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MORPHOLOGICAL AND PHYSIOLOGICAL VARIABILITY IN *BIPOLARIS SOROKINIANA**

AMIT CHAUHAN*AND ALOK. KUMAR SINGH

Department of Plant Pathology,

Udai Pratap College (Autonomous), Varanasi-U.P.-221002

ABSTRACT

Variability among 6 isolates of *Bipolaris sorokiniana* (Sacc. in Sorokin) Shoem. was studied in respect of morphological and physiological variability. The isolates BS-F-5 and BS-P-3 were found to be fast growing and high sporulating. The isolates BS-DWR-K-1, BS-K-4, and BS-V-6 were found to be medium in growth and sporulation, whereas BS-D-2 was slow growing and least sporulating. Among the phosphorus sources, dipotassium hydrogen orthophosphate was best for growth and sporulation, whereas sodium phosphate was inhibitory for growth and sporulation of all isolates. Among the potassium sources, potassium nitrate was best and potassium sulphate was inhibitory for growth and sporulation of all isolates. Among the vitamins, thiamine supported maximum growth of all isolates, whereas yeast extract supported maximum sporulation. Inositol and calcium pentothenate were found to be inhibitory for growth of all isolates.

KEYWORDS:

Morphological, physiological, *Bipolaris sorokiniana*, pH, phosphorus, potash, vitamins

The spot blotch disease of wheat causedby *Bipolaris sorokiniana* (Sacc. in Sorokin) Shoem. [syn. *Helminthosporium sativum* Pamm., King & Bakke; teleomorph: *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur] (Singh *et al*., 2001) is a destructive disease of wheat under rice-wheat system (Singh *et al*., 2004). Pathogen variability is an important component of disease epidemiology. Though a few studies on

variability in the isolates of *B. sorokiniana* both, morphological (Akram and Singh, 2001, Mahto *et al.*, 2001) and physiological (Bidari and Govindu, 1975) have been reported, there is lackof recent studies more so on physiological variability. In view of its being of crucial significance in understanding the pathogenicity and molecular biology of pathogen,present study was undertaken to work out morphological variability and ascertain the effect of pH level, phosphorus, potassium and vitaminon the growth and sporulation of *B. sorokiniana* isolates collected from different north and east Indian states.

* Corresponding author: chauhanamit77@gmail.co.in

MATERIALS AND METHODS

Morphological studies

Morphological character like texture, colour, shape, growth pattern of colony and sporulation were recorded after growing the six isolates on PDA for 8 days at 28±1^{0}C. The colony diameter and sporulation were recorded with the help of linear scale and haemocytometer, respectively. Colour of the colony was observed and recorded with the help of Munsell's soil colour chart (1970).

Physiological studies

Effect of pH levels, phosphorus, potash and vitamins were studied on the Czapek's broth medium. In all the studies 100 ml medium was dispensed in 250 ml conical flasks in three replications. Mycelial discs (5 mm diameter) from 8 days old culture on PDA were used for inoculation. The inoculated conical flasks were incubated at 28 ^{0} ± 1^{0}C for 12 days to study on growth and sporulation. For sporulation count, flasks of each treatment at the end of incubation period were shaken vigorously. The shaken medium was taken with the help of haemocytometer pipette and loaded on haemocytometer, and counted under compound microscope (10X). For weight of dried mycelium, mycelial mat were filtered through Whatman's filter paper No. 42 were dried to constant weight at 60^{0}C prior to filtration. Filter paper containing mycelia mat was washed thoroughly with distilled water to remove traces of chemicals and then dried at 60^{o}C for 72 hours in oven, subsequently cooled in desiccator having anhydrous calcium chloride and accurately weighted. The weight of dried mycelium mat was calculated in mg by subtracting the weight of filter paper from total weight.

pH studies

To study the effect of different pH levels *viz.*, 5.0, 6.0, 7.0 8.0 and 9.0 were determined before seeding by Backman pH meter. The different pH levels were maintained by adding 0.1 NaOH or HCl.

Nutritional studies

Four phosphorus sources (dipotassium hydrogen orthophosphate, single super phosphate, diammonium phosphate and sodium phosphate) and three potassium sources were tested for their suitability for growth and sporulation of six isolates of *B. sorokiniana*. Dipotassium hydrogen orthophosphate was omitted from basal medium to supplement with other phosphorus sources to provide 0.178g of phosphorus. To study the potassium sources, the KCl was omitted from the basal medium to substitute with other potassium sources and to provide 0.262g potassium. The medium contained either KCl or K_2HPO_4 or without any potassium sources served as check. Four vitamins *viz*., thiamine @ 100 µg/liter, biotin @ 5 µg /litre, inositol @ 500 µg/ litre and calcium pentothenete (Singh, 1973) were tested for growth and sporulation of *B. sorokiniana*. Two checks i.e. basal medium and basal medium with yeast extract @ 0.2% were taken. The vitamins and yeast extract were dissolved in deionized distilled water and added to the basal medium before autoclaving to give the final concentrations mentioned.

RESULTS AND DISCUSSION

Morphological variability

Colony characters

The variation in cultural characters and morphological character of different isolates of *B. sorokiniana* indicated that isolate BS-F-5 was fast growing, with brown to black colour hyphae, appressed texture, circular periphery and attained 83.63 mm colony diameter after eight days of incubation. The isolate BS-P-3 was second fast growing with brownish to black colour hyphae with gray margin, appressed texture, circular periphery. Three isolates i.e. BS-DWR-K-1, BS-6 and BS-K-4 were almost intermediate type in growth, whereas, isolate BS-D-2 was comparatively slow growing which attained the colony diameter of 49.5 mm after eight days and had olivaceous brown with whitish dots, fluffy texture and irregular periphery (Table-1).

Sporulation

Among the six isolates, maximum sporulation was observed in BS-F-5 (22.00 x 10^4) followed BS-P-3 (13.33 x 10^4), BS—6 (15.67 x 10^4), BS-DWR-K-1 (14.67 x 10^4) and BS-K-4 (13.33 x 10^4) isolates, whereas isolate BS-D-2) exhibited least sporulation (6.33 x 10^4). Based on radial growth and sporulation these six isolates can be grouped into following three categories.

Table: 1 Variation in colony characters and sporulation of *B. sorokiniana* isolates.

Isolates	Colony characters				Sporulation per 5 mm disc x 10^4
	Size (mm)	Colour	Growth	Shape	
BS-DWR-K-1	68.44	Gray with whitish dots	Semi appressed	Irregular	14.67
BS-D-2	49.50	Olivaceous brown with whitish growth and whitish margin	Fluffy cottony	Irregular	6.33
BS-P-3	82.14	Brownish to black with gray margin	Appressed	Circular	18.33
BS-K-4	65.08	Olivaceous with whitish margin	Fluffy	Irregular	13.33
BS-F-5	83.63	Brown to dark black	Appressed	Circular	22.00
BS-V-6	66.33	Black whitish growth, light brownish margin	Semi appressed	Irregular	15.67
SEm ±	1.55				2.23
CD at 5%	3.37				4.86

Category I- Fast growing and high sporulating: BS-F-5 and BS-P-3

Category II- Moderate growth and sporulation: BS-DWR-K-1, BS-K-4 and BS-6

Category III- Slow growing and less sporulating: BS-D-2

Singh and Singh (2006) have studied the colony character and sporulation of *B. sorokiniana*isolates from wheat, barley and *Phalaris minor* collected from Faizabad. Similar studies from other sites of origin of the isolates studied here are about.

Physiological variability

An investigation was undertaken to establish variability on the basis of physiological parameters such as differences in requirement of hydrogen ion concentration (pH), requirement of carbon sources, nitrogen sources, phosphorus sources, potassium sources and vitamins. The results obtained have been discussed in following paragraphs.

Variation in pH requirement

All the six isolates grew well over a wide range of pH *i.e.* from 5.0 to 9.0. However, the isolate BS-D-2 produced its maximum growth at 6.0 pH whereas rest five isolates produced their maximum growth at pH 6.5. The isolate BS-F-5 was always ahead of all isolates in growth and sporulation at every pH tested. Minimum growth of all the isolates was observed at pH 9.0. All these isolates could sporulate only upto 8.0 pH and no sporulation was observed at 9.0 pH. All these isolates exhibited their maximum sporulation at 6.5 pH and minimum at 8.0 pH. It was interesting to note that among the isolates and different pH levels, the minimum sporulation was exhibited by BS-P-3 at 8.0 pH (Table-2). The acidic pH seems to more congenial for growth and sporulation of all six isolates, whereas alkaline pH seems to be less congenial.

Bidari and Govindu (1975) also reported maximum growth of *H. sativum* at 6.5 pH. However, the *H. sativum* isolates also grew well at 3.3 pH. They also reported that acidic pH was more favourable for growth of *H. sativum* as compared to alkaline pH. Similarly Ahmed *et al.* (1997) reported maximum sporulation of *B. sorokiniana* at 6.5 pH. Rahman (1996) also obtained similar results.

Table: 2 Effect of different pH levels on weight of dried mycelium (mg) and sporulation ($x10^3$) of six isolates of*B. sorokiniana*

pH levels	Isolates												Mean	
	BS-DWR-K-1		BS-D-2		BS-P-3		BS-K-4		BS-F-5		BS-V-6			
	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulatio
5.0	336.67	10.00	323.67	8.70	366.00	13.30	338.33	13.30	430.67	14.00	342.33	10.70	356.28	11.67
6.0	349.67	14.20	388.00	14.00	511.67	20.70	467.67	20.70	527.00	23.30	507.00	21.30	458.50	19.03
6.5	495.00	19.30	370.00	15.30	608.00	25.30	510.00	20.70	612.33	28.70	518.33	25.30	519.00	22.43
7.0	368.67	12.70	330.67	10.70	429.67	15.30	427.33	15.30	516.67	14.70	455.33	14.00	421.39	13.78
8.0	338.67	6.30	311.67	6.00	403.33	4.70	334.33	7.70	408.67	7.70	366.67	6.90	360.56	6.55
9.0	153.67	0.00	95.33	0.00	196.33	0.00	168.00	0.00	201.33	0.00	172.67	0.00	164.56	0.00
Mean	340.29	10.42	303.22	9.12	419.17	13.22	374.33	12.95	449.49	14.73	393.72	13.03		

Factors	***CD at 5%***	
	Weight of dried mycelium	Sporulation
pH	11.60	0.10
Isolate	11.60	0.10
pH x Isolate	28.42	0.24

Variation in phosphorus sources requirement

The results indicate that dipotassium hydrogen orthophosphate was most favourable source of phosphorus which supported maximum mycelial growth and sporulation followed by diammonium phosphate and single super phosphate for each isolates, whereas sodium phosphate was found as inhibitor of growth and sporulation both, because there was poor growth and no sporulation on sodium phosphate incorporated media. When isolate wise variation in mycelial growth and sporulation of different isolates were compared, it was found that isolate BS-F-5 exhibited maximum growth and sporulation on all phosphorus sources, while no sporulation was noticed on sodium phosphate incorporated media in any isolate (Table-3). This finding can be supported by Bilgrami and Verma (1994) that sodium phosphate was unutilizable and apparently toxic to the fungus. They also reported dipotassium hydrogen orthophosphate furnishing utilizable

phosphate and potassium ions, both, these salts act as useful buffers and exert a controlling influence over the pH changes in the medium caused by fungal growth and metabolism. Raghuchander *et al.* (1988) also found the dipotassium hydrogen orthophosphate as best source of phosphorus for mycelial growth and sporulation of *B. sorokiniana*. Thus, the findings of present investigation are well in accordance with the findings of previous workers. It is also clear that all the isolates have the similar preference for the phosphorus sources.

Table: 3 Effect of phosphorus sources on weight of dried mycelium (mg) and sporulation ($x10^3$) of six isolates of *B. sorokiniana*

Phosphorus sources	*Isolates*												Mean	
	BS-DWR-K-1		BS-D-2		BS-P-3		BS-K-4		BS-F-5		BS-V-6			
	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation
Dipotassium hydrogen orthophosphate	454.00	16.66	410.33	12.20	625.00	27.70	449.67	20.40	620.00	29.70	584.00	24.80	523.83	21.91
Single super phosphate	201.67	5.70	190.33	4.00	269.33	7.80	203.33	5.30	302.00	8.10	239.00	7.00	234.28	6.32
Diammonium phosphate	330.00	11.50	296.33	10.00	487.00	14.00	378.33	12.00	487.33	13.90	452.33	10.70	405.22	12.02
Sodium phosphate	189.00	0.00	176.67	0.00	190.00	0.00	188.00	0.00	190.33	0.00	189.67	0.00	187.28	0.00
Mean	293.67	8.47	268.42	6.55	392.83	12.38	304.83	9.43	399.92	12.93	366.25	10.63		

F Factors	***CD at 5%***	
	Weight of dried mycelium	Sporulation
Phosphorus	13.73	0.07
Isolate	15.04	0.09
Phosphorus x Isolate	33.64	0.18

Variation in potassium sources requirement

The potassium nitrate was found to be best potassium source for growth and sporulation of all isolate. The potassium sulphate was found to be inhibitory for mycelial growth as compared to potassium chloride but when it was compared with the medium where no potassium was added, and then it was found to be stimulatory for growth. Sporulation was not exhibited when no potassium was added to the basal medium or even when dipotassium hydrogen orthophosphate was deleted from the basal medium. Addition of K_2HPO_4 exhibited significantly higher sporulation as compared to potassium sulphate. Absence of potassium chloride in the basal medium gave the higher sporulation in BS-P-3, BS-K-4 and BS-F-5 as compared to the potassium chloride containing media alongwith other potassium substitutes (Table-4). Usually potassium is incorporated in the culture media in the form of phosphate and/or nitrate. The essentiality of this element and its physiological role in fungal metabolism has been investigated very little. Some fragmentary data related to the effect of potassium on growth and metabolism of fungi indicate that sub optimum level of this element interferes with sugar utilization (Bilgrami and Verma, 1994) and when completely absent, it causes increased accumulation of oxalic acid , which may lead to inhibition of growth and sporulation of microorganisms. All these information though scanty indicate that potassium has a possible role in carbohydrate metabolism.

Variation in vitamins requirement

Maximum growth of all the isolates was exhibited by Thiamine whereas maximum sporulation was recorded in yeast extract (Check 1) containing medium. Three vitamins *i.e.* biotin, inositol and calcium pentothenate were found to be least effective in stimulating growth as compared to yeast extract supplemented media, rather inositol and calcium pentothenate were found to be inhibitory for growth as compared to basal medium (Czapek's broth) without yeast extract. In case of sporulation, all four vitamins *i.e.* thiamine, biotin, inositol and calcium pentothenate were found to be comparatively less effective in inducing the sporulation as compared to yeast extract, whereas inositol was found to be inhibitory for sporulation as compared to basal medium (Czapek's broth), where no vitamins was supplemented. Vitamins *i.e.* thiamine and calcium pentothenate exhibited stimulatory effect on spore production as compared to basal medium without any vitamins or yeast extract. Biotin did not affect the sporulation in a significant manner. It

was found that isolate BS-F-5 was found to exhibit highest mycelial weight and sporulation and BS-D-2 was found to exhibit least mycelial weight and sporulation on any vitamins (Table-5). Madan and Thind (1998) reported that vitamins are constituents or precursors of co-enzyme and are required in small quantities by the living organisms for their normal growth and reproduction. There is a wide variation in the ability of fungi to synthesize vitamins. Some fungi are able to synthesize all vitamins which they require, others do not have the synthetic machinery and one or more vitamins have to be supplied externally for their normal growth. Jandaik and Kapoor (1972) found that there was poor growth when all the vitamins or thiamine were omitted from the medium. Singh (1973) observed that in case of *Helminthosporium solani* which was grown in synthetic medium enriched with various vitamins or lacking them. Growth was decidedly inhibited on media lacking vitamins. Singh (1973) also reported that *H. solani* has a near total deficiency for thiamine and a partial deficiency for biotin. In present investigation, all isolates to be showed stimulatory trend due to thiamine and biotin, whereas inositol and calcium pentothenate were inhibitory for growth and sporulation. The yeast extract which is a source of multivitamins showed a stimulatory effect on sporulation of all isolates. Thus, all the isolates have similar preference in vitamins requirements for growth and sporulation.

Table: 4 Effect of potassium sources on weight of dried mycelium (mg) and sporulation ($x10^3$) of six isolates of *B. sorokiniana*

Potassium sources	*Isolates*												Mean	
	BS-DWR-K-1		BS-D-2		BS-P-3		BS-K-4		BS-F-5		BS-V-6			
	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation
Potassium chloride	475.00	18.40	440.00	12.40	555.33	22.40	465.67	17.30	626.33	25.50	531.00	21.00	515.56	19.50
Potassium sulphate	295.33	11.50	289.33	7.20	462.67	15.00	368.67	15.10	467.33	14.20	370.00	13.60	375.56	12.76
Potassium nitrate	589.00	25.30	448.00	22.00	449.67	54.90	588.00	37.60	650.00	56.60	597.33	38.00	553.67	40.10
Without KCl	362.67	15.00	297.33	11.70	483.00	22.70	427.33	18.30	499.33	32.20	375.33	13.20	407.50	18.85
Without K_2HPO_4	122.00	0.00	96.00	0.00	130.00	0.00	109.00	0.00	132.23	0.00	129.33	0.00	119.78	0.00
Without K_2HPO_4 + KCl	167.33	0.00	154.33	0.00	185.00	0.00	168.00	0.00	199.67	0.00	182.33	0.00	176.11	0.00
Mean	335.22	11.70	287.50	8.88	377.61	19.17	354.44	14.71	429.17	21.42	364.22	14.30	515.56	19.50

Factors	***CD at 5%***	
	Weight of dried	Sporulation

		mycelium	
Potassium		13.16	0.10
Isolate		13.16	0.10
Potassium	x	32.23	0.25
Isolate			

Table: 5 Effect of different vitamins on weight of dried mycelium (mg) of six isolates of *B. sorokiniana*

Different Vitamins	*Isolates*												Mean	
	BS-DWR-K-1		BS-D-2		BS-P-3		BS-K-4		BS-F-5		BS-V-6			
	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation	Wt. of dried mycelium	Sporulation
Thiamine	659.33	24.00	628.33	14.00	725.00	24.70	642.00	23.30	752.33	28.00	651.67	17.30	676.44	21.88
Biotin	570.33	17.70	478.33	13.30	652.67	22.30	564.67	16.30	658.33	25.30	587.33	18.00	585.28	18.82
Inositol	361.33	6.70	315.67	7.30	427.33	9.40	377.00	9.00	474.67	9.80	377.33	10.00	388.89	8.70
Calcium pentothenate	399.67	21.00	385.67	16.70	468.67	23.00	397.00	18.70	484.67	26.00	417.00	22.00	425.44	21.23
Check I (Yeast extract)	625.33	28.70	497.00	30.70	729.67	41.30	588.33	36.00	735.33	44.70	645.67	37.30	636.89	36.40
Check II (Basal medium)	478.00	17.70	406.00	14.00	607.00	22.70	449.67	18.50	617.33	23.70	557.67	19.60	518.61	19.37
Mean	515.67	19.30	451.83	16.00	601.72	23.90	503.11	20.30	619.78	26.20	539.44	20.70	676.44	

F	Factors	***CD at 5%***	
		Weight of dried mycelium	Sporulation
Vitamins		11.70	0.08
Isolate		11.70	0.08
Vitamins	x	28.67	0.24
Isolate			

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