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Study on Leaf White Blotch of Wheat Caused by *Bacillus* sp.

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Abstract. Leaf white blotch caused by *Bacillus* sp. was recently reported in most wheat fields in the Al-Qassim region of Central Saudi Arabia. Six isolates of *Bacillus* sp. were found pathogenic to one or more of 20 wheat cultivars tested in the greenhouse. Seeds of yecora rojo, sham-2 and Cl 8322 wheat cultivars were surface sterilized, either treatd vitavax, not treated or inoculated with a pathogenic isolate of *Bacillus* sp. and sown in the field in 1988 and 1989 growing seasons. Untreated seeds were also, used as check. The stand, number of tillers per plant, severity of leaf blotch symptoms and grain yields were determined.

The stand was reduced up on infection with the *Bacillus* sp. in yecora rojo wheat cultivar only. Minor symptoms of white blotch were observed on wheat plants grown in the field regardless of seed treatment, cultivar and season. The number of tillers and grain yields were not significantly affected by various seed treatments in all cultivars in 1988 and 1989. It was concluded that the grain yields of wheat and white blotch symptoms were not significantly affected by the seedborne inocula of *Bacillus* sp. in Al-Qassim.

Introduction

About 600.000 ha of agricultural land are annually cultivated with yecora rojo wheat cultivar in Saudi Arabia. Over one third of the wheat farms are located in Al-Qassim region, Central Saudi Arabia [1].

Fungal diseases are considered a serious threat to wheat production in Central Saudi Arabia [2]. Bacterial diseases have also been reported including leaf blight caused by *Pseudomonas syringae* var *syringae,* black chaff caused by *Xanthomonas campestris* pv. *translucens* and basal glum rot caused by *Pseudomonas syringae* pv. *atrofaciens.*[3-5].

The white blotch of wheat incited by *Bacillus megaterium* pv. *cerealis* was reported in U.S.A., Canada and Brazil [6]. the economic importance of the disease in other parts of the World is largely unknown. *Bacillus megaterium* is a heterogenous group of strains found in soil [7] and seeds [8], insects and on uredospores of *Puccinia graminis* [9,10]. This bacterium can survive extreme environmental conditions

[10]. Chlorosis or streaks on wheat leaves have been also attributed to viral diseases, copper deficiency, sulfur dioxide or seedborne *P. syringae* [6].

In a recent survey of wheat diseases in Central Saudi Arabia, the leaf white blotch disease caused by *Bacillus* sp. was detected in most fields affecting up to 20% of the plants [3]. Symptoms of the disease were similar to those described by Hosford [10]. Symptoms were first noted, following the boot stage of wheat plant development as small yellow or white lesions that quickly enlarge into white or very light tan, irregular blotches on the center of the flag leaf. the disease was considered among the most widespread of all foliar diseases in Al-Qassim wheat fields in recent years [3].

Objectives of this study were to determine the susceptibility of several wheat cultivars to leaf white blotch disease caused by *Bacillus* sp. and evaluate the possible role of seedborne inoculum in development of the disease in the field.

Materials and Methods

Isolation of bacteria

Wheat plants with distinct white blotch symptoms were collected from various fields in the Al-Qassim region. Infected leaf tissues were washed with running tap water for 15 min, rinsed in sterilized distilled water and subsequently surface sterilized in 0.5 % NaOCl for 30 sec. The surface sterilized leaf tissues were cut into sections 3 × 3 mm and plated on Potato Dextrose Agar (PDA), Nutrient Agar (NA) and Sucrose Peptone Agar (SPA) media and incubated at 25°C for 24-48hr [10]. Representative colonies of the bacteria associated with the infected leaf tissues were purified by streaking technique and maintained on PDA slants for further studies.

Pathogenicity studies

Surface sterilized seeds of 20 wheat cultivars were treated with vitavax (3 g/l kg seeds). Treated seeds were sown in plastic pots containing sterile sand and vermiculite (2: 1 ratio) at the rate of ten seeds per pot (8 cm diam) and 10 pots were used for each wheat cultivar. Seedlings were grown in the greenhouse during January (average temp. was 12°C at night and 18°C during the day). Six *Bacillus* isolates from different wheat fields were grown on PDA plates at 25°C for 24-48 hr. The bacteria grown was suspended in distilled sterilized water. Bacterial concentration was adjusted at four plates/250 ml;~10^{15} cfu/ml. Three week-old seedlings from each wheat cultivar were sprayed with the bacterial suspension. The leaves of inoculated plants were then rubbed lightly with fingers after inoculation, covered with plastic bags to maintain high relative humidity for 24 hr and subsequently placed in the greenhouse for 2 weeks before the symptoms of the disease were determined. Disease severity was determined according to the key of leaf blotch or scaled of barley [11].

Effect of seedborne inoculum

Seeds of yecora rojo, sham 2 and Cl 8322 wheat cultivars were subjected to the following treatments: 1) Untreated (check 2) sterilized seeds (Seeds were surface sterilized by dipping for 30 sec. in a heated (70°C) mixture of 95% ethanol and 1% NaOCl (1:1) [12]; 3) Seeds were cleaned as above and dipped in a suspension ($\sim 10^{15}$ cfu/m) of Bacillus sp; 4) Seeds were treated with vitavax (3 g/1 kg). The statistical design for this experiment was a split plot with wheat cultivars as main plots. Treated seeds were sown in field plots during 1988 and 1989 growing seasons. Each plot consisted of five rows (3 × 1 m) and the seed rate was 180 kg/ha. The field plots were fertilized according to the fertilizing regime adopted by the college experimental farm [(urea (46% N) 200 Kg/ha and NPK (18: 18: 0) 40 kg/ha]. The field plots were irrigated daily by a sprinkling system until near harvesting. The stand count, number of tillers per plant and symptoms of the bacterial diseases were determined at booting and heading stages of plant development. The middle three rows were hand harvested and mechanically threshed for determination of grain yields.

Results

The isolation from wheat leaf with white blotch symptoms (Fig. 1), revealed that over 90% of the leaves were colonized with *Bacillus* spp. The isolated bacteria grown on PDA plates were as discribed by Hosford [10], white and smooth colonies of chained non-motile cells that average 3.5 um. The cells were gram positive and form ellipsoidal spores.

As shown in Table 1, all the tested isolates were pathogenic to one or more of the wheat cultivars. Isolate No. 35 was the most pathogenic causing severe symptoms on leaves of Lokame (a local cultivar), NKt(s), NS 2699, West bread 911 and yecoro rojo cultivars. The other *Bacillus* isolates caused minor damage to most of all wheat cultivars studied.

Table 2 presents data on the effect of bacterial infection and seed treatments on stand, tillering, leaf blotch symptoms and grain yield of yecora rojo, sham 2 and Cl 8322 wheat cvs. Inoculation of *Bacillus* sp of yecora rojo and sham 2 reduced wheat stand significantly in the field during the 1988 growing season but no such effect was observed in 1989. The stand of yecora rojo cv was not significantly influenced by surface sterilizing or coating seeds with Vitavax. On the other hand, the stands of sham 2 and Cl- 8322 were significantly higher when seeds were surface sterilized or treated with vitavax than in non treated seeds in 1988 growing season.

The number of tillers per plant and the incidence of leaf blotch were generally not significantly affected by seed treatments (Table 2). However, the incidence of leaf blotch symptoms although not significant, was most visible in sham-2 and yecoro rajo in 1988 and was very low in all cultivars in 1989.

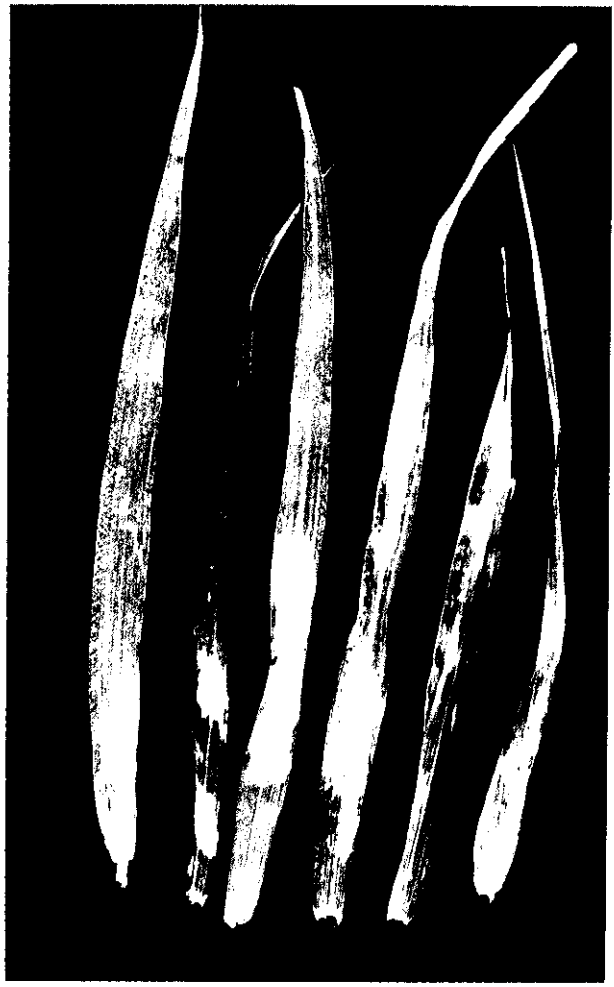

Fig. 1. Symptoms of white blotch disease on flag leaves of yecora rojo wheat cultivar

According to data presented in Table 2 the grain yield of yecora rojo was significantly low where the seeds were untreated and when they were coated with *Bacillus* isolate No. 35 in the 1989 growing season.

The grain yield of sham-2 was not influenced by any of the treatments in the two years of field testing. The lowest grain yield in Cl 8322 was obtained in 1988 when seeds were not treated and when seeds were inoculated with *Bacillus* sp. in 1988 growing season.

Table 1. Pathogenicity of six isolates of *Bacillus* sp. to seedlings of 20 wheat cultivars.

Cultivars	Bacillus isolates					
	10	20	21	31	35	38
	Disease severity[K]					
CL 8322	0.0 A[L]	0.7 AB	0.0 C	0.3 B	0.0 E	0.7 CD
Gonen	0.0 A	0.0 B	0.0 C	0.7 B	0.0 E	0.0 E
Hope (s)	0.0 A	0.0 B	0.0 C	0.0 B	0.0 E	1.0 BC
Jup (s)	0.7 A	0.7 AB	0.7 AB	0.7 B	3.0 BC	0.3 DE
KFU 283*	0.3 A	0.3 AB	0.0 C	0.7 B	0.0 E	0.0 E
KFU 483*	0.7 A	0.0 B	0.0 C	0.3 B	0.3 E	0.0 E
KUZ/Cyn	0.3 A	0.0 B	0.0 C	0.0 B	0.0 DE	1.0 E
Lokame*	0.3 A	1.0 A	0.7 AB	0.7 B	5.0 A	0.7 CD
Maaya*	0.0 A	0.7 AB	0.0 C	0.7 B	0.0 E	0.7 CD
Mexipak 65	0.0 A	0.0 B	0.3 BC	0.0 B	3.0 BC	0.0 E
NKt (s)	0.0 A	0.0 B	0.3 BC	1.0 AB	4.0 AB	0.3 DE
NS 2699	0.7 A	1.0 A	0.3 BC	0.0 B	4.0 AB	0.0 E
Pondera	0.7 A	1.0 A	1.0 A	1.0 AB	3.0 BC	0.3 AB
Probred	0.3 A	0.3 AB	0.0 C	0.3 B	0.3 E	0.0 E
SERT	1.0 A	1.0 A	1.0 A	1.7 A	3.0 BC	1.7 A
SHAM 2	0.7 A	0.0 B	0.0 C	0.3 B	0.0 E	0.0 E
W 3918 A	0.7 A	0.3 AB	0.0 C	0.0 B	2.0 CD	0.0 E
Wanspum	0.7 A	0.3 AB	0.0 C	0.3 B	0.0 E	0.0 E
West bread 911	0.0 A	0.0 B	0.0 C	0.7 B	4.0 AB	0.7 CD
Yecoro rojo	0.0 A	0.0 B	0.0 C	0.7 B	4.0 AB	0.0 E

K = Severity was based on an arbitrary key 1 to 5 (0 no infection and 5 = severe symptoms and total collapse of the leaf).

* = Native or locally developed wheat cultivars.

L = Means within the same column followed by the same letter(s) are not significantly different according to Duncan's multible range test (P = 0.05).

Discussion

The 20 wheat cultivars tested in this study included those recommended by the Saudi Ministry of Agriculture and Water Resources and those potentially promising wheat cultivars under test in various Experimental Stations of the Kingdom. The wheat cultivars differ in their susceptibility to *Bacillus* sp in greenhouse tests. This is in agreement with previous reports [10]. Further work is needed for determining their tolerance to white blotch in the field.

The insignificant effect of *Bacillus* sp. on most of wheat cvs in 1989 compared with 1988 could be due to the discrepancy of field conditions. The effect of environ-

Table 2. Effect of inoculation with *Bacillus* sp. isolate 35 and fungicidal treatment of wheat seeds on growth, leaf blotch and grain yields of three wheat cultivars in 1988 & 1989.

Cultivar	Seed treatment	Stand (plants/20 cm length)		Tillers (No./plant)		Leaf blotch % leaf area		Grain yield (kg/ha)	
		1988	1989	1988	1989	1988	1989	1988	1989
Yecora rojo	Untreated check	16 A*	17 A	2.5 A	1.2 A	7.1 A	0.5 A	1,620 B	1,820 A
	Sterilized	24 A	13 A	2.5 A	1.7 A	4.5 A	0.8 A	5,610 A	2,200 A
	Vitavax	20 A	15 A	2.6 A	1.6A	6.5 A	1.2 A	5,890 A	1,910 A
	Baccilus No. 35	10 A	13 A	2.0 A	2.0 A	9.3 A	0.5 A	2,100 B	2,050 A
Sham 2	Untreated check	13 B	13 A	2.0 A	1.9 A	10.1 A	1.4 A	5,480 A	2,640 A
	Sterilized	27 A	16 A	2.6 A	2.0 A	14.2 A	0.6 A	4,790 A	2,560 A
	Vitavax	27 A	15 A	2.6 A	2.4 A	12.6 A	2.1 A	4,480 A	2,480 A
	Bacillus No. 35	17 B	12 A	2.9 A	2.0 A	14.2 A	2.6 A	5,160 A	2,420 A
Cl-8322	Untreated check	14 B	12 A	2.3 A	1.7 A	5.9 A	0.0 A	3,700 B	3,040 A
	Sterilized	21 A	15 A	2.4 A	1.9 A	1.2 A	0.6 A	5,610 A	2,910 A
	Vitavax	25 A	12 A	2.4 A	1.9 A	0.6 A	0.2 A	5,540 A	2,870 AB
	Bacillus No. 35	20 A	12 A	2.5 A	2.4 A	1.2 A	0.2 A	4,820 AB	2,510 B

*Means within each column followed by the same letter (s) are not significantly different according to Duncan's multiple range test (P = 0.05).

mental factors and other cultural practices on development of white blotch in al-Qassim wheat fields is largely unknown. The high temperature, long sunny days, application of urea fertilizer through sprinkling irrigation system and infestation with insects during the booting and heading stages of wheat growth may contribute to the development of the white blotch in Al-Qassim fields.

The symptoms of white blotch observed in the field blots may be induced partially by several sources of inocula. The role of seedborne *Bacillus* on development of leaf blotch is not clear. However, the application of *Bacillus* sp. to seeds of some wheat cultivars reduced their stand in one of the trials. Crop residues are the most likely source of inoculum under continuous cultivation of wheat. Several isolates of *Bacillus* sp. pathogenic to wheat were isolated from crop residues from various fields of Al-Qassim region [3]. Crop rotation and selection of disease tolerant cultivars may reduce the incidence of white blotch in Al-Qassim Wheat field.

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دراسة في التلطخ الأبيض لأوراق القمح المتسبب عن نوع من الـ *Bacillus*

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ملخص البحث. شوهد حديثًا مرض التلطخ الأبيض في أوراق القمح المتسبب عن البكتيريا *Bacillus* sp. وذلك في معظم حقول منطقة القصيم بوسط المملكة العربية السعودية. اختبرت ست عزلات من *Bacillus* sp. من حيث قدرتها على إحداث المرض لعدد عشرين صنفًا من القمح الربيعي، وكانت كل عزلة قادرة على إحداث إصابة لواحد على الأقل من هذه الأصناف في الصوبة الزجاجية. تم معاملة بذور ثلاثة أصناف هي yecora rojo و sham 2 و Cl 8322 بالتعقيم السطحي أو Vitavax أو عزلة ممرضة *Bacillus* sp. ثم زرعت البذور في الحقل في ١٩٨٨م و١٩٨٩م. أدت المعاملة بالبكتيريا إلى خفض نسبة الإنبات في صنف واحد، وفي عام واحد، ولم تكن هناك فروق معنوية في أعراض المرض أو في محصول الحبوب نتيجة لباقي المعاملات في الأصناف الثلاثة المختبرة. يستخلص من البحث أن عدوى البذور بالبكتيريا ليست ذات أثر معنوي على انتشار المرض في الحقل كما أنها لا تؤثر عل محصول الحبوب.