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SOME STUDIES ON BACTERIAL LEAF STREAK AND BLACK CHAFF OF WHEAT IN THE KINGDOM OF SAUDI ARABIA.

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Abstract: Bacterial leaf streak symptoms on wheat are elongated translucent, water- soaked lesions were observed in five farms in Al-Karj region, Riyadh, Saudi Arabia Physiological characters of three isolates obtained from naturally diseases wheat plants confirmed as *Xanthomonas translucens* pv. *translucens* Isolates (i.e.XW1,XW2 and XW3). They characterized as Gram negative, short rod-cell with yellow- pigmented colonies tests which were positive were: motility, esculin hydrolysis, hypersensitivity on tobacco and utilizing arabinose and mannose. Tests which were negative were: Kovacs' oxidase, arginine dihydrolases, nitrate reduction and starch hydrolyse. Furthermore, the identification was confirmed by using Biolog system. Pathogenicty tests revealed that all isolates were able to induce leaf streak and black chaff symptoms when the bacterial suspension (10^8CFU) At inoculums density lower than 10^5 CFU/ml, no visible lesions were observed on the leaves segments after 14 days from inoculation. At inoculums concentrations higher than 10^6 CFU/ml, the number of lesions did not increase significantly with the increase in inoculum concentration. Greenhouse tests showed that cultivar Yocora rojo was susceptible to bacterial streak and black chaff. Yield loss was recorded 11.60 % compared with the other cultivars.

Key words: *Xanthomonas translucens* pv. *Translucens*, wheat, bacterial leaf streak, black chaff.

Introduction

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *translucens* (Jones *et al.* 1917) is the major bacterial disease of wheat. It occurs over a range of very different conditions, such as sprinkler-irrigated fields in temperate climates, high-rainfall subtropical highlands and warmer environments characterized by cool nights or frequent climatic changes and sudden temperature variations. It was first reported in 1902 on wheat (*Triticum sativum L.*) in Indiana, USA (Smith,*et al.*,1919).Since then, *X. translucens* pv. *translucens* has been detected in several wheat production areas in

the United States and other countries (Wiese, 1987).This pathogen causes two distinct symptoms on wheat leaf streak and black chaff (Cunfer and Scolari,1982). A yield reduction of 40% has been reported from sprinkler irrigated fields in many wheat-growing regions of the world (Duveiller *et al.*, 1991 and Tubajika, *et al.* 1998). Occurrence of BLS is sporadic from year to year (Bamberg, 1936., Duveiller *et al.*, 1991 and Tubajika, *et al.*1998). Several studies and reports (Hall, *et al.*,1981, Hirano and Upper, 1983, and Sands *et al.*,1986) have contributed to better understanding of the ecology and the epidemiology of *X. translucens* pv. *translucens*, but the reasons for the sporadic occurrence of BLS are still poorly understood. Duveiller *et al.* (19974) reported that environment affected BLS occurrence and that disease severity varied among years and locations.

In the Kingdom of Saudi Arabia, wheat has been cultivated as a commercial crop with a plantation area of about 424000 hectare producing total product 2 million tons (Agriculture Statistical Year Book. 2003). All of the commercial seeds in Saudi Arabia are imported from different countries including Europe and USA. Under the kingdom conditions, the disease were showed only in some areas according to the survey was made by Abo-Swaria (1982) especially in sprinkler-irrigated fields which are mostly common in whole areas.

The present investigation was planned to study the causal organism of wheat leaf streak in Saudi Arabia. Also, to evaluate the resistance of some common commercial cultivars this cultivated in the kingdom to the pathogen.

Material and Methods

1-Isolation of the causal organism:

Wheat plants (cv. Yocora rojo), the cultivar most commonly grown in Saudi Arabia showing typical symptoms of bacterial leaf streak disease (Fig 1) were collected during 2004/2005 growing season in Al-Kharj region, Riyadh, Saudi Arabia Samples were gently washed with running tap water, surface disinfested by immersing in 0.6% sodium hypochlorite solution for 3 min, followed by rinsing in sterilized distilled water. Small portions of the disinfested leaves (approximately1cm long pieces) were macerated in small quantity of sterile water in sterilized mortars. (Demir and Ustun,1992) The obtained suspension was streaked on to nutrient glucose agar medium (NGA). After 48 hr of incubation at 28C, single colonies typical of the *Xanthomonas*, 1-2 mm in diameter, clear, yellow, and smooth, were transferred by loop exhaustion in 3 successive tubes with slanted NGA medium to obtain pure cultures. The obtained isolates were tested for pathogenicity and also after identification.

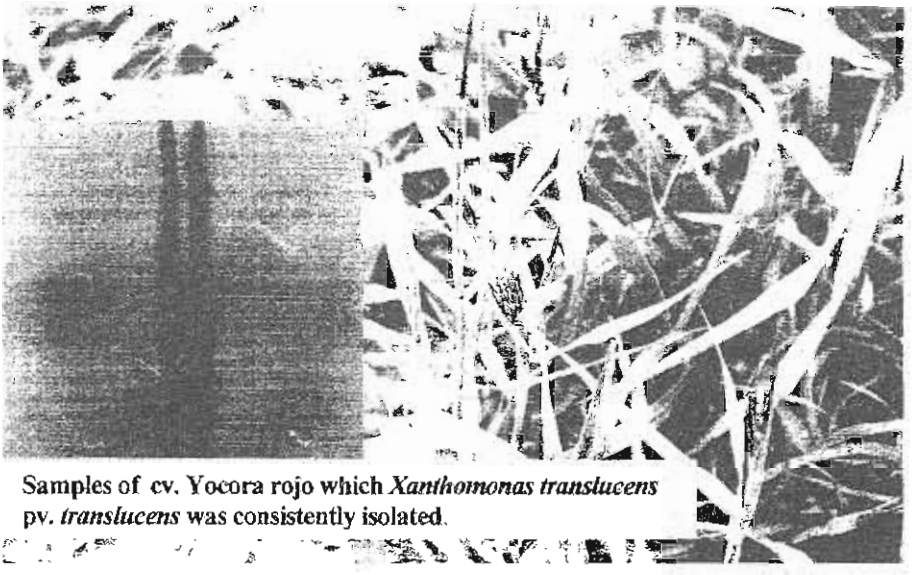

Fig (1): Symptoms of wheat leaf streak disease of natural infection.

2-Pathogenicity tests:

Wheat seeds (cv. Yocora rojo) were chosen from healthy plants with completed nature ripe spikes, there were surface sterilized by dipping in a sodium hypochlorite solution 1% NaOCl for 2 min, and rinsed in sterile distilled water. As described by Galal and Saad (1995), pots (30cm diameter) were sterilized by soaking in 5% formalin for 5 min, and then left for 15 days before planting. The dry wheat grains were planted in the pots filled with soil mixture (peat moss and sand 1:1v/v) at the rate of ten grains per pot.)

Three isolates were used in this experiment. Bacterial suspension prepared by suspending bacteria from 24-hr NGA culture in 10 ml of sterile 0.9% NaCl and colonies were scraped into a test tube (Schaad, 1988). The bacterial cell suspension was vortexed, and turbidity was measured at 640 nm. Concentrations were adjusted to be 10^8 CFU/ml and verified by dilution plating onto NA. After adjusting the concentration, Tween 20 (0.02 percent) was added to the inoculum to facilitate the spread of the liquid over the leaf (Zadoks *et al.*, 1974; Duveiller, 1990). Wheat plants at 12 days old and 10-week old were each spray inoculated until runoffs with each isolate, and sterilized distilled water as a negative control. Plants were distributed in the greenhouse in a completely randomized design. Inoculated

plants were covered with polyethylene bags for 48 hr to maintain high humidity. Plants were then taken out of the bags, put on a greenhouse bench and observed after 4-5 days for initial symptoms. Pots were watered with tap water to maintain soil moisture each day. Flag leaf severity, which represents the percentage of flag leaf area with leaf streak symptoms. Black chaff severity was expressed as percentage of the total plant leaf area diseased using key developed by Duveiller (1994).and on spikes after 2 weeks after inoculation according to James (1973).

3- Physiological characteristics

Reactions of the different bacterial isolates in this study to some selected physiological tests which are diagnostic for *X. translucens pv. translucens* were carried out according to the method described by (Mohan and Mehta, 1985, Lelliot and Stead,1987, Schaad, 1988, and Klement et al.,1990)

The Biolog system (Microlog 2 Release 3.50, Biolog Inc., Hayward, CA), based on patterns of carbon source utilization, was also used to confirm identity of the bacteria.

4- Varietal response

Response of one hybrid L42002 belonging (*Triticum durum L.*) and three cultivars belonging (*T. sativum* L.) namely, Yecora rojo, West bread, Lokame cvs were planted as previously mentioned. The bacterial cell suspension of isolate No.3 was adjusted to 10^8 CFU ml^{-1}. Seedling and adult plants were inoculated, incubation and BLS severity also estimated as previously maintain.

5- Inoculum density

Wheat seedlings (12 days old) cv. Yocora rojo were spray inoculated until runoff with *X. translucens pv. translucens* isolate No 3 at density of (10^4, 10^5, 10^6, 10^7, and 10^8 CFU/ml). Control plants were treated similarly but inoculated with sterile water only. Incubation and BLS severity were also made as previously mentioned.

6- Humidity period

Wheat plants at (12 days old and 10 weeks old) cv. Yocora rojo were each spray inoculated until runoff with isolate No.3 at 10^8 cells/ml. Inoculated plants were covered with polyethylene bags for 24,48,72 and 96 hr to save humidity. Control plants were sprayed with the same isolate without covered. Plants were then taken out of the bags, put on a greenhouse bench and FL and BL severity were estimated.

Statistical analysis

Each treatment was represented by seven pots per

replicate and a complete randomized block design with four replications was used. All recorded data were subjected to analysis of variance procedures and treatment means were compared using LSD. as described by Gomez and Gomez, (1984). Percentages data were transformed to arcsines before subject to statistical analysis.

Results and Discussion

1- Pathogenicity tests:-

Pathogenicity of all isolates was tested on wheat plants cv. Yocora rojo under greenhouse conditions. Three isolates designated as XW1, XW2 and XW3 were pathogenic and caused leaf streak and black chaff symptoms Severity of disease differed between isolates. Table 1 shows that isolate XW3 was highly pathogenic isolate, while, XW1 and XW2 were moderately pathogenic. Isolate XW3 gave the highest disease severity index. 34.54 % on seedling plants and 56.86% on adult plants Isolates XW1 and XW2 reacted as the moderately pathogenic where their caused 22.10, 24.32 25.40 and 26.31 % respectively.

Table (1): Pathogenicity of three isolates of *X. translucens* pv.*translucens* on wheat (cv. Yocora rojo).

Isolates	Disease severity %		Pathogen reisolated
	Seedling plants (12 days old)	Adult plants (10 weeks old)	
XW1	22.10	25.40	+
XW2	24.32	26.31	+
XW3	34.54	56.86	+
Control	00.00	00.00	-
LSD at 5%	1.19	1.56	-

2- Isolation and identification of the isolates:-

Cultural, physiological and biochemical characters of the isolated bacteria are presented in (Table 2.). The isolated bacteria were all rod shaped, motile and Gram negative. Moreover, all the tested bacterial isolates produced a diffusible yellow pigment. They were negative for Kovacs' oxidase, arginine dihydrolase, 2-ketogluconate production, nitrate reduction and starch hydrolyse. Furthermore, the isolated bacteria were positive for esculin hydrolysis and hypersensitivity

on tobacco. All isolates, utilized Arabinose and Mannose, while, they failed to utilize Lactose and Inositol. Accordingly, data suggest that all the bacterial isolates belong to *X. translucens pv.translucens*. Furthermore, the identification was confirmed using biolog system. According to selected biochemical and physiological properties isolates were confirmed as *Xanthomonas translucens pv. translucens* (Mohan and Mehta, 1985, Lelliot and Stead,1987, Schaad, 1988, Klement et al.,1990,Young *et al.*,1991). The identity of bacterial species was confirmed by Biolog analysis (carbon source utilization at 37°C), with a similarity index of 0.75.

Table (2): Physiological and biochemical characters of the bacterial isolates.

Test	Bacterial isolates			
	XW1	XW2	XW3	*X.c.***pv *translucens*** (Duveiller *et al.*, 1997)
Shape	Rod	Rod	Rod	Rod
Motility	+	+	+	+
Gram reaction	-	-	-	-
Spore forming	-	-	-	-
Yellow pigment	+	+	+	+
Starch hydrolysis	-	-	-	-
Nitrate reduction	-	-	-	-
Arginine dihydrolase	-	-	-	-
Kovac,s oxidize	-	-	-	-
2-Ketogluconate production	-	-	-	-
Esculin hydrolysis	+	+	+	+
Hypersensitivity on tobacco	+	+	+	+
Potato soft rot	-	-	-	-
Lactose	-	-	-	-
Inositol	-	-	-	ND
Arabinose	+	+	+	ND
Mannose	+	+	+	ND

+ = Positive reaction - = Negative reaction ND = Not detected

Three replicates / experiment were used and each experiment repeated twice.

3- Reaction of some wheat cultivars to bacterial streak and black chaff isolate XW3:- Differences in bacterial streak severity among cultivars under greenhouse conditions have been recorded in (Table 3). Disease

severity on flag leaves and spikes were differentiated best at 9 days after inoculation (data not shown) and ranged from 16 to 55.70% for the cultivars and strain evaluated. Average disease severity on flag leaves were 16 to 33.56 %. Black chaff severity was greater than bacterial streak severity in all tested cultivars Yecora rojo was the most susceptible to black chaff, bacterial streak symptoms and number of infected spikes followed by West bread cultivar. BLS resistance has been identified globally in wheat (Duveiller, 1990; El Attari *et al.*, 1996; Milus and Mirlohi, 1994; Milus *et al.*, 1996), very little information is available on its mode of inheritance. Recent research conducted in the field in Mexico showed that five genes condition BLS resistance in five wheat lines (Turaco, Alondra, Angostura, Mochis and Pavon). Cultivars Pavon and Mochis showed the highest level of resistance. None of the five genotypes contained the full set of identified resistance genes, which suggests that there are some cultivars more resistant than Pavon and Mochis cvs (Duveiller and Maraite 1993). Differences between genotypes in their susceptibility to bacterial leaf streak have been reported (Duveiller, 1990), but resistance is incomplete. On the other hand, in Table (4), Lokame cultivar was the greatest in black chaff severity comparing with bacterial leaf streak. Fresh leaves ranged from 8.93 to 11.36g / pot. The lowest fresh weight of leaves resulted from wheat cultivar Yecora rojo however, the heaviest value of fresh weight of leaves resulted from un-inoculated plants of West bread cultivar. The dry weight of leaves show that, L42002 had the lowest dry weight of leaves. When bacterial streak and black chaff severity were 33.56 and 55.70 % respectively, the number of infected spikes and 1000 – kernel weight were 184 spike / pot and 29.70g in Yecora rojo cultivar compared with un-inoculated plants. The grain weight (1000-kernel) was reduced by an average of 11.60% They pointed out that wheat cultivars varied in their susceptibility to bacterial leaf streak. This is the first study to evaluate some cultivars against bacterial leaf streak under Saudi Arabia conditions. Little quantitative information is available on losses caused by BLS. Yield losses as high as 40 percent have occurred in the most severely diseased fields in Idaho, United States, although losses are generally 10 percent or less (Forster et al., 1986). Shane *et al.*, (1987) demonstrated that 50% disease severity on the flag leaf resulted in 13% loss in kernel weight and yield reductions are estimated to be as high as 40% in susceptible wheat

cultivars (Schaad and Forster, 1985). In severe cases, 5 to 10 percent of the wheat spikes may be sterile due to infection (Forster and Schaad, 1988). Data from Mexico indicated that, on average, losses below 5 percent could be expected when the percent infected flag leaf area is less than 10 percent. (Duveiller and Maraite, 1993).

Table (3): Reaction of wheat cultivars to bacterial streak and black chaff isolate XW3 on FLS, No.infected spike and BCS.

Cultivars	FLS%	No. infected spike	BCS%
L42002	21.80	17.50	19.57
Lokame	16.00	39.50	27.00
West bread	31.50	177.8	42.30
Yecora rojo	33.56	184.0	55.70
LSD at 5%	1.65	28.21	

FLS = flag leaf severity, which represents the percentage of flag leaf area with leaf streak symptoms.
BCS = Black chaff severity was expressed as percentage of the total plant leaf area diseased using key developed by Duveiller(1994).

Table (4): Reaction of wheat cultivars to bacterial streak and black chaff isolate XW3 on fresh and dry weights and 1000 – kernel weight (g).

Fresh weight (g)			
Treatment	Control	Inoculated	% loss
Cultivars			
L42002	10.68	10.43	2.38
Lokame	10.30	10.23	0.68
West bread	11.36	9.75	11.60
Yecora rojo	9.91	8.93	9.90
LSD at 5%	3.56		
Dry weight (g)			
L42002	1.16	1.12	3.44
Lokame	1.93	1.80	1.18
West bread	1.17	1.25	12.84
Yecora rojo	1.89	1.78	11.46
LSD at 5%	0.50		
1000 – kernel weight (g)			
L42002	41.16	40.67	2.66
Lokame	47.66	47.33	1.33
West bread	41.30	38.46	6.85
Yecora rojo	33.60	29.70	11.60
LSD at 5%	2.24		

4-Effect of inoculum density of *X. translucens pv.translucens* on disease severity of black chaff bacteria:-

With an increase in inoculum density, the number of BLS lesions also increased (Table 5). At inoculums concentrations lower than 10^5 CFU/ml, no visible lesions were observed on the leaves at the end of the experiments, 14 days after inoculation. At inoculum concentrations higher than 10^6 CFU/ml, the number of lesions did not increase significantly with the increase in inoculum concentration. However Bamberg (1936) found that forcing a 10^5 cfu/ml bacterial suspension into the leaf whorl of young plants (four to five leaves) or into the boot of older plants with a hypodermic syringe is a very effective inoculation method for testing pathogenicity. This was confirmed at the International Maize and Wheat Improvement Center (CIMMYT), where plants are usually incubated for five days in a humid chamber after inoculation (Duveiller, 1994).and Milus and Mirlohi (1994) they reported that, a concentration of 10^6 CFU/ ml gave the best differentiation of disease reaction among different concentrations.

5- Effect of humidity period with isolate XW3 on wheat disease severity of cv. Yocora rojo:-

In some cases, water soaking was observed as early as three to four days. A concentration of 10^4 cfu/ml of a young culture (24 hour) on agar medium is usually appropriate. (Duveiller et al., 1997).

Table (5): Effect of inoculum density of *X. translucens* pv.*translucens* on black chaff disease severity.

Isolates (CFU/ml)	WX1	WX2	WX3	Means
10^4	00.00	00.00	00.00	00.00
10^5	21.00	20.10	25.40	00.00
10^6	21.40	21.10	27.00	23.10
10^7	27.70	27.30	31.60	28.80
10^8	29.40	28.20	35.30	30.90
Control	00.00	00.00	00.00	00.00
Means	15.70	15.30	18.70	
LSD at 0.05: Isolates (A) = 2.20 Inoculum(B) =6.28 Interaction (A X B) = 10.77				

With an increase in period of incubation under humidity after inoculation, the number of BLS lesions was increased (Table 6). At 96 hr visible lesions were observed on the leaves at the rate of 34.92% and the black chaff severity was recorded 55.50% on the adult plants (10 weeks old) At 24 hr no visible lesions were observed. At 48 hr, the number of lesions did not increase significantly with the increase in period of humidity after inoculation. Milus and Mirlohi (1994) they reported that, a concentration of 10^6 CFU/ ml at humidity period of 69 hr gave the best differentiation of disease reaction among different concentrations.

Table (6): Disease severity of wheat cv. Yocora rojo as affected by humidity period with isolate XW3.

Humidity period / hours	FLS %	BCS % (at 10 weeks old)
96	34.92	55.50
72	34.67	55.32
48	34.16	54.60
24	00.00	00.00
00	00.00	00.00
Control	00.00	0.000

FLS = flag leaf severity, which represents the percentage of flag leaf with leaf streak symptoms.

BCS = Black chaff severity was expressed as percentage of the total plant leaf area diseased using key developed by Duveiller(1994).

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بعض الدراسات على بكتيريا تخطيط الأوراق وأسوداد القنابع على القمح في المملكة العربية السعودية.

إبراهيم يوسف طرابلسى ، ياسر عيد إبراهيم ، على عبد الله المسرحى

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أعراض بكتيريا تخطيط الأوراق على القمح شوهدت على هيئة بقع مائية مطاولة ونصف شفافة فى خمسة مزارع بمنطقة الخرج بالرياض- المملكة العربية السعودية. أكدت الاختبارات الفسيولوجية لثلاثة عزلات أن البكتريا تنتمي الى زانزوموناس ترانسلونز النوع ترانسلونز .ثلاث عزلات هى(XW1,XW2 and XW3) كانت سالبة لصبغة جرام-عصوية قصيرة وتعطى مستعمرات صفراء تم عزلها من نباتات قمح (صنف يوكورا روجو) تبدو عليها أعراض مرض تخطيط الأوراق واسوداد القنابع فى موسم النمو ٢٠٠٤-٢٠٠٥وأعطت كل العزلات نتائج متشابهة وكانت نتائجها موجبة لكل من اختبارات الحركة وتحلل الاسكيولين وتفاعل شدة الحساسية للدخان واختزالها لسكر الاربينوز والمانوز. وأعطت العزلات الثلاث المختبرة نتائج سالبة لاختبار الأكسدة وتحلل الأرجنين واختزال النترات الى نتريت وتحليل النشا.وتم تأكيد التعريف عن طريق نظام البيولوج.أظهرت العزلات المختبرة قدرتها على إحداث أعراض تخطيط الأوراق واسوداد القنابع على القمح عند الرش بمعلق بكتيري بتركيز 10^{8} وحدة مكونة للمستعمرة وجد أن تركيزات المعلق البكتيري التي أقل من 10^{5} وحدة مكونة للمستعمرة لم تظهر أى أعراض على النباتات حتى بعد ١٤ يوم من العدوى وانه لم تكن هناك فروق معنوية بين التركيزات الأعلى من 10^{6} وحدة مكونة للمستعمرة. أظهرت اختبارات الزراعة فى البيت المحمى أن الصنف يوكورا روجو صنف شديد القابلية للإصابة بأعراض تخطيط الأوراق واسوداد القنابع وسجل نسبة خفض فى المحصول وصلت الى ١١,٦٠% مقارنة بالأصناف الأخرى.