Identification of New Sources of Resistance in Winter Wheat to Multiple Strains of *Xanthomonas translucens* pv. *undulosa*

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Abstract

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Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa*, has re-emerged as an important disease of wheat (*Triticum aestivum*) in the United States. Planting resistant varieties is the best approach to manage BLS in the absence of effective bactericides. However, most of the wheat varieties currently grown in the Upper Midwest of the United States appeared to be susceptible to BLS. From the core subset of the USDA National Small Grain Collection (NSGC), a set of 605 winter wheat accessions of diverse origin and improvement status were initially inoculated with a virulent strain BLSW16 of *X. translucens* pv. *undulosa* from Casselton, ND on the flag leaf of each plant in a greenhouse. Disease reactions were assessed between 7 and 10 days after infiltration using a 0 to 6 rating scale, where ≤ 2.0 was considered resistant and > 2.1 was considered susceptible. Resistance varied with geographic origin and was significantly ($P \leq 0.05$) more frequent in accessions from North America compared to accessions from northern, eastern, and southern Europe and from

south-central Asia. About 8.3% of accessions tested were resistant, and accessions with an improvement status of “cultivar” were significantly more likely to be resistant than were accessions classified as either landraces or breeding lines. Forty-two of the accessions exhibiting resistance in response to the strain BLSW16 in the first screening test were retested utilizing each of the two additional strains (BLS Cr25 and BLS Lb74 of *X. translucens* pv. *undulosa*) collected from Carrington and Lisbon, respectively. Nonparametric data analysis revealed 35 accessions were resistant, one accession, PI 266860, was susceptible to both strains, and six accessions showed differential responses. The majority of the BLS-resistant accessions also were resistant to at least one other important wheat disease based on the Germplasm Resources Information Network (GRIN) data. These results suggest that diverse and novel sources of resistance to BLS identified in this study can be utilized in winter wheat breeding programs.

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa* (3), is an important disease of wheat (*Triticum aestivum* L.) worldwide. The disease was first reported in 1919 (24). The early disease symptoms on the leaves are characterized by translucent water-soaked streaks that are associated with bacterial exudates (7). At the early milk to soft dough stage (Zadok's DC 77–83) (29), black chaff develops as dark purple to black blotches on the glumes (4,24). During the 2008–2009 seasons, five locations (Carrington, Casselton, Fargo, Lisbon, and Prosper) and 20 wheat fields in Casselton, Fargo, and Prosper in North Dakota were surveyed, and an average 80% BLS incidence was observed (T. B. Adhikari and S. Gurung, unpublished data). The infected wheat plants were stunted and chlorotic (7).

Traditionally, BLS has been managed by treating seeds with hot water and mercury or copper fungicides (11–13); however, these methods are insufficient to meet current safety and/or efficacy standards. Although warm and humid environments are conducive for disease progression, the bacterium cannot over-summer in crop debris or in soil (19). The disease is mainly seedborne (13) and potentially transmitted through wheat germplasm exchange (11–13). Yield losses of 30 to 40% due to this disease have been reported from Idaho (12).

Winter wheat cultivation has gradually expanded in the Upper Midwest region of the United States, particularly in North Dakota and Minnesota (28). Anecdotal evidence in North Dakota and neighboring states suggests that BLS has re-emerged as a potential threat to both winter and spring wheat. Recent BLS epidemics

could be related to unusually high rainfall and high humidity, to possible changes in the bacterial populations, and to planting recently released wheat varieties that are susceptible to the disease. In a preliminary study, none of the commercially grown wheat varieties in North Dakota were resistant to BLS (1). Additionally, the impact of BLS on winter wheat appeared to be more severe than on spring wheat (T. B. Adhikari and M. Mergoum, unpublished data). This situation prompted us to search for resistance to BLS in winter wheat. Compared to synthetic and diploid wheat, we hypothesize that some hexaploid wheat landraces could have good agronomic adaptation and resistance to BLS and other foliar diseases of importance in the region. The main objective of this study was to identify novel sources of resistance to BLS among winter accessions in the USDA-ARS National Small Grains Collection (NSGC).

Materials and Methods

Selection of winter wheat accessions. The NSGC core subset represents 10% of the total collection and was chosen to maximize diversity based on geographic origin. There are 1,415 winter habit accessions in the core subset, and from this group 605 were randomly selected for testing. These included accessions of various categories which the Germplasm Resources Information Network (GRIN) refers to as “improvement status”, i.e., breeding lines, released cultivars, cultivated accessions (where it is uncertain if the accession is a landrace or a cultivar), and landraces, which were developed in antiquity through farmer selection and natural selection (2). Nearly all of the selected accessions originated from 11 geographic “macroregions” based on the United Nations designations for World macroregions (27).

Planting of wheat accessions. Plastic cones (3.8 cm in diameter and 20 cm long) (Stuewe and Sons, Inc., Tangent, OR) were filled with Fison sunshine mix #1 (Fison Horticulture, Vancouver, BC) in the greenhouse. Three seeds of each accession were planted in a cone, and each accession was planted in three cones. The seedlings

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were supplied with 2.5 g of multicote slow release commercial fertilizer (14-14-16, N-P-K, Sungro Horticulture Distribution Inc., Bellevue, WA) at planting.

To evaluate resistance to BLS, the 605 accessions were arranged in a randomized complete block design (RCBD) with three replications. Each treatment consisted of three plants per replication (total of nine plants per accession). Each cone was considered an experimental unit, and the flag leaf of each plant was regarded as a sampling unit. Greenhouse temperatures were maintained at 24 to 28°C with 16 h of supplemental light provided by 400 W Lucalox LU400 sodium vapor lamps (General Electric Co., Cleveland, OH) until disease scoring. All experiments were conducted at North Dakota State University (NDSU), Fargo, 26 June 2009 through May 2010.

Inoculum preparation. Based on our preliminary results (1), a highly virulent strain, BLSW16, of *X. translucens* pv. *undulosa* from Casselton (46°54'N, 97°12'W, 282 m asl) was selected to evaluate resistance in winter wheat in this study. The bacterial strain (stored in 30% glycerol at -80°C) was revived on peptone sucrose agar (PSA) (20) plates. The plates were incubated at 28°C for 72 h. The bacterial cells were scraped with a wire-loop, and inoculum was prepared by suspending bacterial cells for 2 to 3 min in 20 ml of sterile deionized water per plate. The bacterial suspension was adjusted to an optical density of 0.2 at 590 nm using a spectrophotometer prior to inoculation, and approximately 1×10^7 CFU/ml were counted by dilution plating after inoculation. The inoculum was transferred to a glass flask, placed on ice, and brought to the greenhouse for inoculation.

Plant inoculation. For each accession, the adaxial surface of a fully expanded flag leaf was infiltrated using a disposable syringe without a needle (18). Approximately 10 to 15 µl of the bacterial cell suspension was gently infiltrated into the middle of the flag leaf of each plant. In each flag leaf, only one area was infiltrated with bacterial cells, and distribution of bacterial cells within the infiltrated area on the flag leaf was uniform. The sizes of the infiltrated areas ranged from 0.2 to 0.4 cm², and they were marked by a nontoxic permanent marker on both ends. The plants were placed in trays of water and left on the bench in the greenhouse.

Disease assessment. Disease reactions within the infiltrated areas on flag leaves were visually estimated between 7 and 10 days after infiltration using a 0 to 6 rating scale (16), where 0 = no visible symptoms, 1 = chlorosis without water-soaked lesions, 2 = water-soaking less than 10%, 3 = water-soaking 10 to 30%, 4 = water-soaking 31 to 70%, 5 = water-soaking 71 to 100%, and 6 = water-soaking extending beyond the infiltrated area. Disease scores of 0 to 2 (or less than 10% water-soaking within the infiltrated areas) were considered resistant (R) (26), and 2.1 to 6 (or greater than 10% water-soaking) were regarded as susceptible (S) reactions. Wheat breeding line ND495 (1) and cultivar Magnum (17) served as susceptible (positive control) and resistant (negative control) check, respectively.

Retesting 42 resistant accessions against the two additional bacterial strains. A subset of the 50 accessions exhibited resistance to strain BLSW16 in the first screening test, and 42 resistant accessions were randomly selected (Table 1) to retest further against the two additional bacterial strains. Based on initial pathogenicity tests, two strains (BLS Cr25 and BLS Lb74 of *X. translucens* pv. *undulosa*) collected from Carrington (47°27'N, 99°7'W, 464 m asl) and Lisbon (46°26'N, 97°41'W, 332 m asl), North Dakota, were selected, and the inoculations repeated with each of the two additional strains on the 42 accessions. Carrington is located in Foster County, while Lisbon is in Ransom County, and these two locations are approximately 204 km apart. All plant growing conditions, inoculum preparation, inoculation, and disease scoring were the same as described above. The experiments were conducted in 2010. Winter wheat accessions were arranged in a RCBD with three replications. The experiment was repeated a second time with each of the two additional strains of *X. translucens* pv. *undulosa* on separate benches in the greenhouse. Each cone was considered an experimental unit. Flag leaves of the

nine plants per accession were rated in each experiment for each strain. In each experiment, replications were regarded as a random effect and wheat accessions were considered a fixed effect.

Statistical analysis. Disease reactions of the nine infiltrated areas on flag leaves (one site on each flag leaf) of each accession were averaged. To examine the presence or absence of association between region of origin and BLS resistance, country of origin of each accession was classified geographically according to the United Nations designations for World macroregions (27). Nonoverlap of the 95% binomial confidence intervals was used to determine significant differences between geographic regions and percent resistant accessions. Association between BLS resistance and accessions improvement status was analyzed using Fisher's exact test (2).

Variance-type statistic was performed using nonparametric analysis (6). The existence of significance ($P \leq 0.05$) of BLS ratings among wheat accessions for each experiment and the interaction between wheat accessions and experiments was determined first. To determine whether or not resistance in the 42 wheat accessions differed against each of the two additional strains (BLS Cr25 and BLS Lb74) of *X. translucens* pv. *undulosa*, a nonparametric analysis (6,22) was performed for ordinal BLS scorings using the MIXED procedure of SAS with the LSMEANS option (Version 9.2, SAS Institute Inc., Cary, NC). Additionally, comparisons were made between individual wheat accessions and between individual bacterial strains. Fixed effects, mean values, median rank (R_{ij}), relative treatment effects (p_{ij}), variances, and their 95% confidence intervals (CI) were calculated using LD_CI macro (6) to determine the significant differences ($P \leq 0.0001$) between wheat accessions and bacterial strains, and to analyze the interactions between the wheat accessions and the bacterial strains using the MIXED procedure of SAS with the LSMEANS option (Version 9.2, SAS Institute).

Results

Distribution of BLS resistance in different geographic macroregions. Among the 605 winter wheat accessions evaluated, average BLS scores ranged from 0.9 to 6.0. Of these, 8.3% showed scores of 0.9 to 2.0 and were rated as resistant (Fig. 1). Among the susceptible accessions, 200 were rated 2.1 to 3.0, 243 were rated 3.1 to 4.0, and 112 were rated between 4.1 and 6.0 (Fig. 1). Resistant accessions were identified from every macroregion except

Table 1. Responses of winter wheat accessions to *Xanthomonas translucens* pv. *undulosa* strain BLSW16 from Casselton, ND evaluated in the greenhouse

Macroregion	Responses of accessions to BLSW16 ^x			
	Total	S	R	% ^y
Northern Africa	2	2	0	0.0 abc
Northern Europe	43	43	0	0.0 a
Eastern Europe	113	109	4	3.5 a
Southern Europe	69	66	3	4.3 a
South-central Asia	170	159	11	6.5 a
Western Europe	85	77	8	9.4 ab
Eastern Asia	32	28	4	12.5 ab
South America	22	19	3	13.6 ab
Western Asia	26	21	5	19.2 ab
Northern America	35	25	10	28.6 b
Australia-New Zealand	5	3	2	40.0 abc
Total	602 ^z	552	50	8.3

^x Accessions were arranged in randomized complete block design with three replications. Each plastic cone contained three plants. Disease reactions within infiltrated areas on flag leaves were scored between 7 and 10 days after infiltration using a 0 to 6 scale (16). Disease reactions were based on average of nine infiltrated areas on flag leaves (one site on each flag leaf). Disease scores of 0 to 2 or water-soaking less than 10% were considered resistant (R) (26), and 2.1 to 6 were considered susceptible (S).

^y In column, percentage of the total wheat accessions from each region that were resistant to bacterial leaf streak and values followed by the same letters are not significantly different ($P \leq 0.05$) by binomial confidence intervals.

^z Three susceptible accessions not included in regions listed in Figure 1.

northern Africa (only 2 tested) and northern Europe, suggesting that BLS resistance is widely distributed geographically (Table 1). Resistance was significantly ($P \leq 0.05$) more frequent in accessions from North America (28.6%) compared to south-central Asia (6.5%), southern Europe (4.3%), eastern Europe (3.5%), and northern Europe (0%) (Table 1). Based on geographic scale, accessions from Europe had a significantly lower frequency ($P = 0.01$) of BLS resistance (15 of 310 or 4.8%) compared to accessions from the Americas (13 of 57 or 22.8%). Improvement status "cultivated" was associated with BLS susceptibility ($P = 0.05$, whereas improvement status "cultivar" was associated with resistance ($P = 0.05$). There were no significant associations between disease resistance and the landrace or breeding categories.

Reactions of winter wheat accessions to *X. translucens* pv. *undulosa* strain BLSW16. There were 50 accessions that initially showed resistance to BLS, and many also showed some resistance to other important wheat diseases (Table 2). Four accessions had exceptionally low disease ratings (scores of 1 or less): PI 178184, Cltr 11731, PI 583786, and PI 326301. PI 178184 is a landrace accession from eastern Turkey collected in 1948 by J. Harlan. Cltr 11731 and PI 583786 are wheat breeding lines from the midwestern United States. PI 326301 is a cultivar developed in the 1960s in Kazakhstan. The highest frequency of resistance to BLS on a country basis occurred among accessions from China, where resistance was found in two accessions (PI 82886 and PI 83248) from Jiangsu Province, one cultivated accession (PI 435113) from Henan Province, and one cultivar (PI 565178) from Hebei Province. Other resistant accessions from Asia included two accessions each from Iran (PI 243759 and PI 243776), Kazakhstan (PI 326301 and PI 572654), Armenia (PI 94374 and PI 591862), and Turkey (PI 172582 and PI 178184) (Table 2).

Wheat accessions from South America varied in their reactions to BLS. Of 22 South American accessions evaluated, two (PI 436469 and Cltr 15942) from Chile and one (PI 351315) from Argentina were resistant to BLS (Table 2). Among the susceptible accessions, 10 were from Chile and the rest were from Cuba, Peru, Uruguay, and Venezuela.

Ten of the accessions (28.6%) from North America were identified as resistant, including wheat cultivar OAC104 (Cltr 11862) from Ontario, Canada. A high frequency of resistance occurred among accessions from the United States. These accessions were

mainly breeding lines and cultivars, and originated from Kansas (Cltr 11731), Nebraska (Cltr 11984), Indiana (Cltr 13701), Ohio (Cltr 14156), Oregon (PI 565361), Illinois (PI 583672), Oklahoma (PI 583786), and Washington (PI 628640) (Table 2). Cltr 11731 was developed by Kansas State University and contributed to the collection in 1935; and PI 583786 originated in Oklahoma, and was derived from a wide cross and has resistance to *Wheat streak mosaic virus* (21,25).

Inoculation of a subset of the 50 to retest 42 resistant accessions utilizing each of the two additional strains (BLS Cr25 and BLS Lb74) of *X. translucens* pv. *undulosa*. No significant interaction between wheat accessions and experiment was observed. Thus, combined analysis over the two experiments was performed and disease scores were averaged for each accession. The nonparametric analysis (6,22) revealed that wheat accessions differed significantly ($P \leq 0.0001$); however, bacterial strains did not have significant effects ($P = 0.6355$) (data not shown). The interactions between wheat accessions and bacterial strains were significant ($P \leq 0.0001$) (data not shown). Thirty-five wheat accessions had lower median ratings (with a median value of 0 to 2.0), mean rank, and relative marginal effects than the remaining wheat accessions (Table 3). In contrast, one accession (PI 266860) from New Zealand was susceptible to both strains. Six wheat accessions, PI 583786, PI 266142, PI 351338, PI 591862, Cltr 11862, and PI 82886, resistant to BLSW16 showed differential responses to the two additional strains (BLS Cr25 and BLS Lb74) of *X. translucens* pv. *undulosa* (Table 3).

Discussion

Current management strategies to reduce the impact of BLS on wheat production include seed treatment with hot water or chemicals (11–13), and planting resistant cultivars (15). The use of antibiotics to control BLS is not practical due to costs and the risk of developing antibiotic resistance in bacterial populations (14). Therefore, it is imperative to develop resistant wheat varieties for managing BLS. In this study, part of the NSGC core subset of winter wheat accessions was randomly sampled to represent different geographic regions and evaluated in the greenhouse to identify accessions that are resistant to BLS. Accessions with high levels of resistance to multiple strains of *X. translucens* pv. *undulosa* were identified, and these could be useful sources of resistance in winter wheat breeding programs, particularly the North American breeding lines and cultivars which are more likely to be better adapted to U.S. growing conditions compared to landrace materials from overseas.

In the previous studies, spring wheat cultivars Pavon 76, Mochis T88, Nanjing 8331, and Thornbird were found to be resistant to BLS in Mexico (8,10), while winter wheat cultivars Magnum, Bayles, Sawyer, and Terral 101 showed resistance to BLS in the United States (17). Despite efforts made in the past decade to evaluate and characterize resistance to BLS, the available pool of BLS-resistant wheat appears to be inadequate, and more materials should be evaluated to identify additional sources of resistance to BLS for use in wheat breeding programs.

Disease reactions (0 to 6 rating scale), an indicator of susceptibility or resistance within infiltrated areas on flag leaves, were used to identify resistance in wheat germplasm and to determine variation in virulence in bacterial strains and appeared to be the best at 9 days after infiltration for wheat cultivars and bacterial strains tested (16,18). Furthermore, disease reactions were converted into disease severities such as 0, 5, 20, 50, 85, and 100% of water-soaking developed within infiltrated areas of flag leaves and were used to evaluate resistance in wheat (16). Although disease reactions within infiltrated sites differed consistently between susceptible and resistant wheat cultivars, disease severities were altered due to differences in inoculum concentrations and environmental factors (16). More importantly, even weakly virulent bacterial strains developed disease symptoms when high inoculum doses and wounded wheat leaves of susceptible cultivars were used and kept at 100% relative humidity (16). Although field screening

Fig. 1. Frequency of winter wheat accessions ($n = 605$) resistance or susceptibility to *Xanthomonas translucens* pv. *undulosa* strain BLSW16 evaluated in the greenhouse, North Dakota. Disease reaction was assessed visually on the flag leaf of each plant between 7 and 10 days after infiltration on a 0 to 6 disease rating scale (16), where 0 = no visible symptoms, 1 = chlorosis without water-soaked lesions, 2 = water-soaking less than 10%, 3 = water-soaking 10 to 30%, 4 = water-soaking 31 to 70%, 5 = water-soaking 71 to 100%, and 6 = water-soaking extending beyond the infiltrated area. Disease scores of the nine infiltrated areas on flag leaves (one site on each flag leaf) were averaged.

was effective in identifying resistance to BLS, disease reactions on wheat accessions were confounded by plant phenology and genotype $\times$ environment interactions (26). Thus, we performed experiments in controlled environment conditions, and used stringent criteria such as disease scores of less than 2.0 (i.e., less than 10%

water-soaking and chlorosis symptoms) and relatively high bacterial inoculum dose (10^7 CFU/ml) for classifying accessions as resistant to BLS between 7 and 10 days after infiltration. Marked differences in disease resistance among accessions were recorded, and this variation in disease reactions is likely due to genetic diver-

Table 2. Responses of the 50 resistant winter wheat accessions to *Xanthomonas translucens* pv. *undulosa* strain BLSW16 collected from Casselton, ND, and some other foliar pathogens

Accession no. ^r	Name ^s	Region	Country	Status ^t	Ave. disease score ^u	Resistance to other foliar pathogens
PI 266860	Aotea	Australia-New Zealand	New Zealand	C	1.2	Stripe rust ^{v,w}
PI 266861	Arawa	Australia-New Zealand	New Zealand	C	2.0	Stripe rust ^w , powdery mildew
PI 435113	Ping Yuan 50	Eastern Asia	China	Cd	2.0	Leaf rust, stripe rust ^{v,w}
PI 565178	Shichiazuang 5144	Eastern Asia	China	C	1.4	Stripe rust (4 races) ^{v,x} , powdery mildew
PI 82886	C5B	Eastern Asia	China	L	1.6	Leaf rust, stripe rust (2 races) ^{v,x}
PI 83248	F24	Eastern Asia	China	L	1.4	Stripe rust (3 races) ^{v,x}
PI 338387	C 48	Eastern Europe	Poland	C	1.7	Stripe rust (1 race) ^{v,x} , powdery mildew
PI 278423	Filipescu	Eastern Europe	Romania	Cd	1.9	Stripe rust ^v
PI 191287	Tejskaia	Eastern Europe	Russian Federation	C	1.7	BYDV, stripe rust ^v
PI 351202	Moskovskaja 02460	Eastern Europe	Russian Federation	C	1.9	Stripe rust ^v
Cltr 11862	OAC 104	Northern America	Canada	C	2.0	NA ^y
Cltr 11731	Kansas No. 343273	Northern America	United States	B	0.9	BYDV
Cltr 11984	Nebr. Sel. 4CS-21	Northern America	United States	B	1.4	Stripe rust ^v
Cltr 13701	Knox 62	Northern America	United States	C	1.9	NA
Cltr 14156	Logan	Northern America	United States	C	2.0	NA
PI 565361	Prohibition	Northern America	United States	C	2.0	NA
PI 583672	Satanta	Northern America	United States	C	1.6	NA
PI 583786	OK65C93-8	Northern America	United States	B	1.0	Stripe rust (1 race) ^x
PI 628640	Finch	Northern America	United States	C	2.0	Stripe rust ^{v,w}
PI 633885	N02Y5057	Northern America	United States	B	1.6	Stripe rust ^{v,w}
PI 351315	38 M.A.	South America	Argentina	C	1.3	NA
Cltr 15942	CAR 16	South America	Chile	B	1.3	Stripe rust ^w
PI 436469	447	South America	Chile	L	1.4	Stripe rust (2 races) ^x
PI 243759	5499	South-central Asia	Iran	L	1.3	Powdery mildew
PI 243774	5515	South-central Asia	Iran	L	1.6	NA
PI 243776	5519	South-central Asia	Iran	L	1.1	NA
PI 621837	IWA8609676	South-central Asia	Iran	L	2.0	Stripe rust (2 race) ^{v,x}
PI 627456	IWA8602425	South-central Asia	Iran	L	2.0	NA
PI 627521	IWA8602602	South-central Asia	Iran	L	2.0	Stripe rust (5 races) ^{v,w,x}
PI 627649	IWA8603072	South-central Asia	Iran	L	1.7	Stripe rust (1 race) ^x
PI 326301	Alabasskaja	South-central Asia	Kazakhstan	C	1.0	Stripe rust ^v
PI 572654	Grekum 3	South-central Asia	Kazakhstan	C	2.0	Stripe rust ^{v,w}
PI 429689	NW86A	South-central Asia	Nepal	L	1.9	Stripe rust (1 race) ^{v,w,x}
PI 565404	Tadzykskaja 13	South-central Asia	Tajikistan	C	2.0	NA
PI 344545	Unknown	Southern Europe	Croatia	Cd	2.0	NA
PI 266142	Diamiano Chiesa	Southern Europe	Italy	C	1.7	Leaf rust, stripe rust (1 race) ^x
PI 345476	473	Southern Europe	Serbia	L	1.6	Stripe rust ^v
PI 591862	Artashati 42	Western Asia	Armenia	C	2.0	Stripe rust ^{v,w}
PI 94374	65BSR	Western Asia	Armenia	L	2.0	Stripe rust (2 races) ^{v,x}
PI 57185	CI 7132	Western Asia	Georgia	L	1.5	NA
PI 172582	8728	Western Asia	Turkey	L	1.3	Stripe rust (1 race) ^x , snow mold ^z
PI 178184	Tir	Western Asia	Turkey	L	0.8	NA
PI 351046	69Z2.179/1380C	Western Europe	Austria	L	2.0	Stripe rust ^v
PI 338358	CAMA	Western Europe	Belgium	C	1.6	Stripe rust (3 races) ^x
PI 340685	Coutiches	Western Europe	France	C	2.0	Stripe rust ^{v,w}
PI 351314	Hybride de la Noue	Western Europe	France	C	2.0	Stripe rust (3 races) ^{v,w,x}
PI 278249	Ostmarker	Western Europe	Germany	L	2.0	Stripe rust ^{v,w}
PI 285939	Dornburger Heils Dickhopf	Western Europe	Germany	C	2.0	Stripe rust ^v
PI 351338	K43 Kienles	Western Europe	Germany	B	1.6	Stripe rust ^{v,w}
PI 350974	69Z2.104/672A	Western Europe	Switzerland	L	1.5	Stripe rust ^{v,w}

^r Winter wheat accession number in USDA National Small Grains Collection, Aberdeen, ID. Data on some other foliar pathogens were retrieved from the GRIN database (<http://www.ars-grin.gov/npgs/verified> 17 June 2009).

^s Entry name for winter wheat accessions in USDA National Small Grains Collection.

^t IS = improvement status, B = breeding line, C = cultivar, Cd = cultivated, and L = landrace. Accessions were arranged in randomized complete block design with three replications. Each plastic cone contained three plants (total nine plants per accession).

^u Disease reactions within infiltrated areas were scored between 7 and 10 days after infiltration using a 0 to 6 scale (16), where 0 = no visible symptoms, 1 = chlorosis without water-soaked lesions, 2 = water-soaking less than 10%, 3 = water-soaking 10 to 30%, 4 = water-soaking 31 to 70%, 5 = water-soaking 71 to 100%, and 6 = water-soaking extending beyond infiltrated area. Disease reactions of the nine infiltrated areas on flag leaves (one site on each flag leaf) were averaged. Disease scores of 0 to 2 or water-soaking less than 10% were considered resistant (R) (26), and 2.1 to 6 were considered susceptible (S).

^v Resistant in the field at adult plant stage at Mount Vernon.

^w Resistant in the field at adult plant stage at Pullman.

^x Resistant at seedling stage to specific races of the stripe rust pathogen.

^y NA = data were not available for other foliar pathogens.

^z Bruehl (5).

sity among wheat accessions tested. The resistant accessions identified in this study have not been reported previously (10,15,18,26) and may represent novel sources of resistance to *X. translucens* pv. *undulosa*.

Landrace accessions in the NSGC were originally collected from farmers' fields or markets either before the era of modern plant breeding or later in less favorable environments where modern cultivars are not grown (2). No significant association between landrace status and BLS resistance was observed. Improvement status "cultivated" is used when it is uncertain if the accession is a landrace or cultivar (2), and in the present study classification as cultivated was associated with BLS susceptibility. However, BLS resistance was significantly associated with accessions classified as cultivars, which were all presumably developed by wheat breeding

programs. Since many of these materials were from North America, this result may indicate that breeding programs in the United States have made some progress toward BLS resistance simply by selecting in a disease conducive environment. The identified resistant cultivars and breeding lines should also possess other good agronomic traits relative to landrace accessions. Perhaps they can be used directly as parents in wheat breeding programs for developing wheat varieties resistant to *X. translucens* pv. *undulosa*.

In this study, resistance differences among wheat accessions were evident when 42 BLS-resistant accessions were further assessed with each of the two additional BLS strains from different geographic origins. Among these, 35 winter wheat accessions were resistant to all three strains and seven accessions were resistant to either one or two strains. This result demonstrates that strains of *X.*

Table 3. Median, mean rank (R_{ij}), and relative treatment effects (p_{ij}) along 95% confidence intervals (CI) relative treatment effects for bacterial leaf streak (BLS) ratings on the 42 resistant winter wheat accessions randomly selected and retested with the two additional strains (BLS Cr25 and BLS Lb74) of *Xanthomonas translucens* pv. *undulosa* from Carrington and Lisbon, ND, respectively

ACPNO ^x	BLS Cr 25				BLS Lb 74			
	Median ^y	R_{ij}	$\hat{p}_{ij}$	95% CI for p_{ij}	Median	R_{ij}	$\hat{p}_{ij}$	95% CI for p_{ij}
PI 344545	1	322.6	0.3	(0.260, 0.572) ^z	0	77.70	0.1	(0.054, 0.170)
PI 278423	1	261.7	0.3	(0.161, 0.558)	1	218.3	0.2	(0.150, 0.449)
PI 350974	1	322.6	0.3	(0.260, 0.572)	1	186.7	0.2	(0.127, 0.394)
PI 435113	1	92.9	0.1	(0.069, 0.190)	2	623.4	0.7	(0.662, 0.872)
Cltr 11984	1	280.4	0.3	(0.229, 0.501)	2	417.6	0.4	(0.395, 0.654)
PI 172582	1	244.0	0.3	(0.169, 0.492)	2	449.2	0.5	(0.456, 0.670)
PI 351202	1	300.2	0.3	(0.205, 0.589)	2	417.6	0.4	(0.395, 0.654)
PI 351314	1	218.3	0.2	(0.150, 0.449)	2	390.7	0.4	(0.390, 0.595)
PI 583672	1	354.0	0.4	(0.229, 0.686)	2	534.7	0.6	(0.568, 0.764)
Cltr 13701	2	471.3	0.5	(0.422, 0.745)	1	264.0	0.3	(0.227, 0.458)
Cltr 14156	2	511.1	0.5	(0.485, 0.776)	1	232.3	0.2	(0.200, 0.406)
PI 94374	2	439.7	0.5	(0.368, 0.725)	1	295.7	0.3	(0.259, 0.501)
PI 178184	2	439.7	0.5	(0.368, 0.725)	1	217.1	0.2	(0.173, 0.403)
PI 243759	2	385.9	0.4	(0.344, 0.631)	1	232.3	0.2	(0.200, 0.406)
PI 243776	2	377.6	0.4	(0.264, 0.696)	1	171.4	0.2	(0.107, 0.388)
PI 338358	2	374.0	0.4	(0.278, 0.673)	1	155.0	0.2	(0.110, 0.324)
PI 338387	2	444.4	0.5	(0.406, 0.703)	1	307.3	0.3	(0.232, 0.570)
PI 429689	2	534.7	0.6	(0.568, 0.764)	1	334.2	0.4	(0.241, 0.625)
Cltr 11731	2	444.4	0.5	(0.406, 0.703)	2	476.1	0.5	(0.468, 0.718)
Cltr 15942	2	479.4	0.5	(0.422, 0.761)	2	296.9	0.3	(0.234, 0.539)
PI 57185	2	449.2	0.5	(0.456, 0.670)	2	417.6	0.4	(0.395, 0.654)
PI 83248	2	390.7	0.4	(0.390, 0.595)	2	385.9	0.4	(0.344, 0.631)
PI 266861	2	354.2	0.4	(0.299, 0.603)	2	354.2	0.4	(0.299, 0.603)
PI 278249	2	296.9	0.3	(0.234, 0.539)	2	312.1	0.3	(0.263, 0.540)
PI 285939	2	449.0	0.5	(0.348, 0.760)	2	296.9	0.3	(0.234, 0.539)
PI 326301	2	354.2	0.4	(0.299, 0.603)	2	370.7	0.4	(0.309, 0.632)
PI 340685	2	327.3	0.3	(0.297, 0.539)	2	370.7	0.4	(0.309, 0.632)
PI 345476	2	460.9	0.5	(0.420, 0.725)	2	281.7	0.3	(0.207, 0.537)
PI 351046	2	400.9	0.4	(0.292, 0.716)	2	385.9	0.4	(0.344, 0.631)
PI 351315	2	359.0	0.4	(0.340, 0.570)	2	449.2	0.5	(0.456, 0.670)
PI 436469	2	343.8	0.4	(0.302, 0.574)	2	507.8	0.5	(0.548, 0.723)
PI 565178	2	483.0	0.5	(0.400, 0.783)	2	561.6	0.6	(0.594, 0.800)
PI 565361	2	422.3	0.5	(0.452, 0.610)	2	507.8	0.5	(0.548, 0.723)
PI 565404	2	390.7	0.4	(0.390, 0.595)	2	359.0	0.4	(0.340, 0.570)
PI 572654	2	359.0	0.4	(0.340, 0.570)	2	422.3	0.5	(0.452, 0.610)
PI 583786	1	248.8	0.3	(0.199, 0.456)	3	615.3	0.7	(0.661, 0.859)
PI 266142	2	354.2	0.4	(0.299, 0.603)	3	596.6	0.6	(0.624, 0.846)
PI 351338	2	561.6	0.6	(0.594, 0.800)	3	679.1	0.7	(0.766, 0.915)
PI 591862	2	417.6	0.4	(0.395, 0.654)	3	601.8	0.6	(0.559, 0.885)
Cltr 11862	3	588.4	0.6	(0.624, 0.832)	2	449.2	0.5	(0.456, 0.670)
PI 82886	3	601.8	0.6	(0.559, 0.885)	2	370.7	0.4	(0.309, 0.632)
PI 266860	3	685.3	0.7	(0.769, 0.923)	3	615.3	0.7	(0.661, 0.859)
Magnum	1	200.7	0.2	(0.182, 0.340)	2	327.3	0.3	(0.297, 0.539)
(Resistant check)								
ND 495	4	776.6	0.8	(0.970, 0.985)	5	780.3	0.8	(0.975, 0.989)
(Susceptible check)								

^x Accessions number in USDA National Small Grains Collection, Aberdeen, ID.

^y In each experiment, disease scores of the nine infiltrated areas on flag leaves (one site on each flag leaf) were averaged. Accessions were arranged in randomized complete block design with three replications. Each plastic cone was considered a replication and contained three plants. Two independent experiments were conducted in the greenhouse. Disease ratings were recorded visually on the flag leaf of each plant between 7 and 10 days after infiltration on an ordinal 0 to 6 rating scale (16), where 0 = no visible symptoms, 1 = chlorosis without water-soaked lesions, 2 = water-soaking less than 10%, 3 = water-soaking 10 to 30%, 4 = water-soaking 31 to 70%, 5 = water-soaking 71 to 100%, and 6 = water-soaking extending beyond the infiltrated area. Median, R_{ij} , p_{ij} , and CI values were estimated (6,22). Median value less than 2 was resistant and above 2 was susceptible.

^z First and second values in parentheses indicate lower limit and upper limit at 95% CI, respectively (6).

translucens pv. *undulosa* interact differently with wheat accessions. Thus, selection of strains can significantly affect the reactions of wheat germplasm to *X. translucens* pv. *undulosa*, and the same sources of resistance in winter wheat to *X. translucens* pv. *undulosa* may be ineffective against predominant virulent strains present in different wheat-growing regions of the United States. The best winter wheat accessions could be those that have the relatively lowest BLS ratings or median values. It appears that the best winter wheat accessions for breeding programs could be identified by combining the results from evaluation of wheat accessions individually inoculated with a set of BLS strains and comparing the median values. Alternatively, the use of an inoculum mixture with high concentration (10^7 CFU/ml) of virulent strains of *X. translucens* pv. *undulosa* from different geographic areas or with known genetic differences may lead to the development of winter wheat cultivars with improved resistance to *X. translucens* pv. *undulosa*.

It has been suggested that identifying BLS-resistant wheat varieties has been hampered because resistance to this disease is incomplete (8), and presumably is governed by quantitative trait loci (10). Field experiments conducted in Mexico indicated that BLS resistance in five wheat cultivars (Turaco, Alondra, Angostura, Mochis, and Pavon 76) is governed by five genes: *Bls1/bls1*, *Bls2/bls2*, *Bls3/bls3*, *Bls4/bls4*, and *Bls5/bls5* (10,11). Among these five genes, *Bls1* is present in wheat cultivars Angostura, Mochis, and Pavon 76 (10). Although there were significant differences in the levels of resistance of the wheat accessions against three bacterial strains used in this study, further studies related to the inheritance of resistance to *X. translucens* pv. *undulosa* in these accessions, including allelism relationships, are necessary to test if these accessions have the same genes as reported earlier (10,15) or if they contain a novel gene(s).

The disease significantly reduces grain yield and quality of both spring and winter wheat. Yield loss due to BLS was estimated as high as 30 to 40% in sprinkler irrigated fields in Idaho (12). Both kernel weight and plumpness were severely affected and negatively correlated with BLS severity on the flag leaf of wheat and barley (23). In Mexico, single tiller studies revealed that 11 to 29% of grain weight per spike was lost when 50% of the flag leaf was affected by the disease (9). BLS has been re-emerging as a potential threat to wheat production, particularly in the Northern Great Plains of the United States. This may be due to lack of effective management tools. Crop rotation, seed certification, seed treatments, bactericides, and host resistance have been investigated in the past decades (7,9,11,13,15,18,26). Although these disease management tactics have shown some promise, an adequate control of BLS has not been provided for wheat producers. Some of the accessions identified in this study may have useful resistance to *X. translucens* pv. *undulosa* and may be a key component of integrated disease management. Deploying wheat varieties with resistance to BLS would not only curtail local carryover of bacteria in seed and regional movement of this seedborne pathogen through exchange of wheat germplasm, but also could reduce yield losses due to the disease.

Among the 50 BLS-resistant accessions, the majority (70%) were resistant to multiple races of stripe rust (Table 2). Four accessions (PI 266861, PI 565178, PI 243759, and PI 338387) were resistant to powdery mildew in addition to some races of stripe rust. Three accessions (PI 82886, PI 83248, and PI 435113) from China were resistant to leaf rust and stem rust. Only one accession (PI 191287) was resistant to both BYDV and stripe rust. One accession (PI 172582) was found previously to be resistant to snow mold (5). Thus, these accessions are likely to carry some other foliar disease resistances useful to wheat breeding programs.

In conclusion, we have used intense disease evaluation methods (e.g., high inoculum dose of multiple virulent strains collected from different geographic areas, stringent disease rating criteria on the flag leaf, and a conducive environment for BLS development) in the greenhouse, and identified potentially diverse BLS-resistant winter wheat germplasm. These panels of genetic resources will provide stable resistance to BLS when different sources are com-

bined and utilized in winter wheat breeding programs. Importantly, several BLS-resistant accessions also possessed resistance to multiple diseases such as BYDV, leaf rust, powdery mildew, snow mold (5), *Wheat streak mosaic virus* (21,25), and different races of stripe rust; and thus, these might be useful to combine resistance genes into an adapted winter wheat cultivar to achieve broad-spectrum resistance to multiple pathogens in wheat.

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