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IN VITRO SCREENING OF SYNTHETIC HEXAPLOID WHEAT LINES AGAINST *BIPOLARIS SOROKINIANA* IN PAKISTAN

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Abstract

Leaf blight caused by *Bipolaris sorokiniana* (*Cochliobolus sativus*) is a world wide economically important foliar disease of wheat. Leaf blight or Spot blotch mainly occurs in warm, humid wheat growing areas. In Pakistan *Helminthosporium* leaf spots (spot blotch) has been noted in different agro-ecological wheat production zones especially where winter temperatures are warmer. *Bipolaris sorokiniana* was identified as predominant pathogen of leaf spot in wheat growing areas of Pakistan during 2003 - 2006. Out of 87 isolates collected from different agro-ecological zones of wheat producing areas, the most aggressive isolate P2 -9 was used to screen the synthetic hexaploid elite I, elite II from *Aegilops tauschii* (2n=2x=14, 44) accessions and durum parents (*Triticum turgidum*) (2n=4x=28, AABB) by *In vitro* technique. Out of 93 accessions of Elite I, none of them was found resistant or moderately resistant in foliar symptoms. Nine out of 32 accessions of synthetic hexaploid (Elite II) and 3 accessions of total 51 durum parents were found moderate resistant to *B. sorokiniana* in two years of *In vitro* study. Total 16 lines of synthetic hexaploid elite 11 and 12 lines of Durum parents having moderately resistance and moderately susceptibility may further be exploited in breeding program.

Introduction

Spot blotch of wheat, caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker; syn. *Helminthsporium satvum* Pammel, C.M. King & Bakke) occurs through out the world and considered as the most severe constraint to the wheat production in the countries of Southeast Asia such as India, Bangladesh and Thailand, where climates are warm and moist. The fungus is one of the constraints for crops in warmer growing areas and causing significant yield losses (Aftabuddin *et al.*, 1991). High temperature and high relative humidity favour the outbreak of the disease (Aggarwal *et al.*, 2000). In north eastern and northwestern plains of India the yield losses ranged from 27% to 56.6% during 1998-99, incited due to the leaf blight caused by *B. sorokiniana* (Satvinder *et al*., 2002).

A severe leaf blight epidemic was observed in Indian Punjab, the dominant pathogen in the blight complex was *Drechslera sorokiniana* followed by *Fusarium* spp. The seed germination and seedling emergence significantly decreases with increasing quantities of black pointed seeds caused by *B. sorokiniana* in studies conducted in Bangladesh (Hossain & Hossain, 2001) and in China (Song *et al*., 2001).

The disease was considered as the major limitation for wheat cultivation in Bangladesh (Alam *et al*., 1994), the sever losses due to this destructive pathogen was estimated up to 15% on several farms over a number of years in the country, 18-22% loss has been observed in India (Singh *et al*., 1997) and 23.8% in Nepal (Shrestha *et al*., 1997). The disease affects all plant parts and can cause up to 100% yield loss (Mehta, 1993). Pathogen has also been reported to be one of the principal fungus involved in the seedling blight and root rot of wheat in Pakistan (Bhatti *et al*., 1986; Hafiz, 1976; Kishawa *et al*., 1992).

The environment friendly approaches could be used for the management of foliar blights. Particular, sources of resistance to spot blotch are known to exist in various germplasm. However, genetic diversity within the conventional wheat gene pool is somewhat limited. Complementing the existing diversity species from wild relatives across the primary, secondary, and tertiary pools may find a significant place in wheat improvement (Mujeeb-Kazi, *et al*., 1996; Mujeeb-Kazi, 2000).

Several sources have been identified as resistant to spot blotch by Duveiller & Gilchrist (1994). Several workers have observed the resistance, like *Aegilops squarrosa* crosses has shown impressive resistance to spot blotch in Mexico (Ginkel & Rajaram, 1997). Among 14000 entries of wheat and related wild species; *Ae. triuncialis, Ae. spelloides, Ae. squarrosa, Ae. triaristata, Triticum dicoccoides, Ae. cylindrical* and *T. boeolicum* have been identified having source of resistance at Punjab Agricultural University in Punjab, India (Dhaliwal *et al*., 1993; Sing & Dhaliwal, 1993). An early Mexican variety, Cocoraqua was found resistant to spot blotch in field (Mehta, 1985). Five germplasm lines have been identified as leaf blight resistant germplasm during a study conducted at hot spot (Poza Rica) of Mexico (Mujeeb *et al*., 1996). Resistance to spot blotch has also been reported in 2001 (Mujeeb *et al*., 2001) in 5 synthetic hexaploid wheat and seven bread wheat line.

Very little information is available in Pakistan on the management of foliar blights; therefore the information about the source of resistance can be helpful in successful management of the diseases.

Materials and Methods

The inoculum of single spore culture of the most aggressive isolate (P2-9) was increased on potato dextrose agar medium. This was selected after aggressiveness analysis of 87 isolates, collected from different agro-ecological wheat production zones.

Test tubes measuring (20 cm x 3 cm) were prepared by filling $1/4^{th}$ of cotton in the bottom of the tube. Distilled water (20 ml) was added in each tube and tubes were covered with aluminum foil. Tubes were autoclaved. These tubes were ready for further experiment.

Screening: The seeds of synthetic hexaploid, Elite I, Elite II and durum parents which were provided by Dr. Mujeeb Kazi (CIMMYT) were screened against the most aggressive isolate (P_2 9) of *Bipolaris sorokiniana* through standardized test tube method (Iftikhar *et al*., 2008).

Ninety-three accessions of synthetic hexaploid (Elite-I), 32 accessions of synthetic hexaploid (Elite-II) from *Aegilops tauschii* (2n=2x=14, 44) and 51 durum parents (*Triticum turgidum*) (2n=4x=28, AABB) with check (Wafaq 2001) were screened.

Three-seeds/ tube were surface disinfected with 1% Clorox solution for one minute and placed on the moist cotton swab in the test tubes. One disc of 5 mm of fungal isolates with the help of cork borer was placed adjacent to the seeds. The tubes were placed in randomized (RCD) manner in the steel racks. After inoculation tubes were again covered with aluminum foil and were placed in growth chamber at 25°C for incubation. The data was recorded upon the appearance of spots on the leaves on 0-5 scale where 0 = no symptoms, 1 = 1-5% spots on leaves, 2 = 6-20% spots on leaves, 3 = 21 – 40% spots on leaves, 4 = 41 – 60%, 5 = more than 60% (Anon., 1996). The scale is considered as 0= resistant, 1-2 = moderately resistant, 3-4 = moderately susceptible and 5= susceptible.

Results and Discussion

The two years (2005 & 2006) data of *In vitro* screening of synthetic hexaploid revealed that out of 93 synthetic hexaploid of Elite-1, none of the accession showed resistance to *B. sorokiniana* and may be reliable for further use in breeding program. In some cases the two years study have variation as they showed moderate resistance in one year but next year they showed susceptibility (Table 1).

Out of 32 synthetic hexaploid of Elite II from *Aegilops tauschii* (2n=2x=14, 44), eleven accessions (arlin-1/ae.squarrosa (218), LCK59.61/ AE.SQUARROSA(693)/, CETA/ AE.SQUARROSA(1025)/, CPI/GEDIZ/3/ GOO/JO/CRA/4/ AE.SQUARROSA (1018)/, CETA/ AE.SQUARROSA(1038), CETA/ AE.SQUARROSA(368), GAN/ AE.SQUARROSA(206), ARLIN-I/ AE.SQUARROSA(335) & GAN/ AE.SQUARROSA (335) showed moderate resistance to *B. sorokiniana* in consecutive two years (2005 & 2006). While 2 accessions DVERD-2/AE.SQUARROSA(214) & STYUS/CELTA// PALS.3/SRM-5/4/AE.SQUARROSA(431) showed moderate susceptibility in both years (Table 2). Three accessions including DOY1/AE.SQUARROSA(1027)\, CETA/ AE.SQUARROSA(1031)\ and CROC_1/AE.SQUARROSA(212) showed resistance in first year screening but 2nd year they showed moderate susceptibility to *B. sorokiniana*. Similarly 2 accessions CETA/AE.SQUARROSA(533)\ and CETA/ AE.SQUARROSA (1046) showed moderate resistance in 2005 but moderate susceptibility in another year to the same aggressive isolate.

Out of 51 Durum (*Triticum turgidum*) (2n=4x=28, AABB) parents, 3 accessions ARAOS, STY-US/CELTA//PALS/3/SRN-5 & FALCIN-1 showed moderate resistance and can be exploited in breeding program. Five lines including LARU, SNIPE/ YAV79/DACK/TEAL, GAN, AGAMI and KAPUDE-1 showed moderate susceptibility to *B. sorokiniana* in both the years (Table 3). Similarly 3 lines (CPI/GEDIZ/ 3/GOO//J0/CRA, TKSN1081 & YAV-3/SCOT//J069/CRA/3/YAV79 showed moderate resistance in one year but moderate susceptibility in 2nd year. YAV-2/TEZ have potential to serve as genetic stock for subsequent use by breeders which showed resistance in 1st year but have moderate susceptibility in 2nd year (Table 3).

Table 1. Screening of synthetic hexaploid (Elite-1) against *Bipolaris sorokiniana.*

E. No.	Genotype	Leaf infection (0-5) Y-2005	Leaf infection (0-5) Y-2006
1.	ALTAR 84/AE.SQUAROSA (188)	5	4
2.	DOY1/AE.SQUAROSA (188)	5	2
3.	ALTAR 84/AE.SQUAROSA (192)	5	-
4.	ALTAR 84/AE.SQUAROSA(193)	5	4
5.	ALTAR 84/AE.SQUAROSA(198)	4	4
6.	CROC-1/AE.SQUAROSA(205)	3	5
7.	ALTAR84/AE.SQUAROSA(205)	-	5
8.	CPI/GEDIZ/3/GOO//J069/CRA/4/ AE.SQUARASA(208)	5	4
9.	ALTAR 84/AE.SQUAROSA(211)	5	-
10.	D67.2/P66.270//AESQUAROSA(211)	5	4
11.	D67.2/P66.270//AESQUAROSA(213)	4	3
12.	ROK/KML//AE.SQUARROSA(214)	5	2
13.	D67.2/P66.270//AESQUAROSA(217)	5	5
14.	YUK/AE.SQUAROSSA(217)	-	3
15.	D67.2/P66.270//AESQUAROSA(218)	5	2
16.	ALTAR 84/AE.SQUAROSA (219)	5	4
17.	ALTAR 84/AE.SQUAROSA (220)	4	3
18.	DVERD-2/AE.SQUARROSA(221)	-	4
19.	ALTAR 84/AE.SQUAROSA (221)	5	4
20.	D67.2/P66.270//AESQUAROSA(221)	3	-
21.	D67.2/P66.270//AESQUAROSA(211)	4	4
22.	D67.2/P66.270//AESQUAROSA(222)	-	4
23.	CROC-1/AE.SQUAROSA(205)	5	4

Table 1. (Cont'd.).

E. No.	Genotype	Leaf infection (0-5) Y-2005	Leaf infection (0-5) Y-2006
24.	ALTAR 84/AE.SQUAROSA (224)	5	5
25.	ACO89/AE.SQUARROSA(309)	5	-
26.	68.111/RGB-U//WARD/3/ AE.SQUARROSA(316)	-	4
27.	68.111/RGB-U//WARD/3/AE.SQUARROSA(326)	5	5
28.	68112/WARD//AE.SQUARROSA(369)	-	5
29.	68112/WARD//AE.SQUARROSA(369)	2	5
30.	DOY1/AE.SQUARROSA (447)	5	5
31.	YAV-3/SCO//JO69/CRA/3/YAV79/4/ AE.SQUARROSA(498)	5	4
32.	DOY1/AE.SQUARROSA (511)	5	4
33.	68.111/RGB-U//WARD/3/AE.SQUARROSA(511)	4	4
34.	DOY1/AE.SQUARROSA (515)	5	5
35.	68.111/RGB-U//WARD/3/FGO/4/RABI/ 5/AE.SQUARROSA(629)	5	5
36.	FGO/USA2111//AE.SQUARROSA(658)	-	5
37.	CROC-1/AE.SQUARROSA(725)	5	4
38.	68.111/RGB-U//WARDRESEL/3/STIL/4/ AE.SQUARROSA (781)	5	4
39.	68.111/RGB-U//WARDRESEL/3/STIL/4/ AE.SQUARROSA (783)	5	5
40.	YAR/AE.SQUARROSA(783)	5	5
41.	YUK/AE.SQUARROSSA(864)	5	4
42.	68.111/RGB-U//WARD/3/FGO/4/RABI/5/ AE.SQUARROSA(878)	5	5
43.	68.111/RGB-U//WARD/3/FGO/4/RABI/5/ AE.SQUARROSA(878)	5	5
44.	CROC-1/AE.SQUARROSA(879)	5	4
45.	68.111/RGB-U//WARD/3/FGO/4/RABI/5/ AE.SQUARROSA(882)	5	5
46.	SORA/AE.SQUARROSA(884)	5	4

Table 1. (Cont'd.).

E. No.	Genotype	Leaf infection (0-5) Y-2005	Leaf infection (0-5) Y-2006
47.	68.111/RGB-U//WARD/3/FGO/4/RABI/5/ AE.SQUARROSA(890)	5	5
48	CROC-1/AE.SQUARROSA(518)	-	2
49	PBW114/AE.SQ	5	4
50	ALTAR 84/AE.SQUARROSA (JABANGOR)	-	4
51	YAV-2/TEZ//AE.SQUARROSA(249)	-	4
52	CETA/AE.SQUARROSA(895)	5	3
53	GAN/AE.SQUARROSA(180)	-	2
54	D67.2/P66.270//AE.SQUARROSA(257)	5	
55	LCK59.61/AE.SQUARROSA(313)	5	3
56	LCK59.61/AE.SQUARROSA(324)	5	4
57	SRN/AE.SQUARROSA(358)	5	3
58	SCOOP_1/AE.SQUARROSA(358)	5	4
59	GAN/AE.SQUARROSA(408)	5	3
60	SCA/AE.SQUAROSSA(518)	5	2
61	YAR/AE.SQUARROSA(518)	5	4
62	BOTNO/AE.SQUARROSA(617)	5	4
63	BOTNO/AE.SQUARROSA(620)	5	4
64	BOTNO/AE.SQUARROSA(625)	-	3
65	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSSA(629)	5	4
66	D67.2/P66.270//AE.SQUARROSA(633)	5	4
67	D67.2/P66.270//AE.SQUARROSA(659)	5	5
68	SNIPE/YAV79//DACK/TEAL/3/AE.SQUAROSSA(700)	5	5
69	TRN/AE.SQUAROSSA(700)	5	4

Table 1. (Cont'd.).

E. No.	Genotype	Leaf infection (0-5) Y-2005	Leaf infection (0-5) Y-2006
70	SNIPE/YAV79//DACK/TEAL/3/AE.SQUARROSSA(877)	5	3
71	GAN/AE.SQUARROSSA(897)	-	3
72	YAV_2/TEZ//AE.SQUARROSA(895)	5	-
73	ARLIN/AE.SQUARROSA(283)	5	2
74	FALCIN/AE.SQUARROSA(312)	5	2
75	RASCON/AE.SQUARROSA(312)	5	4
76	SCOT/MEXI_1//AE.SQUAROSA(314)	-	4
77	DOY1/AE.SQUARROSA (333)	5	4
78	DOY1/AE.SQUARROSA (428)	5	4
79	68.111/RGB-U//WARD/3/AE.SQUARROSA(452)	5	-
80	68.111/RGB-U//WARD/3/AE.SQUARROSA(454)	5	4
81	DOY1/AE.SQUARROSA (458)	5	2
82	GREN/AE.SQUARROSA (458)	5	4
83	CETA/AE.SQUARROSA(174)	4	4
84	DOY1/AE.SQUARROSA (188)	5	4
85	SCA/AE.SQUARROSSA(409)	5	4
86	CPI/GEDIZ/3/GOO//J069/CRA/4/AE.SQUARROSA(409)	5	2
87	STY-US/CETA//PALS/3/SRN_5/4/AE.SQUARROSA(502)	5	3
88	ALTAR 84/AE.SQUARROSA (502)	5	2
89	CROC-1/AE.SQUARROSA(517)	5	1
90	CETA/AE.SQUARROSA(1024)	5	2
91	DVERD-2/AE.SQUARROSA(1027)	5	3
92	CETA/AE.SQUARROSA(1027)	5	4
93	DOY1/AE.SQUARROSA (1030)	5	5

0 -5 Scale: 0= Resistant, 1-2= Moderate Resistant, 3-4= Moderate Susceptible and 5= Susceptible.- = N.A

Table 2. Screening of synthetic hexaploid (Elite-2) against *Bipolaris sorokiniana*.

E. No.	Genotype	Leaf infection (0-5) Y-2005	Leaf infection (0-5) Y-2006
1.	SORA/AE.SQUARROSA(192)	3	4
2.	CROC_1/AE.SQUARROSA (210).	4	4
3.	DVERD_2/AE.SQUARROSA(214)	3	3
4.	ARLIN_1/AE.SQUARROSA(218)	2	2
5.	TKSN1081/AE.SQUARROSA(222)	5	2
6.	GAN/AE.SQUARROSA(236)	5	4
7.	SORA/AE.SQUARROSA(323)	2	N.A
8.	D67.2/P66.270//AE.SQUARROSA(308)	3	5
9.	STYUS/CELTA//PALS.3/SRM_5/4/AE.SQUARROSA(431)	3	3
10.	LCK59.61/AE.SQUARROSA(693)\	2	2
11.	CETA/AE.SQUARROSA(1025)\	2	2
12.	DOY1/AE.SQUARROSA(1027)\	1	3
13.	CETA/AE.SQUARROSA(386)\	2	5
14.	CETA/AE.SQUARROSA(392)\	1	4
15.	CETA/AE.SQUARROSA(533)\	2	3
16.	CPI/GEDIZ/3/GOO/JO/CRA/4/AE.SQUARROSA(1018\	2	2

Table 2. (Cont'd.).

E. No.	Genotype	Leaf infection (0-5) Y-2005	Leaf infection (0-5) Y-2006
17.	CETA/AE.SQUARROSA(1031)\	1	2
18.	CETA/AE.SQUARROSA(1038)	2	2
19.	CETA/AE.SQUARROSA(1046)	2	3
20.	CETA/AE.SQUARROSA(1053)	3	5
21.	CROC_1/AE.SQUARROSA(212)	1	3
22.	CETA/AE.SQUARROSA(368)	2	2
23.	ARLIN_1/AE.SQUARROSA(430)	2	5
24.	D67.2/P66//AE.SQUARROSA(497)	4	4
25.	D67.2/P66//AE.SQUARROSA(10157)	2	4
26.	GAN/AE.SQUARROSA(206)	2	2
27.	ARLIN_1/AE.SQUARROSA(335)	2	2
28.	GAN/AE.SQUARROSA(335)	2	2
29.	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (385)\	4	4
30.	CETA/AE.SQUARROSA(417)\	4	5
31.	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (431)\	3	5
32.	DOY1/AE.SQUARROSA(534)\	1	5

0 -5 Scale: 0= Resistant, 1-2= Moderate Resistant, 3-4= Moderate Susceptible and 5= Susceptible.

Table 3. Screening of Durum parents against *Bipolaris sorokiniana*.

E. No	Genotype	Leaf infection (0-5) Y-2005	Leaf infection (0-5) Y-2006
1.	CROC-1	4	4
2.	ARLIN-1	5	5
3.	ROK/KML	5	4
4.	ALTAR 84	3	
5.	DVERD-2	4	4
6.	LARU	3	3
7.	68.111/RGB-U//WARD RESEL/3/STIL	4	3
8.	68.111/RGB-U//WARD	4	5
9.	68.111/RGB-U//WARD/3/FGO/4/RABI	4	5
10.	6973/WARD.7463//74110	3	4
11.	CPI/GEDIZ/3/GOO//J0/CRA	2	3
12.	D67.2/P66.270	3	4
13.	CERCETA	4	4
14.	STERNA-DW	4	4
15.	RABI//GS/CRA	4	5
16.	SORA	5	4
17.	SCAUP	3	4
18.	SNIPE/YAV79/DACK/TEAL	3	3
19.	TKSN1081	2	3
20.	YAV-2/TEZ	1	3
21.	YARMUK	4	4
22.	DECOY 1	4	4
23.	GARZA/BOY	4	4
24.	68.111/RGB-U//WARD	5	4
25.	**ARAOS**	**2**	**2**
26.	GAN	3	3

Table 3. (Cont'd.).

E. No	Genotype	Leaf infection (0-5) Y-2005	Leaf infection (0-5) Y-2006
27.	SCOOP_1	2	4
28.	**STY-US/CELTA//PALS/3/SRN-5**	**2**	**2**
29.	AGAMI	3	3
30.	YAV-3/SCOT//J069/CRA/3/YAV79	3	2
31.	YAR	4	5
32.	68112/WARD	3	4
33.	FGO/USA2111	4	4
34.	ALG86/4/FGO/PALES//MEXI-1/3/RUFF/FGO/5/ENTE	4	4
35.	BOTNO	4	4
36.	CIT71/CPI	4	2
37.	LCK59.61	3	4
38.	TRINAKRIA	3	4
49.	RASCON-37	3	4
40.	AJAIA-9	3	4
41.	CERCETA	4	4
42.	SCOT/MEXI-1	2	4
43.	**FALCIN-1**	**2**	**2**
44.	GREEN-3	2	4
45.	SHAG-22	4	4
46.	KAPUDE-1	3	3
47.	ARLEQUIN	3	4
48.	CHEN-7	5	5
49.	ACONCHI 89	4	4
50.	ALCATRAZ-3	4	3
51.	LOCAL RED	5	5

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