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Fusarium Wilt of Tomato caused by *Fusarium oxysporum* f. sp. *lycopersici (Fol)*, it's Variability in Telangana State

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ABSTRACT

In India, tomato is the third important vegetable crop grown after potato and onion. The major tomato growing states in India are Odissa, Madhya Pradesh, Karnataka, Chhattisgarh, Andhra Pradesh and Telangana. In Telangana, tomato is cultivated in an area of 4,148 ha, with production 1171.50 Mt and productivity of 12 mt ha^{-1} .The major tomato growing districts in Telangana are Adilabad, Rangareddy and Sangareddy. Tomato is very often affected by several diseases incited by pathogens such as fungi, bacteria, viruses and nematodes. Among the fungal diseases, Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici (Fol)*, is one of the most destructive diseases across the world causing severe economic losses, wherever tomato is grown. Roving surveys were conducted in major tomato crop growing areas of Telangana state viz., Adilabad, Sangareddy and Rangareddy during the year 2017- 18. Fifteen isolates of *Fusarium oxysporum* f. sp. *lycopersici* collected from different tomato growing areas of Telangana were studied for their pathogenic variability and was observed that, *Fol* isolate collected from Adilabad district was found to be more virulent in causing disease incidence and disease severity when inoculated to susceptible tomato cv Pusa Ruby compared to the other fourteen isolates collected from different places of Telangana . All the *Fol* isolates were found to be pathogenic by causing diseased wilt symptoms on cv Pusa Ruby. Further, all the fifteen isolates were characterized at molecular level with species specific primer ITS 1(5'TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3′) and by using ISSR primers which revealed the relationship among the fifteen isolates with varied degree of coefficient.

Keywords Tomato, Fusarium wilt, Pathogenic variability, *F. oxysporum* f.sp. *lycopersici,* Telangana state.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.), a "fruity vegetable", native of Peru, South America, is a popular and widely grown annual vegetable crop

with weak woody stem, growing to a height of 1-3 meters under family *Solanaceae* , also known as Nightshades throughout the world. Due to its tangy flavor contributed to the dish, it is a favorite additive in all the regular cuisines across the world. In India, tomato is the third important vegetable crop grown after potato and onion with an area of 0.78 M ha, and production and productivity with 19,759 Mt and 25.04 Mt ha^{-1} respectively, and was grown mainly as *rabi* in plain areas and summer as well as rainy season crop at hilly areas. Tomato is very often affected by several diseases incited by pathogens such as fungi (Fusarium wilt, early blight, anthracnose, Verticillium wilt etc) bacteria (wilt and canker), viruses (leaf curl and tomato spotted wilt) and nematodes. Among all the fungal diseases that infect tomato, Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.), Snyder and Hans, is one of the most serious and destructive diseases across the world causing severe economic losses, wherever tomato is grown (Sudhamoy *et al.* 2009). Under these circumstances, integration of cultural, chemical and biological methods have played a major role in managing the Fusarium wilt disease of tomato (Singh *et al.* 2015). Adequate information is lacking with respect to variability among the isolates and source of genetic resistance. Knowledge on pathogenic variation among the races of pathogen is very much essential for effective disease management strategy especially for breeding resistant varieties. Keeping in view the importance of tomato crop, the present investigation was carried out to find the pathogenic variability among the *Fol* isolates for effective disease management strategies.

MATERIALS AND METHODS

Wilt diseased tomato plant were identified during roving surveys from major growing tomato growing areas (Table 1) and were brought to laboratory, washed under running tap water to remove adhered soil particles, surface sterilized with 1 per cent NaOCl (sodium hypochlorite) solution for 1 to 2 min followed by rinsing twice with sterile distilled water, then dried between sterile filter papers for 10 to 15 min. Infected and discolored stem portions were cut into small pieces with sterilized knife and again washed with distilled water followed by disinfection for one minute with 1% sodium hypo chlorite solution. They were again washed thrice with distilled water to remove residues of sodium hypo chlorite and then transferred aseptically under laminar air flow system on to sterilized Petri plates containing PDA medium. The plates were incubated at room temperature at 27 + 2 ^{0}C for 10 days for development of typical mycelial growth of associated causal organism.

Table 1. List of *Fol* isolates collected from different villages of Telangana State (2017-18).

Isolate No.	Village	District	GPS Points
Fol 1	Gudihatnoor	Adilabad	19.5293° N, 78.5121° E
Fol 2	Indervelley	Adilabad	19.3014° N, 79.0826° E
Fol 3	Salewada	Adilabad	19.4281° N,78.4026° E
Fol 4	Tosham	Adilabad	19.6578° N, 78.5314° E
Fol 5	Dandumailaram	Ranga Reddy	17.2291° N, 78.7760° E
Fol 6	Raipole	Ranga Reddy	17.2038° N, 78.7150° E
Fol 7	Manchal	Ranga Reddy	17.1605° N, 78.2077° E
Fol 8	Yacharam	Ranga Reddy	17.2044° N, 78.4001° E
Fol 9	Damarigidda	Ranga Reddy	16.8188° N, 77.5032° E
Fol 10	Thadlapally	Ranga Reddy	17.6297° N, 78.0837° E
Fol 11	Gummadidala	Sangareddy	17.6847° N, 78.3686° E
Fol 12	Nallavelli	Sangareddy	17.2751° N, 78.7972° E
Fol 13	Jharasangam	Sangareddy	17.7637° N, 77.7122° E
Fol 14	Zaheerabad	Sangareddy	17.6814° N, 77.6074° E
Fol 15	Chinna hyderabad	Sangareddy	17.6314° N, 78.3326° E

The isolated fungus cultures associated with wilt diseased specimens were identified based on cultural and morphological characters (micro and macro conidial characters and mycelial colors) with the help of monograph: The Fusarium (Booth 1971, Nelson *et al.* 1983), Illustrated Genera of Imperfect Fungi (Barnet and Hunter 1998) and CMI descriptions.

The identified cultures were further purified by single hyphal tipping method and were sub cultured on Petri plates having with PDA medium and were allowed to grow at 27 + 2 ^{0}C for 10 days.

To determine the forma specials of the collected *Fol* isolates and to prove Koch's postulates, fifteen isolates of *Fol* were tested for pathogenicity. On confirmation of pathogenicity by observing wilting diseased symptoms on tomato plants (Fig.1), re isolation of causal organism i.e., *Fol*, was done and the fifteen *Fol* isolates were designated serially from *Fol* 1 to *Fol* 15 and studied further for its pathogenic variability.

Fig. 1. Tomato plant wilted due to inoculation of *Fol.*

The wilt affected tomato plants exhibiting external symptoms viz., drooping of leaves, yellowing, stunted growth, initially yellowing on one side of the plant on lower leaves and branches, browning and death of entire plant at advanced stage of infection. On proper identification the infected plants were uprooted and checked for vascular discoloration by split opening the stem, which is chief characteristic symptom of Fusarium wilt of tomato. Pathogenic variability of *Fol* isolates were assessed by calculating PDI (Per cent disease incidence) and Disease severity separately for all the fifteen isolates.

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Disease severity index

Disease severity was recorded from 15th day onwards for each *Fol* isolate on 0 to 4 rating scale (Weitang *et al.* 2004), at an interval of 10 days up to the age of 45 day old and per cent disease severity was calculated as per the given formula,

$$\text{Disease severity index} = \frac{\text{Sum of all disease ratings}}{\text{Total number of plants observed} \times \text{Maximum disease rating}} \times 100$$

Disease rating scale (Weitang *et al.* 2004).

0 = No symptoms,
1 = Slight infection (< 25% showing wilted symptoms)
2 = Moderate infection (26 to 50 % showing wilted symptoms),
3 = Extensive infection (51 to 75% showing wilted symptoms)
4 = Complete infection (> 75 % of the plant wilted and dead).

Fifteen *Fol* isolates were categorized based on disease severity index into five categories, as follows (Charoenporn *et al.* 2010).

Disease severity	Isolate category
0	Avirulent
< 25%	Low virulent
26 to 50%	Moderately virulent
51 to 75%	Virulent
> 76%	Highly virulent

Root dip inoculation technique

Root dip inoculation technique was employed to find pathogenic variation in fifteen isolates of *Fol* on susceptible cv Pusa Ruby under green house conditions as per the procedure mentioned. Twenty one day old seedlings of tomato susceptible cv Pusa Ruby were uprooted slowly from protrays, abrased gently at root portions, dipped in the spore suspension having spore load of *Fol* @ 1×10^6 /ml for 15 to 20 mins (Sheu and Wang 2006), separately for all fifteen *Fol* isolates. On successful completion of root dip inoculation technique, seedlings were transplanted into pots having autoclaved sterilized soil of two kg each. Three replications were maintained in a Completely Randomized design with five seedlings per each replication. The pots were supplied with essential nutrients along with proper irrigation as per the protocol for proper establishment to express wilt symptoms similar to that of field conditions. The tomato seedlings cv Pusa Ruby were dipped in sterile

distilled water as check were transplanted into pots under similar conditions.

RESULTS AND DISCUSSION

Pathogenic variability among the fifteen *Fol* isolates was ascertained on the basis of the ability to cause disease and the temporal variation in appearance of the specific symptoms viz., drooping, yellowing, wilting or plant death. The results indicated that gradual increase in expression of wilt symptoms started from first fortnight to third fortnight and the appearance of characteristic wilt symptoms varied depending on the virulence level of each *Fol* isolate. Disease severity was calculated as per the appearance of the symptoms caused by each *Fol* isolate on 0-4 rating scale.

All the fifteen *Fol* isolates evaluated in the present study were found to be pathogenic in nature by causing the disease and producing characteristic symptoms viz., yellowing, drooping, netted appearance and wilting susceptible tomato cv Pusa Ruby. Temporal and symptomatic wilt expression from 15th day of inoculation (DOI) to 45th DOI clearly differentiated these fifteen *Fol* isolates under five different groups viz., Avirulent (denoted by DSI=0), low virulent (DSI-1), moderately virulent (DSI-2), virulent (DSI-3) and highly virulent (DSI-4).

Table 2. Pathogenic variability of *F. oxysporum* f.sp. *lycopersici* isolates on susceptible cv Pusa Ruby.

Isolate	PDI (%) and wilting severity (%) by *Fol* isolates					
	PDI (%) (15 DAP)	Disease severity (%)	PDI (%) 30 DAP	Disease severity (%)	PDI (%) 45 DAP	Disease severity (%)
Fol 1	20.00 (26.55)	4.00	40.00 (30.77)	12.00	66.66 (54.96)	33.33
Fol 2	20.00 (26.55)	5.33	26.66 (30.77)	11.66	66.66 (54.96)	50.00
Fol 3	20.00 (26.55)	4.00	66.66 (54.96)	21.66	93.33 (81.13)	43.33
Fol 4	60.00 (50.74)	12.00	100.00 (90.00)	51.66	100.00 (90.00)	90.00
Fol 5	20.00 (26.55)	4.00	66.66 (54.96)	20.00	86.66 (72.27)	51.66
Fol 6	20.00 (26.55)	4.00	26.66 (30.77)	16.66	66.66 (54.96)	45.00
Fol 7	20.00 (26.55)	6.66	40.00 (39.21)	11.66	73.33 (59.18)	60.00
Fol 8	33.33 (34.99)	10.00	80.00 (63.40)	36.66	100.00 (90.00)	83.33
Fol 9	20.00 (26.55)	5.00	40.00 (39.21)	16.66	66.66 (54.96)	53.50
Fol 10	20.00 (26.55)	5.00	80.00 (63.40)	23.30	100.00 (90.00)	81.66
Fol 11	20.0 (26.55)	5.00	33.33 (34.99)	15.00	73.33 (59.18)	58.30
Fol 12	20.00 (26.55)	5.00	73.33 (59.18)	28.33	100.00 (90.00)	78.30
Fol 13	20.00 (26.55)	5.00	26.60 (30.77)	10.00	66.66 (54.96)	40.00
Fol 14	20.00 (26.55)	5.00	73.30 (59.18)	31.66	93.33 (81.13)	83.30
Fol 15	26.66 (30.77)	6.66	73.30 (59.18)	31.60	93.33 (81.13)	78.30
CD (p=0.05)	4.645		10.14	--	14.8	--
SE(m)±	1.59	--	3.48	--	5.08	--
CV	9.52	--	12.14	--	12.35	--

Note: PDI: Percent disease index, DAP: Days after planting. Figures in parentheses are arcsine values.

At 15th DOI (date of inoculation)

The results (Table 2) revealed that per cent disease incidence (PDI) and disease severity index (DSI) varied significantly among fifteen *Fol* isolates which ranged from minimum PDI of 20.00 % (*Fol* -1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14) and grouped under DSI -1 (low virulent) to maximum PDI of 60% (*Fol*- 4) which was grouped under DSI -2 i.e., moderately virulent.

Among the fifteen *Fol* isolates, it was found that *Fol*-4 isolate was the only moderately virulent isolate at 15th DOI.

At 30th DOI

On 30th DOI, results (Table 2) revealed that significant differences were observed among fifteen *Fol* isolates. *Fol* -1, 2 and 6 recorded least per cent wilt disease incidence of 26.66% with DSI 2, while maximum per cent wilt disease incidence (100.00%) was observed in *Fol*- 4 with DSI 4. The other *Fol* isolates recorded per cent incidence of 66.66 % (*Fol* - 3), 40.00 % (*Fol* - 7 and *Fol* - 9), 66.66 % (*Fol* - 3 and *Fol* -5), 73.33 % (*Fol* -12) and 80.00 % (*Fol* - 8 and *Fol* -10) .

At 30th DOI, isolates *Fol* - 4, 8 and 10 were found significantly different from other *Fol* isolates in causing PDI and disease severity and were grouped under DSI 4 i.e., as highly virulent with disease severity of > 75% of the plant being affected.

At 45th DOI

The data recorded on 45th DOI revealed that pathogenic virulence varied significantly among the fifteen *Fol* isolates with minimum PDI of 66.66% (*Fol* - 1, 2, 6, 9 and 13) to maximum of 100% (*Fol* - 4, 8, 10 & 12) followed by per cent disease incidence of 93.33 per cent (*Fol*- 3, 14 and 15), 86.66% (*Fol*- 5) and 73.33 % (*Fol*- 7 and 11).

Isolate *Fol*- 4 (plate 1) was found to be highly virulent among the other *Fol* isolates, based on virulence in causing wilt disease incidence and disease severity on susceptible variety i.e., Pusa Ruby from 15th DOI to 45th DOI. The control plant i.e, uninoculated tomato plant performed well without any diseased symptoms (Fig.2).

Fig. 2. Control (un inoculated).

Grouping of *Fol* isolates based on disease severity

Based on wilt disease severity, the results revealed that (Table 3) fifteen *Fol* isolates were grouped into five groups as follows, Avirulent (DSI=0), Low virulent (DSI=1), moderately virulent (DSI=2), Virulent (DSI =3) and highly virulent (DSI= 4).

Similar variation in the pathogenicity of different isolates were reported by Jaruhar and Prasad (2011), Sumangala *et al.* (2013), Chopada *et al.* (2015) and Sivakumar *et al.* (2018) and based on the disease severity, isolates were grouped as most virulent and least virulent isolates. Similar results were reported by various workers, viz., Abdel *et al.* (2012) and Sundaramoorthy and Balabaskar (2013) which revealed that maximum PDI of 88.80, 78.50 and from 43.40 to 46.50 was observed with virulent isolate under glass house conditions.

Nirmaladevi and Srinivas (2012) also reported that pathogenic variability among 69 *Fol* isolates when tested against five susceptible varieties of tomato by adopting root cut and dipping technique and, based on the mean disease severity (MDS), virulence

Table 3. Grouping of *F. oxysporum* f.sp. *lycopersici* isolates based on disease severity.

DSI	Disease severity	15th DOI	30th DOI	45th DOI
0 (Avirulent)	0	--	--	--
1 (Low virulent)	1-10	*Fol* -1, 2, 3, 5, 6, 7, 8,9,10,11,12,13,14 & 15	--	--
2 (Moderately virulent)	11 - 25	*Fo*l- 4	*Fol* - 3,5,6,7,9, 10,11 & 13	--
3 (virulent)	26 - 50	--	*Fol*- 1,8,12,14& 15	*Fol* -1,3,6 & 13
4 (Highly virulent)	> 51	--	*Fol*- 4	*Fol* -2, 4, 5, 7, 8, 9, 10, 11,12,14 & 15

of *Fol* , isolates were recorded as weak pathogenic with low (MDS: < 25%), moderate pathogenic (MDS:25–50%) or high pathogenic (MDS: > 50%).

Reis *et al.* (2005) reported that the transmission of *F. oxysporum* f. sp. *lycopersici* , can be through by contaminated seeds of tomato which would further aggravate the establishment of different physiological variants with distinct variability from one location to other location even in same regions and districts. Mishra *et al*. (2010) reported that variation among *Fol* isolates may be due to mutation in the genome.

CONCLUSION

Pathogenic variability studies revealed that Isolate *Fol* - 4, collected from Adilabad district was found to be more virulent in causing disease incidence and disease severity when inoculated to susceptible tomato cv Pusa Ruby. Further, all the fifteen isolates were characterized at molecular level with species specific primer ITS 1(5'TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3′) and by using ISSR primers which revealed the relationship among the fifteen isolates with varied degree of coefficient.

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