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PATHOGENIC VARIATION AMONG *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* ISOLATES AND VARIETAL SCREENING OF TOMATO AGAINST WILT UNDER SOUTH GUJARAT, INDIA

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ABSTRACT
Ten isolates of *Fusarium oxysporum* f. sp. *lycopersici* collected from different tomato growing areas of South Gujarat. Pathogenic variability study was carried out on six tomato varieties using root dip inoculation technique. All isolates showed variation in incubation period which was range from 18 days to 25 days. Wilt incidence in six test genotypes were ranged between 30 to 100 %. Isolates SGFOL-7, SGFOL-4 and SGFOL-8 proved highly virulent pathogen showed 72.78%, 63.89% and 61.11% mean wilt incidence on all varieties respectively. Rest of isolates showed moderately to less virulent reaction. Ten tomato varieties were screened for resistance to SGFOL-7 isolate in pot, varieties NS-2535, Heamsona and GT-2 were found moderately resistant with 33.33, 33.33 and 46.67 per cent wilt incidence respectively.

INTRODUCTION

The production of tomato (*Lycopersicon esculentum* Mill.) is of worldwide agricultural importance. India is the fourth largest tomato producer in the world after China, USA and Turkey. In terms of area, it occupies second place after potato at the national level. Many diseases and disorders affect tomatoes during the growing season. Fusarium wilt of tomato is caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen is one of the most prevalent, serious diseases of tomato (Reis *et al.,* 2005; Sudhamoy *et al.,* 2009). It becomes one of the most prevalent and damaging diseases wherever tomatoes are grown intensively because the pathogen can persist indefinitely in infested soils (Agrios 1997). Worldwide poses a major threat in successful cultivation of tomatoes (Jones *et al*., 1991). The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield. There may be a 30 to 40% yield loss (Murthy *et al.,* 2009). However, severe cases it may cause 80% loss in tomato production (McGrath *et al*., 1987).

Management 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). 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 eco-friendly 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 pathogen. In view of this present study was carried out to identify different strain of *F. oxysporum* f. sp. *lycopersici* and to study pathogenic behaviour of isolates collected from different areas of South Gujarat and to found of variety which shows resistance against wilt pathogen.

MATERIALS AND METHODS

Isolation of *Fusarium oxysporum* f. sp. *lycopersici* isolates

Ten isolates of *F. oxysporum* f. sp. *lycopersici* were collected from different areas of South Gujarat namely, Bardoli, Maroli, Olpad, Gandevi, Navsari, Mandvi, Kamrej, Bharuch, Kadod *etc.,* during 2010-11 growing seasons and designated as SGFOL-1 to SGFOL-10. The samples showing characteristic wilt symptoms were brought into laboratory, 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 potato dextrose agar (PDA) medium and incubated at 25ºC for 3-5 days. The resultant fungus was isolated and purified using the hyphal tip method (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) and its selectivity for tomato plant was established through pathogenicity test. The fungus was cultured and maintained on PDA at 4ºC (Vibha and Nidhi, 2014). The fungus was multiplied on sorghum (*Sorghum vulgare* Pers.) grains pre-soaked for 12 hours in water and autoclaved at 1.1 kg/cm^2 for 30 minutes for two days subsequently for sterilization in 500mL flasks. Inoculated flasks were incubated in BOD incubator at 25 ± 1ºC for 15 days (Dubey *et al.,* 2010; Chopada and Singh, 2014).

Pathogenic variation

Pathogenic variation study was carried out on six tomato varieties *viz.,* Pusa ruby, AND-1, GT-1, GT-2, PKM-1 & Heamsona, were employed for testing pathogenic variation following root dip inoculation technique adopted by Elegresma *et al.,* (1972). Spore suspension was prepared by transferring sorghum grain medium containing growth of the isolate into distilled water and filters through double layered muslin cloth. Inoculum concentration of each isolates was adjusted to 1 × 10^6 spores/ml with the help of haemocytometer. Roots of one month old seedlings were clipped from distal one third root system with the help of sterilized razor and dipped in the spore suspension separately for 20 minutes and then transplanted into earthen pots filled with mixture of sterilized sand and soil (with ratio of 1:4 w/w). Ten seedlings per pot for each variety were kept for different isolates and three replications were maintained. Observations on incubation period and per cent wilt incidence recorded periodically after transplanting and per cent wilt incidence was calculated using following formula (Mandhare and Patil, 1993):

$$\text{Per cent wilt incidence (PWI)} = \frac{\text{No of wilted plant}}{\text{Total no of plant}} \times 100$$

Varietal screening

Ten tomato varieties *viz.,* Pusa ruby, AND-1, GT-1, GT-2, PKM-1, Heamsona, Pusa early dwarf, S-22, NS-2535 and DT-11 were screened for their resistance against most virulent isolate SGFOL-7 in pot using root dip inoculation technique. Per cent wilt incidence recorded and varieties were grouped under different degrees of resistance on the basis of disease grades suggested by Mandhare and Patil (1993), if the value of PWI is between 0 to 24 % ; resistant - if 25 to 49%; moderately resistant -if 50 to 74%; moderately susceptible - if 75% and above; susceptible.

Table 1: Pathogenic variability among isolates of *F. oxysporum* f. sp. *lycopersici* on six tomato varieties

Isolate	Tomato varieties												Mean wilt incidence (%)
	Pusa ruby		GT-1		AND-1		GT-2		PKM-1		HEAMSONA		
	1	2	1	2	1	2	1	2	1	2	1	2	
SGFOL 1	23	63.33* (52.75)**	25	36.67 (37.21)	22	50.00 (44.98)	24	33.33 (35.20)	24	33.33 (35.20)	25	10.00 (18.43)	37.77
SGFOL 2	21	86.67 (68.83)	20	50.00 (44.98)	21	83.33 (66.12)	20	43.33 (41.13)	20	56.67 (48.83)	20	26.67 (30.98)	57.78
SGFOL 3	22	83.33 (66.12)	21	50.00 (44.98)	23	63.33 (52.75)	24	30.00 (33.20)	23	53.33 (46.90)	21	20.00 (26.55)	50.00
SGFOL 4	18	86.67 (68.83)	20	73.33 (58.98)	20	73.33 (58.98)	20	50.00 (44.98)	21	63.33 (52.75)	21	36.67 (37.21)	63.89
SGFOL 5	20	80.00 (63.41)	23	43.33 (41.14)	21	56.67 (48.83)	20	26.67 (30.98)	23	43.33 (41.14)	23	20.00 (26.55)	45.00
SGFOL 6	22	60.00 (50.75)	25	30.00 (33.20)	22	53.33 (46.90)	24	36.67 (37.21)	24	46.67 (43.06)	25	10.00 (18.43)	39.45
SGFOL 7	18	100.00 (89.06)	20	80.00 (63.41)	19	90.00 (71.54)	20	60.00 (50.75)	21	66.67 (54.76)	21	40.00 (39.21)	72.78
SGFOL 8	19	96.67 (83.22)	21	50.00 (44.98)	19	83.33 (66.12)	23	46.67 (43.06)	23	60.00 (50.75)	23	30.00 (33.20)	61.11
SGFOL 9	20	83.33 (66.12)	22	46.67 (43.06)	23	73.33 (58.98)	22	53.33 (46.90)	22	40.00 (39.22)	22	23.33 (28.77)	53.33
SGFOL 10	21	63.33 (52.75)	20	63.33 (52.75)	22	66.67 (54.76)	21	40.00 (39.22)	22	56.67 (48.83)	22	30.00 (33.20)	53.33
S. Em ±		2.67		1.77		1.20		1.55		1.75		1.18	
C.D. at 5%		7.89		5.23		5.89		4.57		5.15		3.47	
C.V.%		7.00		6.61		6.07		6.67		6.56		6.96	

*Incubation period in days; **Per cent wilt incidence;* Average of three Replications;** Figures in parenthesis are angular transformed values

RESULTS

Pathogenic variation

Result (Table 1) revealed that wilt incidence in test genotypes were ranged between 30 to 100 %. Isolates SGFOL-7, SGFOL-4 and SGFOL-8 which were highly virulent produced 100.00, 86.67 and 96.67 per cent wilt incidence in Pusa ruby. In moderately susceptible varieties AND-1, PKM-1 and GT-1 wilt incidence by these virulent isolates was 90.00, 73.33 and 83.33; 66.67, 63.33 and 60.00; 80.00, 73.33, and 50.00 per cent, respectively. Wilt incidence in moderately resistance varieties GT-2 and Heamsona by these virulent isolates (SGFOL-7, SGFOL-4 and SGFOL-8) was 60.00, 50.00, and 46.67; 40.00, 36.67 and 30.00 per cent respectively. Other isolates produced wilt incidence ranged from 53.33 to 83.33 per cent in moderately susceptible varieties (AND-1, PKM-1 and GT-1). In AND-1, 33.33 to 56.67 per cent in PKM-1 and 30.00 to 63.33 per cent in GT-1. In moderately resistance varieties (GT-2 and Heamsona) isolates produced wilt incidence from 26.67 to 60.00 per cent in GT-2 and 10.00 to 40.00 per cent in Heamsona.

Highly virulent isolates SGFOL-7, SGFOL-4 produced wilt symptoms after 18 days of incubation but SGFOL-8 produced wilt symptoms after 19 days of incubation on susceptible variety Pusa ruby while other isolates (SGFOL-2, SGFOL-9, SGFOL-10, SGFOL-3, SGFOL-5, SGFOL-6 and SGFOL-1) produced wilt symptoms after 20 to 23 days of incubation. In moderately susceptible varieties AND-1, PKM-1 and GT-1, most virulent isolates showed wilt symptoms after incubation period ranging from 18 to 23 days, while other isolates showed wilt symptoms after 20 to 25 days of incubation. Isolates SGFOL-7, SGFOL-4 and SGFOL-8 produced wilt symptoms after 20 to 23 days of inoculation and other isolates produced wilt symptoms from 20 to 25 days after incubation on moderately resistance varieties GT-2 and Heamsona.

Varietal screening

After categorization of varieties based on per cent wilt incidence (PWI), none of the variety was resistant against SGFOL infection. Cent per cent wilt incidence was recorded in variety Pusa ruby. While S-22 showed 83.67 % hence these both varieties were categorized as susceptible to wilt. GT-1 (73.33%), Pusa early dwarf (66.67%), AND-1 (66.67%), PKM-1 (60.00%) and DT-11 (55.67%) varieties showed wilt incidence between 50.00 to 74.00 per cent and were categorized as moderately susceptible. GT-2 (46.67%), NS-2535 (33.33%) and Heamsona (33.33%) varieties showed wilt incidence between 25.00 to 49.00 per cent and therefore, were categorized as moderately resistant.

Table 2: Evaluation of different tomato varieties against *F. oxysporum* f. sp. *lycopersici* in pot condition

Sr No.	Variety	Total no. of plants sown	Total no. of wilted plants	Wilt incidence (%)	Reaction
1	Pusa ruby	30	30	100.00	S
2	Pusa early dwarf	30	20	66.67	MS
3	S -22	30	26	83.67	S
4	NS-2535	30	10	33.33	MR
5	DT - 11	30	17	56.67	MS
6	Heamsona	30	10	33.33	MR
7	GT-1	30	22	73.33	MS
8	GT-2	30	14	46.67	MR
9	AND-1	30	20	66.67	MS
10	PKM-1	30	18	60.00	MS

DISCUSSIONS

Present study clearly indicated the variation among ten isolates of *F. oxysporum* f. sp. *lycopersici*, collected from South Gujarat region and there may be chance of presence of new race of this pathogen as far as regional occurrence is concern. The phenomenon thus proved that on isolate pathogenic to one variety may not be identical in reaction with another variety. Thus result indicated that the response of isolates varied with genotype. 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). Jaruhar and Prasad (2011) collected ten isolates of *F. oxysporum Schlecht*. f. sp. *lentis* and found that All isolates showed virulent to the host plant but the degree of virulence varies from each other. Tomato varieties and Fol isolates interaction could produce different levels and patterns of defence related biochemical compounds which eventually may cause variation in disease severity (Ozer *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).The phenomenon also endorses supports the observations of Dubey *et al.* (2010); Kumar and Srivatava, (2013); Akram *et al.* (2014).

In conclusion, the use of single pathogen isolate for screening of resistant source against a plant disease is not sufficient. 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 *F. oxysporum* f. sp. *lycopersici* relation dynamics.

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