Journal of AgriSearch, 7(1):08-11
An Open Access International Peer Reviewed Quarterly

ISSN : 2348-8808 (Print), 2348-8867 (Online)
https://doi.org/10.21921/jas.v7i01.17626

Phenotypic characterization in natural population of Spot Blotch (Bipolaris sorokiniana) of Barley (*Hordeum vulgare* L.)

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ARTICLE INFO

Received on	:	09/11/2019
Accepted on	:	18/02/2020
Published online	:	09/03/2020

ABSTRACT

A study was undertaken on phenotypic characterization of different isolates of *Bipolaris sorokiniana* isolated from the different varietal trials and nurseries of barley at the agricultural research farm, Institute of Agricultural sciences, Banaras Hindu University. The isolation was performed by transfer of single spore of the pathogen from diseased leaf in Potato Dextrose Agar media (PDA) under aseptic condition. The experiment revealed five groups of isolates on the basis of colony colour, morphology and number of spores. It was found that (42.2%) of the isolates were black in colour with profuse sporulation and suppressed type growth, (35.71%) of the isolates were greyish in colour and (21.42%) of the isolates possessed whitish colony with full plate growth and lower sporulation. The size of conidia ranged from 24 × 13µm to 36 × 14µm in size, while the number of spores per mm^2 of colony varied from 20.36 to 162.77.

KEYWORDS

Bipolaris sorokiniana, isolates, aseptic, sporulation, PDA

INTRODUCTION

Among three major foliar diseases of barley viz., leaf blight, spot blotch, and net blotch caused by *Alternaria solani, B. sorokiniana, and Drechslera sp.* respectively, the spot blotch disease caused by *B. sorokiniana* has been considered as most important disease of barley. *B. Sorokiniana* (Sacc. In Sorok) Shoemaker (syn: *Helminthosporium sativum,* teleomorph: *Cochliobolus sativus* (Ito and Kurib, Drechsl. Ex Dastur) is a seed and soil borne hemi-biotrophic fungus having both biotrophic and necrotrophic phases. The pathogen has a variable range of host covering both wild and cultivated members of the Poaceae and is extremely variable in pathogenicity towards barley.

The pathogen is now causing worlwide menace to the crop and is still being reported from several new areas (Rehman *et al.*, 2019). Traditionally, *B. sorokiniana* has been defined as a variable fungus on the basis of morphology (Jaiswal *et al.* 2007; Pandey *et al,* 2008, Jahani *et al.* 2014), physiology (Kumar *et al.* 2002) and genetics (de Oliveira *et al.* 2002; Singh *et al.* 2014). The morphological, physiological and biochemical characterization of *B. sorokiniana* have been the aim of many studies (Muller *et al.* 2005; Poloni *et al.* 2009). Conidia of *B. sorokiniana* have two different physiological forms corresponding to infection and survival phases respectively, as suggested by Chand and co-workers (2003). Variability in the population of *B. sorokiniana* on the basis of morphological and pathological (Jaiswal *et al.* 2007) traits has also been well reported. The morphological and physiological variability affects the evolution and aggressiveness of isolates. The correct species identification and recognition of aggressive strains is critical to understand the host-pathogen relationship (Strongman and MacKay, 1993). Researchers have grouped *B. sorokiniana* in three different populations *i.e.* black, mixed and white (Bashyal *et al.* 2010; Chand *et al.* 2003; Bashyal *et al.* 2015). White isolate of *B. sorokiniana* could not form conidia and are morphologically distinct in growth medium (Pandey *et al.* 2005; Jaiswal *et al.* 2007). Since most of the Indian workers remained confined to characterization of *B. sorokiniana* populations isolated from wheat, efforts are made to characterize the population from barley.

MATERIALS AND METHODS

Diseased barley leaf samples showing the typical spot blotch symptoms were collected between heading to dough stage from the different entries of the different nurseries, viz., Elite barley disease screening nursery (EBDSN), Initial Varietal Trial (AVT) -Dual, Initial Varietal Trial (AVT)-Feed barley and Station Varietal Trail (SVT) planted at the Agriculture Research Farm, Banaras Hindu University, Varanasi brought to the laboratory. The leaf samples were washed with running tap water and surface sterilized under aseptic conditions with 95% ethyl alcohol. The surface sterilized leaves were cut into small pieces (3-5 mm) having lesions with the help of sterilized scissor. The leaf bits were transferred into a sterilized moist chamber. The moist chambers were incubated at 25±2ºC.

After conidial crops formation on third day, a single conidium from each leaf bit was transferred separately by a fine needle onto each of several Petri-dishes poured with Potato Dextrose Agar medium (Peeled potato: 200g, Dextrose: 20g, Agar-Agar: 20g, Distilled water: 1000ml) acidified with 4-5 drops of 25% lactic acid. The colony colour was observed and radial growths were noted for 9 days after inoculation. The isolated fungus was identified based on the colony characters and taxonomic features. The colony colour varied from light whitish to light grey to

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black to olive cious black with presence of knotting of the mycelium. The conidia showed bipolar germination and the germ tube was in semi axial position. The number of septa varied from 3-4 (7 days young culture), while its number varied from 7-9 in older conidia. The conidiophores were un-branched, septate, light brown to dark brown in colour and erect.

The young conidia measured 24.5×13µm, whereas, the old conidia were 45×25.5 µm. These morphological characters were confirmed with relevant literature (Asad *et al.*, 2009) and the fungus was identified as *Bipolaris sorokiniana* (Sacc.) Shoemaker. For spore calculation we took a circular bit of 5mm with the help of cork borer from 9 days old culture and added it in 2ml of distilled water to make a fine spore suspension .From this spore suspension we took 50 micro litres with the help of micropipette and mixed with 950 micro litres to make it a 1ml solution. Now from this solution we squeezed about 10 micro litres with help of micropipette and poured on cavity slides and observed and counted the number of spores under the microscope.

If a is the number of spores counted than the no. of spores per mm 2 can be calculated as follows:
No. of spores per mm^2 = a x 2000/10 x πr^2, where r is the radius of the bit.

Table 1: Categorization of the different isolates of *Bipolaris sorokiniana* on the basis of colony colour, number of spores per unit area and size of conidia

Isolate No.	Source of Isolates	Colony colour	No. of spores mm^2	Conidia size (µm)
1	EBDSN-34	Greyish black	69.24	33 x 14
2	IVT FB - 5	Black	54.48	32 x 15
3	IVT DUAL 9	Black	102.84	34 x 13
4	IVT DUAL 7	Black	61.69	36 x 14
5	IVT FB 15	Black	81.59	36 x 13
6	IVT DUAL 8	Black	57.11	35 x 14
7	IVT FB 16	Black	58.81	36 x 14
8	IVT DUAL 11	Grey	38.47	30 x 15
9	SVT 6	Grey	162.77	28 x 13
10	IVT DUAL 1	Grey	84.40	29 x 16
11	IVT FB 27	Grey	48.37	28 x 13
12	SVT 3	Milky white	40.92	26 x 14
13	EBDSN 71	Off white	30.99	24 x 13
14	SVT 13	Milky white	20.67	25 x 14

SVT, station varietal trial; EBDSN, Elite barley disease screening nursery; IVT, Initial varietal trial; FB, feed barley trail

RESULT AND DISCUSSION

The data on phenotypic characterization of the different isolates of *B. sorokiniana* isolated from leaves collected from the different entries of the different barley nurseries viz., Elite barley disease screening nursery (EBDSN), Initial Varietal Trial (AVT) -Dual, Initial Varietal Trial (AVT)-Feed barley and Station Varietal Trail (SVT) planted at the Agricultural Research Farm. Institute of Agricultural Sciences, Banaras Hindu University revealed variation in colony colours, number of spores per unit area and size of conidia (Table 1, Plate 1). The study revealed five groups of the isolates on the basis of colony colour, morphology and number of spores, viz., greyish black, black, grey, milky white and off-white. Out of the 14 isolates, 6 isolates showed black colour colony (Isolate 2, 3, 4, 5 and 6), whereas, only one isolate showed greyish black colony (Isolate 1). It was also found that 42.2% isolates were black in colour with profuse sporulation and suppressed type growth. The Isolate 8, 9, 10 and 11 showed grey colours and Isolate 12 and 14 exhibited milky white colony. However, Isolate 13 was off white in colour (Plate 1).

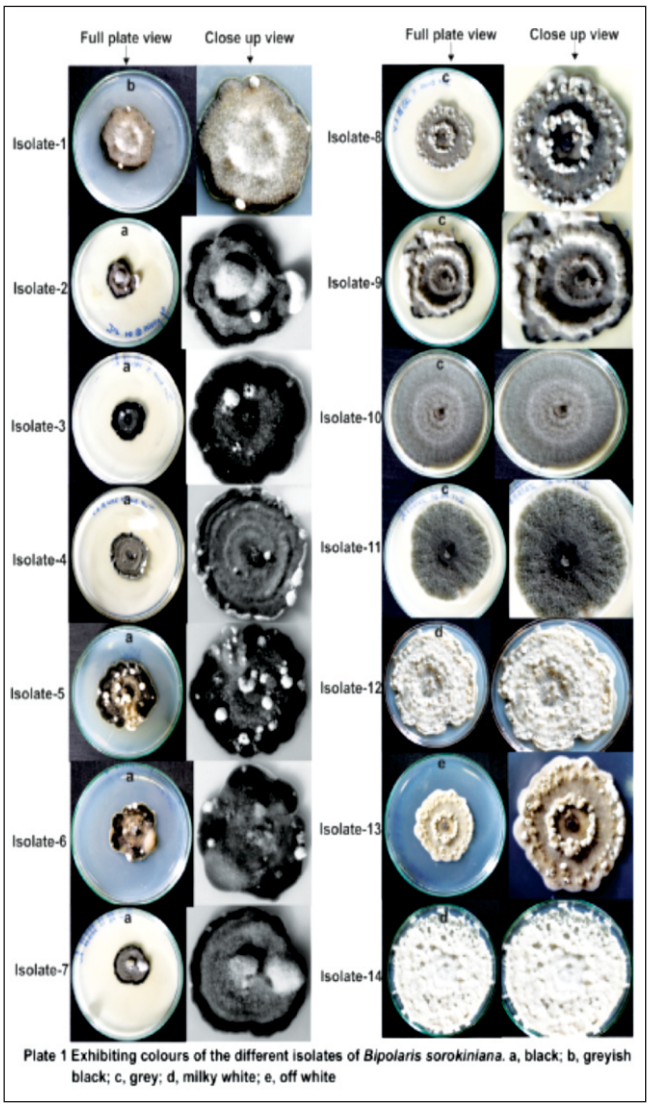

Plate 1 Exhibiting colours of the different isolates of *Bipolaris sorokiniana*. a, black; b, greyish black; c, grey; d, milky white; e, off white

Further it was inspected that black colony had the higher number of spores followed by grey and white colony, respectively. However, Isolate 9 had grey colony depicted the highest number of spores per mm^2 (162.77).The size of conidia varied with respect to colour of colonies. The maximum size of conidia was registered with respect to greyish black to black colour colonies of the isolates 1 to 7 followed by grey, milky

Table 2: Showing radial growth of different Isolates of *Bipolaris sorokiniana* and its relation with the number of spores per mm

Isolate No.	Colony Colour	Radial growth (mm)			Mean(mm)	No. of Spores ($mm^{2)}$
		Days after inoculation				
		3	6	9		
Isolate 1	Greyish black	13.33^{g}	28.67^{h}	38.00^{ef}	26.66	69.24
Isolate 2	Black	19.66^{defg}	22.33^{i}	31.00^{g}	24.33	54.48
Isolate 3	Black	18.66^{fg}	24.00^{i}	33.00^{g}	25.22	102.84
Isolate 4	Black	19.00^{efg}	24.00^{i}	37.00^{f}	26.66	61.69
Isolate 5	Black	20.67^{defg}	30.33^{gh}	40.00^{e}	30.33	81.59
Isolate 6	Black	20.67^{defg}	33.00^{fg}	43.00^{d}	32.22	57.11
Isolate 7	Black	19.33^{efg}	21.67	33.33^{g}	24.77	58.81
Isolate 8	Grey	24.67^{cdef}	38.00^{e}	46.67^{c}	36.44	38.47
Isolate 9	Grey	37.33^{ab}	52.33^{c}	62.00^{b}	50.55	**162.77**
Isolate10	Grey	41.67^{a}	64.00^{b}	89.00^{a}	64.88	84.40
Isolate11	Grey	29.33^{bcde}	46.00^{d}	61.67^{b}	45.66	48.36
Isolate12	Milky white	30.00^{bcd}	44.33^{d}	87.67^{a}	52.66	40.92
Isolate13	Off white	26.33^{cdef}	35.00^{f}	46.00^{c}	35.77	30.99
Isolate14	Milky white	35.00^{abc}	67.67^{a}	89.67^{a}	**64.11**	20.67
C.V		24.57	4.24	**3.19**		**2.39**
C.D(0.05)		10.44	2.69	**2.81**		**2.61**

white and off white of the isolate 8, 9 and 10; 12 and 14 and 13, respectively. Further it was observed that grey and white colonies showed good radial growth but white colonies showed low sporulation as compared to the black and grey population. (**Table 2 and Fig. 1**)

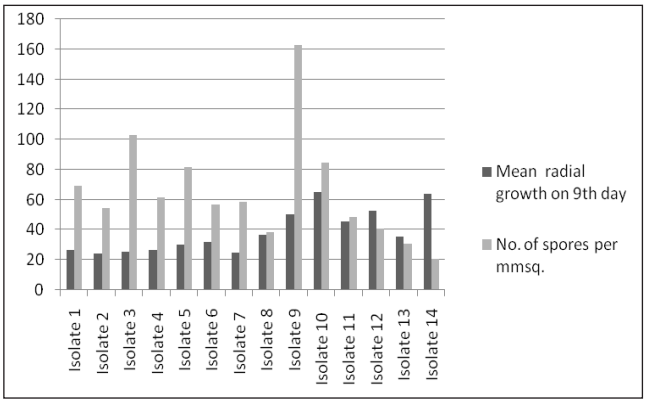

Fig.1: Depicting the mean radial growth of different isolates of *Bipolaris sorokiniana* and its relation with the number of spores mm^{2}

Conclusion

The studies on phenotypic characterization of the different isolates of *Bipolaris sorokiniana* isolated from different barley nurseries disclosed that the isolates fell into five different groups such as greyish black, black, grey, milky white and off white. It was also noticed that the black isolates were predominant over the others with profuse sporulation and suppressed type growth. The grey isolates were comparable to black isolates in terms of sporulation but showed good radial growth as compared to black isolates while the white isolates were very low in sporulation. Thus it can be concluded that the black and grey isolates are useful to multiply because of profuse sporulation for their application as artificial inoculums to test the diverse germplasm against spot blotch of barley following virulence test as well as toxin production ability.

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Citation:

Mazhar, Zeeshan, Vaish Shyam Saran and Akhtar Nishar. 2020. Phenotypic characterization in a natural population of Spot Blotch (*Bipolaris sorokiniana*) of Barley (*Hordeum vulgare* L.) *Journal of AgriSearch* 7(1) 8-11