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Cultural and morphological variability among F*usarium oxysporum* f.sp. *lycopersici* Sacc. (Synder& Hans.,) isolates causing wilt of tomato (*Lycopersicon esculentum* Mill.) under south Gujarat

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An experiment was carried out to study the variability using ten isolates of *Fusarium oxysporum* f.sp. *lycopersici* collected from different tomato growing areas of south Gujarat. Studies were made on cultural and morphological variation like mycelial colour, mycelial growth, dry mycelium weight, sporulation, conidial size and formation of chlamydospores. The isolates produced moderate, profuse fluffy, thin flat to slight fluffy and submerged growth with white, yellow, light pink, dark pink, orange and purple orange pigmentation. Sporulation varied from 2.77 x 10^6 spores/ml to 21.68 x 10^6 spores/ml. The maximum dry mycelium weight was observed in SGFOL-6 (193.33 mg) whereas minimum dry mycelium weight was observed in isolate SGFOL-9 (120.67 mg). Colony diameter varied from 55.33 mm to 88.33 mm. The size of macro conidia ranged from 15.46-21.8 x 4.91-5.45µm to 21.42-44.28 x 7.35-9.14µm with 1 to 6 septa in different isolates. The size of micro conidia varied from 3.57-14.28 x 2.68-4.46µm to 7.14-14.28 x 3.57-5.35µm with 0 to 1 septa in different isolates.

Key words: Tomato, *Fusarium oxysporum* f.sp. *lycopersici,* cultural, morphological characters

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular important commercial vegetable crops rich in vitamins A, B, and C grown throughout the world (Madhavi and Salunkhe 1998). Because of its nutritional importance in India, it is popularly known as "Poor Man's Orange" (Tiwari, 1996). India stands 4th in tomato production and is grown in an area of about 6.34 lakh hectares, with total production of 124.33 lakh tones (Anon., 2010). The crop, is suffering from many fungal diseases (Ketelaar and Kumar, 2002) of which *F. oxysporum* f.sp. *lycopersici* (FOL) is a highly destructive pathogen of both greenhouse and field grown tomatoes in vegetable producing areas of the world. It becomes one of the most prevalent and damaging diseases wherever tomatoes are grown (Agrios 1991, Jones *et al.*, 1991).

The fungus enters tomato plants through the roots, and block the vascular bundles that inhibits water and ultimately leading to plant death (Davies,

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1982). There may be a 30 to 40% yield loss (Murthy *et al.,* 2009). In severe cases it may cause 80% loss in tomato production (McGrath *et al.*, 1987). A lots of variation found within the fungus in different regions. In view of this it become necessary to identify different strain of *F. oxysporum* f.sp. *lycopersici* under south Gujarat condition

MATERIALS AND METHODS

Ten isolates of *F. oxysporum* f.sp. *lycopersici* were collected from different tomato growing regions of south Gujarat during 2010-11 growing seasons and designated as SGFOL-1 to SGFOL-10 (Table 1). Tomato plants that showing characteristic wilt symptoms were uprooted and brought into laboratory for isolation. The roots of such diseased plants were washed with running tap water to remove all adhere soil particles and they were subjected to tissue isolation. The typically infected roots and stem portions from the collar region were cut in to small pieces with the help of sterilized knife and again washed with the sterilized distilled water. These pieces were then disinfected for five minutes with 2.5 per cent sodium hypochlorite solution. To remove residue of 2.5 per cent sodium hypochlorite solution, the pieces were washed thrice in sterilized distilled water for one minute each time and pieces were then transferred aseptically under laminar air flow system on sterilized Petri plates containing 20 ml potato dextrose agar (PDA) medium. The pathogenicity of the isolated fungus was tested on 10 seeds of tomato cultivar Pusa Ruby sown in plastic pots (15 cm) filled with 2 kg autoclaved soil and inoculated (10 g/kg soil) one week prior to sowing with inoculum multiplied on sorghum grains. The fungi were multiplied on sorghum (*Sorghum vulgare* Pers.) grains presoaked for 12 hrs. in water and autoclaved at 1.1 kg/cm^2 for 30 minutes for two days subsequently for sterilisation. Soaked grains (200 g) were filled in 500 ml flasks, sterilised and inoculated with seven-day-old culture. Inoculated flasks were incubated in BOD incubator at 25 ± 1°C for 15 days. Cultures on potato dextrose agar slants were stored at 5°C for use.

The pathogenic isolates were grown on PDA medium incubate at 27±2°C to study the ten days to study the cultural variability. After ten days of incubation period, diameter of the fungal mycelial growth, colony characters, sporulation and pigmentation were recorded. The colony diameters were measured on PDA medium poured into 80 mm Petri dishes (15 ml/plate) with three replications following the methods of Lilly and Barnett (1951). The isolates were also cultured in liquid media in 100 ml flask containing 20 ml of potato dextrose broth (PDB). These flasks were incubated at 27±2°C for fifteen days. In case of liquid media, the mycelial mat was removed by filtering through Whatman No. 1 filter paper after fifteen days of incubation and dried in hot air oven till consistent weight was obtained. The number of macroconidia and microconidia were counted with the help of haemocytometer. Data were analyzed statistically using complete randomized design (CRD) Following the same cultural procedure morphological variability of all the isolates were also measured by using a compound microscope.

RESULTS AND DISCUSSION

The cultural and morphological studies of the ten isolates of *F. oxysporum* f.sp. *lycopersici* were made by growing single spore culture of different isolates on solid and liquid potato dextrose medium at 27±2°C.

On potato dextrose agar medium in Petri plates, colony diameter (mm), cultural characteristics, sporulation and pigmentation were recorded (Table 2). Maximum colony diameter (88.33 mm) was of SGFOL-6 isolate after seven days of incubation at 27±2°C temperature followed by SGFOL-10 (85.33 mm), SGFOL-1 (83.67 mm), SGFOL-4 (83.00 mm), which were statistically at par. Least colony diameter (55.33 mm) was of SGFOL-2 isolate followed by SGFOL-3, SGFOL-8 and SGFOL-5 isolates. Isolates differed in their cultural characteristics, SGFOL-1, SGFOL-2, SGFOL-4, SGFOL-5, SGFOL-6 and SGFOL-8 produced moderate to profuse fluffy dull yellow, light pink, purple orange, dark pink, orange white, pink white with yellowish pattern like mycelium subsequently with white to yellow, dark pink or orange pigmentation, where as SGFOL-1 fail to produce any kind of pigmentation, while three isolates (SGFOL-3, SGFOL-7 and SGFOL-9) produced thin flat to slight fluffy yellowish white to orange mycelium with white to orange or purple orange substrate pigmentation. The isolate SGFOL-10 produced submerged yellowish white mycelium with no substrate pigmentation. Isolates SGFOL-7, SGFOL-4 and SGFOL-8 produced abundant sporulation, while isolates SGFOL-2, SGFOL-3, SGFOL-9 and SGFOL-10

Table 1 : Different isolates of *F. oxysporum* f.sp. *lycopersici* causing wilt in tomato collected from different places and geographical locations of south Gujarat

Isolate	Variety	Place	Geographical coordinates
SGFOL-1	Vaishali	Bardoli	21°07'N 73°07'E
SGFOL-2	GT-1	Regional Horticulture Research Station NAU, Navsari.	20°56'57?N 72°54'49?E
SGFOL-3	S-22	Maroli	28° 0' 30" N, 76° 0' 51" E
SGFOL-4	Vaishali	Olpad	21° 20' 14.56"N 72° 44' 50.82"E
SGFOL-5	Local	Gandevi	20°49'N 72°59'E
SGFOL-6	Local	Kamrej	21° 17' 0"N 72° 59' 0"E
SGFOL-7	Local	Mandvi	21° 15' 11"N 73° 18' 1"E
SGFOL-8	AND-1	Bharuch	21°44' 27.02"N 73°05' 09.53"E
SGFOL-9	Local	Kadod	21° 13' 0" N 73° 14' 0" E
SGFOL-10	GT-2	Regional Horticulture Research Station NAU, Navsari.	20°56'57?N 72°54'49?E

were good sporulators and remaining isolates produced scanty sporulation (Table 2).

In the liquid medium, dry mycelium weight and sporulation was recorded after 15 days of incubation at 27±2°C (Table 3). Maximum dry mycelium weight (193.33 mg) was recorded in SGFOL-6 isolate and which was statistically at par with SGFOL-8 and SGFOL-1 isolates, while SGFOL-5 and SGFOL-3 isolates yielded good mycelial weight 151.33 mg and 176.33 mg, respectively. Least mycelium weight (120.67 mg) was produced by SGFOL-9 isolate followed by SGFOL-7, SGFOL-2, SGFOL-4 and SGFOL-10 . Maximum sporulation (21.68 x 10^6 spores/ml) was observed in SGFOL-7 isolate followed by SGFOL-8, SGFOL-4, SGFOL-2, SGFOL-10, SGFOL-9 and SGFOL-3 isolates. Least sporulation (2.77 x 10^6 spores/ml) was produced by SGFOL-6 isolate followed by SGFOL-1 and SGFOL-5 isolates. Present study in conformity with several workers. Mycelial colour varied from white to dull white with slightly yellowish to pinkish tinge in among twenty isolates of *F. oxysporum* f.sp. *pisi* (Gupta *et al.*, 2011). Singh *et al.* (2011) observed that out of 12 isolates of *F. oxysporum* f.sp. *ciceris*, 7 isolates expressed appressed type growth pattern on PDA. Mycelial of isolates exhibited wide range colour of variation in colour from creamy white to dark purple. Patel *et al.*, (2011) observed that the dry mycelial weight of different isolates of *F. oxysporum* f.sp. *lini* ranged from 221.00 to 494.00 mg.

Morphological studies revealed variation in size of micro conidia, macro conidia and chlamydospores among ten isolates of *F. oxysporum* f.sp. *lycopersici*. The results are presented in Table 3. Macro conidia were straight; spindle as well as sickle shaped and had 1-6 septa. The size of macro conidia ranged from 15.46-21.8 x 4.91-5.45 im in SGFOL-1 isolate to 21.42-44.28 x 7.35-9.14 im in SGFOL-3 isolate. The isolate SGFOL-6 were unable to produce macro conidia. The micro conidia were hyaline, round to oval in shape and had 0-1 septa. The size of micro conidia ranged from 3.57-14.28 x 2.68-4.46µm in SGFOL-2 and SGFOL-6 isolates to 7.14-14.28 x 3.57-5.35µm in SGFOL-4 isolate. Chlamydospores were round, oval, terminal and intercalary in all the isolates. The size of chlamydospores varied from 6.85-7.73 x 6.67-7.90µm in SGFOL-7 isolate to 8.97-13.70 x 8.78-10.18µm in SGFOL-2 isolate. The different isolates showed smaller to higher degree of variation within different parameters like size of macro and micro conidia and chlamydospores. This result was in agreement with several scientists.

It was reported that isolates of *F. oxysporum* f. sp. *ciceris* were variable with respect to their conidial size. Microconidia varied from 5.1-12.8 x 2.5-5.0 im in size, whereas macroconidia were from 16.5-37.9 x 4.0 x 5.9 µm with 1-5 septations most commonly with 2-3 septate conidia. Prasad *et al.* (2008) observed that proportion of macro and micro conidia varied in different isolates of *F. oxysporum* f.sp. *ricini*. Macroconidia were 2 to 7 septate, straight to curve, sickle shaped or linear to broad. The average size of macroconidia ranged from 23.2 x 4.1µm in *For* 22 to 64.5 x 5.4µm in *For* 29. Microconidia were hyaline, round to oval shape ranged from 9.5 x 3.2 in *For* 22 to 23.4 x 6.8µm in *For* 29.

Table 2 : Colony diameter, sporulation and cultural characteristics of ten different isolates of *F. oxysporum* f.sp. *lycopersici* on PDA medium after ten days of incubation and on PDB medium after fifteen days of incubation at 27± 2°C

Isolates	Colony dia-meter* (mm)	Sporulation category**	Cultural characteristics Colony characters	Colour Mycelium	Substrate
SGFOL -1	83.67	+	Thin flat slight fluffy thread like mycelial growth irregular margin	Dull yellow	No colour
SGFOL -2	55.33	+++	Moderate fluffy aerial growth at margin, margin irregular, fluffy aerial mycelial growth at center	Light pink	Pink
SGFOL -3	65.33	+++	Thin flat slight fluffy slight thread like growth regular margin	Yellowish white	Yellow
SGFOL -4	83.00	++++	Profuse fluffy aerial growth with regular margin white, orange and purple mycelium with mosaic like pattern	White, orange and purple	Orange
SGFOL -5	73.00	+	Moderate fluffy, aerial growth margin regular	Dark pink	Dark pink
SGFOL -6	88.33	+	Profuse fluffy aerial mycelial growth , cottony raised mycelium	Pink and white	Light pink
SGFOL -7	75.00	++++	Thin flat, slight fluffy growth, margin regular	Pinkish orange	Orange
SGFOL -8	68.00	++++	Profuse fluffy, cottony raised mycelial growth, margin regular, with yellowish and pinkish mosaic like pattern	White, pink and yellow	Pink
SGFOL -9	79.67	+++	Thin flat, slight fluffy growth, margin regular	Orange	Purple orange
SGFOL -10	85.33	+++	Submerged growth, with irregular margin	Yellowish white	No colour
S. Em.±	1.378				
C.D. at 5%	4.066				

* Average of three repetitions **Sporulation category: - Absent, + Scanty, ++ Moderate, +++ Good, ++++ Abundant (on PDB)

Table 3: Pathogenic variability among different isolates of *F. oxysporum* f.sp. *lycopersici* on six different tomato varieties

Isolates	*Dry mycelium weight(mg)	*Sporulation (million/ml)	Microconidia		Macroconidia		Chlamydospore
			Size (µm)	No. of septa	Size (µm)	No. of septa	Size (µm)
SGFOL-1	181.67	3.13	5.35-12.49 x 3.57-5.35	0-1	15.46-21.8 x 4.91-5.45	2-3	8.08-8.21 x 6.66-7.84
SGFOL-2	131.67	16.79	3.57-14.28 x 2.68-4.46	0	23.25-35.8 x 3.86-5.26	2-3	8.97-13.70 x 8.78-10.18
SGFOL-3	176.33	14.41	6.35-12.50 x 3.57-5.35	0-1	21.42-44.28 x 7.35-9.14	3-6	8.95-11.58 x 5.09-7.38
SGFOL-4	141.33	17.38	7.14-14.28 x 3.57-5.35	0-1	16.40-32.84 x 5.27-6.78	1-2	7.90-8.87 x 7.85- 7.90
SGFOL-5	151.33	5.26	6.35-12.50 x 3.92-4.46	0-1	21.42-39.27 x 3.57-5.35	2-3	8.03-10.19 x 6.07-7.19
SGFOL-6	193.33	2.77	3.57-14.28 x 2.68-4.46	0	Not formed	-	7.67-10.88 x 7.15-7.90
SGFOL-7	124.67	21.68	4.46-12.50 x 3.57-5.35	0-1	17.85-40.82 x 4.35-7.14	3-6	6.85-7.73 x 6.67-7.90
SGFOL-8	189.67	18.09	6.24-14.28 x 2.68-4.46	0	17.18-38.70 x 4.91-5.97	1-3	8.08-9.64 x 7.73-9.13
SGFOL-9	120.67	15.18	5.35-12.50 x 2.68-5.35	0	28.56-43.55 x 6.35-8.19	3-5	7.55-7.83 x 7.02- 7.90
SGFOL-10	144.00	15.74	5.35-14.28 x 3.57-5.35	0	16.65-35.56 x 3. 57- 5.46	1-3	7.55-8.03 x 6.15-7.15
S. Em. ±	1.211	0.309					
C.D. at 5%	3.572	0.910					

* On pdb (average of three repetitions)

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