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SCREENING OF LOCAL WHEAT VARIETIES AGAINST BACTERIAL LEAF STREAK CAUSED BY DIFFERENT STRAINS OF *XANTHOMONAS TRANSLUCENS* PV. *UNDULOSA* (*XTU)*

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Abstract

Wheat is one of the most important food crops of Pakistan, but its yield is adversely affected due to bacterial leaf streak disease caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu)* resulting in 20% decrease in yield. During present investigation 6 wheat varieties including Chakwal-97, Inqulab-91, GA-2002, Aquab-2002, Punjnand-2000 and Tartara-2000 were screened against 10 different strains of *Xanthomonas translucens* pv. *undulosa* (*Xtu)* at various stages of plant growth to check virulence against bacterial leaf streak. Wheat nursery was grown in glass house and pathogenicity of each strain/isolate was tested at seedling stage (4 weeks olds) and at tillering stage (8 week-old) wheat plants by using clipping method of artificial inoculation. The symptom development was rated by counting water-soaked lesion, number, size and progress of necrosis on leaves and the resistance of particular variety against particular strain was determined. It was evident from the results that all the six wheat varieties were susceptible to *Xanthomonas translucens* pv. *undulosa (Xtu)* but Aquab-2002 was highly susceptible to all the exotic/local strains/isolates of *Xanthomonas translucens* pv. *undulosa (Xtu)* with an average maximum percentage (69%) of disease incidence and Inqulab-91 was least virulent (10%). The highest percentage (83%) of disease incidence was for *Xanthomonas* strain UPB-513 at the seedling stage of Aquab-2002. The disease incidence of all the 6 wheat varieties was less at maximum tillering stage (52%) as compared to that of seedling stage (83%) for Aquab-2002 as well as all other varieties. Reactions of bacterial strains were variable (10-83%) to different wheat varieties and the response of bacterial strains/isolates was also found variable (0-72%) within the same wheat varieties.

Introduction

Wheat (*Triticum aestivum* L.) is a leading grain crop of Pakistan and used as a staple food in the country. Almost 9,062 thousands hectares area of Pakistan is wheat cultivated, *i.e*., 51.5 % of total cultivable area of Pakistan (Anon, 2010).

Various conventional methods are being employed for the improvement of wheat to maximize wheat yield by developing improved varieties and to control the effect of various factors that perturb the wheat productivity (Kamil *et al*., 2005). Diseases caused by bacteria and fungi are the major factors to decrease wheat yield. Due to bacterial leaf streak 10-20 % wheat yield is reduced (Aslam & Akhtar, 1986). The pathogen was first identified on barley (Jones *et al*., 1917) and later on wheat (Smith and Towsend, 1919). Bacterial leaf streaks usually considered to be widespread but unimportant. However, account of this disease, particularly on wheat in 1980s have become more frequent and have aroused much concern (Duveiller, 1989). Differenet names have been proposed, depending on the host plant but the name *Xanthomonas translucens* pv. *undulosa* is used here to refer to the pathogen that causes bacterial leaf streak on wheat.

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In Pakistan the disease was recorded for the first time by Akhtar and Aslam (1985 and 1986) later on nineteen wheat varieties under National uniform wheat yield trial in 1985 were tested at 10 locations and its occurrence was noted in almost all provinces of Pakistan (Akhtar & Akram, 1987). Bacterial leaf streak (BLS) of wheat created a serious situation in wheat during the crop year 2002. It occurs at all stages of the crop and shows either creek or leaf streak symptoms. If plant produces panicles, the sterility percentage increases as well as the number of immature grains. When there is heavy infection, no grain formation takes place (Akhtar *et al*. 2003).

The aim of the present study was screening of six wheat varieties viz., Chakwal-97, Inqulab-91, GA-2002, Aquab-2002, Tartara-2000 and Punjnand-2000 by utilizing 10 different strains of *Xanthomonas translucens* pv. *undulosa* to investigate disease susceptibility. In the later part of this study *Agrobacterium* mediated genetic transformation would be done to create disease resistance in susceptible varieties.

Materials and Methods

Seed source: The healthy seeds of 6 wheat varieties were obtained from Wheat Program, Crop Sciences Institute, NARC, Islamabad. These wheat varieties were selected to study the bacterial leaf steak caused by *Xanthomonas translucens* strains.

Nursery maintenance: The healthy seeds of each variety were chosen and separately soaked by placing them on the wet filter paper in the separate Petri plate to break the dormancy and to obtain the synchronous growth. After germination, 15-20 seeds were sown is a separate pot and three such pots were maintained. At three-leaf stage, these seedlings were shifted in separate pots so that one pot contained 2-3 plants. Plant aged 30 days (4 weeks) and 60 days (8 weeks) were used to study bacterial leaf steak of wheat.

Bacterial strains: Ten different strains of *Xanthomonas translucens*. pv. *undulosa* used to study bacterial leaf steak of wheat. These strains include names according to Unit of Phytopathology Bacterial (UPB) strain collection (Bragard *et al*., 1997):

UPB 410, UPB 412, UPB 443, UPB 482, UPB 513, UPB 522, UPB 633, UPB 644, UPB 876, UPB 955

First four strains were obtained in 1993 and remaining during the year 1997 from the International Maize and Wheat Improvement Center (CIMMYT), provided to IABGR, NARC, Islamabad.

All these strains/isolates were in dry preserved form at 4^{o}C in small vials. So to proceed, first these strains had to be revived. Bacteria were grown on Wilbrink's medium because the pathogen's typical yellow mucous colony is best distinguished from saprophytes on this non-selective medium (Sands *et al*., 1986).

Growth conditions: These plates were incubated at 28^{o}C for two to three days depending upon colony development. All the bacterial strains and isolates revived using the same method (Fig. 2.1 a).

Inoculum preparation: When bacterial colony was isolated on agar medium, inoculum was prepared by growing bacterial strain on Wilbrink's medium. Before preparing inoculua, the slants of medium were prepared. The composition of the medium was same

as described before except 20 g of agar was used for slants instead of 15.0 g for proper solidification of slants.

Slant and suspension preparation: Approximately one third of the test tubes were filled with Wilbrink's medium after autoclaving. These were placed upward slanting position to make slants. Then these slants were incubated at 30°C for 24 hours to observe the presence of contamination. Slants free of contamination were used for the streaking of bacteria. These slants were incubated at 28°C for 48 hrs. For the preparation of fresh inoculum 10ml of sterile distilled water was added to the 48 hrs culture of *Xanthmonas translucens* in the slants for the preparation of bacterial suspension

Pathogenicity test: For pathogenicity test, clip method was used for the inoculation of the Wheat plants with *Xanthomonas translucens* pv. *undulosa*.

Clip method: Kauffman *et al*., (1973) reported the clip method. In clip method sterilized surgical scissors dipped in bacterial suspension were used for inoculation. For this purpose a pair of scissors was dipped in bacterial suspension. Leaves of all the three plants in a pot were grasped in one hand and the top 1-3 inches of three leaves were clipped off simultaneously. The same procedure was followed for inoculation of the different strains and isolates to each variety of wheat. The inoculum should be used within two hours after preparation as *Xanthomonas translucens* which quickly loses its viability. A control of each variety was also maintained, by using scissors dipped in sterile for clipping off the leaves. The pathgenicity of each strain was tested on 4 week, 8 week and 12 week old Wheat plants of all three varieties, at 35 ± 2°C in the glass house with a light period of 12-14 hours.

Glasshouse test: Following the inoculation, the plants were surveyed after every 24h time interval to note the appearance of disease symptoms and final data was recorded after 14 days of inoculation. Mean Percent disease incidence was calculated by the help of following formula (Gnanamanickam *et al*., 1999):

$$\% \text{ Disease incidence} = \frac{\text{Total lesion length (cm)}}{\text{Total leaf length (cm)}} \times 100$$

Confirmation of the bacterial leaf steak in infected wheat leaves: The leaves of wheat plants inoculated with *Xanthomonas translucens*, showing the symptoms of bacterial leaf steak *i.e*., yellow lesions were used for isolation of the bacteria. The infected leaves were cut with the help of a pair of scissors from plant grown in the glass-house and taken into the lab. These leaves were then washed with sterile water for 2-3 times. Infected leaves of wheat were cut into small pieces 1-2 cm with the help of sterile cutter. These were then placed on solid Wilbrink's medium in Petri plates under sterile conditions then these plates were incubated at 28°C for 48h. After 48 h these plates were observed for the presence of *X. translucens.* The same procedure was followed for all the wheat varieties inoculated with different strains of *X. translucens* showing the visual symptoms of bacterial leaf steak.

Results

Morphology of Bacterial Culture on Wilbrink's medium: The strains of *Xanthomonas translucens* pv. *undulosa* were incubated on Wilbrink's medium for the revival as well as

inoculum preparation. After 24h yellow smooth, circular and viscous colonies appeared which became somewhat irregular after 48h due to viscous fluid secreted by bacteria (Fig. 1).

Development of symptoms: Disease symptoms first appeared 4-5 days after inoculation in all wheat varieties. The initial symptoms were leaf curling near the cut-off portion. The curling was much more pronounced in Chakwal-97 and GA-2002 in comparison with the other varieties. Soon after curling water-soaked lesions developed from the cut surface and advanced down the leaf (Fig. 2).

Twenty-eight days after inoculation (DAI), status of disease was again checked and noted that disease was spread not only to the whole leaf but to the vascular bundle also (Fig. 2). At tillering stage, that loss of leaved severely cause decrease in yield (Fig. 3).

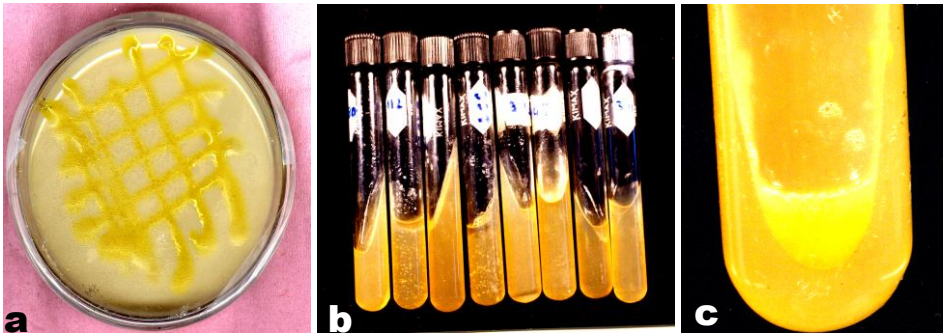

Fig. 1. Morphology of cultures of *Xanthomonas translucens* pv. *undulosa*.

(a) Pure culture of *Xanthomonas translucens* pv. *undulosa*

(b) 48 hrs old culture of *Xanthomonas translucens* pv. *undulosa* on the slants used for inoculum preparation

(c) Bacterial suspension. 10ml sterile distilled water was added to the 48h old culture of *Xanthomonas translucens* pv. *undulosa*

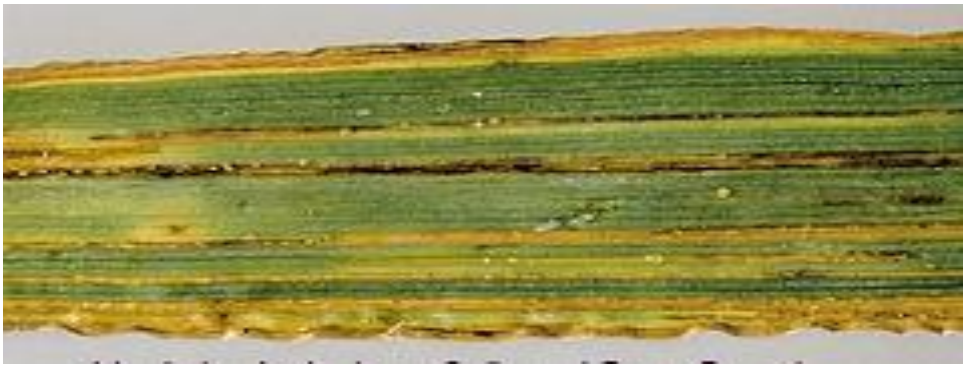

Fig. 2. A close view of the symptoms of Bacterial leaf streak on Chakwal-97.

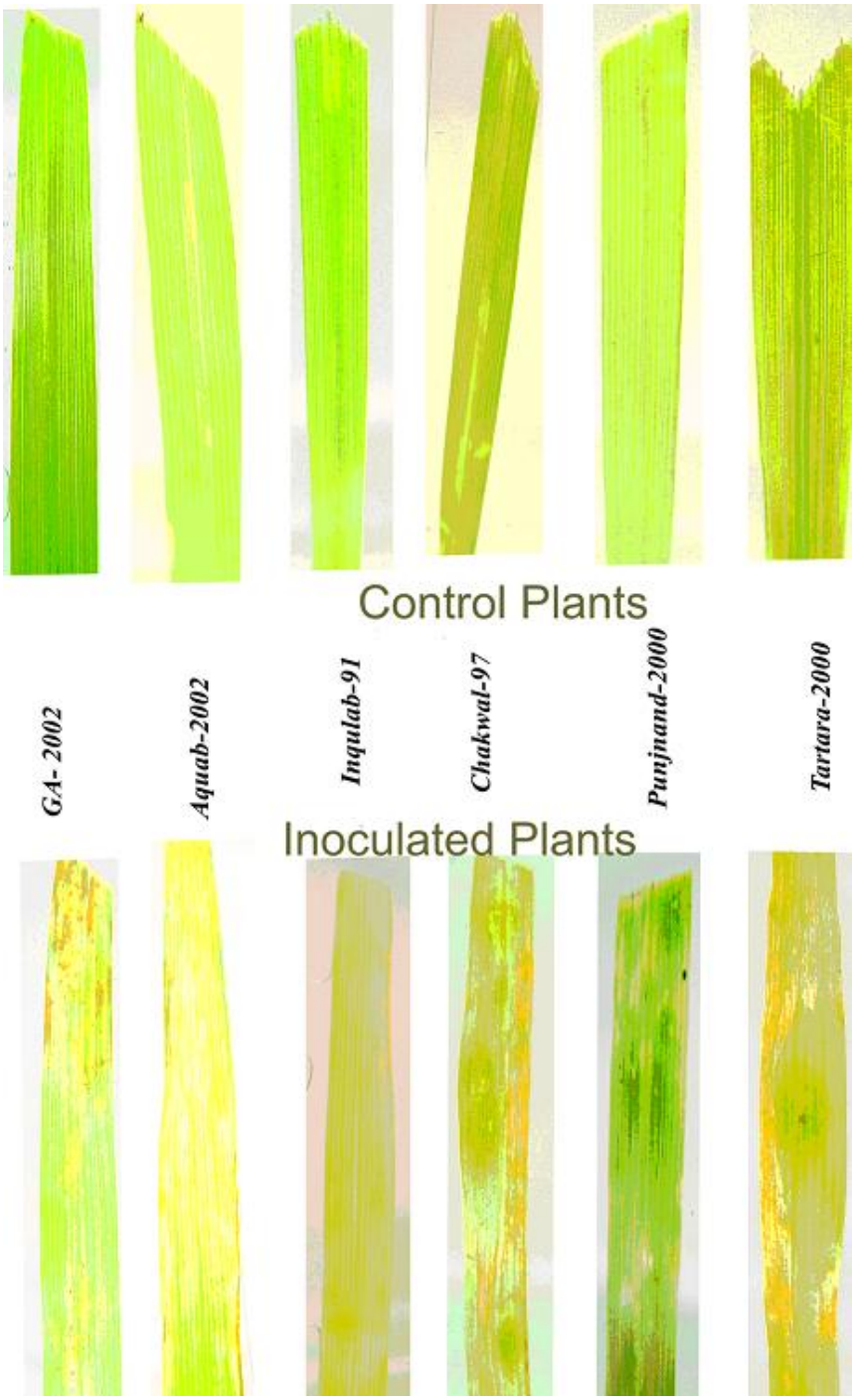

Fig. 3. Comparison between control and inoculated wheat varieties for development of bacterial leaf streak symptoms.

Length of lesion caused by *Xanthomonas translucens* pv. *undulosa* isolates: In each variety, lesions developed uniformly downward from the point of inoculation. However, the length from the leaf tip varied from variety to variety and isolate to isolate. In case of Aquab-2002 length of the lesion was 5-9cm whereas in GA-2002 it was 2-4cm. All the strains were pathogenic but the pathogenicity of local strains were less (30-50%) as compare to strains (70-80%).

Responses of wheat varieties to bacterial isolates: The pathogenicity of 10 different strains of *Xanthomonas translucens* pv. *undulosa* was tested against different variety of wheat under dry conditions. These tests were conducted on two different growth stages of wheat plants *i.e*., at seedling stage and tillering stage.

Pathogenicity of *Xanthomonas translucens* pv. *undulosa* at seedling stage: At seedling stage *i.e*., after 28-30 days of sowing, all the six varieties were susceptible to bacterial strains with percent disease incidence ranging from 0% to 67.72% (Gnanamanickam *et al*., 1999) (Table 1). Aquab-2002 showed maximum percent disease incidence *i.e*., 67.72% (score = 7) followed by Tartara-2000 46.98% (score = 5) at seedling stage for all the strains.

At seedling stage, in comparison, GA-2002 variety was less susceptible (17.42%) than Inqulab-91 (46.38%). Similarly, Chakwal-97 was more susceptible (45.78%) than Punjnand-2000 (41%) UPB 443 was avirulent to all the wheat varieties and caused an average of disease incidence 2% (score = 1). On the other hand UPB 513 was the highly virulent strain and caused highly percentage disease incidence of an average of 74% (score =8). It is defined that at seedling stage GA-2002 and Inqulab-91 found moderately resistant *i.e*., 37.34% and 28.09% respectively (score =3), while remaining varieties were moderately susceptible with score ranging from 5 to 7.

Table 1. Comparison of % disease incidence of 10 different strains of *Xanthomonas translucens* pv. *undulosa* on six different wheat varieties at seedling stage.

Strains	Chakwal-97	Inqulab-91	GA-2002	Aquab-2002.	Punjnand-2000	Tartara-2000	Mean
UPB 410	30.81	54.93	0	51.9	65.6	73.21	46.075
UPB 412	66.17	29.59	45.9	80.81	0	67.86	48.38833
UPB 443	0	0	0	10	2.5	0	2.083333
UPB 482	48.18	64.91	2.16	79.4	0	61.4	42.675
UPB 513	80.85	68.33	65	82.91	75.41	72.05	74.09167
UPB 522	72.03	5.68	5.88	80.3	69.06	57.83	48.46333
UPB 633	70.23	47.25	0	80	69.83	0	44.55167
UPB 644	17.19	64.71	0	75.91	0	70.5	38.05167
UPB 876	72.41	68.18	22.88	60.5	58.33	0	47.05
UPB 955	0	60.24	32.39	75.47	69.38	66.95	50.73833
Mean	45.787	46.382	17.421	67.72	41.011	46.98	
Status	MS	MS	MR	S	MS	MS	

*Data was taken after 30 days of sowing. **Average of ten uppermost leaves of two-three plants

HR=Highly Resistant (Infection 0%, Score 0), R= Resistant (Infection 0-10%, Score 1), MR= Moderately Resistant (Infection 10-30%, Score 3),

MS= Moderately Susceptible (Infection 30-50%, Score 5), S= Susceptible (Infection 50-75%, Score 7), HS=Highly Susceptible (Infection 75-100%, Score 9).

Table 2. Comparison of % disease incidence of 10 different strains of *Xanthomonas translucens* pv. *undulosa* on 6 different wheat varieties at tillering stage.

Strains	Chakwal-97	Inqulab-91	GA-2002	Aquab-2002.	Punjnand-2000	Tartara-2000	Mean
UPB 410	51.43	0	0	71.43	0	59.04	30.31667
UPB 412	45.45	18.95	20.45	74.46	35.38	61.11	42.63333
UPB 443	54.41	19.19	27.78	69.42	0	0	28.46667
UPB 482	0	17.46	16.73	51.45	3.04	0	14.78
UPB 513	0	26.32	14.76	53.66	0	49.37	24.01833
UPB 522	54.07	19.89	18.4	40.08	4.13	0	22.76167
UPB 633	37.69	0	0	43.78	44.58	54.14	30.03167
UPB 644	0	0	0	52.09	7.19	0	9.88
UPB 876	0	24.8	36.17	56.64	28.71	27.59	28.985
UPB 955	0	15.73	32.37	23.5	0	11.47	13.845
Mean	24.305	14.234	16.666	53.651	12.303	26.272	
Status	MR	MR	MR	S	MR	MR	

*Data was taken after 60 days of sowing. **Average of ten uppermost leaves of two-three plants
HR=Highly Resistant (Infection 0%, Score 0), R= Resistant (Infection 0-10%, Score 1), MR= Moderately Resistant (Infection 10-30%, Score 3),
MS= Moderately Susceptible (Infection 30-50%, Score 5), S= Susceptible (Infection 50-75%, Score 7), HS=Highly Susceptible (Infection 75-100%, Score 9)

Pathogenicity of *Xanthomonas translucens* pv. *undulosa* at tillering stage: The results of the pathogenicity test of 10 strains of *Xanthomonas translucens* pv. *undulosa* against 6 different wheat varieties after 58-60 days of sowing (8 week) at maximum tillering stage are summarized in Table 2. All varieties showed moderate resistance except Aquab-2002 (53.65%). Like seedling stage at this stage too, all strains were virulent for Chakwal-97 with an average of 24.30 % (score = 7) disease incidence and least virulent for Punjnand-2000 *i.e.*, 12.30% (score = 2). In general percentage disease incidence of Tartara-2000 varieties were comparatively higher (26.67%) than that of Inqulab-91 (14.23%).

Detection of bacteria from infected wheat leaves: To confirm that the symptoms showed by the inoculated control and transgenic wheat plants were due to presence of *Xanthomonas translucens* pv. *undulosa*, diseased leaves were plated on Wilbrink's media. Plates were monitored between 72-98 h for bacterial colonies. The colonies produced were similar to the bacterial colonies used for inoculation *i.e.*, yellow smooth and viscous.

Discussion

The present study was conducted to evaluate the resistance of 6 different varieties of wheat to 10 different strains of *Xanthomonas translucens* pv. *undulosa* and a comparison of virulence of *Xanthomonas translucens* pv. *undulosa* between Pakistani and Iranian strains/ isolates was made. The wheat seeds of all varieties were soaked in water in order to break dormancy and to achieve the synchronous growth. Bragard *et al*., (1997) reported that when the seeds try to grow are very fragile and any sudden change can cause the wheat plant to die. The soaked seeds were kept at $30^{o} \pm 2^{o}$C for germination. Duveiller, (1990) reported that the favorable temperature for the growth of wheat plant is

28º-30ºC. The isolates and strains of *Xanthomonas translucens* pv. *Undulosa* (*Xtu*) used in the present study were in dry preserved form and therefore firstly they were revived. Wilbrink's medium was used for the revival of the bacterial strains and later for inoculum preparation on slants in our studies. Duveiller and Maraite (1995) used the same medium for the maintenance of bacterial strains collected during the year of 1994. According to Bragard & Maraite (1994) the growth of *Xanthomonas translucens* on medium requires carbon and nitrogen sources. Tu *et al*., (1998), Kauffman *et al*., (1973) and Nelson *et al*., (1994) used the same medium to evaluate the resistance of different wheat varieties to bacterial leaf streak (BLS). Karganilla *et al*., (1973) stated that $FeSO_4.7H_2O$ was also favorable for single colony isolation.

For the revival of the strains and later for the preparation of inoculums on Wilbrink's medium, the incubation temperature for the bacterial growth was maintained at 29ºC for 48 h. After 24 h of incubation, yellow, smooth, convex and circular colonies were observed which became somewhat irregular after 48 h due to the viscous fluid secreted by the bacteria. Similar types of culture characteristics *i.e*., yellow, smooth, circular and viscous colonies on potato semi synthetic agar medium were reported by Wilbrink (1967) and Attari *et al*., (1996). Akhtar & Aslam (1985) reported that the temperature range for the bacterial growth was from 5–40ºC while the optimum temperature was 26-30ºC. Similarly Leach *et al*., (1992) used 28ºC incubation temperature for the growth of *Xanthomonas translucens* pv. *undulosa(Xtu)* on culture medium (Wilbrink's medium), while Attari *et al*., (1996) incubated bacterial culture at 37ºC for 48 h for screening wheat varieties against bacterial leaf streak.

Bacterial culture suspended in 10 ml of sterile distilled water was used to inoculate control as well as transgenic wheat plants in this study. Kauffman *et al*., (1973) reported that the bacterial population of 10^7 cell/ml or greater of PXO 25 in inoculum suspension was adequate to give maximum infection on the wheat varieties IR8 (susceptible) and IR 20 (resistant). The ability of cultural filtrates to induce wilting and yellowing gradually decreased when the filtrate was dialyzed against distilled water. In the present study minimum dilution of suspension was used. All inoculations were made within one hour after preparing the bacterial suspension as suggested by Kauffman *et al*., (1973) that inoculum should be used within one hour after preparation.

The clipping method of artificial inoculation was used to evaluate the resistance of the wheat varieties (control as well as transgenic wheat plants) against bacterial leaf streak (BLS) as it was convenient for the inoculation of wheat plants in greenhouse studies to get 90% pathogenecity. Kauffman *et al*., (1973) used the clipping method for evaluating resistance of wheat varieties against *Xanthomonas translucens* pv. *undulosa(Xtu)* and they stated that clipping inoculation score and natural infection score were highly correlated. Ogawa *et al*., (1990); Tu *et al*., (1998); Kaku *et al*., (1977, 1980); and many other researchers used clipping method of inoculation for evaluating resistance of wheat varieties including transgenic plants and also for genetic analysis of resistance of wheat plants.

In this study the lesions caused by infection of *Xanthomonas translucens* pv. *undulosa(Xtu)* resulted in water soaked lesions at the margin of the cut off portions of the leaves. Lesions started at the margins, a few cms from the tip, as water soaked stripes. The lesions enlarged both in length and in width, had a wavy margin, and turned yellow within a few days, the region adjoining the healthy parts showed water soaking. As the disease advanced, lesions covered the entire blade, turned white and later became grayish as reported by Salim *et al*., (2003).

Initial symptoms in the present study were leaf curling which appeared after 3 days in case of Chakwal-97 and Inqulab-91, as these varieties are highly or moderately susceptible to *Xanthomonas translucens* pv. *undulosa(Xtu)*. While in all the other varieties it appeared after 4 to 5 days of inoculation at seedling stage. This was supported by the work of Satya *et al*., (2005) as they reported that the disease symptoms first appeared 4 to 5 days of inoculation in the form of leaf curling near the cut off portion when they evaluated the resistance of UPB 412 and UPB 522 against UPB 876.

Percentage disease incidence calculated for control and transgenic wheat plants on the basis of lesion length was used to evaluate the pathogenicity of the bacterial strains to wheat varieties and scores were assigned to these (0-9) according to the standard evaluating system for wheat. Gnanamanickam *et al*., (1999) also used percentage disease incidence in a series of experiments to evaluate the performance of *Pseudomonas putida* strain V 14i as a bio control agent to suppress bacterial leaf streak disease in IR24. Mew & Khush (1981) used standard evaluation system (0-9 scale) to evaluate the resistance of different wheat varieties to UPB 410.

All the 6 varieties were inoculated at two different growth stages, seedling stage and maximum tillering stage *i.e*., after 30 and 60 days of germination respectively as the age of the host plant influence development of bacterial leaf streak. Similarly Lihjiuan *et al*., (2005) checked the susceptibility of 4 wheat cultivars at different growth stages at 30, 45 and 60 days after sowing and positions on the disease reaction were analyzed.

At the seedling stage the most susceptible variety was GA-2002 which exhibited susceptible reaction to all the exotic and local strains/isolates of *Xanthomonas translucens* pv. *undulosa(Xtu)* tested. The maximum disease incidence was 82.91% against UPB 410. Earlier, Khan *et al*., (2000) also reported that GA-2002 showed susceptible reaction against the indigenous strain of *Xanthomonas translucens* pv. *undulosa(Xtu)*. Among all the wheat varieties tested Inqulab-91at seedling stage showed susceptible reaction (an average of 51.36%) against Iranian and Pakistani strains/isolates which is against the findings of Khan *et al*., (2000) as they reported that out of five varieties tested by them, only C-591 was found moderately resistant against the bacterial leaf leaf streak (BLS). Satya *et al*., (2005) screened 8 aromatic genotypes against 5 isolates from different parts of northwestern India to identify variability in virulence. All the aromatic genotypes were found to be either moderately or highly susceptible against all the isolates with significant differences in disease progress. All strains and isolates induced moderately susceptible response (average of 37.34%) in Margalla-99 at seedling stage. This was supported by the work of Rademaker *et al*., (2006) as they reported a susceptible response of ornamental asparagus against indigenous strain of *Xanthomonas translucens* pv. *undulosa(Xtu)*. For the other local strains, however, there was a resistance response and the disease incidence ranged from 14-25%. Bhutta & Ahmad (1994) also reported that the disease incidence of C-591 was 10-20 %.

Chakwal-97and GA-2002 showed a moderately resistant response against all strains and isolates with maximum percent disease incidence of an average of 28.09% and 20.58% respectively. Only two strains *i.e*., UPB 410 and local isolate UPB 412 were able to induce susceptible response on Chakwal-97 and GA-2002 at seedling stage. Both these varieties of wheat were more or less resistant to all other bacterial strains. Khan *et al*., (2000) observed a moderately susceptible response of Chakwal-97 against the indigenous strain of *Xanthomonas translucens* pv. *undulosa(Xtu)*.

The bacterial strains which induced susceptible reaction to one variety of wheat was not necessarily able to induce the similar reaction in other varieties and reaction of a

bacterial strain was variable to different wheat varieties as UPB 443 induced highly susceptible response to Punjnand-2000, Tartara-2000 and Aquab-2002, moderately susceptible to Inqulab-91 and highly resistant to Chakwal-97 and GA-2002.

The reactions of different bacterial strains were also found variable against the same wheat variety just as Inqulab-91 showed highly resistant response against PXO-112, X0 - 108 and UPB 482 and susceptible against UPB 410, UPB 482 and UPB 513. Similar type of findings was reported by Yoshimura *et al*., (1985) in which they stated that IR-8 was susceptible to UPB 410 while GA-2002 was resistant to it but was susceptible to UPB-522.

The plants inoculated at the tillering stage, revealed that the young plants were more susceptible than the older ones in this experiment. The disease incidence of all the 6 varieties was less at maximum tillering stage than the seedling stage and most of the varieties, which were susceptible at seedling stage gained resistance up to maximum tillering stage against different bacterial strains and isolates.

There was a direct relation between the resistance and the age of host plant. Plant age greatly influenced the varieties that were susceptible at seedling stage and these varieties showed more pronounced resistance response in the later stages. Parallel to these observation Kauffman *et al*., (1973) reported that all varieties tend to become slightly more resistant towards maturity, with the greatest change occurring in the susceptible plant. Our findings were also supported by the work of Baw & Mew (1988); Zhang *et al*., (1998) and Mazzola *et al*., (1993) as they reported that plants gained resistance with the age against bacterial leaf streak disease. Contrary to our observation Mariappan *et al*., (1980) reported that a variety ASD 5, was resistant until the tillering stage, but became infected at the boot leaf stage (60-80 days). In contrast to this report, Sahu (1987) stated that plants resistant at the seedling stage remained resistant at later growth stages of plant, but reverse was not true.

To confirm that the symptoms showed by the inoculated wheat plants were due to presence of *Xanthomonas translucens* pv. *undulosa*, diseased leaves were plated on Wilbrink's media by two methods, *i.e*., direct plating and dilution methods. Plates were monitored between 24-48 h for bacterial colonies. Our results showed that the colonies appeared in both cases were similar to the bacterial colonies used for inoculation *i.e*., yellow smooth and viscous. Bogdanove & Martin (2000) isolated the bacterium by plating inoculated wheat plants using 10^{-3} dilution and reported that yellow colonies appearing on the media confirmed the presence of *Xanthomonas translucens*.

From our results it was obvious that all the 6 wheat varieties are susceptible to all the exotic strains of *Xanthomonas translucens* pv. *undulosa* tested, on the other hand these varieties were showing more resistance to local strains.

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