International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 902-905

Received: 19-09-2017
Accepted: 22-10-2017

Anita Jat
Dept. of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

SS Sharma
Dept. of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Hansraj Dhakar
Dept. of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Correspondence
Anita Jat
Dept. of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Morphological characterization and biochemical defense studies of *Fusarium verticillioides* (sheldon) causing post flowering stalk rot of specialty corn

Anita Jat, SS Sharma and Hansraj Dhakar

Abstract
Four isolates of *Fusarium verticillioides* were collected from different parts of Rajasthan from rotted Specialty Corn stalks and identified by ITCC, IARI (New Delhi, India). The maximum size of macroconidia (40.24 X 6.67) in isolate Fv SC-02 and microconidia (12.43- 3.09 µm) was observed in isolate Fv SC-01 whereas minimum size of macroconidia (21.40- 4.10µm) and microconidia (5.65 - 4.05 µm) was observed in isolate Fv SC-09. On the basis of cultural and morphological characters of all the four isolates showed variability. In this study, basis of host defense response was analyzed after *Fusarium verticillioides* infection by biochemical. Peroxidase and polyphenol oxidase concentration was higher in infected plants when compared to uninfected samples. In defense related biochemical estimation it was recorded that the Peroxidase level increased maximum in QPM *i.e.* 0.099 units with 90 per cent and Polyphenol oxidases secretion was maximum in QPM with an increase of 0.351 units *i.e.* 87.31 per cent increase over control.

Keywords: specialty corn, *Fusarium verticillioides*, morphology, peroxidase and polyphenol

1. Introduction
The specialty corn viz. Pop corn, Baby corn, Sweet corn and QPM are increasing its hectares because of high economic returns. Post flowering stalk rot (PFSR) complex play a vital role in affecting the productivity of specialty Corn (Zea mays L.) crop in all continents of the world. This complex is caused by *Fusarium verticillioides*, *Macrophomina phaseolina* and *Cephalosporium maydis*, out of which *F. verticillioides* is of economic importance (Kumar and Shekhar, 2005, Dorn *et al*., 2009) [6, 2]. In India, the disease is prevalent in most of the maize growing areas, particularly in rainfed areas viz., Delhi, Rajasthan, Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh, Tamil Nadu and Karnataka (Kaur and Mohan 2012. Most of the commercially grown cultivars have shown a high level of disease incidence at the grain filling stage (Iglesias *et al*., 2010) [3]. Studies on cultural and morphological and 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) [10, 11]. Peroxidase (PO) play role in lignification and subrization of cell wall during plant pathogen interactions. Polyphenoloxidase (PPO) in plants modify endogenous phenolic compounds into O-quinones.

2. Material and Methods
The experiment was conducted at Department of Plant Pathology, Rajasthan College of Agriculture (RCA) MPUAT, Udaipur during year 2015-2016.

Collection and identification of isolates
Specialty Corn rotted stem showing typical PFSR symptoms were collected from different locations (Table 1) of Rajasthan during 2015. Isolations were carried out on potato dextrose agar (PDA) plates and subsequently purified by hyphal tip method (Leslie and Summerell 2006) [7].

The purified culture was incubated at 28±2 °C temperature and used for further studies. The cultures were identified as Fusarium species based on morphological characters with the help of "Laboratory Manual for Identification of Fusarium Species" (Booth 1971) [1] and identification report received from ITCC (Indian Type Culture Collection) IARI New Delhi (Table 1).

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 and size of conidia. Data in relation to morphological and cultural characters like, colony diameter, colony color, pigmentation, zonation, topography of the culture, margins of the culture, septation and size of macro and micro conidia were studied. The size of conidia was measured under light microscope at 40X using micrometer. Fifty observations were taken for conidial measurement and mean values were calculated.

Table 1: Isolates of *Fusarium verticillioides* recovered from samples collected from diseased fields in maize growing areas of Rajasthan

S. No	Isolate designation	Location of collection	Land races/ variety	Identification
1	Fv SC-01	RCA Plant Pathology (Udaipur)	Bajaura Popcorn	Booth, 1971[1]
2	Fv SC -02	RCA Agronomy field (Udaipur)	Sugar-75	Booth, 1971[1]
3	Fv SC -03	Bujhda and Sisarma (Udaipur)	Hissar Babycorn	Booth, 1971[1]
4	Fv SC -04	Madar (Udaipur)	Pratap QPM	Booth, 1971 [1]

Biochemical Studies

Biochemical defense governs in response to pathogen invasion, mechanical injuries as well as abiotic stresses. Changes due to infection of PFSR were studied in different trials. Seeds of these were sown in 4 m rows, maintaining row to row and plant to plant distance as 60 and 20 cm, respectively. Two plots of each variety were inoculated by inserting toothpick having *F. verticillioides* culture and un-inoculated plots of each variety served as check against each treatment. After 25 days of inoculation, the sample was taken from diseased and healthy plants of all trials to estimate the activities of Peroxidase and Poly phenol oxidases. Methods used for various biochemical studies are described here in:-

Sampling

The samples were taken at 25 days after inoculation. The infected leaf samples as well as healthy samples were collected and placed in a thermocol box kept moist and brought to the laboratory for biochemical analysis.

Estimation of Peroxidase activity (POX) by using standard method given by Manoranjankar and Mishra, 1976.

200 mg leaf tissue without mid rib was homogenized in 10 ml of ice-cold phosphate buffer (0.1M, p^H =6.5) in mortar-pestle. The homogenate was centrifuged at 2 °C at 10,000 rpm for 15 min in a refrigerated centrifuge. The clear supernatant was taken as the enzyme extract. 3 ml of the assay mixture for Peroxidase activity estimation comprised of 2.3 ml of 0.1M Phosphate buffer (p^H 6.5), 0.5 ml of Guaicol substrate, 0.1 ml of the enzyme extract and finally 0.1 ml of H_2O_2 (5%) to start reaction. The assay components were quickly mixed and transferred to spectrophotometer cuvette for recording changes in absorbance at 15 seconds interval for a maximum time of 3 minutes. Each observation was recorded for Peroxidase activity against a substrate blank. Peroxidase activity was calculated based on change in absorbance per minute per milliliter of the reaction-mixture.

Estimation of Poly Phenol Oxidases (PPO) by using standard method given by Manoranjankar and Mishra, 1976.

200 mg leaf tissue without mid rib was homogenized in 10 ml of ice-cold phosphate buffer (0.1M, p^H =6.5) in mortar-pestle. The homogenate was centrifuged at 2 °C at 10,000 rpm for 15 min in a refrigerated centrifuge. The clear supernatant was taken as the enzyme extract. 3 ml of the assay mixture for Poly Phenol oxidases activity comprised of 1.5 ml of phosphate buffer (0.1M, p^H=6.5), 1 ml of Catechol (50mm) as substrate followed by 0.5 ml of undiluted enzyme extract added in test tube. The assay mixture was incubated for 5 min at 25°C Thereafter, the reaction was stopped by adding 0.5 ml of 5% H_2SO_4 (V/V) solution. The color of product was read spectorophotometrically at a wavelength of 410 nm using UV-VIS spectrophotometer (Hitachi) against a substrate blank. One unit of enzyme activity was calculated as change in absorbance per min per milliliter of the crude extract.

3. Results and Discussion

Cultural and morphological variability

A total of 4 isolates of *Fusarium verticillioides* was obtained from the stems of diseased maize plants. Based on morphological characteristics, 4 isolates *F.verticillioides* were classified as Fv SC-01, Fv SC-02, Fv SC-03 and Fv SC-04. Identification of the isolates was made on the basis of morphological characters and as per identification report received from ITCC (Indian Type Culture Collection) (Table-1), IARI New Delhi. The isolates of Fusarium spp. collected from different locations showed variations, in basic culture characteristics. All the four isolates differed in cultural characters i.e. Cottony Pinkish white, Purple violet, Dirty white and Pinkish white growth were observed Fv SC-01 to Fv SC-04 respectively. Isolates varied among themselves, with respect to pigmentation viz pink, violet, brown and pink and topography of the isolates varied from fluffy, flat, fluffy and flat respectively (Table-2).

Table 2: Morphological characters of different isolates of *Fusarium verticillioides*

S. No.	Isolate designation	Colony diameter (mm)	Colony color	Margin	Topography of the culture	Zonation	Pigmentation in culture
1	Fv SC-01	90.0	Cottony Pinkish white	Circular irregular	Fluffy	Undulated	Pink
2	Fv SC-02	90.0	Purple violet	Circular	Flat	Undulated	Violet
3	Fv SC-03	90.0	Dirty white	Circular	Flat	Undulated	Brown
4	Fv SC-04	90.0	Pinkish white	Circular	Fluffy	No undulation	Pink

All the four isolates of *F. verticillioides* showed significant variations in conidial morphology. Results presented in Table3 show that mean length and width of macro conidia in different isolates of Fusarium spp. ranged from 21.40– 40.24 x 4.10 – 6.67 µm and the mean length and width of micro conidia in different isolates ranged from 5.65 – 12.43 x 1.50 – 4.05µm. Among the Fusarium isolates, the maximum length and width of macro conidia was recorded in the isolate Fv SC-02 which measured 40.24- 6.67 µm followed by isolate Fv SC- 01 with 38.14- 5.07, Fv SC-03 with 37.44- 4.90 µm, while minimum in Fv SC-04 as 21.4 – 4.10 µm. Among the Fusarium spp. isolates, the maximum length and width of micro conidia was recorded in the isolate Fv SC-01 which measured 12.43- 3.09 µm followed by isolate Fv SC-02 with 10.34- 1.50 µm, Fv SC-03 with 9.86 –1.80, while minimum in Fv SC-04 with 5.65 - 4.05 µm. There was considerable variation with respect to number of septa in the range of 1-5.

Table 3: Variation in conidial morphology, measurement and time taken in conidial production of *Fusarium verticillioides* isolates

S. No	Isolate Designation	Spore size (µm)						Time taken in conidial production (days)
		Macroconidia			Microconidia			
		Length	Width	No. of septation	Length	Width	No. of septation	
1	FvSC-01	38.08	5.07	5	12.43	3.09	1	6
2	FvSC-02	40.24	6.67	4	10.34	1.50	1	7
3	FvSC-03	37.44	4.90	4	9.86	1.80	1	7
4	FvSC-04	21.40	4.10	4	5.65	4.05	1	8
SEm±		0.051	0.013		0.015	0.008		
CD at 5%		0.16	0.04		0.05	0.02		

The conidial morphology of *Fusarium verticillioides* isolates are in agreement with those described (Patil *et al*., 2007; Khokhar *et al*.,) [9]. The survival of pathogen largely depends on variability as it is a continuous feature found in nature. Studies on variability in the pathogen and host are important for documenting resistant sources.

Biochemical Defense Studies

Variation in Peroxidase (PO) activity

Increase in Peroxidase activity has been reported when host tissues are inoculated by pathogen. This increase manifests as a defense response by host as defense enzyme secretion. This oxido-reductase is termed as an antioxidant enzyme and hence, its activity increases, thereby, exhibiting high level of resistance developed in host. The results presented in Table-4 shows an increasing trend of PO activity in diseased samples.

Table 4: Variation in activity of Peroxidase (units/min/100 mg fresh wt. of leaf) in different specialty corn groups under artificially inoculated (Diseased) and uninoculated (Healthy) conditions.

S. No	Group/variety	Peroxidase (PO) activity* (units/min/100 mg fresh wt. of leaf of PO's)		Variation in Peroxidase activity in comparison to control (B-A)	Per cent increase in Peroxidase activity over control
		Healthy (A)	Infected (B)		
1	Pop corn	0.106	0.131	0.025	19.08
2	Sweet corn	0.091	0.126	0.035	27.77
3	Baby corn	0.085	0.124	0.039	31.45
4	QPM	0.011	0.110	0.099	90.00
SEm± CD at 5% C.V. %					
Healthy (A)		0.003	0.008	4.76%	
Infected (B)		0.004	0.011	4.73%	

*****Mean of three replications

However, corresponding increase varies according to the entries were studied. Maximum increase *i.e.* 0.099 units with 90.0 per cent increase over control in infected entries was found in QPM lines followed by Baby corn entries *i.e.* 0.039 units with 31.45 per cent increase over control, in Sweet corn 27.77 per cent increase was recorded over control in infected entries and lowest Peroxidase *i.e.* 0.025 units with 19.08 per cent increase over control was found in Popcorn. The studies indicate that QPM are genetically strong with dominant resistant genes responsible for generation of antioxidant enzymes during pathogenesis and actively imparting resistance to host.

Variation in Poly phenol oxidase (PPO) activity

Poly phenol oxidases are the enzymes which oxidize phenols that offer resistance to infection. This enzyme has also been reported to inhibit mycelial elongation, penetration and colonization, while in spore producing fungi they may even inhibit spore germination. However, there is less corresponding increase as compared to that in Peroxidase, when correlated The maximum differential increase in PPO activity was observed in QPM lines with an increase of 0.351 units *i.e.* 87.31 per cent increase over control followed by Sweet corn *i.e.* 0.270 units with 34.0 per cent increase over control in Baby corn 28.86 per cent increase was recorded over control in infected entries. This positive trend reveals that Popcorn entries had least *i.e.* 0.259 units with 28.24 per cent increase over control in PPO, activity which indicates susceptibility of host tissues to pathogenic attack. The QPM germplasm has been found to exhibit high PPOs activity. These can be used in breeding for developing potentially resistant QPM lines of maize. The high PPOs activity in QPM lines is indicative of presence of dominating gene for Poly phenol oxidase accumulation which is a kind of self-biochemical defense in host. Several reports of this kind of studies are available, Deborah *et al*., 2001, reported that the increase in Peroxidase, Polyphenol oxidase, Phenolics and Lignin's were found to be increased significantly in response to inoculation with non-pathogen compared to inoculation with pathogen. Santiago *et al*., 2007 [12] showed negative correlation of phenolics with severity of disease.

4. Conclusion

The pathogen was isolated and purified by hyphal tip culture technique. Identification of isolates of the pathogen was made on the basis of morphological characteristics of four different isolates of *Fusarium verticillioides.* All the four isolates of *Fusarium verticillioides* were studied for their cultural and morphological characteristics like colony growth, colour, growth pattern, margin, topography, zonation and pigmentation, type of conidia, conidial size and mycelial growth pattern. Significant variations in morphology and cultural characters were found in different isolates of *Fusarium verticillioides viz*. Fv SC-01, Fv SC-02, Fv SC-03 and Fv SC-04.

In defense related biochemical estimation it was recorded that the Peroxidase level increased maximum in QPM *i.e.* 0.099 units with 90 per cent increase over control followed by Baby corn entries *i.e.* 0.039 units with 31.45 per cent increase over control. Likewise Polyphenol oxidases secretion was maximum in QPM with an increase of 0.351 units *i.e.* 87.31 per cent increase over control followed by Sweet corn with 0.270 units with 31.0 per cent increase over control. While, minimum accumulation of these defense enzymes makes the plants more vulnerable to diseases and lead to susceptibility towards disease.

5. Acknowledgements

I would like to thank the Plant Pathology Research farm and Department of Plant Pathology, MPUAT, Udaipur, Rajasthan for providing all possible research facilities while executing the field experiment and laboratory analysis.

6. References

1. Booth C. The Genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England, 1971, 237.
2. Dorn B, Forrer HR, Schurch S, Vogelgsang S. Fusarium species complex on maize in Switzerland: occurrence, prevalence, impact and mycotoxin in commercial hybrids under natural infection. Europial Journal of Plant Pathology. 2009; 125:51-61.
3. Iglesias J, Presello DA, Botta1 G, Lori GA, Fauguel CM. Aggressiveness of Fusarium section L iseola isolates causing maize ear rot in Argentina. Journal of Plant Pathology. 2010; 92:205-211.
4. Kaur H, Mohan C. Status of post flowering stalk rots of maize and associated fungi in Punjab. Plant Disease Research. 2012; 27:165-170.
5. Khokhar MK, Sharma SS, Hooda KS, Roat BL. Morphological and molecular characterization of *Fusarium* spp. causing post flowering stalk rot of Maize. *Vegetos.* 2014; 28:113-121.
6. Kumar S, Shekhar M. Stress on Maize in Tropics Eds. Published by Directorate of Maize Research, Cummings Laboratory, Pusa Campus, New Delhi. Angkor Publisher (P) Ltd. Noida. 2005, 172-194.
7. Leslie JF, Summerell BA. The Fusarium Laboratory Manual. Blackwell Publishing, Ames, IA, USA. 2006, 388.
8. Manoranjankar, Mishra D. Catalase Peroxidase and PPO activities during leaf senescence. Plant Physiology. 1976; 57:315-319.
9. Patil AS, Singh H, Sharma SR, Rao GP. Morphology and pathogenicity of isolates of *Fusarium moniliforme* causing Pokkah Boeng disease of sugarcane in Maharashtra. In**:** Ram R. C. and Sinha A. eds. *Microbial* Diversity: Modern Trends. Daya Publishing House, New Delhi. 2007, 234-63.
10. Rahjoo V, Zad J, Javan NM, Gohari AM, Okhovvat SM, Bihamla MR *et al*. Morphological and molecular identification of *Fusarium* isolated from Maize ears in Iran. Journal of Plant Pathology. 2008; 90:463-68.
11. Sankar NR, Devamma MN, Kumar VK, Giridha D. First Report of *Fusarium moniliforme* causing fruit rot in tinda (Praecitrullus fistulosus) India. New Disease Reports. 2011; 24:24.
12. Santiago R, Reid LM, Arnson JT, Zhu XY, Martinez N, Malavar RA. Phenolics in Maize genotypes differing in susceptibility to Gibberella stalk rot *(Fusarium graminearum Schwabe).* Journal agricultural and Food Chemistry. 2007; 55:5186-93.