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Kulchander
M.Sc., Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Dr. SS Sharma
Professor, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Kalpana Yadav
Ph.D. Scholar, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Kiran Dudi
M.Sc., Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Poonam Yadav
Ph.D. Scholar, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Correspondence
Kulchander
M.Sc., Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Cultural and morphological characterization of post flowering stalk rot of maize caused by *Fusarium* spp.

Kulchander, Dr. SS Sharma, Kalpana Yadav, Kiran Dudi and Poonam Yadav

Abstract
Fusarium is considered as a devastating fungal menace of the most prevalent fungus on maize, particularly in USA, Europe, Africa, Asia and Australia. A experiment was conducted with ten isolates of *Fusarium* spp. (*F. proliferatum* (4 isolates), *F. verticillioides* (5 isolates), and *F. pallidoroseum* (1 isolates) were collected from different parts of Rajasthan from rotted maize stalks and identified by ITCC, IARI (New Delhi, India). The maximum colony diameter (90mm) were observed in isolate Fv-01 (*F. verticillioides*) whereas minimum colony diameter (54.70mm) was observed in isolate Fv-09 (*F. proliferatum*). Isolates varied among themselves with respect to pigmentation by white 3, pink 2, violet 2, Pale Yellow, light vane light brown and brown 1. Margin and topography of the isolates varied from circular 6, semi-circular 3, waivy 1 and flat 5, medium raised 3 and raised 2, respectively. The maximum length and width of macro conidia was recorded in the isolate Fv-02 (*F. verticillioides*) which measured i.e. 40.24- 6.60 µm while minimum in Fv-09 (*F. proliferatum*) of 17.57- 1.80 µm. On the basis of cultural and morphological characters of all the ten isolates showed variability.

Keywords: Maize, PFSR, *Fusarium*, pathogenicity, morphology etc.

Introduction
Post flowering stalk rot (PFSR) complex play a vital role in affecting the productivity of maize (*Zea mays* L.) crop in all continents of the world, including USA, Europe, Africa, Asia and Australia (Nur Ain Izzati *et al.*, 2011) [8]. This complex is caused by *Fusarium verticillioides*, *Macrophomina phaseolina* and *Cephalosporium maydis*, out of which *F. verticillioides*; (Saccardo) is of economic importance (Kumar and Shekhar, 2005, Dorn *et al*. 2009) [16, 2]. In India, the disease is prevalent in most of the maize growing areas, particularly in rainfed areas *viz*., Jammu and Kashmir, Punjab, Haryana, Delhi, Rajasthan, Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh, Tamil Nadu and Karnataka (Kaur and Mohan 2012) [5], where water stress occurs after flowering stage of the crop. Reduction in yield due to PFSR disease in susceptible maize germplasm has been estimated to the tune of 39.5 per cent (AICRIP, 2013) [1]. The water stress at flowering and high soil temperature help in increasing of the magnitude of the stalk rot symptoms at post flowering stage of maize crop (Smith and McLaren 1997, Khokhar *et al*., 2014) [15, 7]. Most of the commercially grown cultivars have shown a high level of disease incidence at the grain filling stage (Iglesias *et al.*, 2010) [4]. Studies on cultural, morphological and molecular variations of predominant pathogen *Fusarium* spp. are important for germplasm evaluation. Morphological identification of plant pathogenic fungi is the first and the most difficult step in the identification process. This is especially true for *Fusarium* species. Although morphological observations may not sufficient for complete identification, a great deal of information is usually obtained on the culture at this stage (Rahjoo *et al.*, 2008, Sankar *et al.*, 2011) [11, 14]. RAPD-PCR has been successfully used to identify strains and races in phyto pathogenic fungi. It has been used for studying inter and intraspecific variability among population from different and same geographic areas (Saharan *et al.*, 2008) [13]. The RAPD pattern visualizes variations in total DNA and thus is suitable for differentiating *Fusarium* spp. isolates. RAPD results obtained in present study enabled fast variability analysis for *Fusarium* isolates. Traditional markers used to study the variability in plant pathogens are based on the differential hosts, cultural characteristics, morphological markers and biochemical tests. There is little information about Post Flowering Stalk Rot disease in maize has not been extensively studied in this country. Additionally, the causal fungi of PFSR disease had not been molecularly identified until the present study.

The aims of this study were to isolate and characterize *Fusarium* spp. associated with PFSR disease of maize using cultural and morphological methods of variability.

Materials and Methods

Survey for occurrence of the disease

The major maize growing areas of Southern Rajasthan are Udaipur, Rajasamand, Chittorgarh, Dungarpur, Banswara, Pratapgarh, Bhilwara, Kota, Bundi, Baran and Jhalawar districts. To determine occurrence and distribution of Post Flowering Stalk Rot (PFSR) on farmer's fields, these areas were surveyed periodically during *Kharif* 2017 which is normally maize growing season in Rajasthan.

Isolation and purification of pathogen

Culture of *Fusarium verticillioides* was isolated from naturally infected stem of maize by splitting under aseptic conditions. The stem of the diseased plants showing typical symptoms of PFSR from farmers' fields were collected, labelled and brought to the laboratory. These internodes were thoroughly washed in running tap water to remove the adhering soil. For this, small pieces showing typical lesions of the PFSR were cut, washed in sterilized water, surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for two minutes, rinsed thrice in sterilized distilled water, dried between blotters and transferred on to potato dextrose agar (PDA) medium in Petri plates. The plates were incubated at 28±1 °C for growth. Sub-cultures were made from the periphery of the mycelial growth, which appeared after 6-7 days.

Cultural and morphological variability

The isolates were grown on PDA at 28±2 °C temperature for seven days to study the morphological characters like width of mycelium, size of conidia and number of transverse and longitudinal septa. Data in relation to morphological and cultural characters like, colony diameter, colony color, pigmentation, zonation, topography of the culture, margins of the culture, rate of sporulation, septation and size of macro and micro conidia were studied. Studies of sporulation were also undertaken using the formula given by Pathak (1984) [10]. The size of conidia was measured under light microscope at 40X using micrometre. Fifty observations were taken for conidial measurement and mean values were calculated.

Results

Cultural Variability: Radial growth and cultural characters of ten isolates of *Fusarium* spp.

The diameter and characteristics of the mycelial growth were recorded after 24, 48, and 72 hrs to know the growth pattern of all the isolates for comparison (plate 2). All the ten isolates differed in cultural characters *i.e.* cottony white, purple, red at bottom with red cottony, white grey, purple violet, cottony pink, cottony white with violet ring at center, cottony white with bluish at center, dirty white cottony and dirty grey color growth were observed in Fv-01, Fv-02, Fv-03 Fv-04, Fv-05, Fv-06, Fv-07, Fv-08 Fv-09 and Fv-10 respectively. Colony diameter ranged between 54.70 to 90 mm. The myeelial growth in isolate Fv-01 completed earlier hence, observations were recorded to know the relative difference in radial growth. The average radial growth of isolate Fv-01 (*F. verticillioides*) was highest i.e. 90.00 mm, followed by Fv-02 (*F. verticillioides)* 86.44, Fv-06 (*F. proliferatum*) 84.65, Fv-08 (*F. verticillioides*) 80.32, and Fv-07 (*F. proliferatum*) 76.40, while, in isolate Fv-03 (*F. pallidoroseum*), Fv-05 (*F. proliferatum*) Fv-04 (*F. verticillioides*), Fv-10 (*F. verticillioides*) and Fv-09 (*F. proliferatum*) it was comparatively less i.e. 72.28, 71.50, 62.80, 60.22 and 54.70 mm respectively at 7th day of incubation under uniform environments and media. Isolates varied among themselves with respect to pigmentation by white 3, pink 2, violet 2, Pale Yellow, light vane light brown and brown 1. Margin and topography of the isolates varied from circular 6, semi-circular 3, waivy 1 and flat 5, medium raised 3 and raised 2, respectively. Conidial morphology in all the collected isolates varied which is a symbol of variation in with the change in climate and geography (Plate-3).

Table 1: Radial growth and cultural characters of *Fusarium* spp. Isolates

S. NO.	Isolate designation	*Fusarium* spp.	Colony diameter (mm)	Colony color	Margin	Topography of the culture	Zonation	Pigmentation in culture
1	UD-Fv- 01	*Fusarium verticillioides*	90.00	Cottony white	Semi-circular (irregular)	Medium raised	No undulation	White
2	UD-Fv- 02	*Fusarium verticillioides*	86.44	Purple	Circular	Flat	No undulation	Violet
3	UD-Fp- 03	*Fusarium pallidoroseum*	72.28	red at bottom with red cottony (superficial)	Circular	Raised	No undulation	Red
4	UD-Fv- 04	*Fusarium verticillioides*	62.80	White grey		Medium raised	No undulation	White
5	UD-Fp- 05	*Fusarium proliferatum*	71.50	Purple violet	Circular	Flat	Concentic rings	Pink
6	UD-Fp- 06	*Fusarium proliferatum*	84.65	Cottony pink		Flat	Concentic rings	Pink
7	UD-Fp- 07	*Fusarium proliferatum*	76.40	Cottony white (superficial) with violet at bottom ring at center	Circular	Medium raised	No undulation	Pale yellow
8	UD-Fv- 08	*Fusarium verticillioides*	80.32	Cottony white (superficial) with bluish at bottom center		Flat	No undulation	Violet
9	UD-Fp- 09	*Fusarium proliferatum*	54.70	Dirty white cottony	Circular	Raised	Concentic rings	White
10	UD-Fv- 10	*Fusarium verticillioides*	60.22	Dirty grey white	circular	Flat	No undulation	Brown

Plate 1: Isolates of different *Fusarium* spp. Recovered from sample collected diseased field in maize area of Rajasthan

F verticilloides	*F verticilloides*
E pallidoroseum	*F verticilloides*
E proliferatum	*E proliferatum*
F. proliferatum	*F verticilloides*
E proliferatum	*F verticilloides*

Morphological Characters

All the ten isolate of *F. verticillioides* showed significant variation in conidial morphology. Results presented in Table-4 show that mean length and width of macro conidia in different isolates of *Fusarium* spp. ranged from 10.90 40.42 x 1.80 - 6.60 µm and the mean length and width of micro conidia in different isolates ranged from 4.20 - 12.43 x 1.50 - 4.5 µm. Macro conidia were produced by all the isolates but their size varied between 40.24 to 6.60 µm. Similarly all the isolates produced micro conidia with considerable variations i.e. 10.92 -2.78 µm. Among the *Fusarium* isolates, the maximum length and width of macro conidia was recorded in the isolate Fv-02 (*F. verticillioides*) which measured i.e. 40.24- 6.60 µm followed by isolate Fv-08 (*F. verticillioides*) with 38.14- 5.04, Fv-01 (*F. verticillioides*) with 37.42- 4.89 µm, while minimum in Fv-09 (*F. proliferatum*) of 17.57- 1.80 µm. Among the *Fusarium* spp. isolates, the maximum length and width of micro conidia was recorded in the isolate Fv-08 which measured 12.43- 3.09 µm followed by isolate Fv-02 (*F. verticillioides*) i.e. 11.33- 1.50 µm, while minimum in Fv-03 (*F. pallidoroseum*) with 4.20- 1.83 µm. Excellent sporulation for macro and micro conidia was found in isolate Fv-01 i.e. 3.4 x 105 and 3.8 x 10^5, followed Fv-02 (*F. verticillioides*) with 2.9 x 10^5 and 3.1 x 10^5 Fv-03 (*F. pallidoroseum*) of 2.6 x 10^5 and 2.8 x 10^5 and Fv-06 (*F. proliferatum*) of 2.5 x 10^5 and 3.2 ×10^5, while minimum sporulation for macro conidia was observed in With respect to number of septa in the range of 2-7 (Table-4).

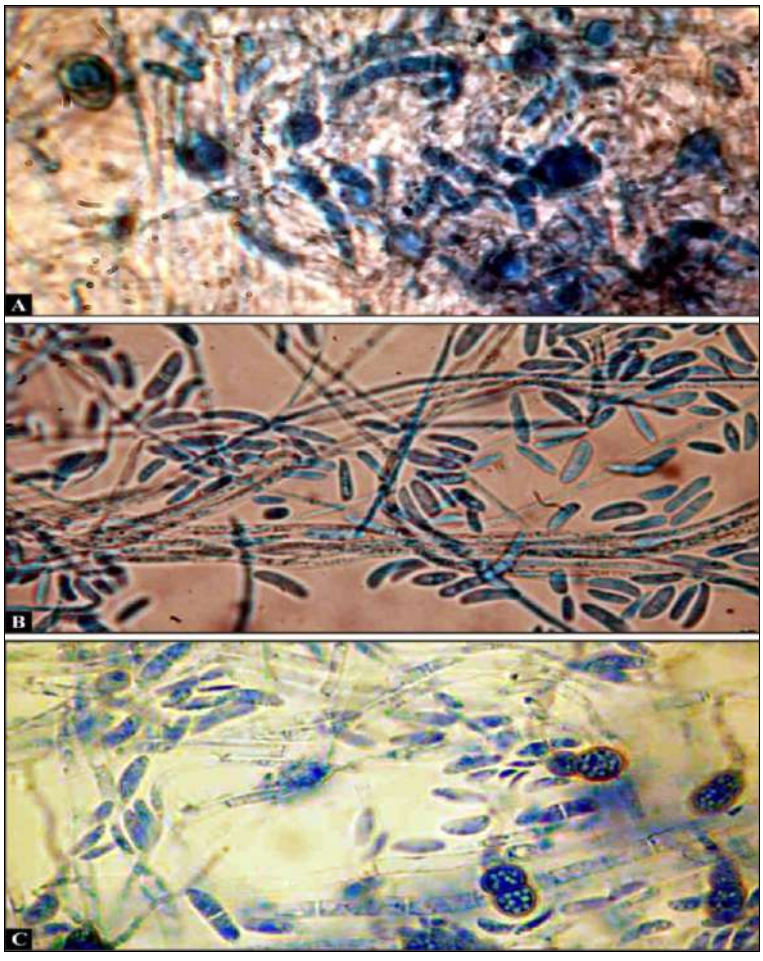

Plate 2: Conidial morphology of *Fusarium* species a). *E verticilloides*; b). *E proliferatum*; c). *E pallidoroseum*

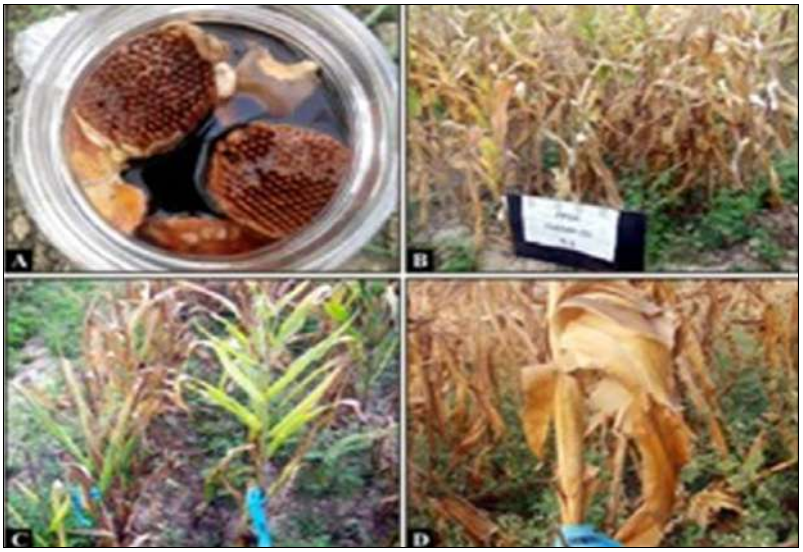

Plate 3: Symptoms of PFSR in field a) Toothpick inoculum, b) Isoculated field, c) Resistant and susceptible lines, d) Tilted Cob

Table 2: Variation in conidial morphology, measurement, septation and spore count of *Fusarium* spp. isolates.

S. No.	Isolate designation	*Fusarium* spp.	Spore size (µm)*				Septation in macro conidia	No. of spore/ml*	
			Macro conidia		Micro conidia				
			Length	Width	Length	Width		Macro conidia	Micro conidia
1	UD-Fv-01	*Fusarium verticilloides*	37.42	4.89	9.82	1.80	5-7	3.4×10^5	3.8×10^5
2	UD-Fv-02	*Fusarium verticilloides*	40.24	6.60	10.33	1.50	4-7	2.9×10^5	3.1×10^5
3	UD-Fp-03	*Fusarium pallidoroseum*	29.50	2.28	4.20	1.83	3-5	2.6×10^5	2.8×10^5
4	UD-Fv-04	*Fusarium verticilloides*	21.04	4.14	5.62	2.44	4-5	2.0×10^5	2.7×10^5
5	UD-Fp-05	*Fusarium proliferatum*	22.55	3.89	10.10	4.05	3-5	2.2×10^5	3.0×10^5
6	UD-Fv-06	*Fusarium proliferatum*	18.83	2.06	6.64	1.60	3-4	2.5×10^5	3.2×10^5
7	UD-Fp-07	*Fusarium proliferatum*	17.66	3.08	4.56	2.96	3-4	1.5×10^5	2.5×10^5
8	UD-Fv- 08	*Fusarium verticilloides*	38.14	5.04	12.43	2.96	4-6	2.2×10^5	3.0×10^5
9	UD-Fp-09	*Fusarium proliferatum*	17.57	1.80	9.09	3.09	3-4	$.08\times 10^5$	1.6×10^5
10	UD-Fv- 10	*Fusarium verticilloides*	10.92	2.78	6.17	1.48	2-3	2.2×10^5	3.0×10^5
S.Em±			0.10	0.016	.03	.01		.008	.010
CD at 5%			0.28	0.045	0.08	0.03		0.024	0.029
CD (P =.01)			0.37	.06	0.11	0.04		0.031	0.038

* Mean no. of 50 conidia and ± S.D. of mean value

Discussion

Similarly, Patil *et al*., (2007) [9] reported colony diameter in different isolates of *F. moniliforme* (*verticilloides*) which varies in range from 85-90 mm after 7 days of incubation. Similarly Siddiqui *et al*., 1995 [12] reported significant differences in growth rate of different isolates of *F. moniliforme* (*verticilloides*). In the present investigations, though some differences exist in growth rate of isolates but no correlation was observed between the colony characters and growth rate of the isolates. Hirata *et al.*, (2001) [3] studied the sporulation of different isolates of *F. moniliforme* (*verticilloides*) and reported that darkness stimulated the production of conidia as well as it had positive effect on conidial length. Siddiqui *et al.*, (1995) [12] also reported significant differences in sporulation of different isolates of *F. moniliforme* (*verticilloides*). Variability is one of the natural phenomenons which largely depends on climatic conditions, geography and cropping pattern. The survival of pathogen is because of variability as a natural phenomenon.

Conclusion

The present study provides information about the causal agents of post flowering stalk rot of maize in India and reveals for the first time genetic and molecular variability among *Fusarium* isolates belonging to three *Fusarium species* (*F. proliferatum*, *F. pallidoroseum* and *F. verticillioides*) obtained from different geographic regions in Rajasthan (India). In addition the present study generated significant information in terms of cultural and morphological variability of *Fusarium verticillioides* which could be further used by breeders for evolving region specific resistant varieties of maize.

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