JOURNAL OF PURE AND APPLIED MICROBIOLOGY, APRIL 2014. Vol. **8**(2), p. 1109-1114

Cultural and Morphological Varaiability among *Fusarium oxysporum* f.sp. *Lycopersici* causing Wilt of Tomato under South Gujarat, India

G.B. Chopada[1]* and Pushpendra Singh[2]

[*1]Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India.
[2]Department of Plant Pathology, Gujarat Agricultural BioTech Institute, Surat, Navsari Agricultural University, Gujarat, India.

(Received: 15 September 2013; accepted: 11 December 2013)

Ten isolates of *Fusarium oxysporum* f.sp. *lycopersici* collected from different tomato growing areas of south Gujarat and designated as SGFOL-1 to SGFOL-10. 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 pigmentation range of white, yellow, light pink, dark pink, orange and purple with orange pigmentation. Sporulation varied from 2.77 × 10^6 spores/ml to 21.68 × 10^6 spores/ml. The macro conidia ranged from 15.46-21.8 × 4.91-5.45 μm to 21.42-44.28 × 7.35-9.14 μm. The micro conidia varied from 3.57-14.28 × 2.68-4.46 μm to 7.14-14.28 × 3.57-5.35 μm. Width of mycelia and chlamydospore dimension also varied in all isolates. 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). Dry mycelium weight varied from 55.33 mm to 88.33 mm.

Key words: Tomato, *Fusarium oxysporum* f.sp. *lycopersici,* Cultural, Morphological characters.

The production of tomato is of worldwide agricultural importance. 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. India stands 4th in tomato production worldwide. In India tomato has been popular since last five decades and is grown in an area of about 6.34 lakh hactares, with total production of 124.33 lakh tones (Annon., 2010)[1]. Many diseases and disorders can affect tomatoes during the growing season. *Fusarium oxysporum* f.sp. *lycopersici* (FOL) is a highly destructive pathogen of both greenhouse and field grown tomatoes in warm vegetable production areas. 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. Management of seed-borne and soil-borne disease such as wilt caused by *Fusarium* specie has always been problematic. In view of this it is necessary to identify different strain of *F. oxysporum* f.sp. *lycopersici* under south Gujarat condition for further management of disease.

* To whom all correspondence should be addressed.
E-mail: Chopada.gopal@gmail.com

MATERIALS AND METHODS

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

Ten isolates of *F. oxysporum* f.sp. *lycopersici* were collected from different tomato growing regions of south Gujarat namely, Bardoli, Maroli, Olpad, Navsari, Mandvi, Kamrej, Bharuch, Kadod during 2010-11 growing seasons. The samples showing characteristic wilt symptoms were

uprooted and brought into laboratory for isolation. The plants were split open and prepared the slide for the microscopic examination. The symptoms and signs on infected plants, observed in nature were critically observed and recorded. Fresh infected plants of tomato showing well developed wilt infection were taken. 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 root 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 (Cabinet Manufactured by Klenzoid Contamination Control Ltd.) on sterilized Petri plates containing 20 ml potato dextrose agar (PDA) medium. These Petri plates were incubated for 5 days at 27±2°C temperature in BOD incubator. The fungal hyphae developed from infected tissues were sub-cultured aseptically on PDA slants. The pure culture was microscopically examined for its purity and was further purified by using hyphal tip method of isolation. The pure culture, thus obtained was maintained on PDA slants at low temperature (4°C) in refrigerator for further study.

Cultural variability

The isolates were separately cultured on Potato dextrose agar (PDA) media, at 27±2°C for ten days. After ten days of incubation period, diameter of the fungal mycelial growth, colony characters, sporulation and pigmentation were recorded. The isolates were also cultured in liquid media in 100ml flask containing 20ml 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. The results were tabulated. Three repetitions were made for each isolates. Data were analyzed statistically using complete randomized design.

Morphological variability

The isolates were also cultured in liquid media in 100 ml flask containing 20ml of potato dextrose broth (PDB). These flasks were incubated at 27±2°C for fifteen days. After incubation, average measurements were taken by the micrometry method. The morphological characters like size (length and width) of macroconidia and microconidia and chlamydospore, were recorded. The observations were recorded in three repetitions within each isolate. The study was carried out using ocular and stage micrometer after mounting them on the slides containing sterile distilled water at magnification of 450X. Data were analyzed statistically using complete randomized design.

RESULTS AND DISCUSSIONS

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 1). 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-

Table 1. Colony diameter, sporulation and cultural characteristics of ten different isolates of *Fusarium 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 2. Growth, sporulation and size of microconidia, macroconidia and chlamydospores of ten different isolates of *F. oxysporum* f. sp. *lycopersici* on PDB for fifteen days of incubation at 27±2°C temperature

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)

7, SGFOL-4 and SGFOL-8 produced abundant sporulation, while isolates SGFOL-2, SGFOL-3, SGFOL-9 and SGFOL-10 were good sporulators and remaining isolates produced scanty sporulation (Table 1). In the liquid medium, dry mycelium weight, sporulation was recorded after 15 days of incubation at 27±2°C presented in Table 6**.** 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 growth 151.33 mg and 176.33 mg, respectively. Least mycelium growth (120.67 mg) was produced by SGFOL-9 isolate followed by SGFOL-7, SGFOL-2, SGFOL-4 and SGFOL-10 (Table 2). 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 (Table 2). The morphological and cultural variation serves as an aid in differentiation of isolates. All the isolates of *F. oxysporum* f. sp. *lycopersici* possessed characteristic feature when culture on PDA. The differences in colony characteristics were observed *viz.*, colony colour, growth, sporulation, and colony diameter.

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 2. 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 µm in SGFOL-1 isolate to 21.42-44.28 x 7.35-9.14 µm 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 2.68-4.46 µm in SGFOL-2 and SGFOL-6 isolates to 7.14-14.28 x 3.57-5.35 µm 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 6.67-7.90 µm in SGFOL-7 isolate to 8.97-13.70 x 8.78-10.18 µm 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.

CONCLUSION

Present study clearly indicated the variation among ten isolates of *F. oxysporum* f. sp. *lycopersici*, collected from South Gujarat region in terms of cultural and morphological characteristics. On the basis of such investigation here we conclude that there may be chance of presence of new race of this pathogen as far as regional occurrence is concern. Further extensive study is required to identify the variation among *F. oxysporum* f. sp. *lycopersici* up to races. Therefore, race specific screening programmes are to be started to identify race specific resistance or multiple race resistant clones of tomato which can withstand wilt disease for long time.

This result was in agreement with several scientists. 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)[2], (*Singh et al.* 2011)[3] observed that out of 12 isolates of *F. oxysporum* f. sp. *ciceris*, 7 isolates expressed appressed type growth pattern on PDA. Mycelial colour of isolates exhibited wide range colour of variation from creamy white to dark purple.

(Prasad *et al.*, 2008)[3] 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 4.1 µm in *For* 22 to 64.5 x 5.4 µm in *For* 29. Microconidia were hyaline, round to oval shape ranged from 9.5 x 3.2 in *For* 22 to 23.4 x 6.8 µm in *For* 29.

(Dubey *et al.*, 2010)[4] observed isolates of *F. oxysporum* f. sp. *ciceris* variable with respect to their conidia size. Microconidia varied from 5.1-12.8 x 2.5-5.0 µm 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.

ACKNOWLEDGMENTS

I am highly thankful to "ASPEE Agricultural Research and Development Foundation, for providing fellowship for research during my M.Sc. (Agri.) study.

REFERENCES

1. Annonymous, National Horticulture Database 2010. All India area, production and productivity of tomato. $2010_{a;}$ pp: 173.
2. Gupta, S. K., Rana, S. and Jarial, K. Variation in morphological, cultural, pathogenic and molecular features of *Fusarium oxysporum* f. sp. *pisi* isolates causing wilt of pea (*Pisum sativum*). *J. Mycol. Pl. Pathol.*, 2011; **41**(2): 275-278.
3. Singh, S. K. Singh, B., Singh, V. B. and Reena. Morphological, cultural and pathogenic variability among the isolates of *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chick pea. *Ann. Pl. Protec. Sci.*, 2011; **19**(1): 155-158.
4. Prasad, S. L., Sujatha, M. and Raoof, M. A. Morphological, pathogenic and genetic variability in castor wilt isolates. *Indian Phytopath*., **61**(1): 18-27.
5. Dubey, S. C., Singh, S. R. and Singh, B. (2010). Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *Archives Phytopathol. Pl. Protec.*, **43**(2): 174-190.