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GENETIC POTENTIAL AND EFFECT OF CHEMICALS AGAINST *BIPOLARIS SOROKINIANA* IN WHEAT

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ABSTRACT

Wheat has been one of the first domesticated food crop and important food grain source for humans. Sixty wheat advanced lines/varieties received from different Research Institutes of the Punjab, Pakistan were screened for genetic potential against *Bipolaris sorokiniana*, a pathogen of wheat leaf blight in two consecutive years under inoculated conditions. Before sowing, the seeds were inoculated with the pathogen. Considering the lower percent of disease and higher vigour index, 12 wheat lines, namely 8C006, 8C007, 9C033; 9C035, 9C036, 7C002; 088186, 088195; 076395, 076309, V. 07142 and V.05068 were identified as tolerant against the pathogen. Among the four fungicides, Score 25% EC and Craze 80% WP were found the most effective as seed treatment to control the disease.

Keywords: Wheat germplasm, leaf blight, spot blotch, chemical control, screening.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the world's most important cereal crop and enjoys the status of staple food for our country with the largest area under cultivation. It plays a significant role in the economic stability of the country. As it occupies an area of 8900.7 hectares with grain yield 25213.8 tones which is more than all the other cereal crops (Anonymous, 2010). Thus high yield of this crop singly can bring prosperity to the country as well as to the farmer's community. But unfortunately average yield taken by our farmers is significantly low as compared to other countries as well as to the potential of this crop. Among the various factors responsible for low yield of wheat in Pakistan, diseases are important one and are the major obstacle in gaining reliable yield with high quality of grains. Continuous research to find out the resistant source against diseases is the need to feed the ever increasing population of the country. Among the diseases attack on wheat crop, leaf blight (*Bipolaris sorokiniana* Sacc.) is economically important foliar disease of wheat in warmer growing areas

throughout the world (Aftabuddin *et al.*; 1991). The fungus *Cochliobolus sativus* is the teleomorph (Sexual stage) of *Bipolaris sorokiniana* (anamorph) which is the causal agent of wide variety of diseases. For the first time *B. sorokiniana* was identified as predominant pathogen of leaf blight/spot in wheat growing areas of Pakistan during 2003 to 2006 (Iftikhar *et al.*, 2006). The disease manifests by developing dark brown necrotic lesions on roots, crown, leaves and lower leaf sheaths. On leaf blades and sheaths, oval to elongated light to dark brown blotches developed. When it severely infects the roots and crown portion, the plants dry out without producing any seed. If the grain formation has been initiated prior to the infection, the infected spike produced shriveled grains. In the prevalence of conducive weather conditions i.e. continuous rain for 5-6 days followed by warmer temperatures (day average of 20–30°C), leaf blight epidemic developed very rapidly (Mehta, 1998; Aftabuddin *et al.*, 1991; Satvinder *et al.*, 2002; Nagarajan & Kumar, 1998; Ruckstuhl, 1998; and Singh *et al.*, 1998). The importance of this disease is evident from the fact that due to this disease, yield losses were estimated at 18-22% in India (Singh & Srivastava, 1997) and 23.8% in Nepal (Shrestha *et al.*, 1997). Leaf blight was of minor

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importance in Pakistan previously (Bhatti & Ilyas, 1986; Hafiz, 1986). However during the last decade, disease prevalence was found predominantly in various districts of Punjab (Ali *et al.*, 2001; Iftikhar *et al.*, 2006). It indicates a further threat for wheat. Resistant source to leaf blight existed in various wheat germplasms in different countries (Duveiller and Gilchrist *et al.*, 1994; Mujeeb *et al.*, 1996; Mujeeb *et al.*, 2001).

The present study was carried out at Plant Pathology Section, Plant Pathology Research Institute, Faisalabad to find out the genetic potential/resistant sources against the disease in our local available varieties/lines and to find out the impact of chemicals for the control of leaf blight.

MATERIALS AND METHODS

Isolation and identification of the pathogen: Disease samples of wheat variety Fareed-2006 showing the symptoms of leaf blight, suspected to be caused by *Bipolaris sorokiniana*, were received from the Deputy District Officer Agriculture (Ext.) Dera Ghazi Khan. For isolation, diseased portion of leaves and roots were excised and placed on sterilized moist blotter paper and on Potato Dextrose Agar (PDA). 25 Petri plates having size 9cm and pour 20ml PDA, were kept in the incubator at 25 ±2°C for seven days. Along with the other fungi recovered from these samples, *B. sorokiniana* was in high frequency. It was identified on the basis of morphological characters having dark brown to black, fusoid, straight or curved thick walled, elliptical conidia with bipolar germination and characterized by 1-6 septa. The colony of the fungus had interwoven hyphae as a loose cottony mass white or light to grey color depending on isolates (Kumar *et al.*, 2002).

Pathogenicity test: Wheat seeds disinfected with 1% Chlorox were sown in the autoclaved soil medium (1:1:1 soil, sand and peat moss) in the plastic pots. Three seeds

/ pot were sown with 5 mm disk of 7 days old fungal culture (approximately containing 3.2×10^4 spores/disk). The inoculum disk was placed adjacent to the seeds. The pots were incubated at 22–25 °C in growth room by placing on the racks randomly. The disease was observed after 30 days in the form of typical spots on leaves. The Pathogenicity was confirmed by following the Koch's postulates.

Genetic potential in wheat against *Bipolaris sorokiniana*: Sixty wheat varieties/lines collected from Wheat Research Institute, Faisalabad; Regional Agriculture Research Institute Bahawalpur, Barani Agricultural Research Institute, Chakwal and Arid Zone Research Institute, Bhakkar along with three check varieties namely Fareed-2006, Bhakkar-2002 and NR-381 were screened for genetic potential against *B. sorokiniana* under inoculated condition in two consecutive years 2011-2012 and 2012-2013. The trial was sown in augmented design. Before sowing, the seeds of each test variety/line coated with pathogen's culture. Each variety/line was sown in 2 rows of 2 meter length with row to row distance of 15cm. All agronomic practices carried out like ploughing, irrigation and weeding were same for all varieties/lines. At the time of tillering, 10 days old culture of *B. sorokiniana* was blended in 5 liter distilled water to make spore suspension (1×10^3) by using heamocytometer. All the entries along with check varieties were sprayed with spore suspension three times at 5 days interval. Plants were frequently sprayed with water by using hand pressure sprayer to create appropriate humidity to make micro-climate conducive for the disease. Data on disease incidence and disease severity was recorded at milking stage following 0-5 scale Table 1 (Anon., 1996).

Disease rating	Disease spots % on leaves	Disease response
0	No symptoms	Immune
1	1-5 % spots on leaf area	Resistant
2	6-20 % spots on leaf area	Moderately Resistant
3	21-40 % spots on leaf area	Moderately Susceptible
4	41-60 % spots on leaf area	Susceptible
5	61 % and above spots on leaf area	Highly susceptible

Efficacy of different fungicides on seed germination: To find out the most effective fungicides as seed treatment against the disease, trial was conducted in plastic trays (18×9) having sand medium. Topsin-M

72% WP, Shincar 50% SC, Score 25% EC and Craze 80% WP were used as seed treatment to check the effectiveness against the pathogen Table 2.

Table 2. Chemical Treatment made on wheat seed.

Treatment	Formulation	Source	Active ingredient	Concentration/Kg seed
T1	Topsin-M 72% WP	Aysta Life Sciences Pakistan Pvt. Ltd.	Thiophenate methyl	2.00 gm
T2	Score 25% EC	Syngenta Pakistan Pvt Ltd.	Difenoconazole	1.00 ml
T3	Shincar 50% SC	FMC Pakistan, Pvt. Ltd.	Carbendazim	2.00 gm
T4	Craze 80% WP	Ali Akbar Group, Pakistan	Mancozeb	2.00 gm

The seeds of check variety i.e. Fareed-2006 were first coated with the inoculum and kept for 48 hours for development of its relationship with the seeds then the inoculated seeds were treated with all the fungicides accordingly. All treatments were arranged in completely randomized design (CRD) and observed the mean comparison by using Duncan's new multiple range test (DNMRT). Forty seeds were sown in four lines in each plastic tray. The effect of different fungicides on pathogen development along with seed germination was recorded at 4, 7 and 14 days after sowing using disease rating scale.

RESULTS AND DISCUSSION

Genetic potential in wheat against *Bipolaris sorokiniana*:

Reaction of all the germplasm along with the three check varieties was assessed against the disease. None of variety/line was found immune or highly resistant to the disease. The data revealed that 12 varieties/lines namely 8C006, 8C007, 9C033; 9C035, 9C036, 7C002; 088186, 088195, 076395, 076309; V 07142 and V05068 exhibited resistance against the pathogen and plant leaf area infected in these varieties/lines ranged from 1-5% only. Similarly 9 Varieties/lines (8C005, 9C034, 9C038; 6C002, 076422, 076377; 076354, WL-1 and V08173) were found to be moderately resistant and leaf area infected in these varieties/lines ranged from 6-20%. On the other hand 27 varieties/lines (8C003, 8C009, 7C003, 6C016; 066253, 076306, 076421; 076408, 066237, V07100, V.07194; V06096, V07102, 05BT014, V076309;

TW57091, V07032, 07BT007, V07076; V07663, FSD.08, LASANI.08, 05082, V07096, V07663, V9272 and V08171) were rated as moderately susceptible and infected leaf area ranged from 21-40%. Other nine varieties/lines (8C002, 7C005, 076325; 088188, 076418, V06018; V07067, Shafaque-2006 and Sehere-2006) followed 41-60% infected leaf area and behaved as susceptible, whereas 3 varieties/lines (076355, 076317 and V076317) were found to be highly susceptible having infected leaf area > 60% according to disease rating scale (Table 3). Low to moderate resistance has been achieved against leaf blight and foliar blight in wheat breeding studies from South Asia (Alam *et al.*, 1994; Devkota, 1994; Bhandari *et al.*, 2003; Sharma *et al.*, 2004; Siddique *et al.*, 2006). Even though, the resistant cultivars of South Asia "Gautam" reported to suffer from substantial grain yield loss under severe epidemic of spot blotch (Duveiller *et al.*, 2005). According to Rajaram (1988) and Dubin and van Ginkel (1991) best sources of resistance reported in the Brazilian and Zambian wheat lines. In studies conducted by Kohli *et al.*, (1991) few Chinese wheat genotypes like SW895422, Chirya1, Chirya3, Chirya7, NL781 and NL785 reported significant resistance levels to spot blotch. On the other hand genetic material exhibiting resistance against the disease indicated the pathway to breeding programmed for the attainment of resistant cultivars against the disease for the purpose of maximum grain yield resulting into prosperity of the farmer's community as well as of the country.

Table 3. Average disease incidence on wheat Germplasm against *B. sorokiniana*.

Sr. No	Varieties/lines	Mean disease incidence	Response
1	076317	86.5a	HS
2	Fareed-06	85.67a	HS
3	V.076317	80.17b	HS
4	076355	76.83bc	HS
5	Bhakkar-2000	75.33c	HS

6	076418	58.5d	S
7	Sehere-2006	58.17d	S
8	076325	55.83de	S
9	8C002	55.83de	S
10	NR-381	54.5e	S
11	Shafaque-2006	54.17e	S
12	V.06018	51.5ef	S
13	7C005	46.83fg	S
14	088188	43.83g	S
15	V.07067	43.17gh	S
16	8C003	38.83hi	MS
17	076421	38.5hi	MS
18	V.06096	38.17i	MS
19	05BT014	38.17i	MS
20	07BT007	38.17i	MS
21	TW.57091	37.17ij	MS
22	8C009	36.83ij	MS
23	076306	36.5ijk	MS
24	V.9272	34.83ijkl	MS
25	V.07194	34.5ijkl	MS
26	V.07076	34.17ijkl	MS
27	Faisalabad 2008	33.17jkl	MS
28	076408	32.5jklm	MS
29	V.07663	31.83klm	MS
30	066253	31.5lm	MS
31	Auqab-2000	31.17lmn	MS
32	7C003	30.83lmn	MS
33	V.07032	30.17lmno	MS
34	V.07102	28.17mnop	MS
35	V.076309	28.17mnop	MS
36	066237	26.5nopq	MS
37	V.07096	25.83opqr	MS
38	05082	25.5opqr	MS
39	V.07194	24.5pqr	MS
40	V.08171	22.83qrs	MS
41	Lasani 2008	21.5rst	MS
42	6C016	18.83stu	MS
43	6C002	18.5stu	MR
44	076354	18.17stu	MR
45	8C005	17.83tu	MR
46	076422	15.5uv	MR
47	9C038	14.5uv	MR
48	076377	12.5w	MR
49	WL-1	9.167wx	MR
50	V.08173	7.833wxy	MR
51	9C034	6.833xyz	MR
52	9C036	6.5xyz	R

53	V.05068	6.167xyza	R
54	8C007	5.833xyza	R
55	7C002	5.5xyza	R
56	076395	4.167yzab	R
57	8C006	3.833yzab	R
58	076309	3.167yzab	R
59	088195	2.5zab	R
60	V.07142	2.167zab	R
61	088186	1.5ab	R
62	9C033	-0.1667b	R
63	9C035	-0.1667b	R

Means sharing similar letters are statistically non-significant ($p>0.05$).

Sensitivity of *B. sorokiniana* towards various fungicides during seed germination and % seedling mortality:

Among all the treatments fungicides used as seed treatment, Score-250EC (Difenoconazole) followed by Craze 80% WP (Mancozeb) was found to be

statistically the most effective; both ensured seed germination and minimized seedling mortality while remaining two test fungicides were comparatively less effective (Table 4 and Table 5).

Table 4. Comparative Efficacy of different fungicides on seed germination.

Treatment	Formulation	Mean seed germination
T1	Topsin-M 72% WP	76.00 bc
T2	Score 25% EC	89.50 a
T3	Shincar 50% SC	74.25 c
T4	Craze 80% WP	84.75 ab
T5	Untreated Control	42.00 d

Mean sharing similar letters are statistically non-significant ($p>0.05$).

Table 5. Comparative Efficacy of different Fungicides in controlling seedling Mortality.

Treatment	Formulation	Mean seedling mortality
T1	Topsin-M 72% WP	20.75 b
T2	Score 25% EC	8.50 c
T3	Shincar 50% SC	14.50 bc
T4	Craze 80% WP	12.00 c
T5	Untreated Control	82.00 a

Mean sharing similar letters are statistically non-significant ($p>0.05$).

Seed treatment with fungicides has proven effective against leaf blight of wheat. Same results were given by Viedma and Kohli (1998). According to Stack and McMullen (1988) and Mehta (1993) many fungicides are used against leaf blight as seed treatment i.e. Captan, Mancozeb, Maneb, Thiram, Pentachloronitrobenzene (PCNB) or Carboxin guazatine plus, Iprodione and Triadimefon and effective in protecting germinating seeds and seedlings from seedling blights. Sharma-Poudyal *et al.* (2005) has reported that seed treatment with Vitavax 200B and Carbendazime improves early plant establishment in heavy soil predominating areas where wheat is cultivated after rice. Studies conducted by Sharma and Duveiller (2006), demonstrated that the fungicide Opus effectively control

spot blotch disease under soil nutrient stressed farmers' field conditions. However, questions have been raised regarding their use and environmental sustainability (Agarwal *et al.*, 2004).

CONCLUSION AND RECOMMENDATIONS

Fungicides like Difenoconazole, Mancozeb, not only control seed borne pathogen *Bipolaris sorokiniana* but also improves seed germination and seedling vigor, ensuring the maximum yield.

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