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Original Article

Study on Virulence Factors of *Mycosphaerella graminicola*, the Causal Agent of Septoria Leaf Blotch and Reactions of some Iranian Wheat Genotypes to this Pathogen in Iran

Mohamad Dalvand[1*], Mohamad Javad Soleimani Pari[1], Dostmorad Zafari[1], Ramin Roohparvar[2], Seyed Mahmoud Tabib Ghafari[3]

Abstract

Septoria leaf blotch disease caused by *Mycosphaerella graminicola* is one of the most important diseases of wheat in Iran and around the world. Evaluation the reaction of wheat genotypes as well as identifying pathogen virulence factors is required in order to achieve a successful breeding program. The aim of this study was to assess the adult plant responses of Iranian bread wheat and durum wheat genotypes (developed by Iranian seed) *Septoria tritici* leaf blotch. Furthermore, this study has aimed surveying the virulence factors of this pathogen in warm and humid weather conditions of the south of Iran. Thus, the study was conducted in one of southern provinces of Iran, Khuzestan, for two sequential years (December 2013-May 2014 and December 2014-May 2015) and included 47 commercial varieties and 26 differential lines with a local pathogen isolate that was collected on a bread wheat variety. Data records were performed with a modified Saari and Prescott method in double-digit scale of 00–99. According to the results, from all commercial varieties, 5 varieties were resistant. Surprisingly, the durum varieties like Behrang and Karkhe were susceptible too. Based on the results of differentiation tests showed that the pathogen isolate of this region has virulence reaction against *stb2*, *stb3*, *stb4*, *stb6*, *stb7*, *stb8*, *stb9*, *stb10*, *stb12*, *stb13*, *stb14*, and *stb18* genes and avirulence reaction against *stb1*, *stb5*, *stb11*, *stb15*, *stb16*, and *stb17* genes.

Keywords: *Mycosphaerella graminicola*, Virulence Factors, Iranian Wheat

1. Department of Plant Protection, Buali university, Hamadan, Iran
2. Seed and plant improvement institute of Iran, Karaj, Iran
3. Safiabad Agriculture Research center, Dezful, Iran

*** Corresponding Author**
Mohamad Dalavand
Department of Plant Protection, Buali university, Hamadan, Iran
E-mail: md5576@yahoo.com

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Introduction

Winter wheat is one of the most important cereal crops in Iran. *Septoria tritici* blotch, caused by the ascomycete fungus *Mycosphaerella graminicola* with anamorph *Zymoseptoria tritici* (old name: Septoria tritici) [15], is one of the most serious foliar disease of wheat [11]. Septoria leaf pathogen survives within straw and seed and of infested wheat and serves as the source of inoculum to start off the disease cycle again with a new crop of wheat. The pathogen is favored by splashing rain, high humidity, and moderate temperatures between 20 degrees to 28°C. The disease agent characteristically moves upward from where infection initiated on the low leaves to the crop canopy. In highly susceptible cultivars, this disease may reduce grain yield by 50% [13]. Where environmental conditions are favorable for disease development, yield losses due to Septoria leaf blotch have been reported ranging from 20% to 43%. It can reduce the economic value of wheat by decreasing both grain's yield and quality [5]. The disease can be controlled by chemical and cultural methods as well as using resistant cultivars. Chemical control of the disease is justifiable only in high performance culture; however, this method is not friendly to the environment and also not entirely reliable. On the other hand, using resistant cultivars is the most cost-effective and healthy control method. *Septoria tritici* blotch (STB) is increasingly the main target as well as the serious concern of the agrochemical and breeding industry in Europe, because of recent outbreaks of disease resistance to strobilurins [17, 20].

Although, it is said that isolates of bread wheat are not capable of infecting durum wheat [10], there are some reports reporting otherwise [16]. STB Increased by using susceptible semi-dwarf cultivars of the International Maize and Wheat Improvement Center (CIMMYT) involved in the green revolution. Afterward, numerous resistant sources from Argentine, Brazilian, Russian, West Europe and China have been found [21]. The Disease agent first was reported from Iran in 1941 by Petrak and Esfandiari and was named *S. graminieum* [14].

The germplasm-derived wheat from CIMMYT, Mexico, (*Triticum aestivum* KavkazK4500L.6.A.4 or KK) is one of the major sources of resistance to *Septoria tritici* blotch (STB). KK is resistant to STB in field conditions in the UK even though a large group of *Mycosphaerella graminicola* isolates show virulence against it [8]. Brading and his colleagues in 2002 reported an isolate-specific resistance of wheat to Septoria tritici blotch suggesting a probably gene relationship. In recent years, 18 major genes (Stb1 toStb18) have been identified which confer resistance to *Septoria tritici* [8, 7]. These genes may be categorized in two classes, although a few may have characteristics of both: 1) qualitative resistance is controlled by genes which control large fractions of genet-

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ic variation, 21 of which have been discovered and mapped so far. Most of them have been shown to be genotype-specific, being effective against the minority of *Z. tritici* isolates which are avirulent, and Stb6 has been shown to control a gene-for-gene relationship. Most qualitative resistances are unlikely to be durable and some formerly effective genes have been overcome by the evolution of pathogen virulence; 2) quantitative resistance is generally controlled by genes with small-to-moderate effects on STB, STB exists in different parts of Iran [7, 19]. Some studies have shown that most cultivars and lines are susceptible to this disease [4, 13].

Differential wheat genotypes carrying known stb genes could be used for determining virulence diversity of *M. graminicola* populations [9]. Reaction of cultivars to *M. graminicola* in adult plant and seedling stages is different. For example, stb17 is a gene with a quantitative effect on disease which is expressed in adult plants but not seedlings [18]. The aim of this study was to determine the response of Iranian cultivars resistant to STB and study of virulence factors of *Mycosphaerella graminicola* isolates in Khuzestan province.

Material and Methods

Isolation, purification and disease inoculums preparation

Leaf samples infected with *S. tritici* showing blotch symptoms were collected from different fields of Khuzestan province. Isolation of fungi was performed by Eyal method [11]. In summary, first, pieces of infected leaves were glued on glass slides and were placed in petri-dishes with a wet filter paper. After 24 hrs, the ejected spores from the mouth of pycnidia were removed with a sterile needle and were transferred to PDA medium.

To prepare inoculums for the inoculation of genotypes, a liquid-medium yeast extract, dextrose, was prepared in 1 L flasks. Then, a little bit of fungal colonies from the solid medium was removed and transformed to flasks. Then, flasks were put in a shaker with150 rpm at 15°C. After a week, spore suspensions were prepared and set for approximately 107 conidia/L determined by hemacytometers.

Evaluation of genotypes

In this study, the reaction of 47 wheat commercial varieties and 26 differential cultivars, each carrying one or more *stb* gene(s) (*Stb1–Stb18*), to STB in the stage of adult plant under an artificial field inoculation in Dezful, Khuzestan-Iran was evaluated for two years (December 2013-May 2014 and December 2014-May 2015). Each genotype was planted in two 1-meterlines on ridges with a width of 30 cm in three randomized replications.

Around the lines, Darab2-susceptible cultivar was planted, as well as field borders were created in order to increase disease incidence. Artificial infection was induced by distributing infected plant debris and spraying spore suspension in the tillering stage. Data were recorded using Saari and Prescott method (1975) modified by Eyal in a 00–99 double-digit scale [11]. The first digit represents the relative height of the disease or its progression from lower to upper leaves and the second digit indicates the severity of the disease. Accordingly, genotypes are classified in the following categories: immune (00), highly resistant (11–14), resistant (15–34), moderately resistant (35–44), moderately susceptible (45–64), susceptible (65–84) and highly susceptible (85–99).

Results and discussion

Evaluation of results from commercial varieties showed that only 5 varieties from 48 cultivars were resistant (Table 1). Even the durum varieties like Behrang and Karkhe were susceptible .These results correspond with the results of Seifbarghi et al., studies [16]. Also, our results show the importance of the bread isolate virulence on durum varieties.

Results of differentiation tests showed that pathogen isolate from this region represent virulence reaction for *stb2*, *stb3*, *stb4*, *stb6*, *stb7*, *stb8*, *stb9*, *stb10*, *stb12*, *stb13*, *stb14*, and is virulence for *stb18* genes and for *stb1*, *stb5*, *stb11*, *stb15*, *stb16*, *stb17* genes (Table 2). Namely, Oasis (carrying *Stb1*), Sullivan (carrying *Stb1*), Cs synthetic (carrying *Stb5*), Ariana (carrying Stb6 and Stb15), M3 (carrying *Stb16* and *Stb17*), 3HD-126 (carrying *Stb11*), KM7 (carrying *Stb16*), and KM41 (carrying *Stb17*) were resistant.

Abrinbana et al., reported Shafir as susceptible cultivar to all Iranian isolates of *M. graminicola* that showed the Stb6 is not a proper resistant source for Iran [1]. The present study on Shafir also showed that the *Stb6* could not be an efficient gene for resistance to STB in other differential genotypes owning this gene. Similar to Shafir, cultivar Flame is the other wheat genotype with only one documented *Stb* gene (*Stb6*); however it showed resistance to Khuzestan *M. graminicola* isolate.

Therefore, it may be concluded that Flame has another resistance gene to *M. graminicola* [12]. Population genetics of *M. graminicola* in Iran has shown that there is strong genetic differentiation between wheat-growing provinces [2]. Therefore, studies on Iranian cultivars reaction and pathogenecity factors in different provinces seem necessary.

Conclusion

Using the *stb* genes to which the pathogen shows virulence reaction is not an effective method for the management of the disease. Instead, our results showed that new resistant sources are required to be integrated into the wheat breeding program in Iran. Breeding aimed to attain resistance to STB can benefit greatly from the long history of breeding crops to control other diseases. These results can be used in wheat breeding programs.

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Table 1. The Responses of Wheat cultivars to *Septoria tritici* Leaf Blotch.

Entry	Cultivar	Source/Note	Growth Habit [a]	Year	
				2013-2014	2014-2015
1	Ofogh			77	77
2	Shotor dandan			0	0
3	Azar2			73	72
4	Pishgam			77	91
5	Pishtaz	Alvd//Aldan/Ias58	S	75	91
6	Marvdasht	HD2172/Bloudan//Azadi	S	0	72
7	Gonbad			75	73
8	Bahar	Bloyka	S	77	91
9	Darab2		S	75	91
10	Bam	MS-87-1	S/F	75	92
11	Niknezhad	F13471/Crow"s"	S	72	73
12	Mihan			75	91
13	Hamoon			51	71
14	Star			73	73
15	Mahdavi			73	77
16	Shiraz	Gv/D630//Ald"s"/3/Azd	S	73	91
17	Sepahan	Azd/5/L2453/1347/4/Kal//Bb/Kal/3/Au//Y50E/3*Kal	S	72	91
18	Shahriar			73	91
19	Sivand	Kauz"S"/Azd	S	77	91
20	Parsi	Dove"S"/Buc"S"//2*Darab	S	75	91
21	Arya	STORK		71	73
22	Arta		S	0	73
23	Toos			75	73
24	Yavarous	YAVAROOS 79	S	0	0
25	Gaspard		S	0	0
26	Marvdasht		S	0	0
27	Morvarid	N-83-3		72	0
28	Behrang	D-79-15 (ZHUNG ZOU/2*GREEN-3)	S	73	72
29	Dena	TARRO-3		72	0
30	Karkhe	SHWA/ MALD		73	75
31	Akbari	MS-87-2		78	92
32	Alvand			73	91
33	Darya			0	91
34	Tajan			73	75
35	Urum	Alvand//Ns732/Her		72	91
36	Shiroodi			75	91
37	Chamran			75	91
38	Moghan			75	75
39	Zareh	130L1.11//F35.70/Mo73/4/Ymh/Tob//Mcd/3/Lira	W	52	73
40	Sistan			77	91
41	Zarrin			75	91
42	MV17			71	75
43	Aflak	S-80-18		75	91
44	Arg			77	91
45	Gascogen			52	75
46	Sirvan	PRL/2*PASTOR	S	0	0
47	Soissons			71	0

a) S: Spring, W: Winter, F: Facultative

Table 2. Rresponse of differential wheat cultivars possessing Stb resistance genes to *Mycosphaerella graminicola*.

No.	Cultivar	Gene	2013-2014	2014-2015
1	Oasis	Carrying *Stb1*	0	0
2	Sulivan	Carrying *Stb1*	0	0
3	Bulgaria 88	Carrying *Stb1* and *Stb6*	0	0
4	Veranopolis	Carrying *Stb2* and *Stb6*	74	75
5	Is. 493	Carrying *Stb3* and *Stb6*	72	71
6	Tadinia	Carrying *Stb4* and *Stb6*	72	71
7	Cs Synthetic	Carrying *Stb5*	0	0
8	Flame	Carrying *Stb6*	0	0
9	Shafir	Carrying *Stb6*	73	72
10	Estanzuela Federal	Carrying *Stb7*	75	73
11	M6 Synthetic	Carrying *Stb8*	73	73
12	courtot	Carrying *Stb9*	0	72
13	Kavkaz -K4500	Carrying *Stb6*, *Stb7*, *Stb10* and *Stb12*	0	72
14	TE9111	Carrying *Stb6*, *Stb7* and *Stb11*	71	0
15	Obelsik	Susceptible check	0	71
16	Taichung 29	Susceptible check	72	77
17	Salamouni	Carrying *Stb13* and *Stb14*	73	75
18	Ariana	Carrying *Stb6* and *Stb15*	0	0
19	Riband	Carrying *Stb15* or another	75	75
20	M3	Carrying*Stb16* and *Stb17*	0	0
21	Balance	Carrying *Stb6* and *Stb18*	0	71
22	Kulm		0	0
23	3HD-126	Carrying *Stb11*	-	0
24	KM7	Carrying *Stb16*	-	0
25	KM41	Carrying *Stb17*	-	0
26	3HD-138	Carrying *Stb18*	-	51
27	Darab2	Local check	78	78

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