Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2020; 9(1): 941-943
Received: 19-11-2019
Accepted: 21-12-2019

Sinijadas K
Department of Plant Pathology, College of Agriculture, Vellayani, Kerala, India

P Asha Devi
Department of Plant Pathology, UBKV, Pundibari, West Bengal, India

AK Chowdhury
Department of Plant Pathology, UBKV, Pundibari, West Bengal, India

PM Bhattacharya
Department of Plant Pathology, UBKV, Pundibari, West Bengal, India

Corresponding Author:
Sinijadas K
Department of Plant Pathology, College of Agriculture, Vellayani, Kerala, India

Extraction and characterisation of fungal toxin (*Bipolaris sorokiniana)* and its effect in different wheat genotypes

Sinijadas K, P Asha Devi, AK Chowdhury and PM Bhattacharya

Abstract
Bipolaris sorokiniana is a hemibiotrophic fungus causing spot blotch of wheat. The pathogen produces phytotoxins in culture that induces necrosis and play role in pathogenesis. Different genotypes of wheat were differentiated into susceptible, moderately susceptible and resistant based on the symptom producing ability of that genotype. Our results indicated susceptibility level of wheat to the spot blotch pathogen based on the different genotype and toxin effect of that pathogen. 5 different genotype of wheat were experimented with toxin isolated from the highly virulent isolate of *B. sorokiniana*, UBS-1.The crude toxin examined under TLC profiling and observed the band morphology. The leaf infiltration bioassay of susceptible, moderately susceptible and resistant varieties of wheat were given the parallel trend of symptom production with toxin treatment.

Keywords: *Bipolaris sorokiniana*, wheat genotype, toxin, TLC plate

Introduction
Spot Blotch of wheat caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker (syn. Helminthosporium sativum teleomorph: Cochliobolus sativus), a hemibiotrophic phytopathogenic fungus infect all parts of the plant and develop variety of symptoms,. Foliar Spot blotch caused by *B. sorokiniana* has emerged as one of the major biotic stresses obstructing the commercial production of barley (Ghazvini and Tekauz 2007) [4]. The disease having importance in Africa, South America, Australia, Canada and Asia particularly to Indian sub-continent having warm and humid environments (Singh and Srivastava, 1997) [10]. Spot blotch has emerged as a major disease in North-western as well as in the Peninsular India and disease acquired the prime focus in national level (Sharma *et al*., 1998) [9]. Ludwig (1957) [6] demonstrated that *B. sorokiniana* (Helminthosporium sativum) produces a toxin that is vital for the development of the diseases in the infected plants. The pathogen produces phyto-toxins which play certain role in pathogenesis (Aggarwal *et al*., 2008) [1]. Spot blotch of wheat was differ in their severity with respect to the different varieties Fourteen diverse genotypes reported for variable disease severity were obtained from the Directorate of Wheat Research Karnal, Haryana (Chand *et al*. 2008) [2]. Keeping this in view, present investigation was undertaken to assess effect of toxin isolated from a virulent strain to five different genotype of wheat.

Materials and Methods
Isolation of pathogen
Isolates of *Bipolaris sorokiniana* (UBS-1) is obtained from the infected wheat leaves collected from the field and maintained in the potato dextrose agar (PDA) plates. The mother culture obtained from above were subsequently incubated at 25^0C under light for mycelial growth and sporulation for 10-15 days. The sporulated cultures were stored at 4^0C refrigerator for further use.

Extraction of crude toxin
The crude toxic extracted from culture filtrate of *Bipolaris sorokiniana* which was produced by inoculating the cultures on minimal medium (Leach *et al*.1982) [5]. Flasks containing 100 ml liquid medium were inoculated with mycelia disc of 5 mm from UBS-1 isolate and placed in an incubator at 25 ^{O}C for 21 days.
Mycelium of *Bipolaris sorokiniana* was filtered with whatman paper No.1 to obtain the cell free culture filtrate. Culture filtrate was adjusted to pH 2 by adding 1N HCl and activated charcoal was added and stirred and kept for overnight.

The charcoal with absorbed toxin was separated by centrifugation (5000 rpm, 5 mints). Discard the liquid phase and suspend the charcoal in ethanol. Ethanol was removed by filtering on a Buchner funnel and the charcoal cake was suspended in chloroform, stirred and filtered through muslin clothes first then filtered through whatman paper N0.1 for getting the pure filtrate.
The process was repeated three times. The chloroform extract were combined with toxin, and the solvent evaporated by hot water bath to yield crude extract.
The residue was dissolved in dichloromethane by thoroughly shaking and crude form of toxin was collected. Crude toxin extracted from UBS-1 isolate (Highly virulent strain), dissolved in dichloromethane was stored in small glass vials in refrigerator at 4 ^{0}C.
This partially purified toxin was used for TLC profiling and for the bioassay of the detached wheat leaf of different genotypes.

Thin Layer Chromatography (TLC)
The toxin isolated from UBS-1 isolate was placed on thin layer chromatograph (TLC) plates to observe their profiles. Aluminium sheet coated with silica gel was cut into 3 cm width sheet and sample were spotted on the plate by using a capillary tube. The development of chromatograms was carried out at room temperature in the following solvent systems;
Methanol: Ethyl acetate {(9:1) v/v}
Acetone: Chloroform {(7:3) v/v}
Chloroform: Hexane {(8:2) v/v}
Benzeen: Acetone {(8:2) v/v}
Choroform: Ethyl acetate {(9.5: 0.5) v/v}

The plates were air dried for 5 minutes and transferred into iodine chamber. Bands were visualized. Rf value of band appearing on the TLC plate were calculated by following formula:

Rf = Distance moved by compound rom origin
Distance moved by solvent front from origin
Test the toxicity of crude toxin on different genotype of wheat
Five genotype of wheat (*Triticum aestivum)* including ISEPTON, LBSN, IPPSN, chirya and sonalika were selected for toxin bioassay studies. Leaf segments of these genotypes were collected from the field and washed thoroughly with sterile water. Surface sterilized by 1% sodium hypochlorite. Detached leaves of 5 different genotype were pricked with needle and the damaged leaf surface were immediately infiltered with partially purified toxin isolated from the highly virulent strain UBS-1. It was incubated in BOD at 27ºC for 48 hours in light in tray and observed the symptom development.

Measuring spots
By using calibrated tripolar fixed NIKON camera was used for taking photographs of toxin as well as pathogen inoculated detached leaves spot measurement. The photographs are analysed by using software Digimizer. This had given the accurate measure of spot area.

Results and Discussion
Morphology of crude toxin
The different isolates of *B. sorokiniana* exhibited a significant variability in their virulence and pathogenesis along with the considerable variability in their cultural characteristics. Earlier, successful attempts have been made to isolate and characterize novel phytotoxin from fungi. As early as in 1961 De Mayo *et al.*, isolated prehelminthosporal from culture filtrate of *Helminthosporium sativum*. The morphological study of crude toxin was observed. The studies revealed that, the crude toxin was yellowish orange with slight oily in nature. The colour was slightly vary from isolate to isolate, Fig 1.

Toxin profiling by TLC
TLC profile showed that solvent system, Chloroform: ethyl acetate (9.5: 0.5) was found to be the best giving a single major band of high resolution in TLC plate it was observed that highest movement of UBS-1. Isolate regarding toxin production as indicated Rf value shows 0.56. The highest mobility (Rf value) was observed for Isolate UBS -1 which was shown broad and thick brownish orange. And it was compared with a toxin isolated from a least virulent isolate UBS-5 with a TLC band. It formed a narrow, thin, yellowish brown band on TLC plate. From TLC profiling it was clear that highest mobile, broad, thick band showing isolate was highly virulent. Observations were recorded in Fig 2.

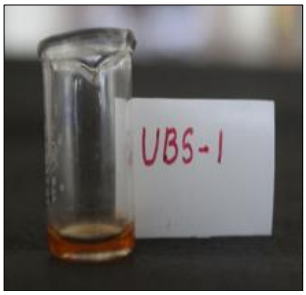

Fig 1: Crude toxin UBS-1

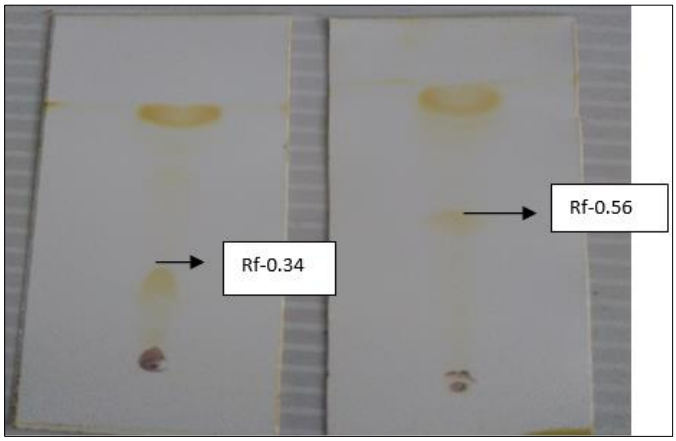

Fig 2: TLC profiling of isolates UBS-5 and UBS-1

Effect of Toxin in symptoms production
Present investigation revealed that, the toxin isolated from different isolates of *B. sorokiniana* shows different Rf value in TLC, so an attempt was made to inoculate the purified toxin on different genotypes of wheat having diverse genetic background. For this purpose, the purified toxin of isolates, UBS-1 was inoculated in different genotypes (IPPSN-152, LBSN-45, Chirya-3, Isepton-152 and Sonalika) having variable disease reactions by detached leaf technique as described earlier. The disease reaction of these genotypes were also observed in double digit scale in flag and flag-1 leaf (0-9, scale: 0= no disease, 9= >80% disease) under natural field condition. The results are presented in Table:-1 and Fig:-3.

Table 1: Effect of purified toxin (UBS-1) on lesion size in different genotype of wheat

Isolate	Genotype	Mean Size of spot blotch lesion in cm after 48 hrs	Disease score (double digit)
UBS-1	IPPSN-152	0.363±0.086	57
	LBSN-45	0.364±0.116	57
	Chirya-3	0.102±0.074	45
	Isepton-152	0.700±0.118	57
	Sonalika	1.413±0.296	97

Fig 3: Effect of UBS-1 toxin in different genotype of wheat

From Table:-2, it indicates that the purified toxin produced typical symptom of spot blotch disease. Lesion size was highest in Sonalika, recording 1.413 cm followed by ISEPTON-152 (0.700 cm) and lowest Chirya-3.Observations made a conclusion that sonalika was showing greater amount of chlorosis when treated with toxin whereas chirya-3 was showing comparatively less chlorosis agains toxin. The symptom produced by the purified toxin is highly co-related with disease in the field under natural conditions. Earlier many workers (Pandey *et al.*, 2005, Ranjan *et al.*, 2017) [7, 8] also used Chirya-3 as a resistant source against spot blotch disease. The present observation confirms their findings. It was observed that toxin isolated from *B. sorokiniana* produced characteristic symptoms of spot blotch disease in different genotype of wheat.

References

1. Aggarwal R, Das S, Jahani M, Singh DV. Histopathology of spot blotch [*Bipolaris sorokiniana* (Teleomorph: *Cochliobolus sativus*)] infection in wheat. Acta Phytopathol. 2008; 43:23-30.
2. Chand R, Sen D, Prasad KD, Singh AK, Bashyal BM, Prasad LC *et al*. Screening for disease resistance in barley cultivars against *Bipolaris sorokiniana* using callus culture method. Indian Journal of Experimental Biology. 2008; 46:249-53.
3. De Mayo P, Spencer EY, White RW. Helminthosporal, the toxin from *Helminthosporium sativum* isolation and characterization. Canadian Journal of Chemistry. 1961; 39:1608-12.
4. Ghazvini H, Tekauz A. Reaction of Iranian barley accessions to three predominant pathogens in Manitoba. Canadian Journal of Plant Pathology. 2007; 20:69-78.
5. Leach J, Lang BR, Yoder DC. Methods for selection of mutants and *in vivo* culture of *Cochliobolus heterostrophus.* Journal of Microbiology. 1982; (128):1719-29.
6. Ludwig RA. Toxin production by *Helminthosporium sativum* and its significance in disease development. *Canadian.* Journal of Botany. 1957; 35:291-303.
7. Pandey SP, Kumar S, Kumar U, Chand R, Joshi AK. Sources of inoculum and reappearance of spot blotch of wheat in rice-wheat cropping systems in eastern India. European Journal of Plant Pathology. 2005; 111:47-55.
8. Ranjan R, Mishra VK, Chand Ramesh, Chowdhury AK, Bhattacharya PM, Joshi AK. Pooling together spot blotch resistance, high yield with earliness in wheat for eastern Gangetic Plains of South Asia. Field Crops Research. 2017; 214:291-300.
9. Sharma AK, Kumar J, Nagarajan S. Disease management strategy in wheat. Indian Farming. 1998; 18:52-53.
10. Singh DV, Srivastava KD, Foliar blights and Fusarium scab of wheat. Present status and strategies for management. In: Management of Threatening Plant Diseases of National Importance. Malhotra Publishing House, New Delhi, 1997, 1-16.