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Screening of wheat genotypes against foliar blight pathogens under artificial inoculation conditions

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Abstract
Foliar blight disease caused by *Bipolaris sorokiniana* (Sacc.) Shoem is most important disease of wheat in North Eastern plain zones (NEPZ) representing warm and humid climate in India. It is also increasing in North Western plains zones (NWPZ), due to climate changes and causes considerable losses in susceptible varieties. A field study was conducted during *Rabi*, 2016-17 crop seasons at Main Experiment Station, Narendradev University of Agriculture and Technology, Kumarganj, Faizabad to test the resistance of 150 genotypes against *Bipolaris sorokiniana* under artificial epiphytotics conditions. Each genotype was sown in last week of November in single row of one meter length. Variety Raj 4015 was used as check and was sown after every 20 genotypes. Pure culture of *Bipolaris sorokiniana* was inoculated on genotypes by using cleaned sprayer, at evening. Disease data was recorded using double digit scale based on per cent blighted area on flag leaf and one leaf just below. Out of 150 genotypes, no any genotype found immune, 9 genotypes were found resistant, 66 were moderately resistant, 69 were moderately susceptible and 5 were found susceptible and no any genotype found high susceptible against spot blotch disease of wheat.

Keywords: Spot blotch of wheat, symptoms, stock culture, varietal screening, yield losses

Introduction
India is the world's second largest wheat producer, behind china and ahead USA. It has revealed from the archaeological records that wheat was cultivated in Mohenjo-Daro and Harappa nearly 5000 year back. The important of wheat as a food of South Asia is well known. It is grown during the mid-winter months of November to April. The common bread wheat, *T. aestivum*, is the most important species, occupying more than 90% of the wheat area and 87% of the total wheat production in the country. In world, Wheat is grown over 224.7 million hectare area with production of 734.80 million metric tons and yield of 3.27 metric tons per hectare. In India, wheat is grown over 31.47 million hectare area with production of 86.53 million metric tons and yield of 2.75 metric tons per hectare (Anonymous, 2016) [5] about 91% of the total wheat production is contributed by northern states. Among them, Uttar Pradesh rank first with respect to area (9.645 m.ha.) and production of (30.00 m.t.) but the average productivity (27.86 q/ha) is much lower as to Punjab and Haryana (Anonymous, 2016) [6]. Spot blotch or Helminthosporium leaf blight caused by *Bipolaris sorokiniana* (Sacc.) Shoem. Is a most important disease of wheat in north eastern plains zone (NEPZ) representing warm and humid climate in India as well as in other South Asian countries. It is also increasing in North western plains zone (NWPZ) due to climatic change and causing losses in susceptible varieties (Singh D.P., 2014) [27].

Bipolaris sorokiniana (Sacc.) Shoemaker is a seed and soil borne pathogen, causes head blight, seedling blight, foliar blight/ spot blotch, common root rot and black point of wheat, barley and other small cereal grains and grasses (Wiese, 1998) [29]. Symptoms mainly develop in the form of dark brown necrotic spots (boat shaped) occur on the coleoptiles, leaves, crowns, stems, and roots with or without yellow halo around these. Darkening of the sub crown internode is a characteristic symptom. Lesions on the leaves start as a few mm that extend as elongated dark brown spots greater than 1-2 cm (Chand *et al*., 2002) [8]. The severity of the disease is directly influenced by tillage operation, irrigation scheduling, soil fertility level, sowing density, crop growth stage, occurrence of late rains during crop cycle, heat stress during grain filling, late planting, high temperature and high relative humidity causing more than 12 hours duration of leaf wetness (Sharma and Duveiller, 2003) [24].

Symptoms description

Symptoms of spot blotch were studied on wheat crop at seedling and adult stage. The crop was regularly observed after germination up to harvesting and a detailed morphological description of spot blotch symptom on crop such as colour, shape and size of spot, presence or absence of yellow halo and zones were recorded from initial to later stages. The disease appears at all the growth stages of the crop starting from the seedling to spikes. The dark brown necrotic spots (boat shaped) occur on the coleoptiles, leaves, crowns, stems, and roots with or without yellow halo around these. Darkening of the sub crown internode is a characteristic symptom. Symptoms mainly develop on sub-crown internodes, stem, leaves, awns, glumes and seeds. The main symptom caused by the pathogen is spot blotch, which is nothing but the disease of leaves. The early lesions on leaves are 1-2 mm long, small and dark brown in colour. There is no sign of chlorotic margin at the initial stage of infection. In the later stage in case of a susceptible genotype the small lesions extends very rapidly and ultimately reach into several centimetres. When the infection occurs into the spikelet; it results into shrivelled grain and the embryo end of the seed becomes dark in colour (Acharya *et al*., 2011.) [2].

(A) (B) (C)

Fig 1: *Bipolaris sorokiniana* (spot blotch) disease of wheat (A), symptoms on leaf (B), conidia of *B. Sorokiniana* (C)

Preparation of medium

PDA medium consisting following composition was prepared and sterilized using method described by Johnson and Booth (1983) [14].

Peeled potato - 200g
Dextrose - 20g
Agar-agar - 20g
Distilled water - 1000 ml

The peeled potatoes were cut in 12 mm cubes. Two hundred grams of potato cubes were rinsed in water and boiled for 20 minutes in 500 ml water. Potato broth was filtered through cheese cloth and kept in measuring cylinder. Agar was melted in 500 ml of water by heating and added to potato broth. Dextrose was added in it. The final volume was made up to 1000 ml by adding distilled water. The pH was adjusted to 7.0. The PDA was poured in test tube for preparation of PDA slant and also in flask. Then these were sterilized at 15 psi for 20 minutes in an autoclave.

Varietal Screening

Early literature on the management of spot blotch as also other foliar spots and blights has been thoroughly reviewed by Singh *et al.* (1986) [16] and Singh. Later literature, specifically on spot blotch management, has been reviewed by Akram *et al.* (2003) [1]. Iftikhar *et al.* (2012) [13] evaluated of 56 commercial wheat varieties against *Bipolaris sorokiniana* was conducted at National Agriculture Research Centre, Islamabad. 2 varieties showed moderate resistance at 2 scale under both conditions. Thirty two varieties showed moderate susceptibility and susceptibility under controlled conditions but had moderate resistance under field conditions, these lines can further be exploited in breeding program. Saadi *et al.* (2002) [23] screened wheat (K65, Cooley, Shawarir and Missani) for resistance to spot blotch (*Bipolaris sorokiniana* [*Cochliobolus sativus*]) disease using a detached leaf method. K65 and Cooley were found resistant. Singh *et al.* (2007) [25] evaluated 78 genotypes, under artificial inoculation conditions, to identify resistant donors against spot blotch diseases and found fifteen genotypes moderately resistant. Kumar *et al.* (2015) [19] Observed based on 0-9 scale were shown that a wide variability was observed for resistance to leaf blight in the wheat lines screened in one year of testing based on maximum leaf score. AUDPC value varies from the 92.6 to 123.5 across the resistant lines. Ojha *et al.* (2016) [20] evaluated of 100 screened entries 20 number of genotype showed highly resistant or Immune to the disease, whereas 28 genotype were resistant, 22 genotypes moderately resistant, 15 moderately susceptible and 15 genotypes susceptible. Indian germplasm lines tended to be more susceptible compression to lines originated from CIMMYT and China.

Preparation of stock culture

To preparation of stock culture, sorghum seeds and wheat straw were socked separately in water for 20 hours and then excess water was removed. The material was sterilized twice subsequently days at 15 pound per square inch for an hour. The flasks were inoculated with bit of 10 days old culture grown on potato dextrose agar and inoculated with periodical shaking to avoid cake formation. After an inoculation of 20 days at 28+ 1^0 C, one set of each pathogen was used for sub - culturing on potato dextrose agar at regular interval. A set of stock culture was store in deep freeze (-40^0C). Fresh culture were made for further experimentation as per needs by taking out few grains from those stock culture. This was done with the view to avoid loss in virulence due to frequent sub-culturing. *Bipolaris sorokiniana* luxuriant growth of the fungus was obtained on potato dextrose agar for fungal sporulation, cultures were placed in an incubator at 21 ^{0}C.

Evaluation of wheat genotypes for disease resistance

The experiment was conducted at Main Experimental Station of Narendra Deva University of Agriculture and Technology, Kumarganj,

Faizabad (U.P.) during Rabi 2016-17. Seeds of 150 genotypes were collected from All India Co-ordinated Wheat and Barley Improvement Project, Department of Genetics and Plant Breeding, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.). Two rows of Raj 4015 and Agara local were sown as border rows around all the sides of experiment as it is susceptible to foliar blight. It was also sown after every 20 entries. All the recommended agronomical and cultural practices were followed for raising the good crop. Details of experiments are as under-

No. of genotypes : 150
Design : Augmented
Plot size : one row of one meter length
Spacing : 20 cm (row to row)
Plant to plant : 5 cm
Fertilizer : 120:60:40 N:P:K (kg/ha)
Sowing date : 25/11/2016

List of genotypes screened in this trial have been given in Table No.-02

Observations recorded

Disease severity following Kumar *et al.* (1998) [18] double digit scale based on per cent blighted area on the flag and flag-1 leaf at flowering, soft dough and hard dough stages. The disease score of each selected plant were recorded twice.

Table 1: The double digit scale, based on per cent blighted area on the flag leaf and one leaf just below given by Kumar *et al.* (1998) [18]

A double digit* scale for appraising blight severity				
S. No.	Severity**		Rating	
	Flag leaf	Flag-1 leaf	Disease response	Range of value
1.	0	0-1	Immune (I)	00-01
2.	1-2	2-4	Resistant (R)	12-24
3.	3-4	4-6	Moderately Resistant (MR)	34-46
4.	5-6	6-8	Moderately susceptible (MS)	56-68
5.	7-8	8-9	Susceptible (S)	78-89
6.	9	9	Highly susceptible (HS)	99

* First and second value respectively, represents per cent blighted area on the flag leaf and flag-1 leaves.
** Values 1,2,3,4,5,6,7,8, and 9, respectively correspond to 10,20,30,40,50,60,70,80 and 90 per cent blighted area.

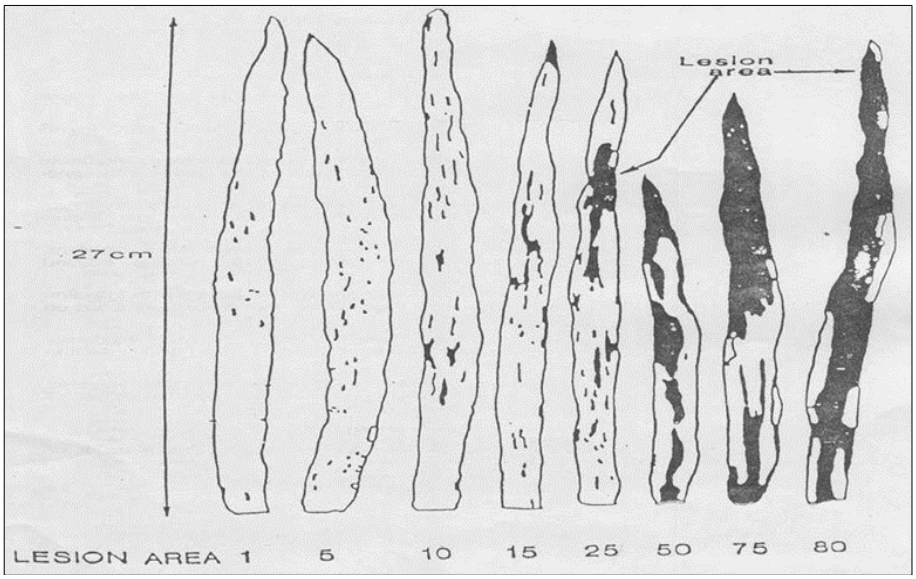

Fig 2: Assessment key of Kumar *et al.* (1998) [18] double digit scale based on per cent blighted area on the flag and flag -1 leaf.

Table 2: Categorization of wheat genotypes against the response of spot blotch disease under artificial disease pressure (2016-2017)

S. No.	Disease reaction	Score	No. of genotypes	Genotypes
1	Immune(I)	00-01	NIL	NIL
2	Resistant (R)	12-24	9	VL 829, HD 2967, DBW 179, HS 646, VL 1011, VL 4003, UP 2942, PBW 778, VL 4001
3	Moderately Resistant (MR)	34-46	66	HS 375, HS 490, HS 507, HS 542, DBW 173, DBW 88, HD 3043,HI 1612, C 306, DBW 39, HD 2733, HD 2888, HD 3171, K 0307, K1317, DBW 110, DBW 168, MACS 6478, NIAW 1415, UAS 304,CoW (W)-1, DBW 71, Kharchia 65, PBW 550, HPW 439, HPW 448, HS 643,HS 645, HS 647,UP 2992, UP 2993, VL 1012, VL 1013, VL 3014, VL 3015, VL 4002, DBW 189, DBW 196, HD 3226, HD 3237, HI 1619, HI 1620, HS 611, MACS 6677, MP 1318, PBW 750, PBW 752, HD 3219, UAS 384, UAS 385, DBW 246, DBW 247, DBW 248, KRL 370, PBW 779, PBW 780, WH 1316, TL 3011, TL 3012, TL 3013, DBW 250, DBW 251, HD 3272, NH-01-VHA, NH-04-VHA, UP 2955
4.	Moderately Susceptible (MS)	56-68	69	HPW 251,VL 892, DBW 90, HD 3059, HD 3086, PBW 644,WH 1021, WH 1080, WH 1105, WH 1124, WH 1142, K 8027, K1006, HD 8627, MP 3288, HI 8777, MACS 4028, UAS 375, GW 322, MACS 6222, NI 5439, UAS 446, HW 2044, HW 5216, DBW 14, DDK 1029, HW 1098,KRL 19, KRL 210, TL 2942, TL 2969, WR

				544, HPW 440, HPW 449, HS 629, HS 630,HS 644, HS 648, VL 3013, BRW 3773, CG 1023, HI 1617,HP1963, WH 1202, DBW 187, BRW 3775, HI 8791, UAS 462, UAS 387, DDK 1052, DDK 1053, KRL 377, KRL 384, KRL 386, TL 3014, TL 3015, DBW 249, HD 3271, HI 1621, PBW 757, PBW 777, WH 1232, WH 1233, NH-02-VHA, NH-03-VHA, NH-05-VHA, NH-06-VHA, NH-07-VHA, NH-10-VHA
5.	Susceptible (S)	78-89	5	AKDW 2997-16(d),MACS 5047, MACS 5049, NH-08-VHA, NH-09-VHA
6	Highly Susceptible (HS)	99	NIL	NIL

Evaluation of genotypes for spot blotch resistance

It is evident from the result presented in Table- 02, that out of these, none of the genotypes/lines were found immune and highly susceptible. Out of 150 genotypes, nine genotypes was found resistant namely VL 829, HD 2967, DBW 179, HS 646, VL 1011, VL 4003, UP 2942, PBW 778 and VL 4001(score 12-24). Sixty six genotypes were found moderately resistant against spot blotch. Some of these were HS 375, HS 490, HS 507, HS 542, DBW 173, DBW 88, HD 3043, HI 1612, C 306, DBW 39, HD 2733, HD 2888, HD 3171, K 0307, K1317, DBW 110, DBW 168, MACS 6478, NIAW 1415, UAS 304, CoW (W)-1, DBW 71, Kharchia 65, PBW 550, HPW 439, HPW 448, HS 643,HS 645, HS 647,UP 2992, UP 2993, VL 1012, VL 1013, VL 3014, VL 3015, VL 4002, DBW 189, DBW 196, HD 3226, HD 3237, HI 1619, HI 1620, HS 611, MACS 6677, MP 1318, PBW 750, PBW 752, HD 3219, UAS 384, UAS 385, DBW 246, DBW 247, DBW 248, KRL 370, PBW 779, PBW 780, WH 1316, TL 3011, TL 3012, TL 3013, DBW 250, DBW 251, HD 3272, NH-01-VHA, NH-04-VHA, UP 2955 (score 34-46). Sixty nine genotypes were found moderately susceptible against spot blotch. Some of these were HPW 251,VL 892, DBW 90, HD 3059, HD 3086, PBW 644,WH 1021, WH 1080, WH 1105, WH 1124, WH 1142, K 8027, K1006, HD 8627, MP 3288, HI 8777, MACS 4028, UAS 375, GW 322, MACS 6222, NI 5439, UAS 446, HW 2044, HW 5216, DBW 14, DDK 1029, HW 1098, KRL 19, KRL 210, TL 2942, TL 2969, WR 544, HPW 440, HPW 449, HS 629, HS 630,HS 644, HS 648, VL 3013, BRW 3773, CG 1023, HI 1617,HP1963, WH 1202, DBW 187, BRW 3775, HI 8791, UAS 462, UAS 387, DDK 1052, DDK 1053, KRL 377, KRL 384, KRL 386, TL 3014, TL 3015, DBW 249, HD 3271, HI 1621, PBW 757, PBW 777, WH 1232, WH 1233, NH-02-VHA, NH-03-VHA, NH-05-VHA, NH-06-VHA, NH-07-VHA, NH-10-VHA(score 56-68). Five genotypes were found susceptible against spot blotch. Some of these were AKDW 2997-16, MACS 5047, MACS 5049, NH-08-VHA, NH-09-VHA (score 78-89), against spot blotch disease and one genotype none germinated.

Discussion

Use of resistant variety is a cheapest and most economical method of disease control. Out of 150 genotypes of wheat, none of the genotypes/lines were found immune and highly susceptible. Nine genotypes were found resistant VL 829, HD 2967, DBW 179, HS 646, VL 1011, VL 4003, UP 2942, PBW 778 and VL 4001. Sixty six genotypes were found moderately resistant against spot blotch, Sixty nine genotypes were moderately susceptible and five genotypes namely AKDW 2997-16, MACS 5047, MACS 5049, NH-08-VHA, NH-09-VHA were found susceptible, against spot blotch disease of wheat. One genotype none germinated. Several scientists have also been reported variable response of different wheat varieties to *Bipolaris sorokiniana* time to time. Kumar *et al.* (2015) [19] screened 147 diverse lines of wheat (*Triticum aestivum*) to determine the variability for leaf blight resistance. Based on over all leaf score of 0-9 scale, 13 lines were found to be resistant. Out of total 147 lines, 34 were moderately resistant. Highest numbers of lines were found moderately susceptible that comprises 60 lines. In susceptibility group there were 40 lines. Ojha *et al.* (2016) [20] reported that out of 100 screened entries 20 number of genotype showed highly resistant or immune to the disease, whereas 28 genotype were resistant, 22 genotypes moderately resistant and 15 moderately susceptible and 15 genotypes susceptible against spot blotch. Singh *et al*. (2007) [25] evaluated 78 genotypes, under artificial inoculation conditions, to identify resistant donors against spot blotch diseases and found fifteen genotypes moderately resistant.

Conclusion

Out of 150 genotypes screened under artificial epiphytotic condition, none of the genotypes/lines were found immune and highly susceptible. Nine genotypes were found resistant namely VL 829, HD 2967, DBW 179, HS 646, VL 1011, VL 4003, UP 2942, PBW 778 and VL 4001. Sixty six genotypes were found moderately resistant, Sixty nine genotypes were noted moderately susceptible and five genotypes were found susceptible, against spot blotch disease, one genotype none germinated. The disease under artificial epiphytotic condition can be utilized in breeding programme to develop high yielding varieties.

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