losses in the tomato fields (Benhamou *et al.*, 1998).

Control of *F. oxysporum* infection in the field is difficult because the pathogen can survive for a long period of time in the form of mycelium in infected plant debris or in the form of chlamydospores in soil (Haware *et al.*, 1996; Agrios, 1997). Chemical control of wilt has not been effective because pathogen is both soil and seed-borne. Some other control strategies against Fusarium wilt include employing antagonistic microbes and applying botanical pesticides (Di Pietro *et al.*, 2003; Djatnika and Hermanto, 2003). Some studies have indicated the ability of antagonistic microbes to control Fol, but their effectiveness in the field has not yet been proven (Bastasa and Baliad, 2005). Genetic resistance in tomato germplasm against this disease is considered as efficient mean of controlling this disease (Medina-Filho and Tanksley, 1983). This approach is also considered as an ecofriendly control measure. The ideal strategy for managing Fusarium wilt disease is by cultivating resistant germplasm.

Sexual mode of reproduction in pathogen provides them new genetic recombination and thus evolving new pathogenic populations (Pushpavathi *et al.*, 2006). For development of resistant plant germplasm against diseases, there is need of complete knowledge of variability in virulence and genetic makeup of different strains of a single

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pathogen. In past, scientists have mostly screened tomato germplasm against fusarium wilt of tomato by using a single pathogen strain of Fol. The objectives of this investigation were to determine pathogenicity extent and genetic polymorphism among different isolates of Fol isolated from different tomato growing areas of Punjab for identification of resistant tomato variety and most virulent strain of Fol that could be helpful in breeding or Fol management programs.

Materials and Methods

Fol Isolation and Identification

F. oxysporum f. sp. *lycopersici* (Fol) isolates were isolated from roots of infected tomato plants collected from tomato fields of Punjab province, Pakistan. Infected plants roots were surface sterilized (5% sodium hypochlorite solution) for 2 min, re-washed several time in sterilized distilled water, dried between sterilized filter papers. Small portions of infected tissues were cut, and plated onto fusarium specific media "PCNB Agar" and incubated at 25^{o}C for 3-5 days. The resultant fungus was isolated and purified using the hyphal tip and/or the single spore methods (Hawker, 1950). Ten Fol isolates were initially identified according to their morphological and microscopic characters as described by Jens *et al.* (1991) Barnett and Hunter (2003) and Leslie *et al.* (2006). Fig. 1 represents different steps of pathogen isolation and identification.

Fol isolates identification was further confirmed by molecular methods by using Fol strain FCBP119 as reference. Fungal Genomic DNA was extracted from mycelium by using methodology as proposed by Lodhi *et al.* (1994). 2x *n*Taq PCR reaction mixture provided by Enzynomics® Korea was used to carry out PCR reaction. PCR was carried out by Fol specie specific primers (EF15´ATGGGTAAGGA(A/G)GACAAGAC-3´) and EF2 5´GGA(G/A)GTACCAGT (G/C)ATCATGTT -3´(Edel *et al.,* 2000). Amplifications were performed in a 25 µL reaction volume. PCR reaction was performed in a 96-well Asco PCR System under the Following cycle program: initial denaturation step for 4 min at 94^{o}C, denaturation at 94^{o}C for 30s x36, annealing at 60^{o}C for 45s and extension at 72^{o}C for 120 s, Followed by a final extension step at 72^{o}C for 7 min. Amplified product at ~700bp were visualized on 1% agarose gel (Fig. 1e).

Screening of Tomato Varieties against Fusarium Wilt

This research was carried out under green house of Institute of Agricultural Sciences, University of the Punjab Lahore. Twenty three tomato varieties, obtained from market and 'Federal Seed Certification and Registration (FSC and RD) Pakistan' were used in this experiment. For inoculum preparation, Fol isolates were grown on MEA broth media. Spore suspensions of these isolates were prepared in dist. sterilized water at concentration of 2000 spores/mL with the help of haemocytometer. Fifty mL of this spore suspension was used for pathogen inoculum. Plastic pots (4 inch diameter) each containing 0.5 Kg sterilized sandy loamy soil was used for pathogenicity test. Each pot was planted with three surface sterilized seeds. Upon emergence of seedlings, pot was thinned to one healthy seedling. Pots were watered to field capacity and left for incubation in green house. Each variety was subjected to all ten Fol isolates separately, leaving behind 230 host pathogen combinations. Three replicates were mad for each treatment.

Scoring of Wilting and Disease Intensities

Response of tomato germplasm against Fusarium wilt was determined by first scoring of wilting symptoms and then by determining disease index based on this scoring. Scoring of wilting symptoms in tested tomato entries due to Fol infection (score 0-3) was conducted by using following criteria developed by Epp (1987) and is provided in Table 1.

Disease Index (DI) was calculated using the following equation:

$$DI = [(ni \times si)/(N \times S)] \times 100\%$$

Where, ni: number of tomato plants with wilt symptoms, si: value of the score of symptoms, N: total number of tested tomato plants, and S: the highest value of score of symptoms (Cachinero *et al.,* 2002).

Overall responses of the tested tomato varieties against Fusarium wilt was established using the following criteria: if the value of DI is equal to 0%; immune – if 1-20%; resistant, if 21-40%; moderately susceptible, if 41-70%; susceptible, if 71- 100%; and very susceptible (dan Sudarsono, 2004). Cluster analysis of tomato varieties was performed by considering disease index values against all Fol strains by using Single Linkage Euclidean Distance method with the help of MYSTAT® program.

Genetic Fingerprinting of Fol Isolates by ISSR Markers

Fungal Genomic DNA was extracted from mycelium by using methodology as described by Lodhi *et al.* (1994). Quantification of isolated DNA was performed by measuring OD at 260 nm (Sambrook *et al.,* 1989). Ten ISSR primers were used in this study. Here also 2X *n*Taq PCR reaction mixture provided by Enzynomics® Korea was used. Amplifications were performed in a 25 uL reaction volume. PCR reaction was performed in a 96-well Asco PCR System equipped with a Hot Bonnet under the Following cycle program: initial denaturation step for 4 min at 94^{o}C, denaturation at 94^{o}C for 30s x36, annealing at 45- 52^{o}C for 45s, and extension at 72^{o}C for 120s, followed by a final extension step at 72^{o}C for 7 min. Amplified bands from each primer were scored as present (1) or absent (0). Here also dendrogram was constructed by using Single Linkage Euclidean Distance method with the help of MYSTAT® program.

Statistical Analysis of Data

All the data were statistically analyzed by performing Analysis of variance (ANOVA) and DNMRT by 'DSSTAT' software (Steel *et al*., 1997).

Results

Screening of Tomato Varieties against Fusarium Wilt

After categorization of varieties based on mean of disease index (MDI), none of the variety was immune or resistant against Fol infection. Three varieties viz: 'Pride Burn', 'Red Power' and 'Sun Grape' were moderately resistant against fusarium wilt disease. Seventeen varieties were susceptible against fusarium wilt with mean disease index of 40-70%. Varieties as 'Early Boy' and 'Fine Star' were very susceptible by showing mean disease index <70%.

Analysis of Variance demonstrated significant interaction between Fol isolates and tomato varieties. Same Fol isolate was unable to cause uniform level of disease for all tomato varieties. Because a same tomato variety represented different disease index with different Fol isolates. 'Red Cloud' and 'Cosmos 101' were having less susceptibility for Fol 2 but more for Fol 3 as represented by disease index values (Table 2). 'Red Stone' was having disease index level of 84.6 for Fol 3 but for Fol 6, disease index level was 22.5. When 'Early Boy' was checked against all Fol isolates, higher disease index values were observed representing that this entry was prone to mostly Fol isolates. Similarly, most striking differences were observed among different Fol isolates for their disease incidence. Isolate Fol 2, Fol 10 exhibited lowest disease index for 'Sun Grape' but highest when infecting 'Early Boy' (Table 2). On the other hand when mean of disease index was taken for single Fol isolates against all tomato varieties, Fol 7 was most virulent strain with 73.87% MDI Followed by Fol 3 with 69.42% MDI (Table 2). We constructed polar dendrogram based on susceptibility level of tomato entries against all Fol isolates by Single Linkage Euclidean Method. Point of maximum dissimilarity divided all tomato varieties into three groups (Fig. 2).

Genetic Fingerprinting of Fol Isolates by ISSR Markers

Seven ISSR primers were able to revel polymorphism among Fol isolates (Table 3). A total of 110 loci were amplified out of which 82 were polymorphic. Primer '841' amplified maximum polymorphic alleles (Table 2). All ten Fol isolates were separated in two main groups (Fig. 3). Isolates Fol1, Fol 2, Fol 3, Fol 6, Fol 4, Fol 8 were in one group and rest of the isolates were in second group (Fig. 3).

Discussion

Pathogenicity test of the different isolates for the isolated fungus was carried out under green-house conditions.

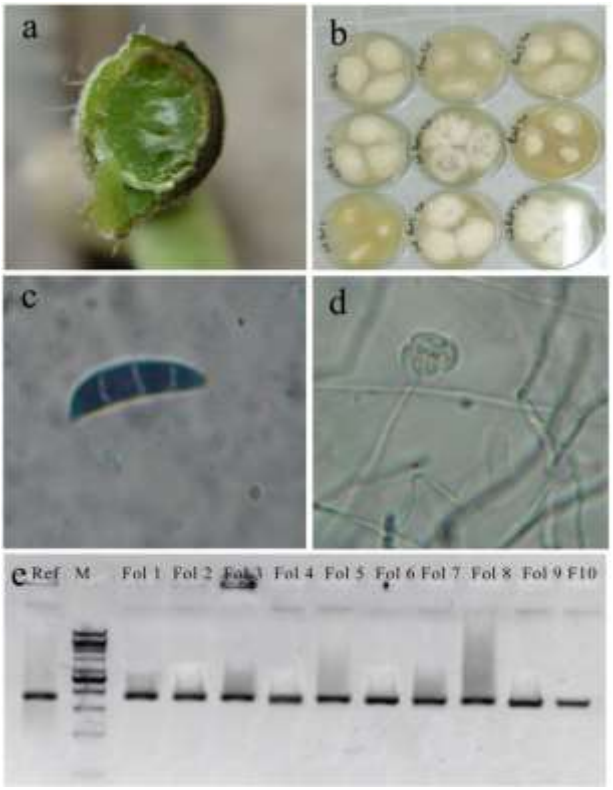

Fig. 1: Isolation and identification of Fol isolates. (a) Infected tomato stem showing vascular browning. (b) Culture purification of Fol isolates. (c) Macrocondia of Fol. (d) Microconidia production by Fol. (e) molecular identification of Fol by specie specific primer

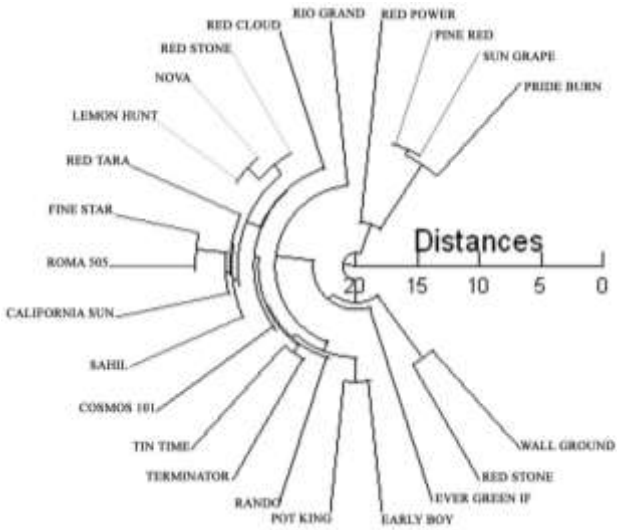

Fig. 2: Dendrogram showing grouping of different tomato varieties based on disease index data

Statistical analysis illustrated a significant interaction between tomato cultivars and Fusarium isolates. Same Fol isolate was unable to infect all tomato varieties uniformly.

Table 1: Scoring of wilt symptoms

Wilt score	Symptoms
0	No wilt symptom
1	Less than 25% plant parts turned yellow
2	Yellowing and browning covered less than 50% plant parts
3	Infected plant parts turned brown and died, hence covered more than 50% plant parts

Table 2: Susceptibility of tomato varieties against different Fol isolates

Varieties	Fol 1	Fol 2	Fol 3	Fol 4	Fol 5	Fol 6	Fol 7	Fol 8	Fol 9	Fol 10	MDI	Response
California Sun	$63.5^{D\text{-}Fde}$	$57.2^{H\text{-}Je}$	$71.6^{D\text{-}Icd}$	$65.4^{E\text{-}Gde}$	$78.3^{B\text{-}Dbc}$	86.5^{Aab}	91.1^{ABa}	37.3^{Cf}	$64.1^{E\text{-}Gde}$	46.4^{DEf}	66.14^{**}	S
Cosmos 101	$55.7^{G\text{-}Ide}$	43.8^{Kf}	$73.2^{C\text{-}Hbc}$	$79.3^{A\text{-}Dab}$	89.1^{Aa}	$64.2^{D\text{-}Gcd}$	$84.7^{B\text{-}Da}$	53.2^{Ae}	57.5^{GHde}	38.2^{EFf}	63.89^{**}	S
Early Boy	71.3^{Cb}	94.5^{Aa}	88.8^{ABa}	70.6^{DEb}	56.2^{Fc}	$67.0^{C\text{-}Fb}$	$88.6^{A\text{-}Ca}$	46.7^{ABc}	84.8^{Aa}	67.9^{Ab}	73.64^{**}	VS
Ever Green IF	85.4^{ABab}	$67.3^{E\text{-}Gc}$	92.9^{Aa}	44.6^{He}	31.1^{Hf}	$49.8^{I\text{-}Kde}$	$53.0^{I\text{-}Ld}$	26.2^{EFf}	81.7^{ABb}	43.5^{Dee}	57.55^{**}	S
Fine Star	71.8^{CDc}	$72.0^{D\text{-}Fbc}$	$85.7^{A\text{-}Ca}$	$81.6^{A\text{-}Cab}$	89.5^{Aa}	$74.2^{B\text{-}Dbc}$	$81.2^{C\text{-}Eab}$	42.1^{BCd}	$72.8^{C\text{-}Ebc}$	51.3^{CDd}	72.22^{**}	VS
Lemon Hunt	91.5^{Aa}	$70.7^{D\text{-}Fbc}$	$63.4^{F\text{-}Icd}$	$73.5^{B\text{-}Eb}$	$72.4^{C\text{-}Eb}$	$68.1^{C\text{-}Fbc}$	$56.1^{I\text{-}Kd}$	00.0^{He}	66.2^{EFbc}	62.0^{ABcd}	62.39^{**}	S
Nova	84.2^{ABa}	$79.1^{B\text{-}Dab}$	$74.1^{C\text{-}Gab}$	$72.6^{B\text{-}Eb}$	69.0^{DEb}	$71.0^{B\text{-}Eb}$	$77.9^{D\text{-}Fab}$	$28.1^{D\text{-}Fd}$	$79.1^{A\text{-}Cab}$	46.8^{DEc}	68.19^{**}	S
Pine Red	19.7^{Je}	25.3^{LMde}	33.3^{Kd}	58.5^{FGb}	45.6^{Gc}	$59.9^{F\text{-}Ib}$	72.8^{FGa}	35.4^{CDcd}	56.0^{GHb}	20.3^{He}	42.68^{**}	S
Pot King	$61.3^{E\text{-}Hd}$	$86.7^{A\text{-}Cab}$	89.6^{ABa}	$71.0^{C\text{-}Ecd}$	$74.1^{C\text{-}Ec}$	$48.6^{I\text{-}Ke}$	93.5^{Aa}	26.4^{EFf}	$78.2^{A\text{-}Cbc}$	63.2^{ABd}	69.26^{**}	S
Pride Burn	13.6^{Jd}	08.2^{Nd}	21.6^{Lc}	34.2^{Hb}	26.1^{Hc}	$51.8^{I\text{-}Ka}$	45.6^{La}	00.0^{He}	24.4^{Ic}	22.9^{Hc}	24.84^{**}	MS
Rando	$55.8^{G\text{-}Id}$	$61.0^{G\text{-}Icd}$	$85.0^{A\text{-}Ca}$	$65.0^{E\text{-}Gbc}$	68.9^{Ebc}	$53.6^{H\text{-}Jd}$	$74.5^{E\text{-}Gb}$	17.8^{Gf}	$67.5^{D\text{-}Fbc}$	31.9^{FGe}	58.10^{**}	S
Red Cloud	$63.2^{D\text{-}Fc}$	48.5^{JKd}	$76.7^{C\text{-}Eb}$	86.6^{Aa}	$73.3^{C\text{-}Eb}$	$75.7^{B\text{-}Db}$	$60.8^{H\text{-}Jc}$	39.3^{BCd}	62.8^{FGc}	26.4^{GHe}	61.33^{**}	S
Red Power	19.5^{Jf}	27.9^{Lef}	45.3^{Jc}	59.3^{FGb}	79.7^{BCa}	41.5^{KLc}	$81.6^{C\text{-}Ea}$	00.0^{Hg}	30.9^{Ide}	38.2^{EFcd}	39.42^{**}	MS
Red Stone	47.1^{Ide}	53.8^{IJcd}	$84.6^{A\text{-}Ca}$	39.4^{Hef}	35.6^{Hf}	22.5^{Mg}	59.3^{IJc}	23.6^{FGg}	$71.8^{C\text{-}Eb}$	41.5^{Eef}	47.92^{**}	S
Red Tara	83.5^{Bab}	$77.9^{B\text{-}Db}$	$81.4^{A\text{-}Cb}$	82.7^{ABb}	87.0^{Aab}	$56.3^{G\text{-}Ic}$	94.8^{Aa}	37.4^{Cd}	83.6^{ABab}	63.7^{ABc}	74.83^{**}	S
Rio Grand	53.2^{Hic}	28.7^{Le}	$81.3^{A\text{-}Da}$	59.7^{FGc}	$73.8^{C\text{-}Eab}$	41.6^{KLd}	63.8^{HIbc}	24.9^{FGe}	54.4^{Hc}	18.2^{HIe}	49.96^{**}	S
Roma 505	$61.9^{E\text{-}Gc}$	$68.1^{E\text{-}Gc}$	$78.5^{B\text{-}Db}$	84.2^{Aab}	91.7^{Aa}	82.9^{ABab}	$87.1^{A\text{-}Ca}$	38.4^{BCd}	$69.5^{D\text{-}Fc}$	39.1^{EFd}	70.14^{**}	S
Sahil	$66.4^{C\text{-}Ecd}$	87.3^{ABa}	$63.7^{F\text{-}Id}$	88.2^{Aa}	83.1^{ABab}	$76.0^{A\text{-}Cbc}$	93.4^{Aa}	52.6^{Ae}	$72.4^{C\text{-}Eb\text{-}d}$	43.9^{DEe}	72.75^{**}	VS
Slumac	81.5^{Ba}	$69.2^{E\text{-}Gb}$	$74.2^{C\text{-}Gab}$	$79.1^{A\text{-}Dab}$	$75.8^{B\text{-}Eab}$	78.9^{ABab}	$81.7^{C\text{-}Ea}$	19.8^{FGc}	$75.9^{B\text{-}Dab}$	22.8^{Hc}	65.89^{**}	S
Sun Grape	15.9^{Jfg}	18.6^{MNef}	23.6^{Le}	$69.6^{D\text{-}Fb}$	58.6^{EFc}	$63.1^{E\text{-}Hbc}$	$77.0^{D\text{-}Fa}$	$33.3^{C\text{-}Ed}$	29.2^{Id}	09.0^{Ig}	39.79^{**}	MS
Terminator	71.6^{Cab}	$80.3^{B\text{-}Da}$	62.1^{Ibc}	51.4^{Gcd}	54.8^{Fc}	$43.4^{J\text{-}Lde}$	68.1^{GHb}	41.9^{BCde}	$70.3^{C\text{-}Eab}$	37.0^{EFe}	58.09^{**}	S
Tin Time	83.5^{Ba}	$65.0^{F\text{-}Hbc}$	$79.8^{B\text{-}Da}$	$62.7^{E\text{-}Gc}$	$74.8^{B\text{-}Eab}$	$49.9^{I\text{-}Kd}$	63.5^{HIc}	$28.6^{D\text{-}Fe}$	66.1^{EFbc}	43.1^{DEd}	61.70^{**}	S
Wall Ground	48.7^{Ibc}	53.2^{IJb}	$66.4^{E\text{-}Ia}$	37.9^{Hcd}	26.9^{Hd}	34.8^{Ld}	49.1^{Lb}	37.9^{Ccd}	54.1^{Hab}	55.9^{BCab}	46.49^{**}	S
MDI	59.55^{**}	58.44^{**}	69.42^{**}	65.96^{**}	65.88^{**}	59.18^{**}	73.87^{**}	30.03^{**}	64.05^{**}	40.57^{**}		

Capital letters shows level of significance in interaction of single Fol isolate against all tomato varieties as governed by DNMRT at p=0.05. Small letters shows level of significance in interaction of single tomato variety against all Fol isolates as governed by DNMRT at p=0.05. MDI= Mean Disease Index. (**)= significant difference among values at p=0.01 as governed by ANOVA. (MS)= Moderately Susceptible. (S)= Susceptible. (VS)= Very Susceptible

Table 3: Details of ISSR Primers used for genetic fingerprinting of Fol isolates

Primer	Sequence (5'-3')	Ann. Tepm.	Total no of bands	Polymorphic bands	%age polymorphism
810	GAGAGAGAGAGAGAGAT	50	17	11	64.70
823	TCTCTCTCTCTCTCTCC	50	23	14	60.86
826	ACACACACACACACACC	51	15	09	60.00
841	GAGAGAGAGAGAGAGAYC	52	16	14	87.50
845	CTCTCTCTCTCTCTCTAGG	52	11	07	63.63
855	ACACACACACACACACYT	50	18	13	72.22
856	ACACACACACACACACCTA	52	10	04	60.00
Total			110	82	74.54

Similarly same tomato variety displayed different levels of susceptibility against different isolates. This suggests that presence of multi–allelic or multi–genic responses towards resistance mechanisms of tomato varieties against Fusarium wilt disease (Saxena and Cramer, 2009). Tomato varieties and Fol isolates interaction could produce different levels and patterns of defense related biochemical compounds which eventually may cause variation in disease severity (Özer *et al.*, 2003).

Pattern of disease occurrence of different isolates of a pathogen for different varieties of a same crop is highly variable phenomenon (Sivaramakrishnan *et al.*, 2003). Thakur and Rao (1997) found variation in virulence among different isolates of *Sclerospora graminicola* against pearl millet varieties. In another investigation, Casela and Ferreira (1995) observed different virulence levels of *Colletotrichum graminicola* against sorghum.

Variable patterns of disease causing ability of Fol isolates of different tomato varieties cannot be easily understood by analyzing the mean values of disease index, because of the nature of interactions between tomato germplasm and Fol. isolates. A complete understanding of this variable disease patterns between different tomato germplasm is necessary for extracting useful information regarding resistance mechanisms. The most striking difference in resistance mechanism was observed between 'Cosmos 101' 'Rando' and 'Red Stone' (Table 1). These were resistant to one isolate of Fol but susceptible to

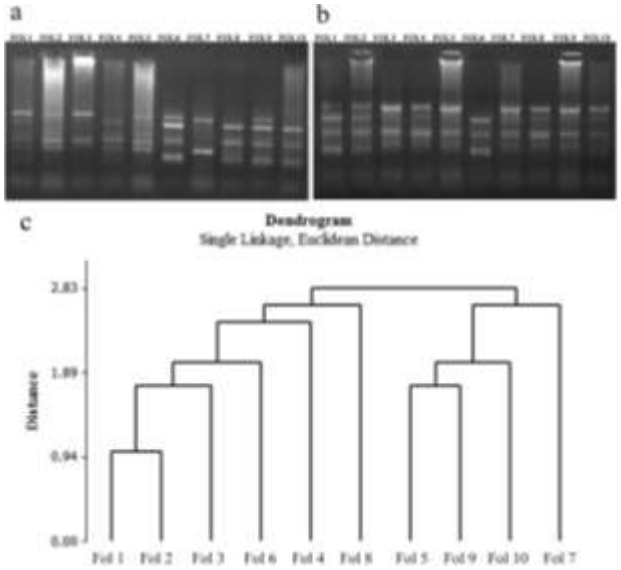

Fig. 3: DNA finger printing of Fol isolates by ISSR markers. (a) ISSR marker profiles of Fol isolates generated by primer. Dendrogram showing relationships between Fol Isolates using ISSR data

other. Same type of difference can also be observed in other tomato entries (Table 1).

The response of different isolates of a pathogen for causing same disease is not surprising, as it has been studied that a few mutations can lead to significant differences between isolates of same species (Evans *et al.*, 1986; Saxena and Cramer, 2009; Thakur *et al.,* 1992). In the same way, differences in virulence by different isolates of same pathogen species are still poorly understood. Even it remains to be explained why only for isolate Fol3 disease development is severe as compared to other two isolates as we observed in our current investigation. These findings prove that there exist differences in virulence levels of different isolates of same pathogen that are needed to be explored at genetic level. Fol1 exhibited lowest disease index when infecting 'Pride Burn' and highest disease index when it infected 'Ever Green IF' (Table 1). In addition, isolates, Fol2 and Fol3 exhibited high disease index when inoculated onto 'Early Boy' and low disease index when inoculated onto 'Lemon Hunt' and 'Nova' (Table 1). Varieties such as 'Early Boy', 'Roma' and 'Red Tara' were very susceptible to all isolates of Fol. Saxena and Carmer (2009) found same type of variations in disease susceptibility when they screened onion varieties against different isolates of *F. oxysporum* f. sp. CEPAE.

Different pathological behavior of Fol isolates in our current investigation can be attributed towards difference in their genetic material as we revealed in this study by using ISSR markers that effectively separated these isolates based on the differences in their genetic material. Chen *et al*. (1993) also described difference in virulence of *Puccinia striiformis* because of polymorphism in their genetic material. However, difference in virulence of Fol isolates along with differences in their genetic makeup provides bases for future studies.

In conclusion, the use of single pathogen isolate for screening of resistant source against a plant disease is not sufficient. Pathogen virulence analysis based on disease development using different varieties of host is more useful as compared to molecular analysis alone. Screening of different tomato varieties by multiple isolates of pathogen will provide useful information for development of resistance source by breeding program. A combination of current approach along with molecular investigations is needed to describe tomato and Fol relation dynamics.

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