IDENTIFICATION AND GENOMIC MAPPING OF RESISTANCE TO BACTERIAL LEAF STREAK IN WHEAT

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Bacterial leaf streak (BLS) of wheat, caused by *Xanthomonas translucens* pv. *undulosa*, is an important disease of wheat worldwide. Use of resistant cultivars is the most preferred way of managing BLS. The objective of this research was to identify highly resistant wheat germplasm and map BLS resistance genes. Two collections of wheat germplasm, including triticale and spring wheat, were evaluated for their reactions to BLS. A wide range of reactions from highly resistant to highly susceptible were observed for both collections. Five triticale accessions and twenty-four spring wheat genotypes with high level of resistance to BLS were identified. Genome wide association studies using the spring wheat collection revealed seven quantitative trait loci on chromosomes 1A, 2B, 3B, 5A, 5B, 6A, and 7A significantly associated with BLS resistance. The identified resistant lines and molecular markers have a potential to be utilized in the breeding programs aiming to improve BLS resistance.

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GENERAL INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most cultivated crops worldwide and serves as a major staple food in many countries of the world. Bacterial leaf streak (BLS), caused by the Gram-negative bacterium *Xanthomonas translucens* pv. *undulosa* (*Xtu*), is a common disease of wheat around the world (Duveiller et al. 1997). The causal bacterium can also infect and survive on several other small grain crops and grass species. BLS is an economically important disease because it has the potential to cause yield losses of up to 40% (Forster and Schaad 1988). In recent years, the disease has become more evident in the Upper Midwest region where the majority of the US spring hard red spring and durum wheat is produced (Adhikari et al. 2012; Kandel et al. 2012).

Currently, it is nearly impossible to control BLS in the field. Seed serves as a source of primary inoculum (Smith et al. 1919). Using clean seeds or applying seed treatments can reduce disease incidence, but cannot stop the inoculum spread from other fields (Duveiller et al. 1997). No chemical has been found to be effective or practical in controlling the bacterium in the field (McMullen and Adhikari 2011). Development and deployment of resistant cultivars appears to be the only option.

Disease evaluations have been conducted on a large number of wheat cultivars/lines in the field or greenhouse and it was found that most of them were susceptible (Tillman et al. 1996; Adhikari et al. 2011; Kandel et al. 2012). Some wheat genotypes were identified with partial resistance; however, resistance in these genotypes has not been further confirmed, and also controversial results were obtained for a few resistant genotypes when they were evaluated in different environmental conditions (Tillman et al. 1996). Wheat germplasm with high levels of resistance has not been identified. However, several triticale lines were reported to possess high

levels of resistance to wheat BLS (Cunfer and Scolari 1982; Johnson et al. 1987). Inheritance of BLS resistance has not been widely studied due to the lack of reliable resistant sources for the utilization in genetic analysis, but a few studies have suggested it is quantitative (Duveiller et al. 1993; Tillman and Harrison 1996). Molecular mapping with statistical analysis can be used to estimate and locate the genes conferring quantitative traits, known as quantitative trait loci (QTL). Few studies have been done to map BLS-resistance QTL using bi-parental or natural populations (Adhikari et al. 2012b; Gurung et al 2014; Kandel et al. 2014)

In this study, we first evaluated a large collection of triticale lines for reaction to the local *Xtu* strains under greenhouse conditions to identify germplasm that has high levels of resistance. Secondly, a subset of worldwide spring wheat core collection was evaluated in the field and used to map resistance QTL through an association mapping approach.

LITERATURE REVIEW

Wheat: Classification, Evolution, and Production

Classification and evolution of wheat

Wheat is a member of the grass family (Poaceae) and belongs to the genus *Triticum*. The family Poaceae also includes other important crops such as rice, maize, barley and rye. The genus *Triticum* is a member of the tribe Triticeae which contains about 300 species (Clayton and Renvoize 1986). Based on the current nomenclature system, genus *Triticum* consists of six species that are at three polyploidy levels, including diploids: *T. monococcum* L. (2n=14, AA genome), *T. urartu* Tumanian ex Gandilyan (2n=14, AA genome), tetraploids: *T. turgidum* L. (2n=28, AABB genome) and *T. timopheevii (Zhuk.) Zhuk.* (2n=28, AAGG genome), and hexaploids: *T. aestivum* L. (2n=42, AABBDD genome) and *T. Zhukovskyi* Menabde & Ericz (2n=42, AAAAGG genome) (Matsuoka 2011). Each species has several subspecies which exist either in a wild or cultivated form. Currently, *T. turgidum* L. subsp. *durum* (desf.) Husn, and *T. aestivum* L. subsp. *aestivum*, known as durum and bread wheat, respectively, are the most widely cultivated wheat.

Archaeological evidence indicates that wheat was originated in the Near East, particularly in the Fertile Crescent region (Matsuoka 2011). Research has strongly suggested that wheat species evolved from the lower to higher polyploidy levels through natural hybridization with *Aegilops* species followed by allopolyploidization (Tsunewaki 2009). The cultivated tetraploid wheat *T. turgidum* L. subsp. *durum* (2n= 28, AABB) arose from crosses between *T. monococcum* L. (AA) and a B genome donor, most likely, *A. speltoides* (Monte et al. 1993). The current common wheat (2n=42, AABBDD) was the result of the natural cross between *T. turgidum* (AABB) and *A. taushii Coss*, a D genome donor (Kihara 1944; McFadden and Sears 1944).

Owing to the advance in tissue culture and chromosomal doubling techniques, the rapid development of hexaploid wheat, known as synthetic wheat, can be made by artificially crossing *T. turgidum* and *A. taushii* followed by embryo rescue and chromosome doubling. This process can be utilized to transfer useful genes from *A. taushii* accessions to common wheat (Jiang et al. 1994).

Wheat production in the world, US and ND

Wheat is one of the most important cereal crops, providing more nourishment and energy for human than any other food crops in many countries (Leonard and Martin 1963). The total harvested area and production of wheat in 2014 was 221.970 million hectares and 725.034 million metric tons (Economic Research Service, USDA, updated on 3/11/2015). The top four wheat producers in the world are the European Union, China, India, and the United States (US). Wheat is the third most planted field crop in the US only behind corn and soybean. The US produced 55.12 million metric tons of wheat from 18.76 million hectares of land in 2014 with about half of its production exported (Economic Research Service, USDA, updated on 3/11/2015). Wheat is cultivated in 42 states of the US with Kansas and North Dakota being the major wheat producers.

Based on the differences in growth habit and other genetic and physiological characters, wheat is divided into six classes, including hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), durum, hard white (HW) and soft white (SW). HRW is the most produced wheat in the US accounting for 40% of production, followed by HRS, SW, SRW and durum wheat at 20%, 20%, 14% and 5%, respectively. Although wheat is one of the most important crops in the US, it has faced many challenges in the past decade, such as weak domestic markets and low profitability.

Agriculture is the leading revenue-generating industry in North Dakota (ND) which accounts for about 25% of the state's economy. Wheat is a major agricultural commodity generating economic revenues estimated at 5 to 7 billion dollars annually (North Dakota Wheat Commission, 2012). Three classes of wheat are grown in ND: HRS (74%), durum wheat (25%) and HRW (1%) (North Dakota Wheat Commission, 2012). In 2012-2013, North Dakota produced 257 million bushels HRS and 43 million bushels durum, which is about 50% and 65% of the US total HRS and durum production, respectively (North Dakota Wheat Commission annual report, 2012).

Bacterial Leaf Streak (BLS)

Distribution and economic importance

BLS was first reported on barley in 1917 (Jones et al. 1917) and then on wheat (Smith et al. 1919). Similar diseases have also been later found in other cereal crops and grass species, such as rye, triticale, oat, brome and quack grass (Hagborg et al. 1942; Fang et al. 1950). These diseases are caused by a group of genetically related bacteria that now are called *Xanthomonas translucens* with different pathovar names (Vauterin et al. 1992). In wheat, the bacterium has been named as *Xanthomonas translucens* pv. *undulosa* (*Xtu*). BLS is a common disease of wheat in many wheat-growing areas in the world. The occurrence of BLS has been reported in many countries from North America, South America, Asia, Africa, Oceania and most parts of Europe (Duveiller et al. 1997). However, the disease has not been established in Western Europe, which might be due to unfavorable environmental conditions and extensive quarantine efforts (Paul and Smith 1989; Duveiller et al. 1997)

Since the first report by Smith et al. (1919) in Indiana, BLS has been found in many places of the US and the outbreak and epidemic of the disease mostly occurred in the

southeastern regions (Milus and Mirlohi 1994; Tubajika et al. 1999). In recent years, the incidence of BLS has increased in the Upper Midwest region of the US including North Dakota, Minnesota and South Dakota (McMullen and Adhikari 2011; Adhikari et al. 2012a; Kandel et al. 2012). Three possible reasons were speculated as the contributing factor to the increasing BLS incidence, including increased acreage of fall sown winter wheat which may serve as the source of primary inoculum for spring wheat, the wide use of Fusarium head blight resistant varieties which are susceptible to *Xtu*, and favorable climatic conditions during the late stage of growth season (Adhikari et al. 2012a).

BLS has the potential to cause significant yield losses. Yield losses due to BLS are generally 10% or less, but severe infection can lead to a loss up to 40% on highly susceptible cultivars (Waldron 1929, Foster 1982, Forster and Schaad 1988). In extreme situations, the disease can cause sterile spikes resulting in complete crop losses (Burton 1931; Forster and Schaad 1988). Studies have indicated that yield loss due to BLS generally correlate with disease severity on flag leaves (Shane et al. 1987; Duveiller and Maraite 1993). Among yield components, grain test weight is most affected by BLS infection (Shane et al. 1987; Tillman et al. 1999). In addition, BLS infection can also affect protein content of the grain resulting in quality reduction (Shane et al. 1987)

Symptoms

The bacterium mainly infects leaves and spikes. On leaves, the disease is characterized by the longitudinal stripes that can extend several centimeters between the veins, and the production of milky exudates can also be seen under humid conditions (Duveiller et al. 1993). The early symptoms of BLS are translucent water-soaked streaks that become dry and later develop into brown or necrotic longitudinal lesions. In the later growing season, the lesions

become larger and come together to form large necrotic areas which are sometimes confused with leaf spot diseases caused by fungi (McMullen and Adhikari 2011). When the bacterium infects the spikes, dark purple to black streaks can be seen on the glume, which has been referred to as black chaff (McMullen and Adhikari 2011).

Epidemiology

The disease cycle for BLS remains largely unknown and there are many questions that need to be addressed. These questions include what are the major sources of primary inoculum are, how the pathogen can rapidly spread, and which environmental conditions are favorable for disease. Seed serves as a source of primary inoculum because the bacterium was readily recovered from the seeds that were harvested from the infested field. In addition, the disease occurred on the seedling if the planted seeds were infected (Smith et al. 1919; Boosalis 1952; Forster and Schaad 1988; Milus and Mirlohi 1995). The survival rate of bacteria on the seeds and possibility of transmission to seedlings were dependent on the storage conditions, length of storage, and the level of susceptibility of genotypes (Boosalis 1952; Forster and Schaad 1990; Milus and Mirlohi 1995). The bacterium may also survive on grasses and weeds. Some weeds and grasses are natural hosts for the bacterium and the others may serve as the place for the bacterium to overwinter as epiphytic populations (Wallin 1946; Fang et al. 1950; Boosalis 1952; Thompson et al. 1989). Contradictory results were obtained on if the bacterium can survive in the soil and crop debris (Boosalis 1952; Cunfer 1988; Milus and Mirlohi 1995).

Warm and humid conditions are thought to be important for BLS development because it was more often found in wet seasons or in sprinkler irrigated fields in combination of warm temperature; however, the exact conditions conducive to BLS development are not well known. Epidemic of BLS has been reported to be sporadic and vary from year to year (Bamberg 1936;

Duveiller et al. 1991; Tubajika et al. 1998). Artificial introduction of disease in field plots was not easy (Boosalis 1952; Duveiller et al. 1997), which made it difficult to observe the relationship of weather conditions with the BLS epidemics. Duveiller and Maraite (1995) showed that temperature is more important than other factors to initiate epidemics. Other factors implicated in BLS epidemics include damage from wind, hail and frost, dew period, and host genotypes (Duveiller et al. 1997). Under extremely humid conditions, yellow bacterial exudates form on the surface of the water-soaked streaks and the bacteria can spread to healthy leaves by rainfall and winds (Duveiller et al. 1997).

Management

Since seed is considered a source of primary inoculum, use of clean seed might be a way of reducing BLS incidence. Different methods have been developed to detect the bacterium in seeds, including dilution plating with use of selective media, seedling infection assays, serodiagnostic assays, and PCR amplification (Schaad and Forster 1985; Bragard and Verhoyen 1993; Maes et al. 1996). Seed disinfection methods have also been developed to eliminate the bacterium by using different chemicals or physical ways (Forster 1982; Forster et al. 1990); however, the effectiveness of seed treatments is still questionable because of contradictory results obtained from different studies (Duveiller et al. 1997). It was shown that if the bacterium population in the seeds is lower than 1000 cfu per gram seed, there would be no field epidemics; therefore, seed treatments are not needed.

Role of cultural practices in BLS disease control is not well understood. Crop rotation is not considered a major control strategy because the bacterium cannot survive in debris for a long time (Milus and Mirlohi 1995). Some grasses and weeds may provide a place for overwintering; therefore, removing a ‘Green Island’ can also reduce the level of primary inoculum. As soon as

the disease initiates in the field, there is no way to stop its progression and spread. No chemicals other than antibiotics have been shown to effectively inhibit the growth of the bacterium in the field; however, application of antibiotics is expensive and impractical due to the persistence of inoculum in nature (McMullen and Adhikari 2011). Using resistant cultivars is the most preferred way and the only way of controlling BLS; however, current cultivars are susceptible to BLS (Kandel et al. 2012). In general, the management of BLS is nearly impossible at present.

The pathogen and its classification

Jones et al. (1917) first reported BLS in barley and named the bacterial pathogen as *Bacterium translucens*. Later, BLS was reported in wheat by Smith et al. (1919), and the name given for the pathogen was *Bacterium translucens* var. *undulosum* because it morphologically resembled the barley pathogen. Cross inoculation with two pathogens indicated that the barley pathogen was only weakly pathogenic to wheat while the wheat pathogen was able to infect both hosts (Smith et al. 1919). Reddy et al. (1924) isolated a similar bacterium from rye and named as *Bacterium translucens* var. *secalis* indicating the host specificity of this bacterium to rye. Bamberg (1936) found that the strains isolated from wheat, barley and rye were also able to infect bromegrass, einkorn and oat. The genus for this group of bacteria was renamed several times from *Bacterium*, to *Pseudomonas*, and to *Phytomonas*. Dawson (1939) created the genus of *Xanthomonas* and included *X*. *translucens*. Hagborg (1942) classified *X. translucens* into five formae speciales based on the natural host and the hosts by artificial inoculations, including f. sp. *hordei* (naturally occurs on barley, produces disease only on barley), f. sp. *undulosa* (naturally occurs on wheat and rye, produce disease on wheat, barley and rye), f. sp. *secalis* (naturally occurs on rye, produces disease only on rye), f. sp. *hordei-avenae* (naturally occurs on barley, produces disease on barley and oat), and f. sp. *cerealis* (naturally occurs on wheat, produces

disease on wheat, barley, rye and oat). Fang et al. (1950) argued that f. sp. *cerealis* and *hordei-avenae* should be combined with f. sp. *undulosa* and *hordei*, respectively, and given the name of f. sp. *cerealis* for strains that naturally occur on smooth brome grass and quack grass but can produce disease on wheat, barley, rye and two grasses by artificial inoculations. Classification by Fang et al. (1950) also included f. sp. *phleipratensis* which was originally identified by Wallin and Reddy (1945) from timothy grass. The overlap of host ranges between different f. sp. was reported by Dye and Lelliott (1974), but their names were retained and placed into *X*. *campestris* as different pathovars, including *X*. *campestris* pv. *cerealis*, pv. *hordei*, pv. *secalis*, *translucens*, and pv. *undulosa*.

Vauterin et al. (1992) systematically analyzed Xanthomonades from cereals and grasses using protein profiling, fatty acids analysis and DNA hybridization. It was found that the five *X*. *campestris* pathovars *cerealis*, *hordei*, *secalis*, *translucens*, and *undulosa* were highly related, and described as the "translucens group". This group was phylogenetically related to the bacteria that cause bacterial wilt on forage and pasture grasses, referred to as the "graminis group". In the subsequent reclassification made by Vauterin et al. (1995), a new species name *X*. *translucens* was proposed to encompass all pathovars from the translucens and graminis groups. Bragard et al. (1997) specifically analyzed 68 *X*. *translucens* strains from small grains or grass species using a pathogenicity test, AFLP and fatty acid analysis. The study suggested that *X*. *translucens* pv. *translucens* is a synonyms of *X*. *translucens* pv. *hordei*, and pathovars *cerealis*, *translucens* and *undulosa* may be true separate biological entities with pv. *translucens* only pathogenic on barley, pv. *undulosa* pathogenic to both barley and wheat and pv. *cerealis* pathogen to barley, wheat, oat and bromegrass. Cunfer and Scolari (1982) also found that the strains from triticale are pathogenic to wheat, barley and triticale, while strains from barley are only virulent on barley.

Recently, 33 bacterial strains isolated from diseased ornamental asparagus were identified as *Xtu* using DNA fingerprinting and cross inoculation (Rademaker et al. 2006). It is extraordinary because the hosts described to date for *X. translucens* have been limited to *Gramineae* and *Poaceae* but the asparagus belongs to a distant family *Liliaceae*.

Host specificity and variation in virulence

As mentioned above, the nomenclature and classification of *X. translucens* has been difficult largely due to the fact that strains vary greatly in host range. Some strains have a broad host range infecting several cereal species whereas others only infect one or two (Mellano and Cooksey 1988). In many bacterial disease systems, a narrow host range is determined by bacterial avirulence genes that do not allow bacteria to cause infection on other hosts. Co-inoculation of a broad host range and a narrow host range *X. translucens* strain led to the wide host range reaction suggesting host specificity for *X. translucens* is not determined by avirulence genes, rather genetic factors that provide function for pathogenicity to specific host (Mellano and Cooksey 1988). In two mutation studies using Tn5 insertion or chemical reagents, shift from a broad host range to narrow host range was usually observed, but not the other way around, further indicating that the broad host range strains possess pathogenicity factors for different hosts (Mellano and Cooksey 1988, Waney et al. 1991).

X. translucens strains differ in host specificity, but evidence for cultivar specificity is not strong. Milus and Chalkley (1994) analyzed pathogen virulence of 81 *Xtu* strains by inoculating on 19 wheat cultivars. Significant differences were observed among strains for virulence and also among the wheat cultivars for resistance/susceptibility. However, no significant interaction was detected between strains and cultivars indicating no race differentiation for *Xtu*. However, Adhikari et al. (2012a) detected a significant strain-cultivar interaction in 226 strains from North

Dakota on 12 wheat cultivars. Furthermore, the authors also revealed high levels of genetic diversity in these 226 strains using rep-PCR techniques. It is very interesting to note that a few narrow host range mutants described in Mellano and Cooksey (1988) also showed loss of pathogenicity towards certain cultivars in other hosts. More research is needed to determine the cultivar specificity of wheat-*Xtu* interaction by using more pathogen strains and host genotypes.

Genetics and genomics of host-pathogen interaction

Pathogenicity/virulence of *Xanthomonas* species are mainly determined by two sets of genes, one for type III secretion system (T3SS) and the other for T3SS effectors (T3Es). T3Es are secreted through T3SS into plant cell and function as important avirulence or virulence factors (Bogdanove et al. 2011). Waney et al. (1991) obtained 12 Tn5 insertion mutants of *X. translucens* strains that lost its ability to cause disease on all hosts and failed to induce any HR symptoms on cotton. These mutants likely had mutations on T3SS, and were unable to secret T3Es into plant cell to cause disease. The recent genomic sequences of two *X. translucens* strains, including Xtg29 of *X. t.* pv. *graminis*, and DAR61454 of *X.t.* pv. *undulosa*, have shown that the complete set of T3SS genes are presented and fully functional in *X. translucens* genomes (Wichmann et al. 2013; Gardiner et al. 2014). Although there are small inversions and rearrangements, the organization of T3SS genes in both *X. translucens* strains were highly comparable to the related *Xanthomonas* sp., such as *X. oryzae* pv. *oryzae*, *X. campestris* pv. *vesicatoria*, and *X. axonopodis* pv. *citri*. Both genomic sequences revealed 22 to 35 effector proteins that are homologous to T3Es previously identified in other *Xanthomonas* spp. These effectors were hypothesized to play a role in the disease, but no functional analysis has been conducted for these effector genes. Recently, a type of highly conserved T3SS effectors, called transcription activated like effectors (TALEs), have been identified in *Xanthomonas* spp. After

being secreted into plant cells, these effectors are transported inside nuclei and act like transcription factors to activate host gene expression. TALEs can serve as an avirulence factor, or virulence factor, depending on the host genes they bind to and activate (Bogdanove et al. 2010). Structurally, all TALEs contain variable numbers of peptide repeats with each repeat consisting of 28 to 34 amino acids and the amino acids are nearly identical among the repeats except for the position of 12 and 13, known as repeat variable di-residues (RVDs) that bind a specific nucleotide base and thus dictate binding specificity (Moscou and Bogdanove 2009). TALE genes are likely presented in all *X. translucens* pathovars, but they were missing in the two published genome sequences. This is largely because TALE genes contain repeat sequences and are difficult to sequence and assemble using the next generation sequencing techniques. The traditional Sanger sequencing method and manual assembling will be needed to identify TALEs from *X. translucens* strains, which is the case for other *Xanthomonas* genome sequencing project (Bogdanove et al. 2011).

Disease evaluation and germplasm screening for BLS resistance

Different inoculation methods have been developed and used for BLS evaluation under field and greenhouse conditions. Direct spraying with a high concentration of bacterial inoculum suspension was commonly used in field screening for adult plants (Duveiller et al. 1993; Tillman et al. 1996; Kandel et al. 2012); however, field inoculations were not always successful due to unfavorable environmental conditions. Another common issue with field inoculations is the non-uniformity of disease development resulting in inconsistent results among different replications. Therefore, greenhouse evaluation protocols with different inoculation methods have been developed for characterizing pathogen virulence or host resistance. There are basically three ways of inoculating plants, including direct spraying (Bamberg 1936; Fang et al. 1950; Cunfer

and Scolari 1982), wounding inoculation (Bamberg 1936; Fang et al. 1950; Cunfer and Scolari 1982; Johnson et al. 1987; Mello and Cooksey 1988) and infiltration (Milus and Mirlohi 1994). Direct spraying requires a long period of misting for bacteria to enter and colonize whereas wounding inoculation using needles and other techniques is time-consuming. The infiltration method is relatively simple and is done by using a needleless syringe to infiltrate a small amount and low concentration of bacterial solution into leaves of seedling or adult plants.

Numerous disease rating scales also have been proposed. Saari and Prescott (1975) developed a 0 to 9 scale for appraising wheat foliar disease. This scale is basically applicable for field evaluations and is based on the disease progress on the whole plant. Kandel et al. (2012) used a double digit scale (00 to 99) to score the BLS under field conditions. The first digit in the scale was used to rate the height of disease progress in the plant canopy (0 = no disease and 9 = disease progress up to top) and second digit was used to rate the disease severity (0 = no disease and 9 = disease on 100% leaf area). Milus and Chalkley (1994) developed a 0 to 6 scale for BLS evaluation on seedlings based on the percentage of water-soaking developed in the infiltrated area where 0 means no visible symptoms observed and 6 means water soaking extended beyond the infiltrated area. Duveiller (1992) found significant correlation between lesion length and degree of exudate production and proposed a 0 to 4 scale to evaluate exudates production where 0 means no symptoms observed and 4 means a large area of water-soaking with abundant exudate.

Several disease screenings have been conducted to identify resistant sources in wheat cultivars and breeding lines and led to the identification of partial resistance in some genotypes (Duveiller et al. 1993; Milus et al. 1996; Tillman et al. 1996; Adhikari et al. 2011; Kandel et al. 2012). A total of 327 CIMMYT bread wheat lines were evaluated by Duveiller et al. (1993) in

the field of Mexico and it was reported that only three lines, 'Pavon 76', 'Mochis T88', and 'Angostura F88', were moderately resistant to BLS. Tillman et al. (1996) screened 5000 accessions of bread wheat under field conditions and reported that only 26 accessions were resistant. Another study by Milus et al. (1996) using a combination of spring and winter wheat cultivars reported that most of them were susceptible and only a few were partially resistant including 'Magnum', 'Bayles', and 'Terral 101'. From a set of 605 winter wheat accessions collected worldwide, Adhikari et al. (2011) identified 8.3% of them to be resistant to three North Dakota *Xtu* strains. Among 45 spring wheat breeding lines from South Dakota screened in the field for the reaction to a local *Xtu* strain, only one was shown to be less susceptible (Kandel et al. 2012). Overall, disease screenings have revealed that the percentage of resistant lines is usually low and there is no immune or highly resistant material. In addition, resistance in some wheat genotypes is still not affirmed due to the controversial results reported among different research (Tillman et al. 1996; Kandel et al. 2012). However, a few triticale lines were identified to be highly resistant to wheat BLS (Cunfer et al. 1982; Johnson et al. 1987).

Genetics and QTL mapping of BLS resistance

Due to the lack of reliable resistant genotypes, investigation of the genetics of resistance to BLS is scarce. In wheat, resistance to BLS was shown to be quantitative (Duveiller et al. 1993; Milus and Chalkley 1994; Tillman and Harrison 1996; Kandel et al. 2012). A study conducted in Mexico by Duveiller et al. (1993) revealed that five genes (*Bls1*, *Bls2*, *Bls3*, *Bls4*, and *Bls5*) condition BLS resistance in wheat based on the analysis of the diallel crosses between five parents including two susceptible cultivars (Alondra and Turaco) and three partial resistant cultivars (Mochis T88, Pavon 76, and Angostura F88). The five genes were found to have different effect on disease resistance. *Bls1* was present in all three partial resistant wheat

cultivars and had the largest effect. Milus and Chalkley (1994) concluded the polygenic nature of resistance in wheat cultivars because of the continuous distribution in the reaction of 19 wheat cultivars to 81 strains of *Xtu*. Based on the genetic analysis on the three F_2 populations derived from Terral 101 (resistant), Coker 9877 (moderately resistant), Pioneer 2548 (susceptible), and Coker 9766 (susceptible), Tillman and Harrison (1996) also found that resistance to BLS in wheat cultivars was quantitative. However, different result was obtained for triticale. A few triticale lines were reported to be highly resistant to BLS in the greenhouse (Cunfer et al. 1982) and the subsequent genetic analysis using highly resistant and susceptible triticale showed that a single dominant gene conditions resistance in these triticale lines (Johnson et al. 1987).

Recently, molecular markers and QTL mapping were used to estimate the genes conferring BLS resistance and locate these QTL in to specific genomic regions. Adhikari et al. (2012b) conducted association mapping on 566 spring wheat landraces using diversity arrays technology (DArT) markers and identified five genomic regions significantly associated with BLS resistance on chromosomes 1A, 4A, 4B, 6B, and 7D. Using the same wheat mapping panel and phenotyping data as in Adhikari et al. (2012b), but different marker system (single nucleotide polymorphism markers), Gurung et al. (2014) identified four QTL significantly associated with BLS resistance, two of which were on the similar genomic regions as reported by Adhikari et al. (2012b). Kandel et al. (2014) identified two simple sequence repeat (SSR) markers on chromosomes 2A (*Xwmc522*) and 6B (*Xbarc134*), respectively, significantly associated with BLS resistance using a mapping population derived from a cross between the partial resistant spring wheat genotypes ‘SD4205’ and a susceptible genotype. QTL mapping for resistance to BLS was also done in barley by Attari et al. (1998) using119 double haploid population derived from the cross between ‘Morex’ (moderately resistant parent) and ‘Steptoe’

(Susceptible parent), which revealed three genomic regions, two on chromosomes 3H and one on 7H, significantly associated with BLS resistance.

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PAPER 1: EVALUATION OF TRITICLAE ACCESSIONS FOR RESISTANCE TO WHEAT BACTERIAL LEAF STREAK DISEASE CAUSED BY *XANTHOMONAS TRANSLUCENS* PV. *UNDULOSA*

Abstract

Wheat bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), has become prevalent in the Northern Great Plains of the United States. A few studies have been conducted to evaluate various wheat germplasm for resistance to wheat BLS, but the results indicated sources of resistance were limited and only partial resistance was identified. However, several triticale accessions were reported to possess high levels of resistance to wheat BLS strains. In this study, we evaluated a collection of 502 triticale accessions from diverse geographic origins as well as the major North Dakota hard red spring and durum wheat cultivars for reaction to two virulent *Xtu* strains collected from North Dakota. All the tested wheat cultivars showed a susceptible reaction but a wide range of reactions were observed among triticale accessions. Out of the 502 accessions tested, 45 and 10 accessions were rated as highly resistant to BLS-LB10 and BLS-P3 strains, respectively. Among them, five accessions, including 8A-95, 8A-312, 6A-405, M86-6121, and T-M-AD-252, were highly resistant to both strains. The highly resistant reaction was characterized by a weak chlorosis and/or very low water-soaking within the inoculated area. Statistical analysis showed significant difference between the accessions, strains, and the accession by strain ($P < .0001$). Bacterial population growth was highly correlated with the level of resistance in triticale accessions. The identified resistant triticale accessions will be valuable in the development of wheat germplasm with high levels of BLS resistance.

Introduction

Bacterial leaf streak (BLS) can occur on a wide range of cereal crops and other grass species. They are caused by several genetically related *Xanthomonas* bacterial pathogens, collectively known as 'translucens group' (Vauterin et al. 1995; Bragard et al. 1997). In wheat, BLS is caused by *X. translucens* pv. *undulosa* (*Xtu*). However, some *Xtu* strains can also infect barley, triticale, rye, oat, and bromegrass (Cunfer and Scolari 1982; Bragard et al. 1997; Adhikari et al. 2012a). Very surprisingly, the bacterium has been recently isolated from diseased ornamental asparagus which is genetically distant from cereal species (Rademaker et al. 2006).

BLS is a common disease on both bread (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L.) worldwide, with most severe outbreaks in warm and semitropical areas (Duveiller et al. 1997). Recently, BLS has re-emerged as an important disease in the Upper Midwest region of the US where it is usually cool during the growth season (McMullen and Adhikari 2011; Adhikari et al. 2012a; Kandel et al. 2012). The increase of disease incidence has caused great economic concerns because this region is a major producer of the US hard red spring and durum wheat. Significant yield losses can result from BLS, which has been reported ranging from 8% to 34% depending on the disease pressure and cultivar susceptibility (Shane et al. 1987; Duveiller et al. 1997). Highly susceptible cultivars could suffer up to 40% of yield losses (Forster and Schaad 1988). In North Dakota, the disease has been recorded to cause 10.5% reduction in test weight of wheat (Waldron 1929). Although the bacterium can survive in the crop debris, weeds and grasses, seed has been implicated as the most important source of primary inoculum (Smith et al. 1919; Cunfer 1987; Duveiller et al. 1993). However, the effect of seed treatments on disease incidence is still uncertain (Duveiller et al. 1997). Furthermore, there is no other

chemical method or cultural practices to control BLS in the field, and thus utilization of genetic resistance appears to be the only option (McMullen and Adhikari 2011; Kandel et al. 2012).

Studies have been conducted to evaluate wheat cultivars and breeding lines for resistance to BLS under field or greenhouse conditions. Duveiller et al. (1993) conducted a field evaluation of 327 CIMMYT bread wheat lines and reported that only three lines, including 'Pavon 76', 'Mochis T88' and 'Angostura F88', were moderately resistant. From 50 CIMMYT spring wheat genotypes and 24 local winter wheat cultivars, Milus et al. (1996) identified a few lines, such as 'Magnum', 'Bayles', and 'Terral 101' as resistant based on the development of water-soaking in the inoculation sites and disease severity. A field evaluation conducted by Tillman et al. (1996) using 5000 accessions of bread wheat led to the identification of only 26 resistant accessions (0.52%) with ≤ 10% of BLS symptoms development on the flag leaves. Furthermore, the study also revealed a negative correlation between BLS resistance and plant maturity. From a total of 605 accessions of winter wheat genotypes from the USDA National Small Grain Collection (NSGC), Adhikari et al. (2011) found 8.3% of the accessions were resistant or moderately resistant in greenhouse evaluations. Kandel et al. (2012) evaluated 45 spring wheat cultivars and breeding lines from the upper Great Plain region under field conditions and found that all the tested genotypes were highly susceptible with only one being less susceptible. All these results indicated that sources of resistance are very limited in current wheat germplasm. In addition, controversial results have been obtained for some resistant wheat genotypes from different evaluations, and even within one study (Tillman et al. 1996). Nevertheless, some triticale accessions were reported to possess high levels of resistance to diverse *Xtu* strains including 'Siskiyou', 'M2A-Beagle' and 'OK 77842' (Cunfer and Scolari 1982; Johnson et al. 1987). Genetic analysis suggested that resistance in these triticale accessions is controlled by a single

dominant gene (Johnson et al. 1987). However, the collection of triticale in the NSGC has not been extensively evaluated for reaction to BLS and the reactions of these resistant accessions to North Dakota strains are unknown.

In this work, we requested a collection of triticale accessions from NSGC and evaluated them in the greenhouse for reaction to two *Xtu* strains collected from North Dakota. The objectives were to determine if previously reported resistant triticale accessions are effective against the North Dakota *Xtu* strains and to identify additional sources of resistance from triticale that can be used in the development of wheat germplasm with high levels of resistance to BLS.

Materials and Methods

Plant materials

A total 2025 accessions of triticale are deposited in National Small Grain Collection (NSGC), Aberdeen, ID and a subset of this collection, 502 accessions, were selected based on their geographic origins for the evaluation. These triticale accessions were originated from 29 different countries located in six continents. This subset also included 'Siskiyou', 'M2A', and 'GA 21' (Siskiyou-derived line) that have been reported to be highly resistant to BLS. Most of the triticale accessions used in this evaluation were spring-type, but winter and facultative-type accessions were also presented. In addition, 31 cultivars of hard red spring and durum wheat currently grown in North Dakota were also included in our evaluation. Previously reported resistant lines 'Magnum' and 'Pavon 76' (Duveiller et al. 1993; Milus et al. 1996) and susceptible line 'ND495' (Adhikari et al. 2009) was also tested for their reactions to the ND strains.

Disease evaluation

The disease evaluations were conducted in the North Dakota State University (NDSU) Agricultural Experiment Station greenhouse. The greenhouse room had a temperature setting from 15 to 21°C with a 16 hour light (5:00 am to 9:00 pm). All plant materials were grown in cones (4 × 13 cm) with two plants per cone. Cones were filled with Sunshine LC1 mix soils (Sun Gro Horticulture Distribution Inc., Agawam, MA), followed by the application of Osmocote 15-9-12 fertilizer after planting (Everris NA Inc., Dublin, OH). Each cone was considered as an experimental unit. All cones were arranged in a rack that can hold a total of 98 cones. The inoculation was done at the three-leaf seedling stage (about two weeks old) using two local *Xtu* strains BLS-LB10 and BLS-P3 (Adhikari et al. 2012a). These two strains were selected because they were found to be highly virulent based on our preliminary experiment.

The inoculum was prepared according to the descriptions by Adhikari et al. (2011) with minor modifications. The bacterial strains were streaked from stock culture onto Wilbrink's agar (WBA) plates (Duveiller et al. 1997) and grown at 28°C for 48 hours. To prepare the bacterial inoculum, the bacterial cells were collected using a sterilized metal loop and suspended in sterile distilled water. The concentration of bacterial inoculum was measured using a spectrophotometer (Eppendorf BioPhotometer D30) and adjusted to an optical density of 0.2 at 600 nm, approximately 1×10^7 cfu/mL, before inoculation. A spot infiltration method developed for the evaluation of rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Reimers et al. 1991) was adopted with some modifications. The bacterial suspension was infiltrated into the secondary leaf using a needleless syringe and multiple infiltrations were performed on each leaf. The amount of inoculum in every infiltration was controlled as much as possible so that solution did not extend outside the infiltrated spot. After infiltration, the plants were left in a water-filled

pan to avoid being watered from the top. Disease progress was monitored from the first day of inoculation (DAI) until 7 DAI on a diversity of triticale and wheat lines. Reactions at 5 DAI were found to be the best to detect the difference among these accessions. Therefore, a 0 to 5 scale was developed based on the disease reactions at 5 DAI (see results).

Bacterial population counting

This experiment was conducted to test if the bacterial growth in triticale accessions correlated with the level of resistance. Three triticale accessions with different levels of resistance to *Xtu* strains were used. Accessions 'Siskiyou' (highly resistant to BLS-LB10 and moderately resistant to BLS-P3), 'M2A' (moderately resistant to BLS-LB10 and highly susceptible to BLS-P3), and 'UC 38' (highly susceptible to both the strains) were selected, and infiltrated with the bacterial suspension (OD = 0.2 at 600 nm) into the secondary leaf as described above. At 0, 3, 5, and 7 DAI, two leaves from each genotype were harvested and the infiltrated area of the leaves (spots) were cut using sterile scissors into the same-size of fragments (2 cm). The leaf fragments from each time point and the same accessions were combined followed by grinding with sterile water using mortar and pestle. Serial dilutions from 1×10^{-1} to 1×10^{-8} of the leaf suspension were made and 10 µl of dilutions 1×10^{-5}, 1×10^{-6}, 1×10^{-7}, and 1×10^{-8} were plated on the WBA plates. The plates were incubated at 28°C for 48 hours or till the colonies can be counted. In each experiment, each dilution was plated on three plates and values were averaged as one replication. Experiment was repeated three times. The number of colonies counted at each time point was converted to log10 value and the average log10 value from three experiments was used to compare the bacterial growth at different time points in three different genotypes.

Genomic *in situ* hybridization (GISH)

In order to confirm the presence of rye chromosomes in triticale accessions, we selected two highly resistant and two susceptible accessions (Siskiyou, 6TA210, UC 38, and Villax St. Jose), and performed Genomic *in situ* Hybridization (GISH). The GISH process followed the protocol described by Yu et al. (2010). Briefly, the genomic DNA from the rye line 'Gazelle' was extracted and used as the probe DNA. The probe DNA was labeled with biotin-16-dUTP using the Nick Translation Kit (Enzo life sciences, Inc.). The blocking DNA from the wheat cultivar 'Chinese Spring' was prepared following the protocol described by Zhang and Friebe (2009). For the chromosome preparation, seeds of triticale accessions were germinated and 1 to 2 cm long root tips were used to prepare metaphase cells on glass slide. The slides were treated with 70-100 µl RNase A (100 µg/mL in 2 × SSC) and hybridization mixture was added. The hybridization mixture contained 100% deionized formamide, 50% dextran sulfate, 20 × SSC (pH = 7.0), 10 mg/mL sperm ssDNA, probe DNA, and blocking DNA. An aliquot of 20ul denatured mixture was added to each slide and the hybridization was performed at 37°C in an incubator for overnight. The following day, the slides were washed with saline sodium citrate (SSC) to remove excess hybridization solution. The slides were then incubated with 80 to 100 µl FITC-avidin (FITC-avidin: 5% BSA = 1:200 dilution) for 1 hour followed by washing. Anti-fade mounting medium was applied to the slide for counterstaining. Images were observed under a Zeiss Axioplan 2 Imaging Research Microscope (Carl Zeiss Light Microscopy, Germany) and captured using a CCD (charge coupled device) camera.

Statistical analysis

The triticale accessions were arranged in a randomized complete block design with three replications. The average disease score from three replications was used to classify the reaction

of individual accessions. To determine the significant difference between accessions, we performed analysis of variance using a nonparametric method and calculated relative treatment effects and their 95% confidence intervals (Shah and Madden 2004; Eskridge 1995; Brunner and Puri 2001). Non-overlapping of CI indicates a significant difference. All analyses were run using GLM procedure of SAS program (Version 9.3, SAS Institute).

Results

Disease reaction and development in wheat and triticale

All of the wheat cultivars used in the evaluation developed large translucent lesions with extended water-soaking areas to both the *Xtu* strains showing susceptible to highly susceptible reactions. Magnum and Pavon 76 also showed a susceptible reaction to both *Xtu* strains with average disease score of 3.0 to 4.0. However, a wide range of reactions were observed for triticale accessions, which allowed us to develop a rating scale. Under greenhouse conditions, no reaction was seen at the first day after infiltration (1 DAI), but water-soaking started to develop at 2 DAI in susceptible accessions. At 3 DAI, susceptible genotypes developed a clear water-soaking in the infiltrated areas (spots) in the susceptible genotypes. In highly susceptible reactions, water-soaking rapidly expanded on both sides of the lesions, and by 5 DAI, they coalesced covering a large area on the leaf surface. In contrast, a resistant reaction was characterized by a very low amount or no water-soaking within the infiltrated area at 5 DAI. Little difference was observed in disease development between 5 DAI and 7 DAI except in the slight expansion of water-soaking. Based on the pattern of disease development at 5 DAI, we developed a rating scale for the BLS evaluation under greenhouse conditions (Figure 1.1). The scale consists of a 0 to 5.0 score, where 0 = no water-soaking developed within the infiltrated area, 1.0 = low amount of water-soaking developed within the infiltrated area, 2.0 = high amount

of water-soaking developed in the infiltrated area without extension, 3.0 = high amount of water-soaking developed within the infiltrated spots and also started extending from the infiltrated spots 4.0 = water-soaking developed further from each spot and started joining together between two adjacent spots, and 5.0 = high amount of water-soaking developed and extended to the whole leaf. The disease score below 2.0 was considered highly resistant or resistant, 2.1 to 3.0 as an intermediate, and 3.1 to 5.0 to be susceptible or highly susceptible. This evaluation protocol and rating scale was used in the subsequent experiments to evaluate the large number of triticale accessions and wheat genotypes under greenhouse conditions.

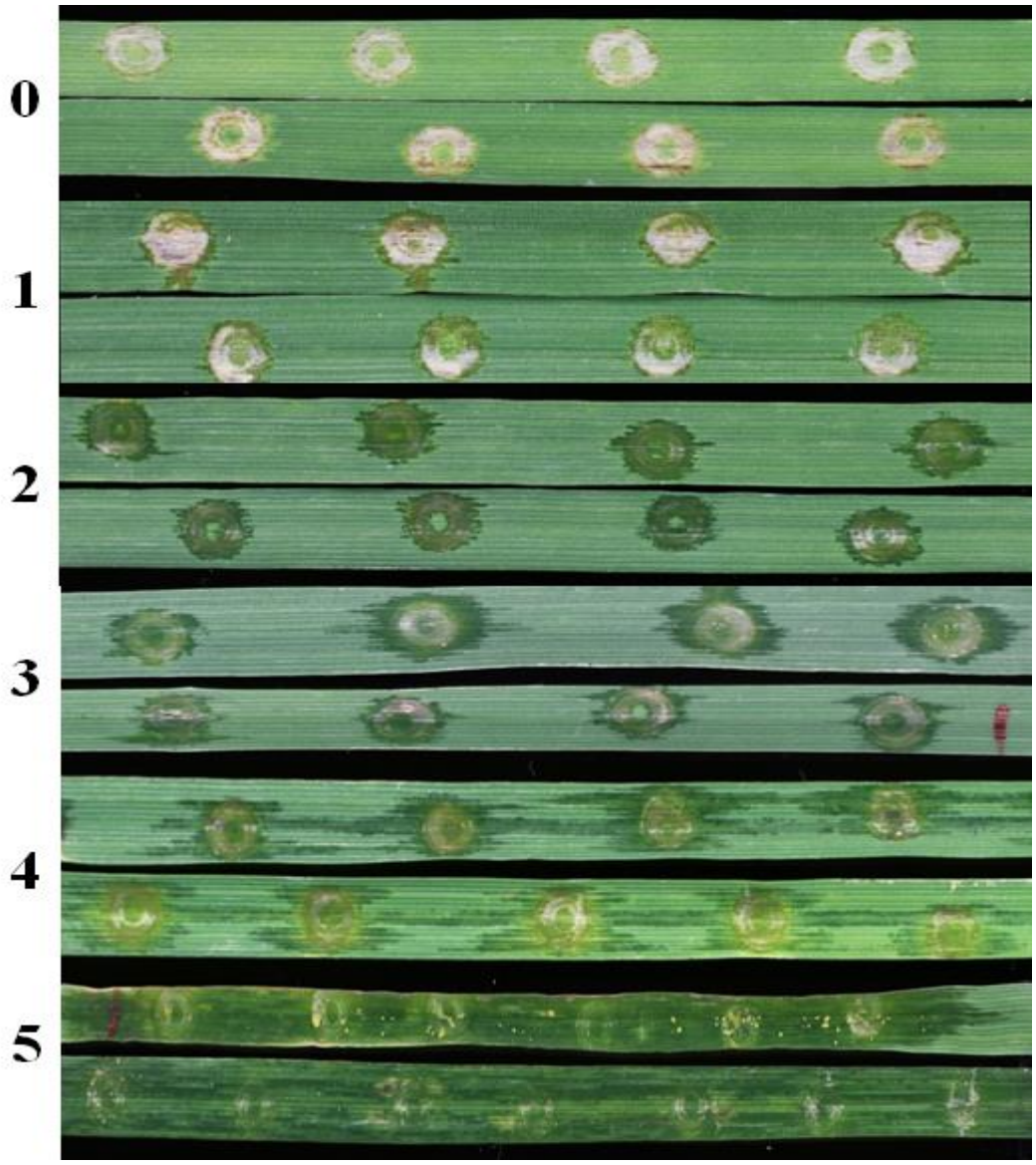

Figure 1.1. A rating scale developed for wheat bacterial leaf streak (BLS) disease assessment under greenhouse conditions. The rating scores of 0 to 1.0 were considered as highly resistant reaction and scores of 4.1 to 5.0 were considered as highly susceptible reaction.

Evaluation of worldwide collection of triticale accessions to *Xtu*

Disease scores ranged from 0.0 to 5.0 in the triticale accessions. For both BLS-LB10 and BLS-P3, the majority of accessions had a disease score between 2.1 to 4.0 indicating an intermediate reaction or susceptible reaction and only a small number of accessions had disease scores either lower than 2.0 as resistant or higher than 4.0 as highly susceptible (Figure 1.2). The

distribution of the 502 triticale accessions in each category (average disease score of 0 to 1.0 = highly resistant, 1.1 to 2.0 = resistant, 2.1 to 3.0 = intermediate, 3.1 to 4.0 = susceptible, and 4.1 to 5.0 = highly susceptible) were as follows, 45 (8.96%), 84 (16.73%), 222 (44.22%), 111 (22.11%), and 40 (7.96%) for BLS-LB10 and 10 (1.99%), 34 (6.77%), 246 (49.00%), 163 (32.47%), and 49 (9.76%) for BLS-P3 (Figure 1.2). More accessions were observed for BLS-LB10 than for BLS-P3 in the categories of lower than 2.0, while more accessions were observed in the categories of above 2.0 for BLS-P3. Most accessions showed similar reactions, either resistant or susceptible to both BLS-LB10 and BLS-P3. However, some accessions had different reactions to the two strains. Five accessions, 8A-95, 8A-312, 6A-405, M86-6121, and T-M-AD-252 originating from Canada, Russian Federation, Canada, US, and Bulgaria, respectively, were found to be highly resistant to both *Xtu* strains (Table 1.1). Five accessions, including 860-62-65, UC 17, M86-6070, 8A-282, and PRAG 60/1 were more resistant to BLS-P3 than to BLS-LB10. Forty accessions that were highly resistant to BLS-LB10, had intermediate or susceptible reaction to BLS-P3. Nonparametric analysis revealed significant differences ($P<.0001$) among the triticale accessions (Table 1.2). The relative treatment effect with 95% confidence interval (CI) was calculated for each accessions using nonparametric method and significant differences were detected among the resistant and susceptible accessions (Appendix A).

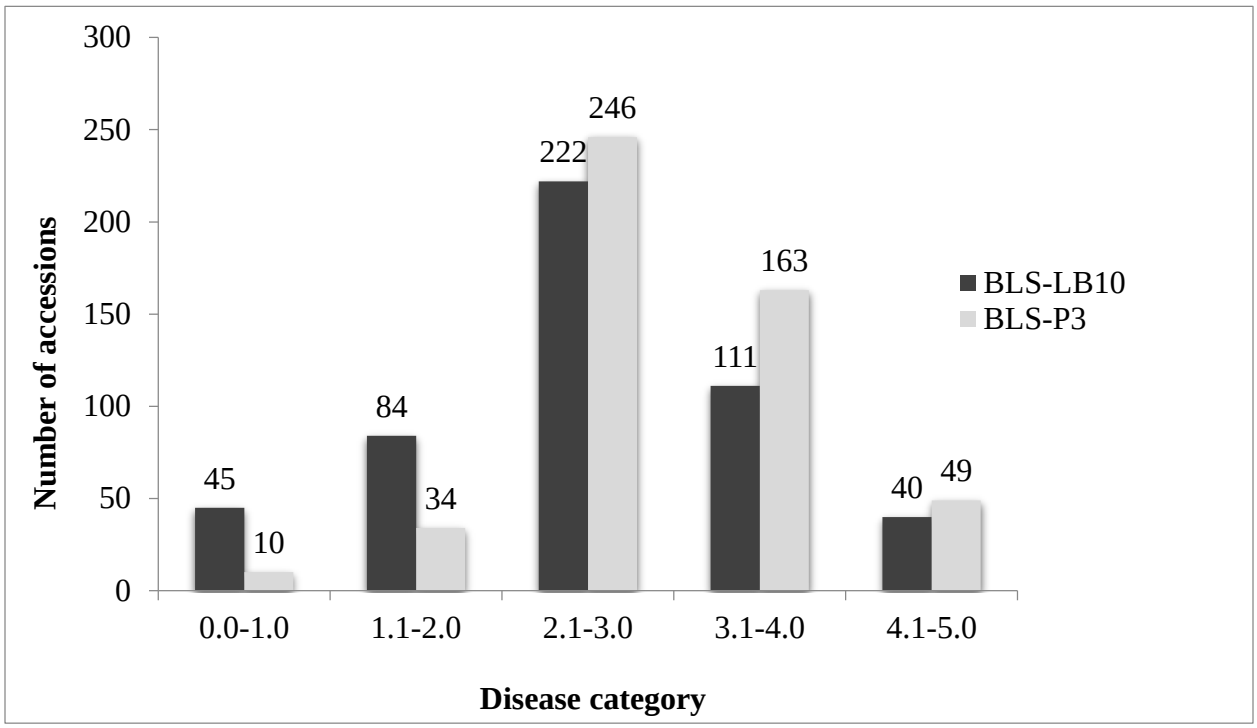

Figure 1.2. Frequency distribution of disease score for 502 triticale accessions to *Xanthomonas translucens* pv. *undulosa* strains, BLS-LB10 and BLS-P3 evaluated under greenhouse conditions.

Table 1.1. List of triticale accessions that are highly resistant to both *Xanthomonas translucens* pv. *undulosa* strains, BLS-LB10 and BLS-P3.

Name	Country	Habit‡	Pedigree§	Ave. disease score	
				BLS-LB10	BLS-P3
8A-95	Canada	S	Rescue / Prolific	0.00	0.83
8A-312	Russian Fed.	S	Triticale F5 Hostianum 23FX / Voronezshaja SHJ	0.33	0.83
6A-405	Canada	S	UM70 / SFS	1.00	0.00
M86-6121	United States	W	Male sterile B219 / A876 // Hyslop Dwarf / Rufus Rye	0.50	0.83
T-M-AD-252	Bulgaria	S	N/A	0.67	0.83
521-5	Canada	S	P4N-30 / Prolific	0.00	1.67
169-1	Canada	S	T4N / Prolific	0.00	1.67
6A-410	Canada	S	RD10-4 / 2D187	0.00	1.83
6TA-210	United States	S	T. aestivum NP 710 /S. cereale Asosan /3/T. persicum / S. cereale //T. durum Stewart /S. cereale Prolific	0.00	1.83
6TA-522	United States	F	N/A	0.00	2.00
X78-5925-1	Australia	S	72UM-2038 / 6-ITSN-3 // Drira	0.00	2.00
6A-250	Russian Fed.	S	T. persicum /S. cereale	0.00	2.00
6A-404	Canada	S	UM70 / Lost Tag rye	0.00	2.33
MTE-120	Spain	S	N/A	0.00	2.33
8A-106	Japan	N/A*	N/A	0.00	2.33
Koala	Mexico	S	CID94979 / Merced rye /3/ Tcl Dur CRLT / Tcl Dur Giza	0.00	2.67
8A-278	Russian Fed.	S	Budde / Petkus	0.00	3.33
M86-6060	United States	W	Daws / Antelope Rye // A876	0.17	2.50
6A-190	Canada	S	Stewart (durum)/ Prolific (rye)	0.17	1.83
MTE-93	Spain	S	N/A	0.17	2.33
6A-274	Russian Fed.	S	AABBCCRR / AABBRR	0.17	2.33
PRAG 46/1	Russian Fed.	N/A	N/A	0.17	2.67
6272-M-1-2-M	South Africa	S	6TA299 / A238	0.17	2.67
6278-M-2-2-M	South Africa	S	6TA312 / 6TA207	0.17	2.83
UC 33	United States	S	(D7069 , II 22234-6M - 1Y-0M , (TM-Tc *2)//(Z-B /W)*2) //PI 243741 / Snoopy	0.17	2.83
Korog	South Africa	S	N/A	0.33	2.00
AD 307	Ukraine	S	N/A	0.33	2.17
6A-64	Spain	S	T. dicoccum /S. cereale	0.33	3.67
6TA427	United States	S	T. persicum /S. cereale //T. durum Stewart /S. cereale Prolific	0.33	3.17
UC 43	United States	S	Mayo 54 // Norin 10 / Brevor /3/ Nainari 60 /4/ PI 243741	0.33	2.67
6272-M-1-1-M	South Africa	S	6TA299 / DL68-119	0.33	3.00
Siskiyou	United States	S	Selection from CIMMYT population T-903 (F3-Masa-101Y).	0.50	3.00
6TA213	United States	S	T. persicum /S. cereale //T. durum Stewart /S. cereale Prolific	0.50	2.17
UC 69	United States	S	(D7191 , II Ganso 'S'/D, Buck * TME-TC / Lak)/Snoopy-126	0.50	2.50
6299-3-1-2-M	South Africa	S	6TA340 / 6TA210	0.50	2.67
UC 19	United States	S	Castelporziano / PI 243741	0.67	2.50
UCF 45	United States	S	Quilafen / Snoopy-E	0.67	1.67
UC 68	United States	S	Mayo 54 // Norin 10 / Brevor /3/ Nainari 60 /4/ Snoopy-126	0.67	2.50
II81-216	Australia	S	Condor / Snoopy	0.67	3.33
P61-124-3	United States	S	Chinese / Imperial	0.83	3.83
Meksitol 1108	Bulgaria	S	N/A	0.83	2.33
8A-219	Germany	W	Trumball 03 / Heines Heilkorn	1.00	4.00
8A-377	Canada	S	T. sphaerococcum / self-fertile spring rye	1.00	2.00
Satu	Australia	S	Maya /2* Armadillo	1.00	2.17
M86-6106	United States	W	M75-8064 / A876 // EMSA876	1.00	2.00
860-62-65	Hungary	N/A	N/A	1.17	1.00
UC 17	United States	S	(D7069 , II 22234-6M - 1Y-0M , (TM-Tc *2)//(Z-B /W)*2)/ Snoopy-24	2.00	0.83
M86-6070	United States	W	A876 / M76-6269	2.00	1.00
8A-282	Canada	N/A	6B259 / Dakold	2.33	0.83
PRAG 60/1	Russian Fed.	N/A	N/A	3.17	1.00

‡ Growing habit of triticale accessions (S = spring, W = winter, and F = facultative).
§ Information about the lineage of triticale accessions in USDA National Small grain Collection.
* N/A = Information was not available.

Table 1.2. Analysis of variance (ANOVA) table calculated on ranks of disease severity median.

Source	Degree of freedom (DF)	Sum of Square	Mean Square	*F*-Value	Pr>*F*
Accession	501	1050588619	2096983	5.61	<.0001***
Strain	1	63871926	63871926	170.86	<.0001***
Acc*Strain	501	313929371	626606	1.68	<.0001***

*** Significant at $p < .0001$

Comparison of *in planta* growth of *Xtu* in resistant and susceptible triticale

For BLS-LB10, three accessions Siskiyou, M2A, and UC 38 were highly resistant, moderately resistant, and highly susceptible, respectively. No significant difference was observed at 0 DAI, and the bacterial population for three accessions were approximately 1 x 10^7 cfu/ml (Log10 value at 7.00), close to the initial concentration of the inoculum (Figure 3.1A). This indicated that the infiltrations were relatively uniform across all accessions. A rapid increase was observed in all three accessions from 0 DAI to 3 DAI. In addition, a significant difference was detected among the three accessions at 3 DAI with Siskiyou, M2A, and UC 38 having log10 value at 9.23, 9.97, and 10.41, respectively. The bacterial population increased at a much slower rate from 3 to 5 DAI in all three accessions compared to that from 0 to 3 DAI but the numbers of bacterial colony were still significantly different among the three accessions (Log10 value at 9.67, 10.09, and 10.62 for Siskiyou, M2A, and UC 38, respectively). A slight increase in the bacterial population was observed at 7 DAI for M2A and UC 38 while a slight decrease was detected for Siskiyou (resistant); therefore, difference among them became larger (Log10 value at 9.61, 10.21, and 10.77 for Siskiyou, M2A, and UC 38, respectively) (Figure 1.3 A). Siskiyou was moderately resistant to the BLS-P3 while M2A and UC 38 were highly susceptible. At 3 DAI, the populations in all three accessions rapidly increase with log10 value at

11.02, 11.49, and 11.37, respectively. The log10 values of the numbers were much higher than those for BLS-LB10. Less difference was observed among these accessions compared to BLS-LB10, but significant differences were detected between Siskiyou, and M2A or UC 38, but not between M2A and UC 38. Similarly, a much slower increase in bacterial population occurred from 3 to 5 DAI and from 5 DAI to 7 DAI for BLS-P3 in all three accessions. The difference in bacterial population among three accessions was quite small, but was significant at 7 DAI (Figure 1.3B).

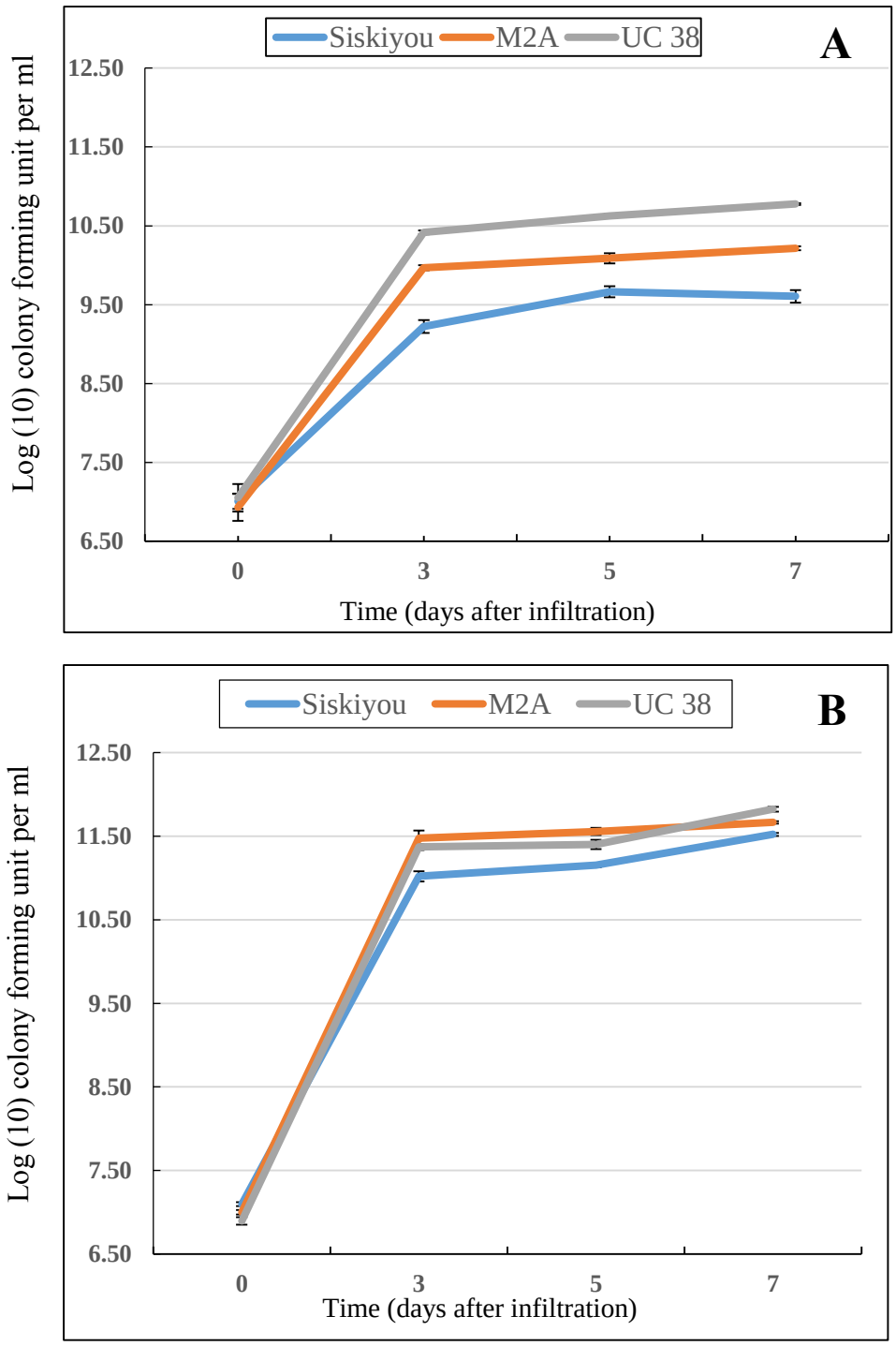

Figure 1.3. Bacterial growth over time during the infection of triticale accessions, (A) Bacterial population counting for BLS-LB10, (B) Bacterial population counting for BLS-P3. Three triticale accessions, including Siskiyou (highly resistant to BLS-LB10 and moderately resistant to BLS-P3), M2A (moderately resistant to BLS-LB10 and highly susceptible to BLS-P3), and UC 38 (highly susceptible to both strains) were used. Each time-point is the average of three biological replicates and the error bars represents the standard error of three replications.

Confirmation of triticale accessions by GISH

GISH analysis revealed four triticale accessions, including Siskiyou (highly resistant to BLS-LB10 and intermediate to BLS-P3), 6TA210 (highly resistant to BLS-LB10 and resistant to BLS-P3), UC 38 (highly susceptible to both the strains), and Villax St. Jose (highly susceptible to BLS-LB10 and susceptible to BLS-P3), contained 42 chromosomes indicating a hexaploid level of the genome. In all GISH images, rye chromosomes were visualized in orange color with green dots formed in telomere or sub-telomere regions of chromosomes and all wheat chromosomes were in red. GISH also revealed 14 rye chromosomes (orange in color) and 28 wheat chromosomes (red in color) in three triticale accessions, Siskiyou, UC 38, and Villax St. Jose, but 12 rye chromosomes and 30 wheat chromosomes in 6TA210 (Figure 1.4). In addition to the variation in rye chromosome numbers, these triticale accessions also harbors small variations in rye chromosome structure based on the pattern of these green dot signals. For example, a pair of rye chromosomes without obvious green dot signals was observed in triticale accession Siskiyou, but not in other lines. Similarly, a pair of rye chromosome with a weak green dot on the sub-telomere region of the long arm is unique to Villax St. Jose (indicated by arrows).

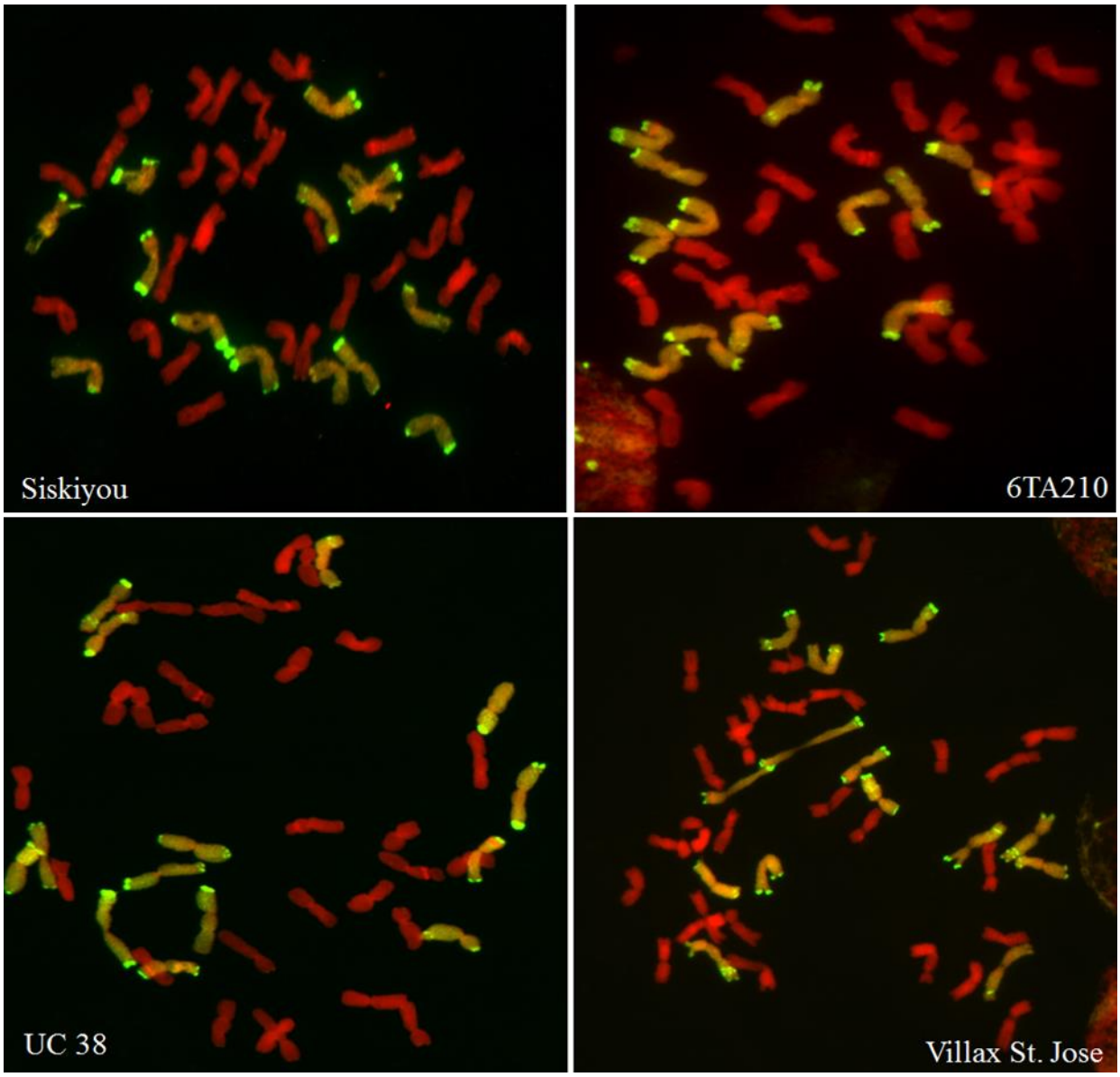

Figure 1.4. Genomic *in situ* hybridization (GISH) on four triticale accessions. The rye genomic DNA and wheat genomic DNA were used as a probe DNA and blocking DNA, respectively. Rye chromosomes were visualized in orange color with green dots on telomere or sub-telomere regions and wheat chromosomes were counterstained with PI as red. Arrows in the figure was used to show the difference observed in rye chromosomes.

Discussion

Disease screening for resistance to BLS is important not only because a reliable source of resistance is currently lacking, but also because development and use of resistant cultivars is the only way to manage this disease. In the current study, we first evaluated the North Dakota major HRSW and durum wheat cultivars for reaction to BLS and found they were all susceptible. This agrees with previous evaluation studies using wheat cultivars and breeding lines (Kandel et al. 2012; Duveiller 1990; Duveiller et al. 1993). To search for sources of resistance, we conducted a large scale of disease evaluation on triticale germplasm because it has been shown to contain high levels of resistance to wheat *Xtu* strains. A total of 502 triticale accessions from different geographic origins were evaluated in the greenhouse for resistance to two local highly virulent strains. From the evaluation, we identified a total of 45 accessions that are highly resistant to BLS-LB10, and 10 accessions that are highly resistant to BLS-P3. Five triticale accessions, 8A-95, 8A-312, 6A-405, M86-6121, and T-M-AD-252, were found to be highly resistant to both strains. Previously reported resistant accession Siskiyou was found highly resistant to BLS-LB10 with average disease score of 0.50 and moderately susceptible to BLS-P3 with average disease score of 3.0. Two other accessions GA 21 and M2A showed moderately resistant reactions to BLS-LB10 but highly susceptible reaction to BLS-P3. Since these triticale accessions are susceptible to the North Dakota strains, they cannot be used as sources of resistance in North Dakota. This work not only confirmed the high level of resistance to BLS is presented in triticale, but also identified additional triticale accessions that are effective against North Dakota strains. The five accessions identified to be highly resistant to both strains can serve as a starting material for developing wheat germplasm with high level of resistance.

Partial resistance to BLS has been identified in wheat (Kandel et al. 2012; Duveiller et al. 1993; Milus et al. 1996), but wheat genotypes with high levels of resistance to BLS have not been reported. We speculate that the gene conferring the high level of resistance in triticale accessions is likely located in the rye genome. Rye (*Secale cereale*) has been proven to be a good source of resistance to biotic or abiotic stresses for wheat (Zeller and Hsam 1983), and the resistance genes in rye genome have been transferred to wheat with the help of triticale as a bridge (Saulescu et al. 2011). Resistance genes for wheat bunt (*Tilletia* sp.) and Barley yellow dwarf virus (BYDV) identified in the rye chromosome have been successfully introgressed into wheat lines using triticale as a bridge (Saulescu et al. 2011). Additionally, resistant gene(s) for some diseases such as, leaf and stem rust (Driscoll and Jensen 1964; Stewart et al. 1968), and powdery mildew (Zeller 1973), were also transferred from rye to wheat via the development of wheat-rye chromosome translocations. Wheat- rye 1RS/1BL translocation line carrying powdery mildew and leaf rust resistance gene has been widely employed in wheat cultivars (Rabinovich 1998). Therefore, the identified five triticale accessions with high levels of resistance to two strains will be useful to develop BLS resistant wheat germplasm, which in turn can be used to develop resistant cultivars.

Several studies have indicated that resistance in wheat is controlled by multiple genes. Duveiller et al. (1993) conducted diallel crosses and genetic analysis with five wheat cultivars that differed in the reaction to BLS and found a total of five genes conditioning partial resistance with each cultivar carrying one or three minor genes. Tillman and Harrison (1996) used three populations derived from partially resistant and susceptible wheat genotypes and concluded that resistance is quantitative and heritability is low. Recent association mapping studies also revealed four to five genomic regions associated with resistance to BLS (Adhikari et al. 2012b;

Gurung et al. 2014). In contrast, genetic analysis showed that a single dominant gene conditions resistance in triticale accessions, Siskiyou, M2A-Beagle, and OK 77842 (Johnson et al. 1987). We have developed several populations derived from highly resistant and susceptible triticale accessions identified in this study. Disease evaluations in two F_2 populations have indicated a single dominant resistance gene in these triticale lines. The population is being advanced to F_6 to develop a recombinant inbred line (RIL) population to map the resistance gene. The identified markers will be very useful in the transferring of this dominant resistance gene into a wheat background.

The two *Xtu* strains we used were selected from a collection of the bacterial population in North Dakota (Adhikari et al. 2012a). This collection consisted of hundreds of strains from five different locations in North Dakota that differ in virulence and genetics. Based on the pathogenicity test on 12 wheat cultivars, BLS-P3 was virulent on more genotypes than BLS-LB10 and any other strains (Adhikari et al. 2012a). Therefore, both strains were used in the evaluations of triticale accessions. Although some accessions showed differential reaction to two strains (Table 1.1), BLS-P3 generally induced more water-soaking symptoms on the inoculated leaf for the majority of accessions than BLS-LB10. Forty-five accessions showed a high level of resistance to BLS-LB10, but there were only ten accessions highly resistant to BLS-P3. On the other hand, more accessions had a disease score in the susceptible or highly susceptible categories for BLS-P3. Furthermore, bacterial growth for BLS-P3 in three different genotypes was at a much higher rate leading to the development of a much larger bacterial population compared to BLS-LB10. All these results indicated that strain BLS-BLS-P3 is more virulent than BLS-LB10. Although BLS-P3 was found to be more virulent on most accessions, there were some accessions that developed more disease with BLS-LB10 than BLS-P3, indicating the

specificity of wheat-*Xtu* interaction. However, the use of only two strains is not appropriate to determine the specificity of host genotype-pathogen interaction and more research is needed to validate this result. A study conducted by Milus and Chalkley (1994) indicated that there is no significant interaction between wheat cultivars and *Xtu* strains based on the reaction of 69 strains of *Xtu* on 19 wheat cultivars. In contrast, Adhikari et al. (2012a) reported significant interaction between wheat cultivars and *Xtu* strains using more than 200 strains. It is possible that significant cultivar-strain specificity can be detected using larger number of bacterial strains and wheat genotypes.

Using GISH techniques on four triticale accessions that differed in disease reaction, we confirmed the genome identity of triticale for these accessions. Very interestingly, we also identified some variations in the number and structure of rye chromosomes for these triticale accessions. One accession has only 12 rye chromosomes compared to 14 chromosomes in a normal triticale. Furthermore, differences in rye chromosome karyotype were also observed. This variation in chromosome number and karyotype might be due to different donor rye accessions used in developing these triticale. It is also possible that triticale accessions have undergone chromosome structure changes because of genetic instability (Seal and Bennett 1981). Lukaszewskim and Apolinarska (1981) analyzed the rye chromosomes in 85 triticale accessions and found that 92% of them had a complete rye genome but 8% had only six pairs of rye chromosomes. Merker (1975) reported that the number of rye chromosomes varied from one to seven pairs in 50 hexaploid triticale accessions. All 14 rye chromosomes were present in 8 winter-type triticale accessions (Seal and Bennett 1981), but in this study, differences in the size of the rye chromosomes were observed. The genes determining resistance or susceptibility might

lie on the absent chromosomes or on the regions that have the variation. This may be an important reason why triticale accessions showed different reactions to BLS.

In conclusion, we have identified a number of triticale accessions highly resistant to wheat BLS confirming that a high level of resistance is presented in triticale. It was found that the rate bacterial growth in triticale correlates with their level of susceptibility. The identified triticale accessions could serve as a good source of resistance to develop BLS-resistant germplasm.

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PAPER 2: IDENTIFICATION AND GENOMIC MAPPING OF RESISTANCE TO BACTERIAL LEAF STREAK IN WHEAT

Abstract

Bacterial leaf streak (BLS) of wheat, caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), has been established as an economically important disease in North Dakota and the surrounding regions. To search for sources of resistance for breeding programs, a field experiment was conducted to evaluate a subset of a spring wheat core collection obtained from the USDA National Small Grain Collection (USDA-NSGC) for reaction to BLS. This subset consisted of 299 genotypes and included cultivars, breeding lines, cultivated, and landraces from different geographic regions around the world. For disease evaluation, plants were inoculated with a single *Xtu* strain collected in North Dakota (BLS-LB10) and rated with a 0 to 9 scale based on the height of disease progress in the plant canopy. The genotypes exhibited a wide range of variation both in disease severity and plant maturing stage. Correlation analysis indicated that disease resistance is negatively correlated (r = -0.64, $p < 0.0001$) to early plant maturing stage. However, a few genotypes with early maturing stage but high levels of resistance to BLS were identified. The disease data were then employed with the available molecular data to identify QTL associated with BLS resistance using a genome wide association study (GWAS). It was found that the markers around *VRN* locus on chromosome 5A were also highly associated with disease reaction. After the elimination of those markers, GWAS revealed seven additional QTL significantly associated with BLS resistance. These QTL were located on chromosomes 1A, 2B, 3B, 5A, 5B, 6A, and 7A. The identified resistant spring wheat genotypes and SNP markers have the potential to be utilized in breeding programs aiming to improve BLS resistance.

Introduction

Bacterial leaf streak (BLS) disease, caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), is a common and economically important disease in many wheat-growing areas all over the world (Duveiller et al. 1997). Yield loss due to BLS has been reported to be as high as 40% under sprinkler-irrigated field conditions in Idaho (Forster and Schaad 1988). It was also found that kernel weight and seed plumpness are negatively correlated with leaf streak severity in barley and wheat (Shane et al. 1987). The typical symptoms of this disease include water-soaked streaks on the leaves, which subsequently develop into translucent necrotic lesions with surrounding chlorosis. The bacterium can also infect the glumes and peduncles causing dark purple to black streaks, which is referred as black chaff (McMullen and Adhikari 2011; Smith et al. 1919). As with most bacterial diseases, there is no chemical method for effectively managing BLS and use of resistant cultivars is the most preferred way (Milus and Mirlohi 1995; Schaad and Forster 1985; McMullen and Adhikari 2011; Tillman et al. 1996). However, breeding for BLS resistance is difficult for several reasons, including the lack of reliable resistance sources, the quantitative nature of resistance to BLS, and poor understanding of host-pathogen interactions (Duveiller et al. 1993; Kandel et al. 2012).

Disease evaluations to identify BLS resistant sources have been conducted on different wheat germplasm, including spring wheat (Akhtar and Aslam 1986; Duveiller et al. 1993; Hagborg 1974), winter wheat (Milus et al. 1996), bread wheat (Duveiller et al. 1993), which has led to the identification of a few genotypes with partial resistance. However, some reported resistant genotypes, such as ‘Pavon 76’, showed controversial results in different evaluations or locations (Duveiller et al. 1993; Tillman et al. 1996). Furthermore, the available amount of resistant sources is limited. A few triticale lines were reported to have a high level of resistance

to BLS (Johnson et al. 1987; Cunfer and Scolari 1982). In the first study, we evaluated a broader collection of triticale lines for reaction to BLS under greenhouse conditions and identified a few triticale lines that were highly resistant to BLS (paper 1). Because triticales are not genetically close to the cultivated wheat, they are not suitable to be directly applied in breeding programs. Therefore, it is better to search sources for resistance in adapted wheat germplasm that are more suitable for the breeding programs.

Advancements in the field of molecular biology have led to the development of molecular markers and statistical analysis, which have greatly facilitated the dissection of quantitative traits (Tang et al. 2000). Linkage and association mapping (AM) which are based on bi-parental populations and natural population, respectively, are the commonly used techniques to dissect these complex traits (Lander and Schork 1994; Zhu et al. 2008). Although the identification of molecular markers linked to the resistance gene/QTL has been successfully conducted using bi-parental mapping populations such as F_2, recombinant inbreed lines (RIL), and double haploid populations, AM or linkage disequilibrium (LD) mapping is an alternative technique to investigate complex traits using genetically diverse germplasm (Flint-Garcia et al. 2003; Ersoz et al. 2009). One important advantage of AM is that it uses natural populations originated from diverse geographic regions reducing the research time needed to develop a mapping population (Zhu et al. 2008; Neumann et al. 2011). Additionally, recombination events that occurred during the evolutionary history of natural populations increases the mapping resolution in AM (Collins and Morton 1998; Zhu et al. 2008). AM was extensively used to study human diseases, but its application has been extended to plants and other organisms (Flint-Garcia et al. 2003; Kerem et al. 1989).

A few studies have shown that partial resistance to BLS is controlled by multiple genes. Duveiller et al. (1993) conducted diallel crosses and genetic analysis with five wheat cultivars that differed in reaction to BLS and found a total of five genes conditioning partial resistance with each cultivar carrying one or three minor genes. By analyzing reaction in three wheat population among 'Terral 101' (resistant), 'Coker 9877' (partially resistant), 'Pioneer 2548' (susceptible), and 'Coker 9766' (susceptible), Tillman and Harrison (1996) concluded that resistance to BLS is quantitative and heritability is relatively low. Recently, molecular markers and genetic mapping have been used to determine the number of genomic regions conferring resistance to BLS as well as their effects. Adhikari et al. (2012b) used Diversity Arrays Technology (DArT) markers to identify QTL associated with BLS resistance in spring wheat landraces with diverse geographic origin and reported five genomic regions on chromosomes 1A, 4A, 4B, 6B, and 7D which are significantly associated with BLS resistance. By using a large number of SNP markers, Gurung et al. (2014) conducted association mapping on the same materials and disease evaluation data reported by Adhikari et al. (2012b) and found four QTL on chromosomes 1A, 5A, 5D, and 6B significantly associated with BLS resistance. Kandel et al. (2014) reported two genomic regions on chromosomes 2A and 6B significantly associated with BLS resistance under both greenhouse and field evaluations using a mapping population developed from a partially resistant spring wheat line 'SD4205'. In barley, three QTL associated with BLS resistance were identified using a double haploid population derived from the cross between 'Morex' (resistant) and 'Steptoe' (susceptible) (Attari et al. 1998). The objective of this study is to identify sources of resistance to wheat BLS by evaluating a subset of the world spring wheat core collection and map potential QTL associated with BLS resistance by conducting a GWAS in this spring wheat collection.

Materials and Methods

Plant materials and experimental design

A subset of spring wheat core collection was obtained from the USDA National Small Grain Collection (USDA-NSGC) located in Aberdeen, ID, USA. This subset consisted of 299 genotypes and included 97 cultivars, 109 breeding lines, 55 cultivated, and 38 landraces that were originated from 72 countries representing diverse geographic regions of the world (Figure 2.1, Appendix D). Triticale accessions 'Siskiyou' and 6TA210' were used as resistant checks, and 'UC 38' and 'Villax St. Jose' were used as susceptible checks (paper 1). The experiment was conducted at NDSU field plot sites in Fargo, ND during the 2014 growing season and the previous crop was potato. A total of 299 entries were planted in the field as hill plots with four hills in each row. For each hill plot, 10-15 seeds were planted. A randomized complete block design with four replications was used. The plants were inoculated at the early jointing stage (Feekes 4.0).

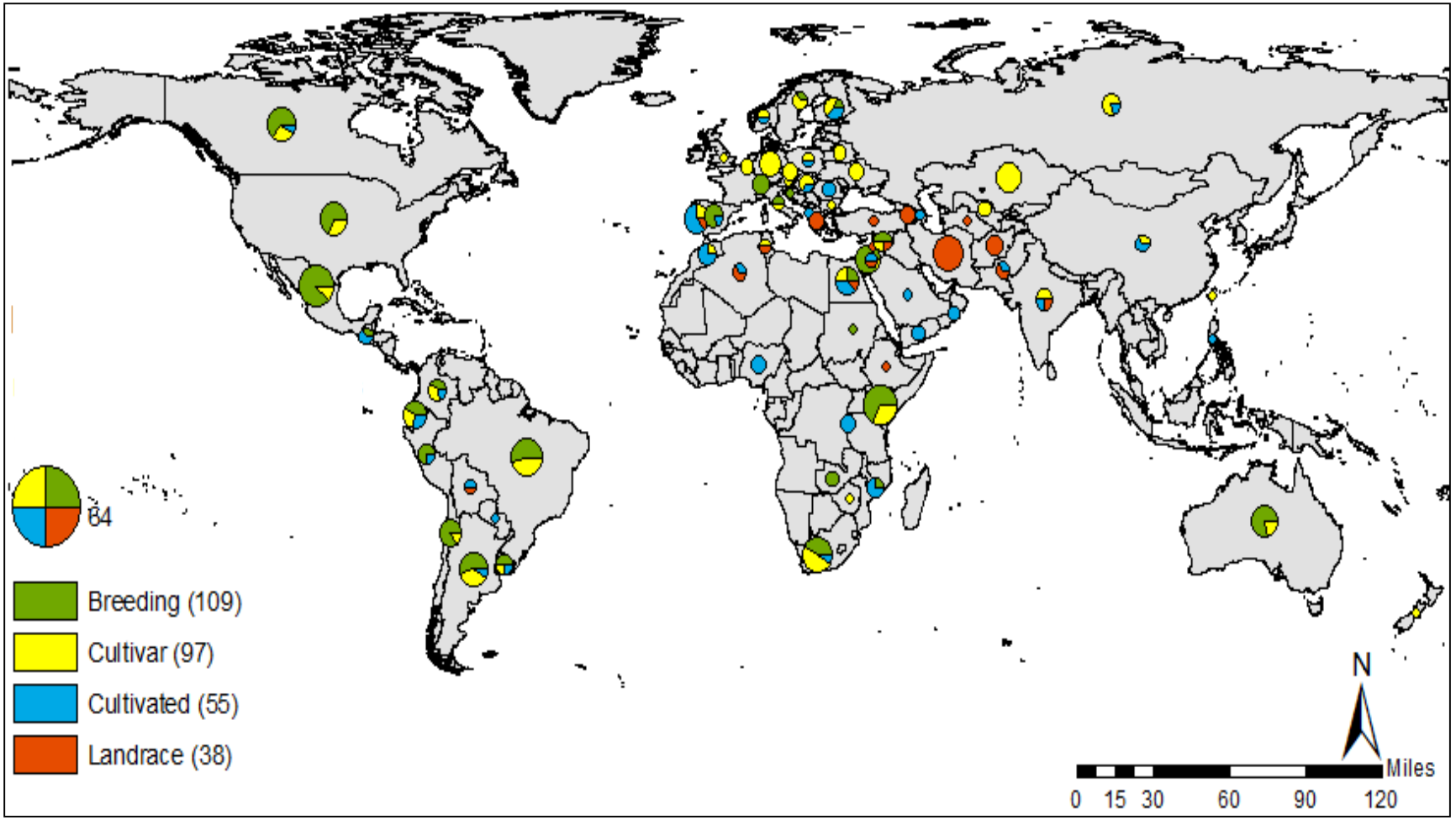

Figure 2.1. Country-specific distribution of the improvement status in the subset of the NSGC spring core collection. The size of the pie chart is proportional to the sample size and colors within each pie chart are reflective of the percentage of samples in each ACIMPT (i.e. breeding line, cultivar, cultivated, and landrace).

Bacterial strain, disease inoculation and evaluation

The bacterial strain BLS-LB10 collected in North Dakota (Adhikari et al. 2012a) was used in the disease evaluation. To prepare the inoculum, bacterium was streaked on Wilbrink's agar (WBA) plates (Duveiller et al. 1997) and incubated at 28°C for 3 days. After incubation, the bacterial cells were collected by using a sterilized loop and suspended into 0.85% saline solution to a concentration of approximately 1×10^7 cfu/mL. Before spraying, carborundum powder (Fisher Scientific) was added (3 g/l) to the inoculum suspension. The bacterial inoculum was sprayed onto the plants using a gas-powered Solo backpack sprayer with a rate of approximately 3L/800 square feet. To promote infection in the field, the moisture was created using an overhead misting system. The mist ran during the nights from 10:00 PM to 8:00 AM for two minutes every hour. No fungicides were applied to the field during the time of experiment because leaf spotting diseases were at very low levels. The plots were rated when most plants were at the soft dough stage (Feekes 11.2) with a 0 to 9 scale developed for wheat foliar disease evaluation (Saari and Prescott 1975). This scale was based on the disease progression over the whole plant canopy with 0 being no disease symptoms observed at all leaves and 9 being severe infections observed from low to flag leaves. The wheat genotypes evaluated in this study were classified into four different categories based on average disease score; resistant with an average disease score ≤ 3.0, moderately resistant with an average disease score of 3.1 to 5.0, moderately susceptible with an average disease score of 5.1 to 7.0, and susceptible with an average disease score of 7.1 to 9.0.

To understand the relationship between plant maturing stage and disease resistance/ susceptibility, we also recorded all entries for growth stage at the disease-reading date. A 1 to 5 scale was used, where 1= more than 50% of spikes in the hill at the ripening stage (Feekes 11.3,

11.4), 2 = more than 50% of spikes in the hill at soft dough stage (Feekes 11.2), 3 = more than 50% of spikes in the hill at flowering stage (Feekes 10.5), 4 = more than 50% of spikes in the hill at booting stage (Feekes 10), and 5 = plants at leaf sheaths erecting stage (Feekes 4, 5).

Statistical analysis

The data obtained from four replications were used in statistical analysis and average disease score was used to classify the reaction of wheat genotypes. To detect if significant difference exists among the genotypes, analysis of variance was conducted using a nonparametric method. Additionally, we calculated relative treatment effects and their 95% confidence intervals to check if there was a significant separation between each genotype (Shah and Madden 2004; Brunner and Puri 2001). Simple linear correlation was calculated between the average disease score and the growth stage score to detect if there is a significant correlation between the two traits. Disease means were compared across accessions based on the grouping of accession improvement status (ACMIPT) as well as continental origin. All analysis were done using a MIXED procedure of SAS program (Version 9.3, SAS Institute).

Genome wide association studies (GWAS)

The core collection of worldwide spring wheat was genotyped with 9K-SNP array by USDA Triticeae Coordinated Agriculture Project (T-CAP), and the genotypic data is publicly available on the Triticeae Toolbox website (triticeaetoolbox.org). Correlation between four replications were relatively high (0.7 to 0.8), the best linear unbiased estimate representing the replicated data was calculated and used in GWAS. The distance-based clustering and the model-based quantitative assessment of subpopulation membership of the accessions were performed using JMP genomics 6.0 and STRUCTURE v 2.3.4 (Pritchard et al. 2000). All GWAS analysis were performed using JMP Genomics 6.0 (SAS Institute Inc.). In a preliminary GWAS done

using a compressed mixed linear model (CMLM) with a kinship matrix model, markers on chromosome 5A near the *VRN* locus were significantly associated with BLS reaction in the field condition indicating that plant maturity is correlated with BLS resistance. Therefore, those markers associated with early growth stage were excluded from the dataset and GWAS was re-performed. Linkage disequilibrium (LD) between pairs of SNP markers ≤ 20.0 cM apart was calculated as D', a normalising *D*, in which D is divided by the theoretical maximum for the observed allele frequencies. To define LD blocks, we used the default settings of 0.1 for minor allele frequency (MAF) and set *D*' confidence limits between 0.98 and 0.70 at α=0.05 for strong LD, 0.9 *D*' upper confidence limit for historical recombination. Out of 4158 mapped SNPs, 3279 were assigned to 792 LD blocks in all 21 chromosomes. For GWAS, these LD blocks instead of individual SNPs were tested for association with the disease. Multiple SNP-trait association was performed using the Principal Component (PC) regression and logistic Kernel Method (KM) models (Zhao et al. 2012; Segura et al. 2012; Want et al. 2014). PCs were calculated from all SNPs within the same LD block. The PCs with the largest eigenvalues were included in a regression model, with SNP genotypes replaced by the PCs. The maximum number of PCs was set to 10; and a 0.85 cumulative proportion of variation was used to determine the final number of PCs. For the logistic KM, all SNPs within the same LD block were included in the model as random effects. Statistics for the corresponding covariance parameters were reported for each LD block within a chromosomal linkage group. *P*-values from these tests, with adjustments applied, were plotted along the marker map, using the location of the first SNP in each LD block.

Results

Reaction of wheat genotypes to *Xtu*

A wide range of variations from highly resistant (score = 1.0) to highly susceptible (score = 9.0) was observed in the field for reaction to BLS. The highly resistant genotypes exhibited only low levels of disease symptom on the lower leaves of the plants and the flag leaves were devoid of any disease lesions. In contrast, highly susceptible genotypes had severe infections on all the leaves with flag leaves being >75% covered with necrotic lesions and lower leaves were all dead. Out of 295 genotypes evaluated (4 genotypes were excluded because of no germination), 24 (8.13%), 93 (31.52), 108 (36.61%), and 70 (23.72%) were found to be resistant, moderately resistant, moderately susceptible, and susceptible, respectively, based on the categories defined in the methods (Figure 2.2). Significant differences ($p < 0.001$) were detected among the genotypes tested based on one factorial ANOVA. The wheat genotypes used in the evaluation also varied greatly in their growth stage from the plants at ripening stage (growth stage score of 1) to plants mostly at leaf sheath erecting (growth stage score of 5). Correlation analysis showed a significant negative correlation ($r = -0.64$, $p < .0001$) between disease resistance and early maturing stage. The most resistant genotypes were late in maturity with a growth stage score >3.0 indicating they were most at flowing stage or later; however, four genotypes, including 3781, H 19 D 12716, 3777-50, and CIGM98.412, originated from Sweden, Portugal, Brazil, and Mexico, respectively, were found to be at soft dough stage (growth stage score =2) and also have a high level of resistance to BLS (Table 2.1).

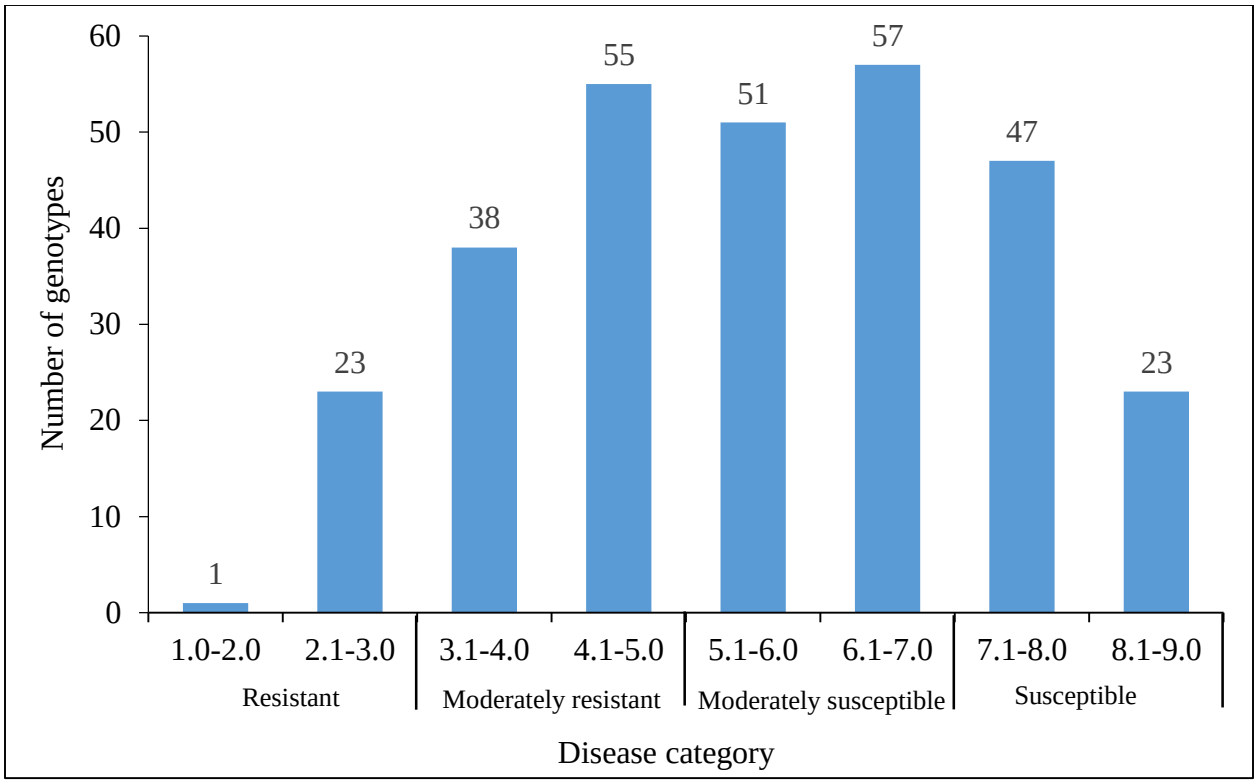

Figure 2.2. Distribution of 295 spring wheat genotypes in each disease category. A subset of NSGC spring wheat core collection was evaluated for resistance to *Xanthomonas translucens* pv. *undulosa* strain BLS-LB10 under field conditions and disease reaction was rated using a 0 to 9 scale. Genotypes with average disease score of 0 to 3.0 were classified as resistant, 3.1 to 5.0 as moderately resistant, 5.1 to 7.0 as moderately susceptible, and 7.1 to 9.0 as susceptible.

Table 2.1. List of the most resistant spring wheat genotypes in the subset of spring wheat core collection evaluated for resistance to a *Xanthomonas translucens* pv. *undulosa* strain BLS-LB10.

Accession no.	Name	Country	Status[†]	Ave. disease score[‡]	Growth stage score[§]
CItr 8442	Favorito	Argentina	C	3.00	3
PI 41032	Allorca	Tunisia	C	2.75	3
PI 184598	Uruguay	Uruguay	Cd	2.50	3
PI 185932	II-888	Mexico	B	3.00	3
PI 191882	BxRA8 10142	Argentina	B	2.75	4
PI 192123	Cjlugas	Mozambique	Cd	2.75	3
PI 192169	Precoce	Portugal	Cd	2.75	4
PI 192312	3781	Sweden	L	3.00	2
PI 192539	H 19 D 12716	Portugal	B	2.00	2
PI 207104	65o	Iran	L	2.25	3
PI 210866	3777-50	Brazil	B	2.50	2
PI 213601	D.I.V. 6722	Argentina	B	2.75	3
PI 225412	51-491	Uruguay	N/A	3.00	3
PI 234968	N/A	Italy	N/A	2.75	3
PI 237658	Rhodesian	Kenya	C	3.00	3
PI 266148	Leone	Italy	C	3.00	3
PI 271129	Mult 760	Peru	B	2.25	3
PI 271130	Mult 764	Peru	B	2.25	3
PI 282922	I-1039	Argentina	N/A	2.25	3
PI 344190	Missioneiro	Brazil	C	2.75	4
PI 410954	N/A	South Africa	N/A	3.00	3
PI 576639	2262-12	Tunisia	L	3.00	3
PI 613317	CIGM98. 412	Mexico	B	2.75	2
PI 638576	99CF 635	United	L	2.50	4
CIxt 20 (R. check)	6TA210	United	B	2.00	3
CIxt 33 (R. check)	Siskiyou	United	C	3.00	2
CIxt 31 (S. check)	UC 38	United	N/A	7.00	3
PI 428848 (S. check)	Villax St. Jose	Morocco	C	5.75	3

[†] Improvement status, (C = cultivars, Cd = cultivated, L = landrace, B = breeding lines, N/A = data not available)

[‡] Wheat genotypes were inoculated with BLS-LB10 strain of *Xtu* and disease reactions were scored using a 0-9 scale. This scale was developed to evaluate wheat foliar disease based on disease development on whole plant canopy (Saari and Prescott 1975).

[§] Wheat genotypes were classified into five categories (0-5 scale) based on growth stage where, 1 = very early and more than 50% of heads in a hill had seeds at ripening stage and 5 = very late and were at leaf sheaths erecting stage.

Correlation of disease with improvement status and geographic regions

All entries were grouped into four categories based on their improvement status (ACIMPT) including, breeding lines, cultivars, cultivated lines, and landraces. The disease means for each ACIMPT groups were 5.8, 6.2, 5.5 and 4.5, respectively. Mean comparison demonstrated no significant difference among them as well as between these group means and population mean (Figure 2.3A). In addition, all entries were grouped according to geographic origin, including Africa, Asia, Europe, North America, Oceania, and South America and the means for each geographic group were compared. A very similar result was obtained and no significant difference was detected among different geographic groups (Figure 2.3B).

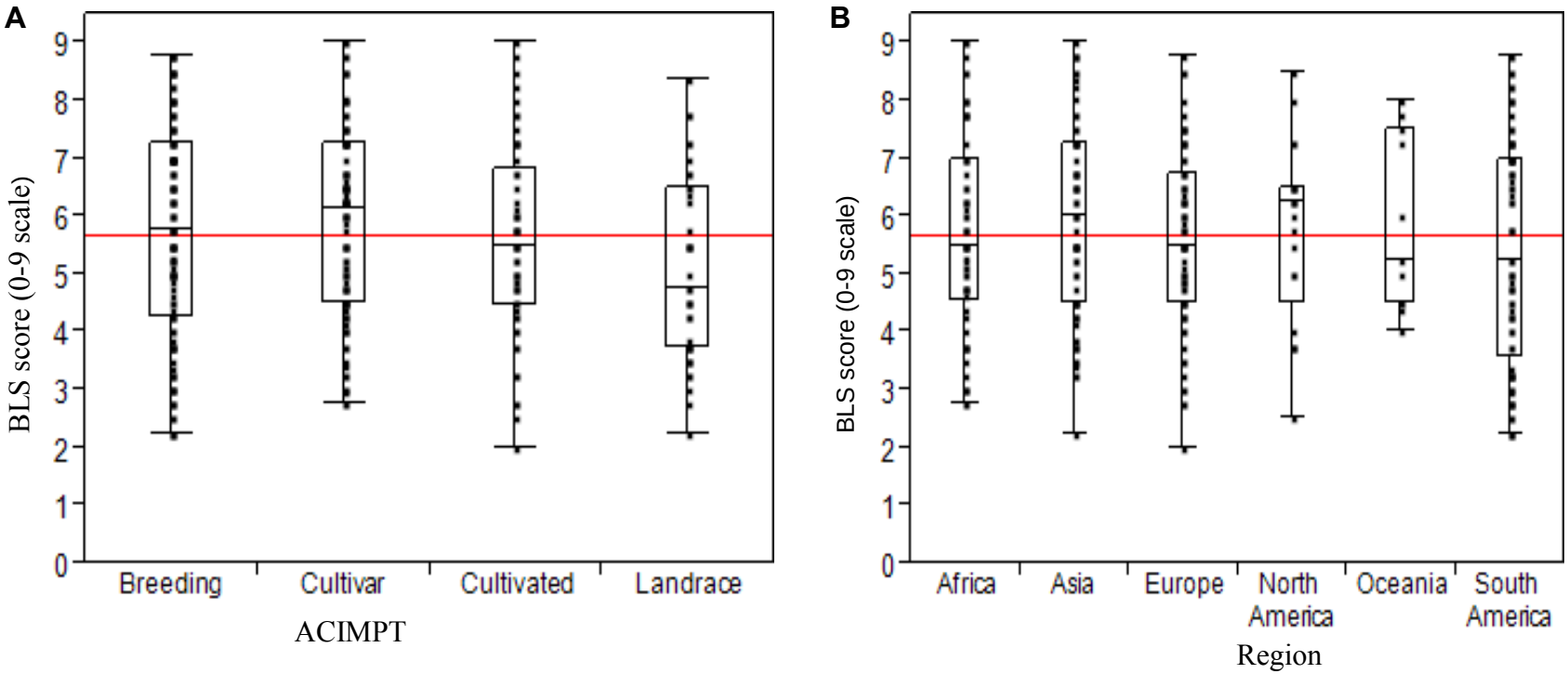

Figure 2.3. Disease mean comparison among different groups of spring wheat genotypes. (A) Spring wheat genotypes were grouped according to improvement status (breeding lines, cultivars, cultivated lines, and landraces), and (B) Spring wheat genotypes were grouped according to geographical regions (Africa, Asia, Europe, North America, Oceania, and South America). Red line represents the population mean.

Population structure and relatedness analysis

The ward clustering and cryptic relatedness analyses in the association mapping panel revealed the presence of subpopulations as well as clusters. Two major clusters were found within the panel (subpopulation group 1 and 2), which are separated by horizontal dashed line in Figure 2.4 A, B. The group 2 can be further divided into three sub-groups (subgroup 2A, 2B, and 2C) indicated by horizontal dotted lines. STRUCTURE analysis also suggested 2 to 4 hypothetical subpopulations, which correlates the grouping based on the cluster and cryptic relatedness analyses (Figure 2.4 C).

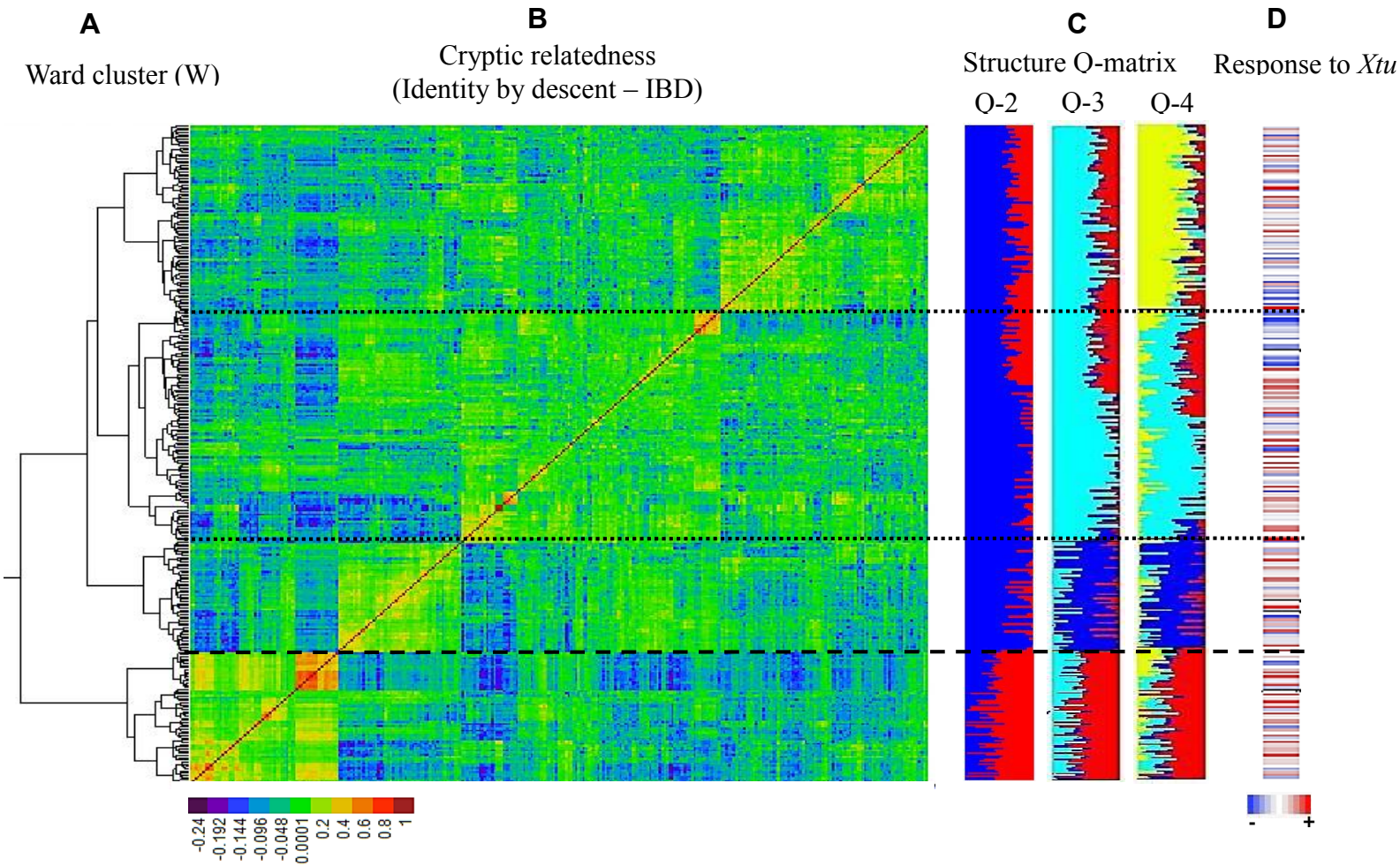

Figure 2.4. Population structure and relatedness analysis for the subset of the NSGC spring wheat core collection. (A) Ward clustering of the 299 accessions from the NSGC spring core collection, (B) Cryptic relatedness matrix based on genetic distance (IBD: identity by descent). Horizontal dashed line separates the two major groups, whereas the horizontal dotted lines separate the subgroups (2A, 2B, and 2C), (C) Matrices of membership coefficients corresponding to 2 to 4 hypothetical subpopulations derived from the STRUCTURE analysis, (D) Heat map for reaction of the 295 accessions to *Xtu* in the field. Blue lines indicate resistance and red lines susceptibility.

Identification of QTL associated with BLS resistance

Result from the preliminary GWAS analysis showed that markers on chromosome 5A *VRN* locus were highly associated with BLS reaction, which might be due to the association of disease reaction with plant growth stage. The *VRN* loci may be tightly linked to the genes conditioning plant maturity or *VRN* also has effect on plant maturity in common wheat. Therefore, GWAS was re-performed after removing all markers on 5A at the *VRN* locus and all marker showing association with plant growth stage. A total of 28 putative loci with adjusted *p*-value (FDR) <0.05 were identified, which are considered as significant QTL. These QTL were located on chromosomes 1A, 2A, 2B, 3A, 3B, 4B, 5A, 5B, 6A, and 7A.

All genotypes were plotted in an area with X-axis being the number of favorable alleles at these 28 markers and Y-axis being the BLS score (Figure 2.5). Regression analysis revealed a linear relationship between the BLS score and the number of alleles with a regression coefficient of -0.22. The more the favorable allele presented in a genotype, the lower BLS score it had. However, some genotypes had larger numbers of favorable alleles, but had a high disease score and the reverse was also true. These might be due to the effect of different genetic backgrounds in each genotype or the fact that some identified QTL were false positive.

After the validation with multiple regression analysis, only seven out of 28 QTL reached the significant threshold. The information on these seven QTL is listed in Table 2.2. These seven QTL were located on chromosomes 1A, 2B, 3B, 5A, 5B, 6A, and 7A (Table 2.2). The seven QTL explained a total of 28% of the observed phenotypic variation for disease resistance.

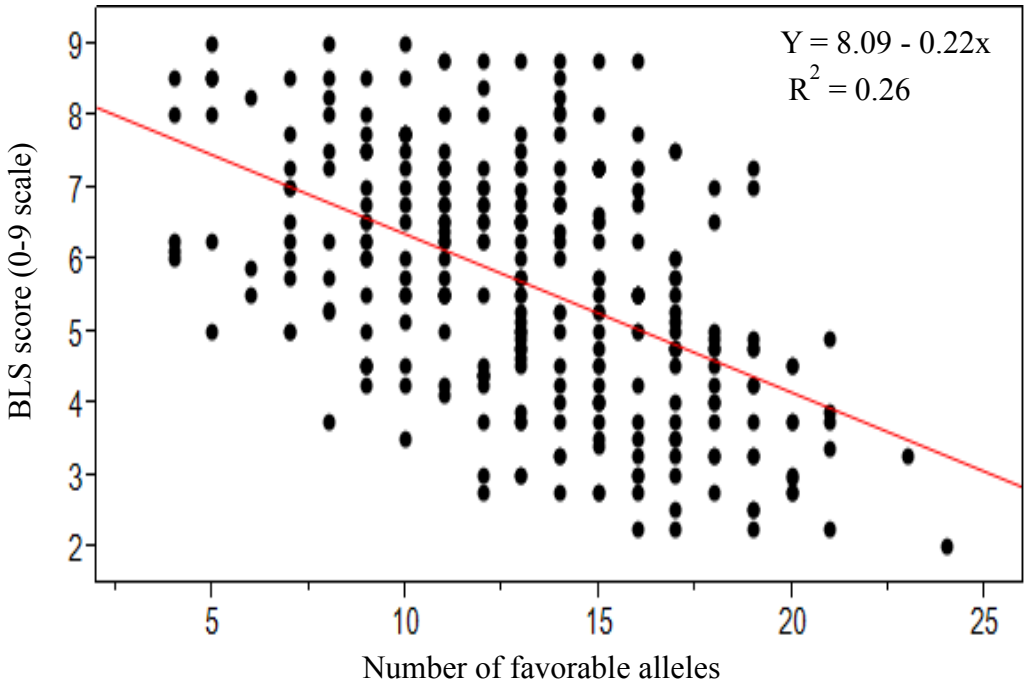

Figure 2.5. Regression of BLS disease score and the number of favorable alleles at the quantitative trait loci significantly associated with BLS resistance in spring wheat collection. The coefficient for the regression was -0.22 indicating that BLS score is expected to decrease by an average of 0.22 for every additional favorable allele.

Table 2.2. SNP markers significantly associated with BLS resistance based on principal component (PC) regression and kernel machine (KM) approaches to multi-locus association studies.

SNP					FDR adjusted *p*-value	
SNP index	**Alleles**	**Frequency**	**Chromosome**	**Position(cM)**	**PC Regression**	**Logistic KM**
3115	A/G	0.29	1A	56.97	2.60**	2.72**
2846	T/G	0.53	2B	19.44	3.26***	2.72**
5014	T/G	0.36	3B	138.95	2.29**	1.83*
2013	T/C	0.84	5A	150.84	2.76**	2.21**
2453	A/G	0.61	5B	126.34	2.76**	2.21**
4961	A/C	0.45	6A	36.84	2.43**	2.02**
3674	T/C	0.11	7A	54.37	2.25**	2.55**

*, **, and *** = *p* value < 0.05, 0.01, and 0.001, respectively.

Discussion

Currently, controlling BLS disease is nearly impossible due to the lack of chemical methods and cultural practices. Development and deployment of resistant cultivars serves as the only choice for managing this disease; however, developing resistant cultivars is a big challenge due to lack of resistant sources in wheat germplasm (Kandel et al. 2012; Duveiller et al. 1997). In addition, developing BLS resistant cultivars or germplasm has been difficult because of the polygenic nature of resistance (Duveiller et al. 1993; Kandel et al. 2012) and resistance being incomplete (Duveiller et al. 1990). Therefore, it is an urgent need to evaluate wheat germplasm for the identification of highly resistant sources. In order to expedite the incorporation of resistance in identified germplasm, resistance gene/QTL as well as linked molecular marker need to be identified. In this study, we evaluated a subset of a world spring wheat collection under field conditions and conducted an association mapping of resistance in this collection. This subset consisted of 299 genotypes were selected from the entire world spring wheat core collection in order to capture the maximum diversity of the larger collection based on their genotypic data (Dr. Mike Pumphrey, personal communications). From this effort, we have identified 24 genotypes with high levels of resistance to BLS with disease score lower than 3.0 and identified seven genomic regions associated with BLS resistance at a high confidence level. The identified wheat genotypes with high levels of resistance could be used to develop resistant cultivars with the aid of the molecular marker identified for these resistance QTL.

Disease evaluations have been conducted previously to identify resistant sources against BLS disease, but the percentage of resistant genotypes was usually low (Tillman et al. 1996; Adhikari et al. 2011; Adhikari et al. 2012b; Kandel et al. 2012). Tillman et al. (1996) evaluated approximately 5000 accessions of bread wheat from the NGSC and found only 0.52% of them as

resistant. From the 605 accessions of winter wheat, Adhikari et al. (2011) found that 8.3% of them were resistant. Kandel et al. (2012) reported only one genotype with partial resistance from the evaluation of 45 spring wheat cultivars and breeding lines. However, when using 566 landraces of spring wheat, Adhikari et al. (2012b) identified a much higher percentage (31.9%) of lines with resistance. In this work, we identified 24 genotypes (8.13%) to be highly resistant to BLS under field conditions, which is relatively higher than most studies, but lower than then study reported by Adhikari et al. (2012b). Among 24 genotypes identified, two including '65o' and '2262-12', were also reported by Adhikari et al. (2012b) as resistant lines. The remaining 22 genotypes from our study have not been reported from the previous studies and they could carry different resistance gene/QTL.

BLS evaluation of adult plants in the field can be affected by many factors, including plant maturity. Therefore, identification of maturity ranges within wheat materials before screening for BLS resistance has been recommended (Tillman et al. 1996). The negative association of plant maturity with BLS resistance was first noticed by Milus et al. (1996) who reported that three susceptible winter wheat genotypes, 'Savannah', 'Andy', and 'Florida 304' matured much earlier than other genotypes. Tillman et al. (1996) evaluated 5000 common wheat and detected a negative correlation (r = -0.55) between BLS reaction and heading dates. A much higher negative correlation (-0.71) was observed in a field study conducted in South Dakota (Kandel et al. 2012). In our field evaluation, we also observed a negative correlation (r = -0.64) between early growth stage and BLS resistance. When the entire marker data set was used in GWAS, markers on chromosome 5A around *VRN* locus were found to be highly associated with disease reaction further confirming the correlation between the two traits. The negative association of plant maturity with other diseases were also common which could be due to

several reasons, but it is unlikely that two sets of genes are linked. The majority of resistant genotypes identified in our study were slightly late in growth stage, but four resistant genotypes had a similar level of growth stage as local cultivars. Therefore, BLS is not always negatively associated with plant maturity and these four genotypes could serve as good germplasm for developing resistant cultivars.

QTL mapping of BLS resistance has been conducted previously using either association mapping or bi-parental mapping approaches (Adhikari et al. 2012b; Gurung et al. 2014; Kandel et al. 2014). In this study, we identified a total of 7 confident QTL that distributed on seven chromosomes, including 1A, 2B, 3B, 5A, 5B, 6A, and 7A. Based on the approximate map locations, two QTL on chromosomes 1A and 5A, respectively, could be the same as those previously reported by Gurung et al. (2014) who used 528 spring wheat landraces and SNP markers. The QTL on the chromosome 1A was also detected in the study by Adhikari et al. (2012b) who used the same mapping panel, but with DArT markers. This QTL on chromosome 1A has been identified in three study suggesting that it could be a QTL with a larger effect. Five out of seven QTL identified on chromosomes 2B, 3B, 5B, 6A, and 7A have not been reported before, which might represent novel genes for BLS resistance. After confirmation using bi-parental mapping, these QTL could be used in breeding programs to develop wheat cultivars with durable BLS resistance through QTL pyramiding.

In summary, a total of 295 spring wheat genotypes with diverse geographic origins were evaluated against a virulent strain of *Xtu* from North Dakota and 24 of them were identified with a high level of resistance. BLS resistance was found to be negatively correlated with plant early maturing stage. GWAS revealed 7 QTL significantly associated with BLS resistance. Two of them map to the locations that have been previously reported, but five were identified at different

genomic locations indicating new resistance genes/QTL. These QTL could be transferred to cultivar wheat with the aid of the identified SNP markers for developing durable BLS resistant germplasm through QTL pyramiding.

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APPENDIX A. COMPARISON OF TRITICALE ACCESSIONS FOR THEIR REACTIONS TO TWO *XANTHOMONAS TRANSLUCENS* PV. *UNDULOSA* STRAINS USING NON-PARAMETER METHOD

	BLS-LB10[a]			BLS-P3[b]		
Name	Median[‡]	R.E[§]	95% CI for R.E	Median	R.E	95% CI for R.E
8A-95	0	0.015	0.013, 0.017	1	0.056	0.040, 0.078
8A-312	0	0.032	0.011, 0.087	1	0.056	0.040, 0.078
6A-405	1	0.066	0.061, 0.070	0	0.015	0.013, 0.017
M86-6121	0.5	0.039	0.019, 0.080	1	0.056	0.040, 0.078
T-M-AD-252	1	0.049	0.025, 0.094	1	0.059	0.027, 0.12
Siskiyou	0.5	0.039	0.019, 0.080	3	0.59	0.38, 0.77
6TA210	0	0.015	0.013, 0.017	1.5	0.25	0.054, 0.67
UC 17	2	0.24	0.11, 0.46	0.5	0.095	0.021, 0.33
6A-190	0	0.023	0.012, 0.042	2	0.18	0.11, 0.29
GA 21	2	0.17	0.092, 0.31	4.5	0.89	0.82, 0.94
M2A	1.5	0.25	0.054, 0.67	5	0.95	0.94, 0.95
UC 115	3	0.56	0.23, 0.84	2	0.29	0.19, 0.42
M AD 341	3	0.68	0.51, 0.81	3	0.59	0.38, 0.77
PRAG 42	2	0.17	0.092, 0.31	2	0.17	0.092, 0.31
8A-129	2	0.3	0.089, 0.65	1.5	0.13	0.059, 0.26
UC 127	2	0.47	0.12, 0.85	3	0.72	0.45, 0.88
H 390	5	0.83	0.49, 0.96	5	0.91	0.81, 0.96
UC 38	5	0.89	0.70, 0.96	5	0.95	0.94, 0.95
Villax St. Jose	5	0.95	0.94, 0.95	4	0.76	0.57, 0.88
AD 114	4.5	0.87	0.72, 0.94	4	0.76	0.57, 0.88
Mizar	2	0.29	0.19, 0.42	5	0.95	0.94, 0.95
Triticale H	5	0.83	0.49, 0.96	4	0.88	0.79, 0.93

a, b *Xanthomonas translucens* pv. *undulosa* strains collected in North Dakota (Adhikari et al. 2012a). These two virulent strains were used to evaluate wheat and triticale germplasm in this study.

‡ Median value of disease score obtained from three different disease readings.

§ Relative treatment effect and 95% confidence interval was calculated for each accession using a nonparametric method.

APPENDIX B. LIST OF ALL TRITICALE ACCESSIONS EVALUATED IN THE STUDY AND THEIR AVERAGE DISEASE SCORE TO TWO *XANTHOMONAS TRANSLUCENS* PV. *UNDULOSA* STRAINS

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
CIxt 2	Alberta I-27-11	Canada	2.67	4.00
CIxt 4	H 390	United States	4.33	4.67
CIxt 5	6TA202	United States	4.00	5.00
CIxt 23	Rosner	Canada	2.67	5.00
CIxt 24	Tcl 6437	Canada	3.67	3.67
CIxt 26	Graze Grain 70	United States	4.33	4.67
CIxt 28	Chinese Triticale 1	China	4.00	3.33
CIxt 31	UC 38	United States	4.50	5.00
CIxt 33	Siskiyou	United States	0.50	3.00
Clxt 37	-	United States	3.33	2.17
PI 428861	T 196	France	3.17	4.00
PI 428884	860-61-65	Hungary	3.33	3.00
PI 428885	860-62-65	Hungary	2.50	3.17
PI 428888	KORAI 1-65	Hungary	3.33	4.67
PI 428896	P61-124-3	United States	0.83	3.83
PI 428932	1	China	4.00	3.17
PI 428826	8A-269	Russian Fed.	1.33	2.50
PI 428848	Villax St. Jose	Morocco	5.00	3.67
PI 428849	Villax Maria	Morocco	4.33	4.33
PI 428851	Villax Elvas 13	Morocco	4.33	4.67
PI 428857	62-108 Secalotrica	Germany	3.17	3.67
PI 428799	AABBDD No. 2	Japan	3.50	4.67
PI 428803	57D29	Canada	3.67	4.33
PI 428806	8A-215	United States	4.67	3.33
PI 428809	8A-219	Germany	1.00	4.00
PI 428815	MTE-4	Spain	3.33	3.67
PI 428936	A-439	Sweden	3.00	3.00
PI 428971	8A-511	Canada	5.00	4.50
PI 527339	GA 21	United States	1.67	4.50
PI 590945	AC Alta	Canada	3.67	3.83
PI 611362	-	Mexico	4.00	3.83
PI 308880	-	Spain	4.33	4.50
PI 340744	Riebesel 47/51	Germany	4.67	4.83
PI 340749	Salzmunder Bartweizen	Germany	3.50	4.67
PI 351662	Hybrid 46-131	Switzerland	4.33	4.50
PI 358312	Triticale 1	India	2.67	3.00
PI 386114	NAD 34	Russian Fed.	2.17	3.17
PI 386150	Oktoploid Derzhavina	Russian Fed.	3.50	4.17

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 386151	CXST-56/212	Sweden	3.33	4.00
PI 388655	5943-M-M-4-1-M	South Africa	3.17	2.83
PI 388684	7543	South Africa	4.00	4.17
CIxt 103	ARK 2014	United States	3.33	4.33
CIxt 107	OK 78828	United States	2.67	4.33
PI 70609	8180	China	4.33	3.33
PI 94602	38	Russian Fed.	4.67	5.00
PI 218251	-	Japan	3.67	3.33
PI 410802	LT 259/72	Poland	3.33	3.17
PI 410906	Mahissa 26	Spain	4.33	5.00
PI 414626	2	China	3.67	4.67
PI 414943	71-1756	Russian Fed.	2.67	3.83
PI 256032	Triticale 6	Spain	3.33	3.33
PI 271074	Pisarevs 1	Russian Fed.	3.33	3.67
PI 280457	Wheatgrass Hybrid 599	Russian Fed.	4.67	4.33
PI 282899	B-4081	Argentina	4.67	4.00
PI 285753	M 1	Poland	4.33	4.17
PI 368166	Salmon	Japan	4.00	3.83
PI 381429	1	Hungary	3.50	4.33
PI 383408	BF-64R	Poland	2.00	1.83
PI 386000	1 AD 545	Russian Fed.	2.33	2.50
PI 414971	Zitnica 1	Ukraine	2.67	3.00
PI 422259	Beagle 'S'	Mexico	4.17	3.67
PI 422262	Inia-Arm 'S'	Mexico	2.00	4.17
PI 422265	M2A	Mexico	2.00	5.00
PI 422267	M2A	Mexico	1.83	5.00
PI 422268	M2A 'S'	Mexico	2.67	3.83
PI 422288	Maya II-Tel 'S	Mexico	2.17	3.50
PI 428728	6A-64	Spain	0.33	3.67
PI 428733	8A-91	Canada	4.67	4.33
PI 428774	Triticale A	Sweden	3.50	3.17
CIxt 3	Alberta I-27-12	Canada	3.67	3.33
CIxt 6	6TA204	United States	2.67	2.17
CIxt 8	6TA131	United States	2.83	3.67
CIxt 9	6TA386	Hungary	2.83	3.17
CIxt 11	6TA203	United States	3.67	3.00
CIxt 13	6TA204-38	United States	2.83	3.67
CIxt 14	6TA205-21	United States	3.33	3.00
CIxt 16	6TA206-20	United States	3.67	3.33
CIxt 17	6TA208	United States	3.67	3.83
CIxt 18	6TA209-19	United States	4.00	3.00
CIxt 19	6TA209-22	United States	3.33	3.33
CIxt 20	6TA210	United States	0.00	1.83
CIxt 21	6TA213	United States	0.50	2.17
CIxt 22	6TA427	United States	0.33	3.17

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
CIxt 25	Tcl 6804	Canada	2.17	3.67
CIxt 29	Chinese Triticale 2	China	3.17	3.33
CIxt 30	Chinese Triticale 3	China	2.50	3.00
CIxt 35	AM 2147	United States	2.33	2.67
CIxt 39	UC 17	United States	2.00	0.83
CIxt 40	UC 19	United States	0.67	2.50
CIxt 41	UC 20	United States	2.67	2.83
CIxt 43	UC 25	United States	1.83	3.33
CIxt 45	UC 33	United States	0.17	2.83
CIxt 47	UC 40	United States	2.67	2.33
CIxt 48	UC 45	United States	0.67	1.67
CIxt 49	UC 46	United States	2.17	2.83
CIxt 50	UC 47	United States	1.50	3.17
CIxt 51	UC 49	United States	2.00	2.67
CIxt 52	UC 50	United States	2.50	2.50
CIxt 54	UC 52	United States	2.50	2.67
CIxt 55	UC 53	United States	1.50	3.17
CIxt 57	UC 55	United States	2.67	2.50
CIxt 58	UC 56	United States	2.67	2.50
CIxt 60	UC 60	United States	2.00	3.00
CIxt 62	UC 66	United States	2.50	3.33
CIxt 63	UC 69	United States	0.50	2.50
CIxt 64	UC 70	United States	3.17	2.17
CIxt 65	UC 72	United States	2.17	3.00
CIxt 66	UC 73	United States	2.17	3.67
CIxt 70	UC 21	United States	1.50	2.83
CIxt 71	UC 42	United States	1.50	2.83
CIxt 72	UC 43	United States	0.33	2.67
CIxt 73	UC 48	United States	2.00	2.67
CIxt 74	UC 61	United States	2.33	2.33
CIxt 75	UC 62	United States	3.33	3.83
CIxt 76	UC 63	United States	2.33	2.50
CIxt 77	UC 68	United States	0.67	2.50
CIxt 82	UC 102	United States	2.33	2.67
CIxt 83	UC 103	United States	2.83	2.83
CIxt 85	UC 105	United States	2.17	3.50
CIxt 91	UC 115	United States	3.00	2.17
CIxt 93	UC 118	United States	2.17	3.00
CIxt 96	UC 122	United States	3.83	4.67
CIxt 98	UC 125	United States	2.50	3.67
CIxt 100	UC 127	United States	3.00	3.67
CIxt 101	CI 101	United States	3.83	3.67
CIxt 102	UC 109	United States	3.17	3.00
CIxt 104	ARK 2301	United States	2.17	3.00
CIxt 105	ARK 2307	United States	2.33	2.17

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
CIxt 106	ARK 2309	United States	2.50	1.83
CIxt 107	OK 78828	United States	3.33	3.33
PI 94606	182/783	Russian Fed.	4.17	4.33
PI 94610	223/845	Russian Fed.	2.50	2.50
PI 197132	17	Japan	3.50	2.33
PI 197133	18	Japan	3.83	3.50
PI 235640	N/A	Spain	2.33	3.00
PI 256030	Triticale 9	Spain	2.00	2.50
PI 256033	Triticale 1	Spain	2.33	3.33
PI 285754	Pszensyto 218	Poland	3.83	3.00
PI 295999	Riebesel 47/51	Germany	4.00	3.67
PI 308881	-	Spain	4.67	4.17
PI 320250	-	Spain	4.67	3.00
PI 320251	-	Spain	4.83	3.83
PI 355949	42-XP-LW	Russian Fed.	1.83	4.00
PI 355950	56XP9-AG20	Russian Fed.	3.00	2.50
PI 355951	25 AD 20	Russian Fed.	3.50	3.00
PI 355952	AD 114	Russian Fed.	2.83	3.67
PI 355953	NAD 236	Russian Fed.	4.50	3.17
PI 355954	M AD 341	Russian Fed.	3.33	3.00
PI 381430	20	Hungary	3.00	3.50
PI 381431	30	Hungary	3.50	3.33
PI 381432	57	Hungary	2.83	3.33
PI 381433	64	Hungary	2.83	3.83
PI 381434	Bokolo	Hungary	2.50	2.67
PI 381435	D-27-F1	Hungary	2.67	3.00
PI 381436	Tomzsi 787-72	Hungary	2.17	2.17
PI 381437	Tomzsi 801-72	Hungary	1.67	2.83
PI 383409	BF-A3	Poland	3.00	2.83
PI 386001 2	AD 325	Russian Fed.	3.17	3.67
PI 386002	25 AD 20/1-60	Russian Fed.	3.33	3.00
PI 386003	31 AD 72	Russian Fed.	3.50	2.83
PI 386004	45 AD 137	Russian Fed.	2.50	2.33
PI 386005	AD 236	Russian Fed.	3.33	2.50
PI 386113	AD 1	Ukraine	3.67	4.33
PI 386115	AD 61-13-AM	Armenia	2.33	3.17
PI 386116	31 AD 72	Russian Fed.	3.67	2.33
PI 386117	AD 114	Russian Fed.	4.33	3.67
PI 386118	AD 196	Ukraine	1.83	2.33
PI 386119	NAD 236	Russian Fed.	3.67	3.00
PI 386120	AD 240A	Russian Fed.	3.17	3.00
PI 386121	AD 349	Russian Fed.	4.50	3.33
PI 386122	AD 762-7	Ukraine	2.50	3.33
PI 386123	AD 767-16	Ukraine	2.83	3.17
PI 386124	AD 2083-2	Ukraine	2.17	2.67

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 386125	AD 2384	Russian Fed.	3.67	3.17
PI 386126	AD 2468	Russian Fed.	1.67	3.17
PI 386127	AD K2	Ukraine	1.83	3.67
PI 386128	AD R-6/9	Ukraine	2.50	3.00
PI 386130	3L 34-AD	Russian Fed.	4.50	3.17
PI 386131	NAD 120	Russian Fed.	3.50	3.33
PI 386132	25 AD 20	Russian Fed.	3.33	3.50
PI 386133	25 AD 20/I 60	Russian Fed.	3.00	3.17
PI 386134	L AD 130	Russian Fed.	3.67	3.33
PI 386135	NAD 329	Russian Fed.	2.50	2.83
PI 386136	NAD 137	Russian Fed.	2.00	3.67
PI 386137	NAD 325	Russian Fed.	2.00	3.67
PI 386138	NAD 333	Russian Fed.	3.00	3.67
PI 386139	NAD 335	Russian Fed.	3.00	3.67
PI 386140	M AD 341	Russian Fed.	4.00	3.00
PI 386141	NAD 430	Russian Fed.	2.67	2.83
PI 386142	NAD 435	Russian Fed.	2.50	3.17
PI 386144	AD 206	Ukraine	1.67	2.50
PI 386145	ADL 2	Ukraine	3.17	2.67
PI 386146	ADL 3	Ukraine	2.67	2.33
PI 386147	-	Azerbaijan	3.50	2.50
PI 386148	-	Russian Fed.	3.83	2.67
PI 386149	N 1186	Russian Fed.	3.00	3.17
PI 386152	LV-1	Ukraine	2.83	4.17
PI 386154	STNIISM-1	Russian Fed.	3.33	2.67
PI 386155	STNIISM 2	Russian Fed.	2.50	2.67
PI 386156	STNIISM N 3	Russian Fed.	3.33	3.17
PI 386157	V-02	Ukraine	2.33	2.50
PI 388656	5954-M-3-1-M	South Africa	2.17	3.67
PI 388657	5950-M-M-19-M	South Africa	1.67	3.33
PI 388658	5954-M-M-2-1-M	South Africa	2.67	3.67
PI 388659	5957-M-1-1-M	South Africa	2.83	3.00
PI 388660	6171-M-5-2-M	South Africa	3.33	3.00
PI 388661	6213-1-1-1-M	South Africa	1.33	2.33
PI 388662	6219-M-5-1-M	South Africa	2.33	3.33
PI 388666	6231-3-1-2-M	South Africa	3.33	3.50
PI 388667	6237-6-2-1-M	South Africa	3.33	3.17
PI 388668	6259-13-1-2-M	South Africa	1.67	2.33
PI 388669	6272-M-1-1-M	South Africa	0.33	3.00
PI 388670	6278-M-2-2-M	South Africa	0.17	2.83
PI 388673	6299-3-1-2-M	South Africa	0.50	2.67
PI 388674	6305-2-4-2-M	South Africa	2.67	3.00
PI 388675	6369-1-3-2-M	South Africa	2.50	2.33
PI 388677	6387-3-1-3-M	South Africa	2.67	3.33
PI 388683	7539	South Africa	3.33	3.67

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 388686	A797-M-M-7-M	South Africa	2.33	2.67
PI 388687	A802-M-M-M-2-1-M	South Africa	1.33	2.50
PI 388689	A805-M-M-2-7-1-M	South Africa	2.33	3.67
PI 388695	A808-M-M-3-3-1-M	South Africa	2.00	2.67
PI 388696	A810-M-M-8-2-M	South Africa	3.50	3.50
PI 388697	A812-M-M-20-1-M	South Africa	2.50	2.50
PI 388698	A814-M-M-7-1-M	South Africa	2.67	3.17
PI 388699	A815-M-M-1-1-M	South Africa	4.17	3.00
PI 405020	Joseph	South Africa	3.50	4.00
PI 405023	5950-M-6-2-M	South Africa	2.83	3.33
PI 405025	6259-6-5-1-M	South Africa	2.83	3.00
PI 405026	6272-M-1-2-M	South Africa	0.17	2.67
PI 405029	6309-M-5-1-M	South Africa	2.50	3.50
PI 405032	6369-7-5-1-M	South Africa	3.67	2.83
PI 405033	6391-6-1-2-M	South Africa	2.83	2.50
PI 410803	LT 344/72	Poland	2.67	2.17
PI 410804	LT 378/72	Poland	3.83	3.67
PI 410805	LT 484/72	Poland	3.17	4.33
PI 410806	LT 97/73	Poland	2.17	3.17
PI 410808	LT 173/73	Poland	2.67	3.50
PI 410809	LT 188/73	Poland	3.33	2.50
PI 410904	954-17	Spain	3.17	2.33
PI 413008	Korog	South Africa	0.33	2.00
PI 414627	3	China	2.00	2.67
PI 414947	AD 307	Ukraine	0.33	2.17
PI 414950	PRAO 6/1	Russian Fed.	4.33	4.00
PI 414951	PRAO 6/2	Russian Fed.	3.50	3.17
PI 414952	PRAO 7	Russian Fed.	3.33	3.00
PI 414954	PRAO 39/1	Russian Fed.	2.00	2.33
PI 414959	PRAG 42	Russian Fed.	1.67	1.67
PI 414960	PRAG 45/1	Russian Fed.	2.50	3.17
PI 414961	PRAG 46/1	Russian Fed.	0.17	2.67
PI 414963	PRAG 49/1	Russian Fed.	1.83	3.00
PI 414965	PRAG 56/1	Russian Fed.	2.67	3.67
PI 414966	PRAG 58/3	Russian Fed.	2.67	2.83
PI 414967	PRAG 59/2	Russian Fed.	2.67	3.17
PI 414968	PRAG 60/1	Russian Fed.	3.17	1.00
PI 414969	PRAG 64/1	Russian Fed.	2.50	2.33
PI 414970	PRAG 65/2	Russian Fed.	2.83	2.67
PI 414972	AD 201	Ukraine	2.33	3.33
PI 422258	Armadillo 'S'	Mexico	2.33	3.33
PI 422260	Drira	Mexico	1.33	2.00
PI 422263	Joco 'R'	Mexico	2.33	2.33
PI 422264	Koala	Mexico	0.00	2.67
PI 422269	Rahum	Mexico	1.83	2.67

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 428729	6A-66	Spain	1.67	2.17
PI 428730	6A-67	Japan	2.00	2.33
PI 428731	6A-69	Japan	1.33	1.67
PI 428732	6A-71	Japan	2.00	3.33
PI 428736	8A-95	Canada	0.00	0.83
PI 428738	8A-100	Canada	4.50	3.50
PI 428739	8A-101	Sweden	3.17	2.67
PI 428742	8A-105	Japan	2.67	2.67
PI 428743	8A-106	Japan	0.00	2.33
PI 428745	8A-109	Japan	2.83	3.00
PI 428748	8A-113	Japan	2.17	2.17
PI 428750	8A-116	Canada	3.00	4.33
PI 428753	55D2.330	Canada	2.67	2.50
PI 428754	8A-120	Canada	2.17	2.17
PI 428755	8A-122	Canada	2.67	2.17
PI 428756	8A-123	Japan	4.33	2.67
PI 428757	Veselopodolyanskaya	Russian Fed.	3.67	3.17
PI 428758	8A-125	Russian Fed.	2.67	2.50
PI 428759	8A-126	Canada	3.83	3.33
PI 428760	8A-127	Russian Fed.	3.33	3.17
PI 428761	8A-129	Russian Fed.	2.00	1.50
PI 428763	8A-131	United States	3.00	2.67
PI 428765	10A-135	Canada	4.33	5.00
PI 428766	8A-142	United States	3.33	3.67
PI 428768	W.R. 4608	United States	3.00	4.00
PI 428770	8A-148	United States	3.17	3.67
PI 428771	8A-154	India	2.67	3.33
PI 428775	Triticale B	Sweden	3.67	4.50
PI 428776	Triticale C	Sweden	3.67	3.33
PI 428777	Triticale F	Sweden	2.50	3.00
PI 428778	Triticale H	Sweden	4.33	4.33
PI 428780	Triticale J	Sweden	1.83	2.17
PI 428781	Triticale K	Sweden	2.00	2.67
PI 428782	Triticale L	Sweden	2.67	1.67
PI 428784	Triticale N	Sweden	3.33	2.50
PI 428785	Triticale O	Sweden	2.67	2.67
PI 428787	Triticale Q	Sweden	3.17	2.67
PI 428792	8A-179	Sweden	3.00	2.50
PI 428794	8A-181	Sweden	2.33	3.00
PI 428795	6A-190	Canada	0.17	1.83
PI 428796	8A-192	Canada	2.83	2.00
PI 428798	8A-195	Canada	2.67	2.50
PI 428800	AABBDD No. 3	Japan	3.50	2.67
PI 428801	AABBDD No. 5	Japan	3.67	3.17
PI 428804	57D2.12	Canada	2.83	2.83

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 428805	56G8.23	Canada	2.67	2.67
PI 428807	8A-216	Canada	3.67	3.83
PI 428810	8A-222	Germany	3.67	3.17
PI 428811	Muncheberg 1	Germany	3.17	2.67
PI 428812	6A-248	Canada	2.67	2.67
PI 428813	8A-249	Russian Fed.	2.83	2.00
PI 428814	6A-250	Russian Fed.	0.00	2.00
PI 428816	MTE-10	Spain	1.67	2.33
PI 428818	MTE-41	Spain	2.83	3.33
PI 428819	MTE-48	Spain	2.17	1.50
PI 428820	MTE-50	Spain	2.50	3.17
PI 428821	MTE-58	Spain	2.67	2.67
PI 428822	MTE-63	Spain	2.33	2.67
PI 428823	MTE-83	Spain	2.67	2.50
PI 428824	MTE-93	Spain	0.17	2.33
PI 428825	MTE-120	Spain	0.00	2.33
PI 428827	6A-272	Russian Fed.	2.33	2.17
PI 428828	6A-273	Russian Fed.	2.33	2.17
PI 428829	6A-274	Russian Fed.	0.17	2.33
PI 428831	6A-276	Russian Fed.	1.67	2.17
PI 428832	8A-277	Russian Fed.	3.50	2.67
PI 428833	8A-278	Russian Fed.	0.00	3.33
PI 428834	8A-279	Russian Fed.	2.67	3.33
PI 428836	8A-282	Canada	2.33	0.83
PI 428839	8A-285	Canada	4.17	2.83
PI 428841	6A-297	Canada	2.17	1.33
PI 428842	6A-298	Canada	2.17	2.33
PI 428845	29 H AD 127	Russian Fed.	1.83	2.67
PI 428846	92 H AD 137	Russian Fed.	2.50	2.67
PI 428853	2 H AD 121	Russian Fed.	2.67	2.50
PI 428854	8A-312	Russian Fed.	0.33	0.83
PI 428855	HAD 259	Russian Fed.	4.33	5.00
PI 428858	8A-318	Canada	5.00	5.00
PI 428860	AD 322	Russian Fed.	3.00	2.33
PI 428865	T 201	France	2.00	2.33
PI 428866	T 204	France	2.33	2.67
PI 428868	T 206	France	3.50	2.50
PI 428869	T 207	France	2.67	2.33
PI 428870	T 212	France	3.50	2.50
PI 428871	T 213	France	4.17	3.67
PI 428873	T 216	France	3.83	4.00
PI 428875	T 275	France	3.00	3.00
PI 428880	H AD 435	Russian Fed.	2.00	2.33
PI 428882	AD 322	Russian Fed.	2.67	2.00
PI 428883	AD 350	Russian Fed.	3.17	3.17

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 428885	860-62-65	Hungary	1.17	1.00
PI 428886	860-63-65	Hungary	2.00	2.33
PI 428887	860-67-65	Hungary	1.67	2.00
PI 428889	KORAI 2-65	Hungary	2.50	2.50
PI 428890	KORAI 3-65	Hungary	2.17	3.00
PI 428891	KORAI 4-65	Hungary	2.33	3.17
PI 428892	KORAI 5-65	Hungary	2.00	3.17
PI 428893	Triticale No. 30	Hungary	1.33	2.33
PI 428894	AD 349	Hungary	2.33	2.33
PI 428895	8A-377	Canada	1.00	2.00
PI 428897	Sel. 4	Spain	2.33	1.67
PI 428898	Cachiruls	Spain	2.50	2.33
PI 428899	6A-388	Canada	2.17	1.33
PI 428900	521-5	Canada	0.00	1.67
PI 428901	381-3	Canada	2.17	2.50
PI 428902	169-1	Canada	0.00	1.67
PI 428903	8A-395	Canada	1.33	2.50
PI 428904	8A-396	Canada	2.33	2.33
PI 428905	8A-397	Canada	3.33	3.00
PI 428907	8A-399	Canada	2.83	3.33
PI 428908	8A-400	Canada	2.83	3.33
PI 428909	8A-401	Canada	3.33	2.33
PI 428911	8A-403	Canada	1.67	3.00
PI 428912	6A-404	Canada	0.00	2.33
PI 428913	6A-405	Canada	1.00	0.00
PI 428914	6A-406	Canada	2.17	1.67
PI 428915	6A-407	Canada	2.17	4.00
PI 428916	6A-408	Canada	2.00	2.00
PI 428917	6A-409	Canada	3.50	3.83
PI 428918	6A-410	Canada	0.00	1.83
PI 428919	6A-411	Canada	2.50	2.67
PI 428920	6A-412	Canada	2.00	2.33
PI 428921	6A-413	Canada	1.33	3.00
PI 428923	6A-415	Canada	2.00	4.17
PI 429185	8TA-164	United States	5.00	5.00
PI 429186	6TA-385	United States	2.33	2.50
PI 429188	8TA-036	United States	2.17	2.67
PI 429189	8TA-083	United States	1.83	2.00
PI 429190	8TA-119	United States	2.67	2.67
PI 429221	UC 38	United States	2.83	3.33
PI 429227	6TA-418	United States	2.00	2.67
PI 429232	6TA-522	United States	0.00	2.00
PI 429233	6TA-386A	United States	3.67	2.67
PI 491409	Beagle 82	United States	2.17	2.33
PI 495821	Toort	Australia	2.00	2.33

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 495869	Marval	United States	2.33	1.83
PI 508249	Whitman	United States	2.50	3.67
PI 519232		Pakistan	2.17	2.50
PI 520419	OC 731070	Brazil	2.17	2.67
PI 520420	F.S. 3972	Brazil	2.00	2.67
PI 520431	Satu	Australia	1.00	2.17
PI 520432	Corong	Australia	1.33	2.17
PI 520433	Panda 'S'	Brazil	2.33	3.00
PI 520434	Panda 'S'	Brazil	1.50	2.17
PI 520435	PFT 811	Brazil	2.50	3.33
PI 520436	Delfin 20S	Brazil	2.33	2.33
PI 520437	Delfin 80	Brazil	2.00	2.67
PI 520438	PFT 80380	Brazil	2.00	2.17
PI 520439	PFT 817	Brazil	2.17	3.33
PI 520440	PFT 8112	Brazil	1.67	2.33
PI 520441	PFT 8116	Brazil	2.17	3.17
PI 520442	PFT 8128	Brazil	2.33	3.00
PI 520443	PFT 80110	Brazil	2.17	2.50
PI 520460	Iniap Mana	Ecuador	2.00	2.83
PI 525197	Muir	Australia	1.67	2.00
PI 542509	M86-6018	United States	2.17	3.17
PI 542512	M86-6027	United States	2.33	2.33
PI 542513	M86-6030	United States	2.17	2.50
PI 542514	M86-6032	United States	2.50	2.67
PI 542517	M86-6037	United States	3.33	4.00
PI 542520	M86-6046	United States	3.00	2.83
PI 542521	M86-6047	United States	2.17	2.33
PI 542522	M86-6051	United States	1.83	2.33
PI 542523	M86-6052	United States	2.17	3.17
PI 542524	M86-6054	United States	2.00	3.00
PI 542525	M86-6055	United States	2.33	3.00
PI 542526	M86-6057	United States	2.00	2.33
PI 542527	M86-6060	United States	0.17	2.50
PI 542528	M86-6064	United States	2.67	2.83
PI 542532	M86-6068	United States	2.17	3.00
PI 542533	M86-6070	United States	2.00	1.00
PI 542534	M86-6071	United States	3.00	3.17
PI 542535	M86-6078	United States	1.67	1.67
PI 542536	M86-6081	United States	2.33	2.67
PI 542538	M86-6089	United States	2.33	2.50
PI 542539	M86-6106	United States	1.00	2.00
PI 542541	M86-6109	United States	3.17	3.17
PI 542542	M86-6116	United States	3.00	2.67
PI 542545	M86-6121	United States	0.50	0.83
PI 542546	M86-6139	United States	2.33	2.83

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 542550	M86-6171	United States	2.33	3.33
PI 542551	M86-6174	United States	2.67	3.50
PI 542554	Jenkins	United States	2.83	2.83
PI 542561	H85-633	United States	5.00	5.00
PI 542564	-	United States	3.33	3.50
PI 542565	-	United States	2.33	2.50
PI 547079	Stan II	United States	4.67	5.00
PI 550576	Sunland	United States	2.17	2.50
PI 559373	Karl	United States	2.17	3.00
PI 561844	GA-SRT	United States	2.83	2.50
PI 564431	1176-163	Bulgaria	2.50	3.17
PI 564432	1346-62	Bulgaria	2.33	3.67
PI 564433	1465-366	Bulgaria	2.33	4.33
PI 564434	1775-570	Bulgaria	2.17	4.67
PI 564435	2333-22	Bulgaria	3.67	3.33
PI 564436	579-447	Bulgaria	2.00	3.67
PI 564438	968-600-132	Bulgaria	2.17	3.33
PI 564440	Meksitol 1108	Bulgaria	0.83	2.33
PI 564441	Persenk	Bulgaria	2.17	3.17
PI 564442	Presto	Bulgaria	2.00	2.50
PI 564443	Vichren	Bulgaria	2.33	2.33
PI 564484	MT 7291	Bulgaria	2.17	3.50
PI 564485	Perun	Bulgaria	2.33	2.83
PI 564486	Mizar	Italy	2.17	5.00
PI 564727	Bob	United States	1.83	2.17
PI 564738	Celia	United States	1.67	1.33
PI 564760	Parma	United States	2.00	2.17
PI 565486	2700	United States	2.33	2.33
PI 572235	Gaucho	United States	2.67	3.00
PI 572949	Uzor	Uzbekistan	2.33	2.50
PI 574285	CT 353-79	United States	2.33	2.33
PI 574286	VT082464	United States	3.33	3.67
PI 574287	VT082478	United States	2.67	2.83
PI 574288	81T211	United States	2.33	3.67
PI 574289	83T103	United States	2.33	3.00
PI 587224	TF 3	Romania	2.33	3.17
PI 587225	Cambridge Rosner	United Kingdom	2.33	3.17
PI 587227	T-M-AD-1160	Bulgaria	1.67	2.67
PI 587228	T-M-AD-1171	Bulgaria	2.17	2.50
PI 587229	T-M-AD-252	Bulgaria	0.67	0.83
PI 587230	T-M-AD-490	Bulgaria	2.00	2.33
PI 587231	T-M-AD-414	Bulgaria	2.33	3.33
PI 587232	Mexitol 1	Bulgaria	2.33	3.67
PI 587244	6A1330	United States	2.17	3.17
PI 587259	II81-210	Australia	2.33	3.33

Accession no.	Accession name	Country of origin	BLS-LB10	BLS-P3
PI 587260	II81-216	Australia	0.67	3.33
PI 587261	81-220	Australia	2.00	2.83
PI 587262	81-221	Australia	2.00	2.67
PI 587263	81-223	Australia	2.67	3.17
PI 587264	81-224	Australia	2.67	2.67
PI 587265	X78-532S-20	Australia	1.67	2.17
PI 587266	X553-2	Australia	2.33	3.00
PI 587267	X78-5715-13	Australia	2.17	2.67
PI 587268	X78-5925-1	Australia	0.00	2.00
PI 587269	X78-5983-3	Australia	3.33	3.33
PI 587270	X78-605-3	Australia	2.83	2.67
PI 587272	8A1394	Australia	2.33	2.33
PI 587273	6A1419	United States	2.50	2.67
PI 587275	8A1424	United States	2.33	2.33

APPENDIX C. LIST OF 31 WHEAT CULTIVARS AND CHECKS USED IN THE EVALUATION, AND THEIR AVERAGE DISEASE SCORE TO TWO *XANTHOMONAS TRANSLUCENS* PV. *UNDULOSA* STRAINS

Name	Class	BLS-LB10	BLS-P3
Briggs	HRS	3.00	3.83
Pierce	Durum	3.50	3.33
Mountrail	Durum	3.67	3.33
Steele	HRS	3.67	4.67
Ben	Durum	3.67	4.00
Tioga	Durum	3.67	4.00
Faller	HRS	3.83	4.00
Sy-Soren	HRS	3.83	3.67
Brennan	HRS	4.00	4.00
Sy-Tyra	HRS	4.00	3.33
Barlow	HRS	4.00	4.50
Alsen	HRS	4.17	4.50
Select	HRS	4.17	3.67
Alkabo	Durum	4.17	4.00
Mott	HRS	4.33	5.00
Divide	Durum	4.33	3.33
Jenna	HRS	4.33	4.00
Lebsock	Durum	4.33	3.67
Kelby	HRS	4.33	4.17
Grenora	Durum	4.50	3.67
Carpio	Durum	4.50	3.67
Rollag	HRS	4.67	4.17
Prosper	HRS	4.67	4.33
Joppa	Durum	4.67	4.33
Glenn	HRS	4.67	4.17
Elgin	HRS	4.67	4.00
Howard	HRS	4.83	4.50
Vantage	HRS	5.00	4.67
Velva	HRS	5.00	5.00
RBO7	HRS	5.00	5.00
Dilse	Durum	5.00	4.00
Magnum (R.	-	3.67	3.33
Pavon 76 (R.	-	3.00	3.50
ND495 (S. check)	-	4.33	4.50

APPENDIX D. LIST OF ALL SPRING WHEAT GENOTYPES AND THEIR AVERAGE DISEASE SCORE TO A *XANTHOMONAS TRANSLUCENS* PV. *UNDULOSA* STRAIN

Accession no.	Accession name	Country	Ave. disease score	Growth stage score
CItr 3008	Wol Koren	S. Africa	4.38	3
CItr 8442	Favorito	Argentina	3.00	3
CItr 12170	S 691B	Canada	3.75	2
CItr 12302	RL 1527	Canada	5.50	2
CItr 12691	Poso 48	United States	7.25	2
CItr 12814	III-46-15	United States	8.00	1
CItr 12835	RL 2661	Canada	5.00	2
CItr 14346	Betmark	S. Africa	5.50	2
CItr 14362	2809-2B-4B-1B-3T	Chile	7.00	1
CItr 14371	8475-59	Brazil	5.00	2
CItr 14400	3 - 18 -57	Peru	4.50	2
CItr 15088	Sr 13	Canada	7.25	2
CItr 15136	American 378	Sudan	6.50	1
CItr 15212	Deir Alla	Jordan	4.25	3
CItr 15634	II-62-71	United States	6.50	2
CItr 15850	II-18889-4M-1Y-4M-2Y-	Mexico	6.75	2
CItr 17750	RL 4314	Canada	6.00	2
PI24486	131	Turkmenistan	3.88	3
PI 41032	Allorca	Tunisia	2.75	3
PI 68281	331	Azerbaijan	7.25	1
PI 83729	Magyarovar 81	Hungary	6.50	3
PI 86198	Krasnaya Ostistaya No. 65	Ukraine	4.88	2
PI 94341	9	Armenia	3.50	4
PI 94367	61BP	Armenia	4.50	3
PI 94757	310	Armenia	7.75	1
PI 106202	G 124-15-0	Australia	7.50	2
PI 107608	Firwhill	Australia	5.25	1
PI 117757	3085	Australia	8.00	1
PI 124847	G-29-14-0-3-1-0	Australia	7.75	2
PI 126822	Kenya Crossbred	Kenya	5.75	2
PI 129518	O.S. Jakowski	Poland	5.50	2
PI 130647	81005	Australia	4.00	3
PI 131273	C 10444	Australia	4.50	2
PI 131401	0-27-5-0	Australia	7.25	2

Accession no.	Accession name	Country	Ave. disease score	Growth stage score
PI 134348	AD 21/1-3-5	Australia	5.00	2
PI 163571	1017	Guatemala	4.75	1
PI 170907	153-M-M-3	South Africa	7.00	1
PI 175517	Sampo	Finland	5.50	2
PI 176325	10056	India	7.75	1
PI 177167	184 P.2.A.1.F	Kenya	8.50	1
PI 180617	Carstens Sommerweizen	Germany	7.25	3
PI 181470	Progress	Finland	5.75	3
PI 182673	Salamouni	Lebanon	4.50	3
PI 184575	Aussie	Australia	4.38	3
PI 184598	Uruguay	Uruguay	2.50	3
PI 184631	Webster	Russian	4.00	3
PI 184634	Heines Kolben	Germany	6.00	3
PI 184845	Criollo	Guatemala	7.25	2
PI 184993	M-36	Norway	5.50	2
PI 185272	H836 SEL 47 259	Argentina	7.25	2
PI 185356	Florence 193	Portugal	6.75	2
PI 185713	Ideal	Portugal	3.50	3
PI 185836	BH 3145	Brazil	6.67	3
PI 185909	II-1989	Mexico	8.50	1
PI 185932	II-888	Mexico	3.00	3
PI 186033	II-1442-4C-1C-11C	Mexico	7.25	2
PI 189384		Finland	4.88	2
PI 189794	Sel. 49-2825 H557	Argentina	6.25	3
PI 189799	Sel. 49-4807 H603	Argentina	3.75	2
PI 189826	Sel. 49-2810 H1070	Argentina	3.25	2
PI 190450	Jo 3	Norway	4.50	2
PI 190914	Pondus	Sweden	4.50	3
PI 191261	Blando 588	Spain	6.25	2
PI 191323	Fylgia	Sweden	7.25	2
PI 191453	Hundi	Spain	5.75	2
PI 191576	H 3 B 12699	Portugal	3.25	2
PI 191638	Equator KTI	Kenya	4.00	3
PI 191882	BxRA8 10142	Argentina	2.75	4
PI 191961	Magvarovax	Hungary	5.00	2
PI 192014	Serrano	Portugal	4.25	2
PI 192027	3430	Mozambique	8.00	2
PI 192123	Cjlugas	Mozambique	2.75	3

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PI 192131	Renew	Mozambique	8.00	2
PI 192169	Precoce	Portugal	2.75	4
PI 192208	Rumanien	Romania	6.00	2
PI 192282	Hatvani 5612	Hungary	8.50	1
PI 192299	Indiskt	India	8.50	1
PI 192312	3781	Sweden	3.00	2
PI 192348	Kadolzer	Czechoslovakia	4.25	5
PI 192539	H 19 D 12716	Portugal	2.00	2
PI 192557	H N B C 13739	Portugal	4.88	3
PI 192623	Hindi 12	Egypt	6.50	2
PI 193937	Inter-Generic	Colombia	8.75	1
PI 202672		Finland	7.00	2
PI 205738	Mariache 50	Argentina	4.50	2
PI 207104	65o	Iran	2.25	3
PI 210866	3777-50	Brazil	2.50	2
PI 210972	T.A. 622-3 Sasl 2176	Egypt	7.75	2
PI 213601	D.I.V. 6722	Argentina	2.75	3
PI 214396	Colotana 2107/50	Brazil	4.25	3
PI 221361	Leda	Belgium	7.50	2
PI 223185	Atacatzo No. 1	Ecuador	5.75	2
PI 225412	51-491	Uruguay	3.00	3
PI 230652	Jhiaveva	Paraguay	4.00	3
PI 231120	II-2809-1c1xmx45x1x	Guatemala	5.75	2
PI 234163	Industrial Argentino	Peru	7.00	2
PI 234236	Idaho 1877 NR HD	Zambia	8.50	2
PI 234239	Idaho 1880 NR BB	Zambia	5.50	3
PI 234968		Italy	2.75	3
PI 235221	Himekei No. 428-1	Japan	3.88	3
PI 237655	Kenya Governor	Kenya	6.25	2
PI 237658	Rhodesian Sabanero	Kenya	3.00	3
PI 238403	358-P.6.A	Kenya	5.13	2
PI 241596	Taichung No. 23	Taiwan	8.00	1
PI 243679	5401	Iran	3.50	3
PI 245394	3064	Afghanistan	4.25	4
PI 247907	Andes 55	Colombia	8.75	2
PI 247914	Maipofen	Chile	5.25	1
PI 249817	NTF 5-1	Isrel	8.50	1
PI 253803	K1761	Afghanistan	6.50	2

Accession no.	Accession name	Country	Ave. disease score	Growth stage score
PI 254124	505.M.I.D.7	Kenya	7.00	2
PI 254126	559.L.2.C.1	Kenya	3.50	3
PI 254128	559.O.1.L.2	Kenya	3.75	3
PI 254132	604.L.1.B.4	Kenya	5.50	2
PI 254824	Janetzkis Jabo	Germany	6.25	2
PI 255140	Loosdorfer Manfred	Austria	4.25	3
PI 260805	1406-3683	Egypt	7.75	1
PI 266148	Leone	Italy	3.00	3
PI 268009	Lir	Portugal	4.50	2
PI 268305	86	Iran	6.75	2
PI 270044	C591	Pakistan	6.00	2
PI 271129	Mult 760	Peru	2.25	3
PI 271130	Mult 764	Peru	2.25	3
PI 272331	IAS-43	Brazil	4.38	3
PI 276705	Krasnozernaja	Russian	5.88	2
PI 278213	Sinai 1	Egypt	3.25	4
PI 278375	Kenya 131	Kenya	3.75	4
PI 278545	Aleppo 28	Syria	5.75	3
PI 278655	Sarrubra	Russian	8.50	2
PI 279454	Ritchie	United Kingdom	6.75	3
PI 282922	I-1039	Argentina	2.25	3
PI 283147	Dorziyeh Karak	Jordan	4.25	3
PI 283874	Hilgendorf 1961	New Zealand	4.50	5
PI 284547	Fylby	Belgium	6.50	3
PI 285944	Gorzowska Sztywna	Poland	5.50	3
PI 286544	Colorado	Ecuador	4.25	2
PI 294911	Karnobatska Ranasreika	Bulgaria	4.00	3
PI 294970	Krasnaja Zvezda	Kazakhstan	3.50	5
PI 297021	184P	Kenya	6.25	1
PI 298603	Betana	South Africa	4.75	3
PI 299414	1013B.1.KJ	Kenya	4.63	1
PI 306529	Harison Barbu	Romania	7.50	2
PI 308674	White Spitzkop	South Africa	4.75	3
PI 312115	Kwarta	South Africa	6.75	1
PI 312116	Rheebok	South Africa	8.00	1
PI 315837	Opal	Germany	7.50	2
PI 321700	Kolben II	Germany	6.25	3
PI 321889	B-858	Turkey	7.25	2

Accession no.	Accession name	Country	Ave. disease score	Growth stage score
PI 323607		Australia	6.00	2
PI 324151	Catcher	Kenya	6.00	1
PI 326331	Khami	Zimbabwe	9.00	1
PI 326336	Sugamuxi 68	Colombia	5.00	2
PI 337147	Magnif 96	Argentina	4.75	2
PI 338417	NP 757	India	9.00	1
PI 343730	Kolibri	Germany	6.50	2
PI 343737	Atacazo	Ecuador	5.75	2
PI 343738	Ruminahui	Ecuador	6.75	2
PI 344154	Ijui	Brazil	6.75	2
PI 344170	IAO 4	Brazil	4.88	2
PI 344190	Missioneiro	Brazil	2.75	4
PI 344203	Anhanguera	Brazil	3.25	3
PI 345693	Minskaja	Belarus	6.25	2
PI 347171	FAO 26.430	Afghanistan	3.25	4
PI 351504	Massaux No. 3	Argentina	7.25	2
PI 351536	Ottawa 2780 E	Canada	5.00	2
PI 351704	Belorusskaja 15	Belarus	4.75	3
PI 351758	Fasan	Germany	5.13	2
PI 351870	T - 2089	Burundi	5.75	2
PI 351874	T - 2093	Burundi	4.75	2
PI 351878	Kiska 9	Burundi	5.50	2
PI 351903	B 205	Switzerland	5.75	2
PI 351994	Z.88.116	Switzerland	6.75	1
PI 352183	Mex 16	Mexico	8.00	2
PI 352204	B 580	Switzerland	5.00	3
PI 352206	B 669	Switzerland	7.50	3
PI 352250	Napo	Ecuador	7.75	1
PI 358339	2020/70	Croatia	5.50	3
PI 366063	Giza 156	Egypt	5.00	2
PI 366923	1196	Afghanistan	5.50	3
PI 372137	Lutescens 491	Ukraine	8.00	2
PI 378910	13340	Colombia	8.25	1
PI 378915	18127	Philippines	6.75	1
PI 382162	16-52-3	Brazil	3.38	3
PI 384025	Ein Dor	Isrel	4.50	2
PI 384352	Dikwa 7	Nigeria	8.75	1
PI 384378	Dikwa 33	Nigeria	7.25	1

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PI 384379	Dikwa 34	Nigeria	6.13	1
PI 387594	IAR/W/128-5	Ethiopia	7.00	2
PI 388036	Line 99	Isrel	5.25	2
PI 388037	539/21	Isrel	8.75	1
PI 388038		Isrel	5.00	2
PI 388082	FAO 33.218	Pakistan	3.75	4
PI 410899	Gloria	Morocco	7.75	1
PI 410914	3297	Morocco	9.00	2
PI 410954		South Africa	3.00	3
PI 411132	Gogatsu Komugi	Japan	6.25	1
PI 412985	Red Bobs	Canada	6.25	2
PI 414538	Sibiriacka 4	Russian	6.00	3
PI 414625	3311	China	3.75	4
PI 418575	Buriatskaja 34	Russian	7.00	2
PI 422282	HI 588	India	7.50	1
PI 422440	Mysegeja	Albania	3.75	3
PI 427285	Sakigake Komugi	Japan	3.25	1
PI 428666	Detenicka Vouska	Czech Republic	7.25	2
PI 428668	Dvorskeho Zoro	Czechoslovakia	7.25	2
PI 428690	Leucurum 3	Uzbekistan	5.00	2
PI 429318	Mesri	Yemen	4.50	2
PI 434987	Estanzuela Young	Uruguay	8.00	1
PI 438961	Al'borubrum 50	Kazakhstan	8.75	2
PI 438966	Pavlodarskaja I	Kazakhstan	4.50	3
PI 438967	Pirotriks 28	Kazakhstan	6.00	3
PI 438968	Snegurka	Kazakhstan	7.25	2
PI 438969	Shortandinskaja 25	Kazakhstan	6.50	2
PI 447353	Lung Chun No. 6	China	6.50	2
PI 447384	Xin Chun No. 1	China	6.50	2
PI 449296	MT-7	Spain	8.75	1
PI 449298	7020	Spain	5.25	2
PI 462111	280	Yemen	6.00	2
PI 468988	MG 27041	Greece	4.75	3
PI 468990	MG 27043	Greece	6.38	2
PI 469072	MG 27959	Greece	5.00	3
PI 480480	R-124	Bolivia	3.25	3
PI 502627	Red Star	Uzbekistan	4.13	3
PI 508385	V764-14-J2-B2-J2	Isrel	8.25	1

Accession no.	Accession name	Country	Ave. disease score	Growth stage score
PI 508387	V882-F22-F2-F3-F2-J2	Isrel	8.75	1
PI 508388	1108/83	Isrel	7.00	1
PI 518648	Laura	Canada	8.50	1
PI 519011	N-1254-5C-2C-2C	Chile	4.00	2
PI 519357	Bluebird 'S'	Mexico	5.25	2
PI 519418	VI-36-2-30B-3T-2B-2T	Ecuador	4.75	2
PI 519421	VI.106-5B-2T-1B-1T-1B	Ecuador	8.50	2
PI 519484	12584-8B-2T-3B1T	Colombia	6.25	1
PI 519503	L 1360-3838	Egypt	6.00	2
PI 519512	ND 71-12-111	United States	5.75	2
PI 519580	CH-7790-12P-9P-1P-1P	Chile	7.25	1
PI 519612	A5292-23P-1P-1P	Chile	7.50	1
PI 519683	A4920-52P-2P-2P	Chile	5.00	2
PI 519792	QP 330-1C-1C-1C-1C	Chile	3.25	3
PI 519805	LE 2096	Uruguay	7.00	1
PI 519842	F8-4	Mexico	7.00	2
PI 519904	W 5865-2-M-3-LM	South Africa	5.33	5
PI 519908	W 5672-3-M-6-TM	South Africa	7.75	1
PI 519912	W 5885-2-M-1-LM	South Africa	4.25	3
PI 520033	Kenya 4135-H3D5	Kenya	5.50	2
PI 520108	CM 39714-5S-2AP-OAP	Mexico	8.00	1
PI 520265	ND 598	United States	6.50	1
PI 520282	CEP 75336	Brazil	3.25	3
PI 520350	ND 587	United States	6.50	1
PI 520374	CM 2281-13M-1Y-3M-	Mexico	7.00	2
PI 520375	CM 23091-1M-2Y-OY	Mexico	7.00	1
PI 520377	SE 381-4S-1S-6S-OS	Syria	5.50	2
PI 520386	CM 32285-3S-4AP-OAP	Syria	6.75	2
PI 520557	Bobwhite 'S'	Mexico	8.25	1
PI 525282	1130	Morocco	5.25	2
PI 525326	1359	Morocco	4.75	2
PI 532056	163	Egypt	5.75	3
PI 532070	1014	Egypt	6.25	2
PI 532255	Mufsegha	Oman	7.25	1
PI 532284	7442	Oman	5.50	2
PI 534448	MG 18237	Algeria	3.75	3
PI 542661	Bourba	Algeria	5.00	3
PI 559704	Uljbinka 25	Kazakhstan	7.75	2

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PI 565222	Gabo	Bolivia	6.50	1
PI 572630	Kazahstanskaja 4	Kazakhstan	7.25	1
PI 572632	Ural'skaja Jubilejnaja	Kazakhstan	7.50	2
PI 572636	Ranjaja 73	Ukraine	5.50	2
PI 572730	Chapingo VF74	Mexico	6.38	1
PI 572822	86PK1311-001.02	Pakistan	8.33	2
PI 574348	15040	Saudi Arabia	5.25	2
PI 576639	2262-12	Tunisia	3.00	3
PI 577777	MG 18017	Algeria	3.75	3
PI 583670	Spinkcota	United States	6.25	2
PI 583705	Coxilha	Brazil	3.75	2
PI 583715	Obregon	Mexico	6.50	1
PI 591942	SST 124	South Africa	5.13	2
PI 593658	AC Barrie	Canada	6.25	2
PI 592983	Pomerelle	United States	4.00	5
PI 595661	8644-057-I	Canada	6.50	2
PI 596367	P8921-Q4C5	Canada	3.75	2
PI 610755	CIGM90.483	Mexico	4.25	2
PI 620714	OR9630064	United States	3.75	4
PI 613317	CIGM98. 412	Mexico	2.75	2
PI 614012	CIGM98. 748 - 1	Mexico	7.00	1
PI 614040	CIGM98. 752 - 1	Mexico	7.50	1
PI 623352	IWA8603039	Iran	7.25	1
PI 623820	IWA8607143	Iran	4.75	3
PI 624156	IWA8607793	Iran	6.25	2
PI 624226	IWA8607919	Iran	3.75	4
PI 624292	IWA8608045	Iran	4.50	3
PI 624883	IWA8609067	Iran	5.50	2
PI 624979	IWA8609318	Iran	6.75	2
PI 625571	IWA8611725	Iran	5.50	2
PI 625642	IWA8611964	Iran	3.50	3
PI 625778	IWA8612362	Iran	5.50	3
PI 638576	99CF 635	United States	2.50	4