GENETIC MAPPING OF RESISTANCE TO BACTERIAL LEAF STREAK IN TRITICALE

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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

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ABSTRACT

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), is an important disease of wheat worldwide. The best way to control this disease in the field is the use of resistant cultivars. Although source of resistance is lacking in wheat, several triticale accessions have high levels of resistance. However, resistance in triticale has not be investigated. The main objective of this project was to map the BLS-resistance gene in triticale. A high density genetic linkage map was constructed in a triticale recombinant inbred line population covering all wheat and rye chromosomes. QTL mapping revealed a single locus on the chromosome 5R significantly associated with resistance to BLS. The resistance reaction of F_1 hybrids indicated the dominance resistance. This is the first study to map a major resistance gene to BLS and will facilitate the introgression of this rye-derived BLS resistance into wheat genome through molecular marker-assisted chromosome engineering.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

GENERAL INTRODUCTION

Wheat is one of the most important crops in the world, and it serves as the staple food for the people in many countries. However, its production can be affected by a number of diseases, and bacterial leaf streak (BLS) caused by *Xanthomonas translucens* is one of them. This disease is economically important in many places because the yield losses due to BLS can reach up to 40% under severe conditions (Forster and Schaad 1988). In recent years, BLS has become evident in the upper Midwest regions of the United States including Minnesota, North Dakota, and South Dakota (Adhikari et al 2012, Kandel et al. 2012). Because North Dakota is the most important state producing hard red spring and durum wheat in US, it is a great need to find ways to manage this disease.

Currently, there are no efficient ways to manage BLS except using clean seeds which helps to reduce the disease incidence in the field (Duveiller et al. 1997). Therefore, development and deployment of resistant cultivars appears to be the only option to manage this disease. Although a number of studies have been carried out to search source of resistant sources in wheat, no immune material is found and only partial resistance exists in limited number of lines (Tillman et al 1996, Millus et al. 1996, Adhikari et al. 2011). Furthermore, controversial results have been obtained for some resistant wheat genotypes from different evaluations (Tillman et al. 1996, Adhikari et al. 2012). However, a few triticale lines have been shown to highly resistant to BLS, and resistance is likely controlled by a single gene (Cunfer and Scolari 1982, Johnson et al. 1987).

Because no major resistance gene has been identified in wheat, the resistance gene found in these triticale lines will be very useful for developing resistant cultivars and controlling BLS. To transfer and utilize the resistance gene from triticale, the genetics and genomic location of

resistance gene needs to be determined and the molecular marker linked to the gene needed to be identified. Therefore, the objective of my research was to develop a genetic linkage map in a triticale population which segregates in the reaction to BLS and determine the genetic locations of resistance gene. This research will facilitate the process of developing wheat germplasm with high level of resistance to BLS, which in turn can be used in the development of resistant wheat cultivars.

LITERATURE REVIEW

Wheat: classification, evolution, and production

Wheat is a group of grass species that are classified in the genus *Triticum*. In a broader view, they belong to Poaceae family (grass family), a huge family of monocots also containing other important cereal crops, such as rice, maize, barley, rye, and millet. The goatgrass species, which played important roles in *Triticum* evolution (see below), are in *Aegilops*, another genus in Poaceae. Because wheat, goatgrass, barley, rye and some other grass species are closely related, they are usually collectively called Triticeae, a tribe of grass family (Clayton and Renvoize 1986). Currently, there are six *Triticum* species recognized, which are at one of the three polyploidy levels (diploid, tetraploid or hexaploid), and either cultivated or grown in nature as wild species (Feldman and Levy 2012).

Wheat is believed to first evolve in the Near East, particularly in Fertile Crescent region according to archeological evidences (Matsuoka 2011). It was believed that the earliest progenitors of *Triticum* and *Aegilops* were evolved from a common diploid ancestor (2n=2x=14) (Faris 2014). Research evidences strongly suggest that the evolution of wheat occurred from lower to higher polyploidy levels via natural hybridization with *Aegilops* species followed by spontaneous chromosome doubling of hybrids (Tsunewaki 2009, Matsuoka 2011). Studies also showed that the cultivated species were derived from their wild relatives at each level through domestication along with human civilization (Faris 2014). Two *Triticum* species are at the diploid level (2n=2x=14), including *T. monococcum* (genome: AA) and *T. urartu* (AA). *T. monococcum* has two subspecies: ssp. *monococcum* (A^mA^m) and ssp. *aegilopoides* with the former as the cultivated species having non-brittle rachis (Faris 2014). About half a Million Years Ago (MYA), *T. uratu* (AA) hybridized with a goatgrass, *A. speltoides* (SS) or a close

relative thereof which has disappeared, to give rise to the wild tetraploid wheat (AABB), *T. turgidum* ssp. *dicoccoides* or ssp. *araraticum*. It is around 10,000 years ago that domestication was taken place in wild tetraploid wheat resulting in the formation of the modern cultivated tetraploid wheat, such as emmer wheat (*T. turgidum* ssp. *dicoccum*) and durum (*T. turgidum* ssp. *durum*) (Dvorak et al. 1993, Matsuoka 2011, Kihara 1944, McFadden and Sears 1944). Hexapliod wheat appeared around 8000 years ago when wild tetraploid wheat crossed with another goatgrass *A. tauschii* (2n=2x=14 DD genome) followed by chromosome doubling (Monte et al. 1993, Kihara 1944, McFadden and Sears 1944, Matsuoka 2011). Similarly, the first hexaploid wheat underwent mutations and domestication to become the modern cultivated hexaploid wheat (*T. aestivum*, 2n=6x=42, AABBDD) after acquiring non-brittle rachis and free threshing traits, also known as common or bread wheat (Faris 2014). Common wheat (*T. aestivum*) and durum (*T. turgidum* ssp. *durum*) are two commonly cultivated wheat crops with common wheat taking up 95% wheat production worldwide.

Wheat is one of the major food crops in the world providing one fifth of calorie need for the world population. In 2016, the harvested areas and production of wheat in the world were above 200 million hectares and 700 million tons, respectively (Economic Research Service, USDA, updated on 9/20/2017). There are five major classes of wheat grown in the United States, including hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), white and durum. Wheat ranks the third among crops cultivated in the United States in term of production. In 2016, wheat was cultivated over 50.2 million acres across 42 states in the United States with the total production over 820 million bushels, about 8% of world production (Economic Research Service, USDA, updated on 8/9/2017). Wheat is the chief agricultural commodity in North Dakota contributing a very important share of economic revenue (~$5-7 billions) for the

state. North Dakota produces mainly HRS and durum with a small percentage of HRW. In 2016, the harvested acreage and total production of wheat in North Dakota were 7 million acres and 330 million bushels, respectively, ranking No.1 in the United States (NAAS/ USDA, 2016).

Triticale: evolution, classification and production

Triticale (× *Triticosecale*) is a synthetic hybrid between wheat (*Triticum* spp.) and rye (*Secale* spp.). Rye is a diploid species (2n=2x=14) and belongs to the grass family Poaceae, tribe Triticea and genus *Secale* (Salamini et al. 2002). Rye is closely related to wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Bauer et al. 2017, Bushuk 2001, Crespo-Herrera et al. 2017). Rye is a versatile crop and can be used as food grain, a livestock pasture, and green manure in crop rotation. Furthermore, rye is a good source of many useful genes for wheat breeding regarding disease resistance, higher vigor, and high tolerance to abiotic stresses. The useful genes in rye can be transferred into wheat background by using triticale as a bridge and then developing wheat-rye chromosome translocation lines (Bushuk 2001, Saulescu et al. 2011, Crespo-Herreditas 2017).

The development of fertile triticale was first reported in 1884 (Carman 1884). However, it has not been realized for a long time that the purpose of developing triticale is to combine the valuable qualities of wheat and rye, and the triticale has not started gaining popularity until the last 50 years (Ammar et al. 2004). Triticale is an amphiploid developed by hybridizing wheat and rye species followed by chromosome doubling with colchicine treatment (Ma and Gustafson 2008). Triticale is either octaploid (2n=56=AABBDDRR) or hexaploid (2n=42= AABBRR) dependent on the use of hexaploid wheat (AABBDD) or tetraploid wheat (AABB) during the crossing. Although both types of triticale have been successfully developed, hexaploid triticale is

cultivated in more widely areas and often used in wheat improvement due to its more genetic stability and less numbers of chromosomes to handle (Ammar et al. 2004).

Triticale is mainly cultivated for animal feeds today, to less degree for human food in the regions with less fertile soil and dry climates which are not suitable for wheat. Recently, triticale has also been proposed to use as a biomass source for fuel production (Hills et al. 2007, Badea et al. 2011). In 2014, the world production of triticale was over 16 million tons, and Poland, Germany, Belarus, France and Russia are the leading countries for triticale production (Food and Agriculture Organization, USA). In the United States, triticale is usually grown in Southern Great Plains and West Coast as a forage crop (Blount et al. 2013). However, diverse triticale accessions have been created not for cultivation but for transferring useful genes from rye to wheat (Mergoum and Gomez-Macpherson 2004, Zeller and Hsam 1983).

The disease: bacterial leaf streak (BLS)

Bacterial leaf streak (BLS) or bacterial blight was first reported on barley in 1917 (Jones et al. 1917), and later, it was reported on wheat in Indiana (Smith et al. 1919). The similar diseases were then found on a number of other small grain crops including triticale, barley, rye, oat as well as some grasses including brome grass (Bamberg 1936, Hagborg 1942, Wallin 1946, Fang et al. 1950, Cunfer and Scolari 1982). Recently, it has been identified on genetically distant Liliaceae family species including asparagus (Rademaker et al. 2006). These diseases are caused by genetically related *Xanthomonas* bacterial group named as “translucens group” and there are different pathovars under this group based on their host range. *Xanthomonas translucens* pv. *undulosa* is the causative agent on wheat and triticale (Vauterin et al.1992).

Symptoms

The bacterial pathogen can infect leaves and the spikes causing distinctive symptoms. On leaves, the initial symptoms are characteristic of translucent water soaking streaks with several centimeters long along and between the leaf veins. Under humid conditions, milky bacterial exudates can appear on the water-soaking streaks (McMullen and Adhikari 2011, Milus and Chalkley 1994). Later, the streaks become larger and coalesce to form large brown necrotic areas (McMullen and Adhikari 2011). When the pathogen infects the spikes, the symptoms appeared as dark purple to black lesions on the glume, making the disease to be called black chaff. However, black chaff is not easy to differentiate from Stagonospora nodorum glume blotch symptoms in the field (McMullen and Adhikari 2011).

Distribution and economic importance

Before the BLS was formally reported, it had been noticed in several places in the United States (Jones et al. 1917). By 1917, eight states in US had been observed to have BLS on barley. Smith et al. (1919) reported the occurrences of wheat BLS in all the wheat states of the Middle West. Later, BLS was reported from almost all wheat and barley growing regions in US with outbreaks often being occurred in warm and semi-tropic regions (Milus and Mirlohi 1994, Tubajika et al. 1999). Recently, wheat BLS has become increasingly evident in the Upper Midwest of the United States, including North Dakota, South Dakota, and Minnesota, which is likely due to the favorable environmental conditions, buildup of primary inoculum and high susceptibility of current major cultivars (Adhikari et al. 2012). Wheat BLS has also reported in many other places of the world, and now it is considered to be a major disease of wheat in the world (Duvellier et al. 1997). However, in some parts of Western Europe and Australia, the

disease has not been observed; therefore, the causal bacterium is recommended as a regulation pest for these areas.

The importance of BLS varies in regions depends on the levels of susceptibility in cultivars and environmental conditions. Studies have showed that the yield losses due to BLS can range from negligible to as much as 40% (McMullen and Adhikari 2011). Generally, the yield loss is correlated with the disease severity on flag leaf where the photosynthesis takes place. It was estimated that 50% of diseased leaf area can lead to from 11%-29% yield losses dependent on the cultivars tested (Tillman et al. 1999). Under the wet and irrigation conditions, highly susceptible could suffer as much as 40% of yield loss (Froster 1982, Froster and Shaad 1998). Yield losses are usually due to the reduction of grain test weight per spike and/or the number of grains per spike (Tillman et al. 1999, Froster 1982, Froster and Shaad 1998). In North Dakota, reduction in grain test weight by 10.5% on spring wheat cultivars has been reported (Waldron 1923). The disease is also capable of affecting the grain quality by altering protein content, which is a problem for the malting barley (Shane et al. 1987). In addition, infection on spike may also lead to discoloration of kernels, thus reducing grain quality in wheat and barley (McMullen and Adhikari 2011, Shane et al. 1987).

Epidemiology

In most areas, the epidemics of BLS have been reported to be sporadic over years and it was difficult to connect the climate conditions and other factors to the development of BLS in the fields (Tubajika et al. 1998, 1999). Seed has been implicated as the most important primary inoculum because the bacterial pathogen is readily detected in the seeds which were harvested from infected plants (Boosalis 1952, Timmer et al. 1987, Milus and Mirlohi 1995, Tubajika et al. 1998, Rashid et al. 2013). However, it was shown that the period of the bacterium surviving in

the seeds is short and dependent on the storage conditions, and also the transmission of the bacterium from seeds to seedling was relatively low (Milus and Mirlohi 1995, Tubajika et al. 1998). It was estimated that if the bacterial population is lower than 1,000 colony-forming units (CFU) in the seeds, no foliar symptom would be developed in the following seedling plants (Duvellier et al. 1997). Although studies have reported that the bacterium can survive on crop residues, in the soil and weedy hosts, it is still not well documented that these sources of inoculum play an important role in disease (Milus and Mirlohi 1995, Wegulo 2012, Stromberg et al. 2000).

The bacterium enters plant tissues using natural openings, for example, stomata or through wounds created by insects, storms and frost damage. The bacterium has been shown to have an ice-nucleation activity, which makes plants amiable to frost damage, thus creating wounds for the bacterial entry (Kim et al. 1987, Azad and Schaad 1988). It is generally believed that warm and humid environmental conditions favor the development of BLS in the fields (Duveiller et al. 1997). In a BLS epidemics study, rainfall, temperature and wind speed have been found significantly related to disease development at the local scales (Tubajika et al. 1999). It was observed that disease is usually severe in the fields under wet or irrigation conditions (Froster and Shaad 1988). The exchange of germplasm with infected seeds allows the disease to spread to other countries or continents.

Management of BLS

Controlling and management of BLS is very difficult or impossible due to the lack of effective chemicals. Because infected seeds are the foremost primary inoculum, it is important to avoid using infected seeds when planting and using pathogen-free seeds can reduce disease incidence (Forster and Schaad 1988, Duveiller et al. 1997). Because the pathogen has been found

to survive in residues or soil, it is not enough just to use clean seeds (McMullen and Adhikari 2011, Mew and Natural 1993). There are several methods that have been developed to detect the bacterial pathogen in seeds, such as dilution plating on selective media, and serodiagnostic assays (Forster and Schaad 1985, Duveiller 1990, Duveiller and Bragard 1992, Bragard and Verhoyen 1993). For dilution plating method, seeds can be washed with a NaCl plus tween 20 solution, which is then diluted up to 10^{-3} and spread onto semi selective agar medium (XTS). The number of bacterial colony can be counted and calculated as colony forming units (cfu) (Forster and Schaad 1985). Duveiller (1990) developed Wilbrink's boric acid-cephalexin agar (WBC) semi-selective medium and showed it was better than XTS for using in dilution planting method. Immunofluorescence and dot immunobinding assays were later developed to detect the *X. translucens* pathogens in seeds in a more specific and sensitive way. For this method, the rabbit or rat monoclonal antibodies are needed to first generate using a reference *Xanthomonas* strain (Bragard and Verhoyen 1993). The immunology-based method is easier to perform and less time consuming than dilution plate analysis (Duveiller and Bragard 1992, Bragard and Verhoyen 1993).

If the seeds are detected for the pathogen, several seed treatment methods can be tried to eliminate the bacteria from the seeds. Soaking infected seeds with acidified cupric acetate solution for 20 minutes has been shown to be an effective way (Schaad et al. 1980, Duveiller 1990). Fourest et al. (1990) found heating seeds at 70-85 $^{\circ}$C for 11 days could be used to significantly reduce pathogen in heavily infected barley seeds. However, the efficiency of these methods needs to be further tested.

Antibiotics can effectively inhibit bacterial growth in plate and the application of antibiotics in the field helped to reduce the disease in spring wheat, the results were inconsistent

(McMullan and Adhikari 2011). Furthermore, using antibiotics is very expensive and impractical due to the persistent nature of the pathogen (McMullan and Adhikari 2011, Kandel et al. 2012). Mercury-based chemicals have been shown to be quite effective in seed treatment for this disease, but these chemicals have been banned since 1970s (Duveiller et al. 1997). There is also a minor effect on control the disease from cultural practices such as crop rotation because the bacterial pathogen does not survive well in crop debris and soil (Milus and Malhori 1995). Therefore, development of resistant genotypes is the most efficient, practical and economical way to control BLS (McMullan and Adhikari 2011, Kandel et al. 2012).

The pathogen

The causal agent of BLS was first identified in barley by Jones et al. (1917) as a bacterium, and later this bacterium was also confirmed to leaf streak on wheat (Smith et al. 1919). The bacterium is a small, rod shape cell with a single flagellum in the end and actively motile as a single cell, but forms a mucous, yellow color colonies on nutrient agar plates. Later, the similar bacteria were found to cause leaf streak disease on other cereal crops and grass species. Due to the lack of morphological, biochemical difference as well as the overlapping host range, classification and taxonomy for these bacteria were very confusing in the history and has undergone several major changes (Vauterin et al. 1992). Recently, DNA marker, genome sequencing and molecular manipulations have provided powerful tools to classify these bacterial strains and investigate bacterial pathogenesis.

Host range

The bacterial pathogen was first reported on barley, and then on wheat (Jones et al.1917, Smith et al. 1919). However, it was found that the bacterial strains isolated from wheat could cause disease on both wheat and barley whereas the strain from barley only caused disease on

barley not on wheat (Smith et al. 1919). Later, the bacterial pathogens were also isolated from other crops, such as triticale, rye, oat, and grass species: timothy, brome grass, quack grass and sudan grass (Reddy et al. 1924, Hagborg 1942, Wallin and Reddy 1945, Fang et al. 1947). Similarly, some bacterial strains caused disease not only on the host where they were isolated, but also on one or more other hosts. Therefore, the bacterial pathogens were given different pathovar names based on host (s) they can cause disease (Hagborg 1942, Fang et al. 1947). Crossing infection and overlapping host range were also reported after 1950s causing a great confusion in bacterial classification and taxonomy (summarized in Vauterin et al. 1992). Although some issues still remain in the classification, all bacterial pathogens are currently classified in a single species *Xanthomonas translucens* with different pathovars (Vauterin et al. 1995, see below). The 'translucens group' in this species has three pathovars including *Xanthomonas translucens*. pv. *translucens* (*Xtt*) causes BLS only on barley while *Xanthomonas translucens*. pv. *undulosa* (*Xtu*) infects barley, wheat, triticale and pv. *cerealis* (*Xtc*) causes BLS on wheat, barley, rye, oat triticale and bromegrass. *X. translucens* has another group, known as 'graminis group' which can cause the bacterial wilt on forage and pasture grasses (Vauterin et al. 1995). Recently, some bacterial strains were isolated from the water-soaking lesions on stems of ornamental asparagus tree fern (*Asparagus virgatus*) and identified as *X. t. undulosa* (Rademaker et al. 2006). These strains were able to infect wheat and barley, and vice-verse, the *Xtu* strains from wheat could also infect asparagus (Rademaker et al. 2006). It is very surprising because the host range of *X. translucens* has been thought to be within Gramineae and Poaceae, but *A. virgatus* belongs to the unrelated Liliaceae family.

Genetically, host range is determined by two possible sets of genes 1) genes having positive functions on pathogenicity and 2) genes recognized by host for avirulence functions

(Loper and Kado 1979, Thomashow et al. 1980). In *Xanthomonas translucens*, the co-inoculation of the strain having wide host range strain with the narrow host range strain resulted in a wide host range reaction (Waney et al. 1991). This strongly suggested that the system involves positive factors allowing some bacteria to infect more hosts, rather than being determined avirulence gene-resistance gene interactions. This speculation was further confirmed by the study where it was found that gene mutations (loss of gene function) leads to the reduction of host range (Waney et al. 1991, Mellano and Cooksey 1988). This was very similar to that in *Agrobacterium* system (Loper and Kado 1979, Thomashow et al 1980).

Nomenclature history and the current classification

When the bacterial pathogen was first isolated from barley, it was named as *Bacterium translucens*. Later, the same pathogen was identified from wheat and rye which were named as *Bacterium translucens* var. *undulosa* and *Bacterium translucens* var. *secalis*, respectively (Smith et al. 1919, Reddy et al. 1924). Dowson (1939) created the genus of *Xanthomonas* and ranked *X. translucens* as a species. Hagborg (1942) accepted *Xanthomonas translucens* species but established five forma specialis (f. sp.) based on their pathogenicity on different hosts, including f. sp. *hordei* (barley), f. sp. *undulosa* (wheat, barley and rye), f. sp. *secalis* (rye), *hordei-avenae* (barley and oat), and f. sp. *cerealis* (wheat, barley, rye and oat).

In the classification and nomenclature of *Xanthomonas* done by Dye et al. (1980) all *Xanthomonas* species were grouped into a single species *X. campestris*. The bacteria causing BLS in small grains were named as different pathovars of *X. campestris*, including pv. *hordei*, *undulosa*, *secalis*, *cerealis* and *translucens*, largely corresponding to f. sp. described by Hagborg (1942). However, the newly established pv. *translucens* was not clearly defined in this nomenclature system. The genus of *Xanthomonas* was later reclassified and the bacteria causing

BLS in small grains were placed in the species of *X. translucens* (*Xanthomonas campestris* pv. *hordei, cerealis, secalis, translucens* and *undulosa*), which is commonly known as 'translucens group' (Vauterin et al. 1992). Later in 1995, a common species name, *X. translucens* was given to all pathovars in 'translucens group' (Vauterin et al. 1995). Stead (1989) has done the fatty acid fingerprints for 14 *Xanthomonas campestris* species and it gave rise another group related to 'translucens group' named as 'graminis group' which can cause the bacterial wilt on forage and pasture grasses. Later in 1992, it was found that the 'graminis group' is phylogenetically related to 'translucens group' (Vauterin et al. 1992). Using pathogenicity test and protein, fatty acid and Amplified Fragment Length polymorphism (AFLP) marker analysis, Braggard et al. (1997) conducted the classification and grouping the 'translucens group' and recognized three true biological entities (pv.), including pv. *translucens,* pv. *undulosa,* and pv. *cerealis. X. t.* pv. *cerealis* was not grouped with pv. *undulosa* because they have distinctive AFLP marker patterns.

Virulence and host-pathogen interactions

The overall virulence mechanism of this bacterial pathogen remains largely unknown but molecular and genomic tools have started revealing the genes underlying bacterial pathogenesis and virulence regarding to *X. translucens*. As mentioned above, some genes determining the wide host range have been identified and are likely to have a function analogous to the host specific nodulation genes of *Rhizobium* (Mellano and Cooksey 1988, Waney et al. 1991). However, the functional characterization of these genes has not been published. Many Gram-negative phytopathogenic bacteria, for example, *Pseudomonas syringae,* are known to produce an arsenal of effector proteins as important pathogenicity or virulence factors. These effector proteins are usually delivered through bacterial type III secretion system (T3SS) directly into plant cells to take action; therefore, these proteins are called T3SS effectors (He et al. 2000).

Genomic sequencing of several *X. translucens* have revealed that the bacterial pathogen has functional T3SS and 20-30 T3SS effectors like other Gram-negative bacteria (Wichmann et al. 2013, Gardiner et al. 2014, Peng et al. 2016).

Many *Xanthomonas* pathogens, such as *X. oryzae* pv. *oryzae* (causal agent of rice bacterial leaf blight), *X. oryzae* pv. *oryzicola* (causal agent of rice bacterial leaf streak), *X. citri* ssp. (the causal agent of citrus canker) were shown to have a unique set of T3SS effectors, known as transcription activator like effectors (TALEs). Once TALEs are delivered inside plant cells through T3SS, they are localized to nucleus and bind to specific DNA sequences of a particular host gene and then activate its transcription (Bogdanove et al. 2010). The upregulation of some host genes, for example, sugar transport gene, benefits the bacterial growth thus leading to susceptibility. A typical TALE consists of T3SS secretion signal in N terminus, the nuclear localization signal (NLS), an acidic activation domain (AAD) in C terminus, mostly important, the central repeat region (Bogdanove et al. 2010). All TALEs are similar in N and C-terminus sequences, but differ in the central repeat region which is used to bind host DNA (Mahfouz et al. 2010, Mak et al. 2012). The TALE central region usually has 17-18 repeats with each repeat typically containing 33-34 amino acid (aa). The amino acids for each repeat are almost identical except the 12^{th} and 13^{th} aa, known as repeat variable diresidue (RVD) which dictate the DNA binding specificity of each TALE (Bogdanove et al. 2010, Mak et al. 2012). Recently, the crystal structure of PthXo1, one of TALE from *X. oryzae* pv. *oryzae*, has been revealed through high-throughput computational structure (Mak et al. 2012). Genome sequencing have shown the presence of several copies of TALEs in *X. translucens* strains and gene knockout of individual TALE indicated some play a significant role in virulence (Peng et al. 2016, Falahi-Charkabhi et al. 2017).

Genomics of *Xanthomonas translucens*

The genome sequences of several *Xanthomonas* pathovars and strains from different origins have been reported, which has provided us with a better understanding of biology, virulence mechanism and genetic relationships of this important bacterial pathogen (Table 1). Wichmann et al. (2013) reported the first draft genome sequence of *Xanthomonas translucens* pv. *graminis* (Xtg29) using illumina sequencing, which identified a complete T3SS system and putative 35 T3SS effector genes, but no TALEs like due to the short reads which is hard for repetitive sequence assembling. Since then, genome sequences of more than 25 different *X. translucens* strains representing three pathovars have been reported (Gardiner et al. 2014, Pesce et al. 2015, Jenicke et al. 2016, Peng et al. 2016, Falahi Charkhabi et al. 2017, Table 1). Because illumina short-read sequencing technology was used for most studies, no or incomplete set of TALE genes were identified. However, two *X.t.* pv. *undulosa* strains: Xt4699 (from Kansas, USA, Peng et al. 2016) and ICMP11055 (from Iran, Falahi Charkhabi et al. 2017) were published with a complete circular genome by using both high coverage of illumina short reads and single molecule real time (SMRT) sequencing method. Eight and seven TALEs were identified from Xt4699 and ICMP11055, respectively, and four of them are identical (Falahi Charkhabi et al. 2017). In the study done by Peng et al. (2016) several *X.t.* pv. *translucens* (*Xtt*), *X.t. undolusa* (*Xtu*) from different regions were sequenced with illumina sequencing and compared to each other and to Xt4699. The phylogenetic analysis reveals that *Xtu* strains can be clearly separated from *Xtt* strains and XT4699 is closer to LG48, a strain from North Dakota. Falahi Charkhabi et al. (2017) also compared ICMP11055 sequence to those of XT4699 and other available *Xtu* and *Xtt* strains, and it was found that ICMP11055 harbors unique two-major rearrangements and nine genomic regions and is close to other *Xtu* strains, but is separated from

North American strains. More recently, Langlois et al. (2017) reported the genome sequence of 15 *Xanthomonas translucens* strains representing 6 pathovars. The genome based phylogeny of the sequenced *Xanthomonas* strains reveals that there are three main clusters among *Xanthomonas* where cluster 1 contains *Xanthomonas translucens* pv. *cerealis*, cluster 2 has both *X. t*. pv. *undulosa* and *translucens* whereas cluster 3 comprises the combination of pathovars *arrhenatheri*, *graminis*, *phlei*, and *poae*.

Table 1. List of different *Xanthomonas translucens* pathovars or strains that have been sequenced for their genome

***Xanthomonas* strain**	**Isolation**			**Sequencing method**	**Number of T3SS/ TALEs identified**	**Genome size (bp)**	**Reference**
	Year	**Host origin**	**Place**				
CFBP2541	1941	Bromegrass	USA	Illumina	24 T3SS effectors and 2 TALEs	4,515,938	Pesce et al. 2015
DSM18974	1933	Barley	MN, USA	Illumina	25 T3SS effectors	4,463,577	Jaenicke et al. 2012
DAR61454	1988	Wheat	Australia	Illumina	26 T3SS effectors	4,452,091	Gardiner et al. 2014
Xtg29	NA	Forage grass	Switzerland	Illumina	35 T3SS effectors	4,100,864	Wichmann et al. 2013
XT4699	1999	Wheat	KS,USA	Illumina and SMRT	25 T3SS effectors and 8 TALEs	4,561,137	Peng et al. 2016
XT-Rocky	2009	Wheat	KS,USA	Illumina	26 T3SS effectors and > 7 TALEs	4,459,068	Peng et al. 2016
XT8	1942	Barley	Canada	Illumina	25 T3SS effectors	4,617,556	Peng et al. 2016
XT123	1952	Barley	Canada	Illumina	24 T3SS effectors and 2 TALEs	4,284,749	Peng et al. 2016
XT130	1939	NA	Canada	Illumina	25 T3SS effectors	4,654,290	Peng et al. 2016
XT5523	1966	Wheat	Canada	Illumina	26 T3SS effectors	4,665,768	Peng et al. 2016
XT5770	NA	NA	Canada	Illumina	26 T3SS effectors	4,617,837	Peng et al. 2016
XT5791	1969	Wheat	Canada	Illumina	25 T3SS effectors	4,719,363	Peng et al. 2016
B1	2013	Barley	ND,USA	Illumina	25 T3SS effectors	4,824,098	Peng et al. 2016
B2	2013	Barley	ND,USA	Illumina	25 T3SS effectors	4,503,259	Peng et al. 2016
P3	2009	Wheat	ND,USA	Illumina	25 T3SS effectors	4,522,131	Peng et al. 2016

Table 1. List of different *Xanthomonas translucens* pathovars or strains that have been sequenced for their genome (continued)

***Xanthomonas* strain**	**Isolation**			**Sequencing method**	**Number of T3SS/ TALEs identified**	**Genome size (bp)**	**Reference**
	Year	**Host origin**	**Place**				
LW16	2009	Wheat	ND,USA	Illumina	25 T3SS effectors and >4 TALEs	4,600,125	Peng et al. 2016
LB5	2009	Wheat	ND,USA	Illumina	26 T3SS effectors	4,766,161	Peng et al. 2016
LB10	2009	Wheat	ND,USA	Illumina	26 T3SS effectors	4,543,985	Peng et al. 2016
LG48	2009	Wheat	ND,USA	Illumina	25 T3SS effectors	4,486,555	Peng et al. 2016
LG54	2009	Wheat	ND,USA	Illumina	26 T3SS effectors	4,623,672	Peng et al. 2016
CS2	2009	Wheat	ND,USA	Illumina	26 T3SS effectors	4,722,832	Peng et al. 2016
CS22	2009	Wheat	ND,USA	Illumina	26 T3SS effectors	4,605,395	Peng et al. 2016
CR31	2009	Wheat	ND,USA	Illumina	26 T3SS effectors	4,720,715	Peng et al. 2016
CS4	2009	Wheat	ND,USA	Illumina	26 T3SS effectors	4,779,534	Peng et al. 2016
ICMP11055	1983	NA	Iran	SMRT	25 T3SS effectors and 7 TALEs	4,561,583	Falahi Charkhabi et al. 2017

NA means not available.

Molecular marker development, genetic linkage map and QTL analysis

DNA marker development

Genetic markers can be used to map and track a trait of interest, which is highly desirable to genetic study and breeding programs. The first generation of genetic marker is morphological markers that usually obtained from a mutation (dwarfing, albeno needles) in the seedlings (Franklin 1970, White et al. 2007). The first genetic map was developed in 1913 containing six morphological markers in *Drosophila melanogaster*. Although morphological markers can be easily monitored, they can be affected by environment and/or an epistatic effect from another morphological marker, and also the number of marker available is very limited (Adresen and Lubberstedt 2003). Biochemical markers based on allozymes was the first type of molecular markers showing much better stability than traditional morphological markers. However, number of biochemical markers was still very limited. DNA-based markers were then developed and due to its abundance, they have been widely used in genetic mapping. The first DNA-based marker was restriction fragment length polymorphisms (RFLP) that was introduced in 1960 by Smith and Nathans (Schlötterer 2004). After that, several DNA marker systems without using radioactive isotope were developed, including random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeats (SSR), diversity arrays technology (DArT) and single nucleotide polymorphism (SNP). At present, SSRs, DArTs and SNP marker systems are the most commonly used in development of genetic maps (Mason 2015).

RFLP, AFLP and RAPD

RFLP needs a single or low copy DNA as a radioactive probe to hybridize the completely digested fragments of genomic DNA (separated on gels) to view polymorphism among different

individuals (Tanksley et al. 1989). RFLP method is very robust, but involves multiple steps as well as radioactivity. RAPD is the first PCR based molecular markers that were generated by using a pair of short DNA oligo primers to randomly amplify genomic regions of different individual (Kumar and Gurusubramanian 2011). Due to the short oligo primer used, the repeatability is not very high. AFLP is another PCR-based marker system, but requires the pre-digestion of the template DNA with restriction enzymes and ligation of the adaptors to both ends, which are then used as template for amplification with different sets of primer homologous to adaptor denaturing using gel electrophoresis (Vos et al. 1995, Meudt and Clarke 2007). Compared to RAPD, AFLP is more reproducible with much better high-throughput ability, thus it had been widely used before SNP-based marker method to generate large number of marker for mapping.

SSR markers

SSRs/microsatellites are a piece of DNA containing mono, di, tri, tetra and penta nucleotide units which are arranged as tandemly repeats and they are widely distributed in the eukaryotic organism genome (Tautz and Renz 1984, Powell et al. 1996). The traditional method to isolate SSRs from genomic DNA involves several sequential steps, including construction of a small insert genomic library, screening of the library by hybridization, sequencing positive clones, PCR analysis and detection of polymorphism (Powel et al. 1996). Since it is time consuming and labor intensive, new SSR method was developed by taking advantage of the availability of a large number of expressed sequence tags (ESTs) for many eukaryotic organisms. Because these SSRs are derived from transcribed regions of the genome, they tend to be more conserved (site specific) (Powell et al. 1996, Li et al. 2008). Because SSRs are site-specific, they

have been used as anchor points for different genetic maps in the same species (Bell and Ecker 1993, Akkaya et al. 1995, Powell et al. 1996).

In wheat, SSR marker development has used both methods. The development of genomic SSR markers was first reported by Devos et al. (1995) and Röder et al. (1998) in hexaploid wheat, 'Chinese Spring' and they were distributed on all linkage groups of all A, B and D genomes. Pestsova et al. (2000) developed wheat D genome specific SSR markers from diploid, *Aegilops tauschii*. At present, more than 2,500 genomic SSRs have been mapped in wheat genome (Gupta et al. 2008). Nicot et al. (2004) developed 3,530 EST-SSRs from 46,510 contigs for hexaploid wheat lines CS. More EST-derived SSR markers were reported later for both durum wheat and bread wheat in several studies (Eujayl et al. 2002, Kantety et al. 2002, Li et al. 2008). Some wheat genomic or EST SSR markers have been shown to be transferable to their closely related species such as rye and triticale (Kelung et al. 2004, Tams et al. 2004, 2005, Kelung et al. 2006).

Saal and Wricke (1999) developed 27 specific SSR markers from rye (*Secale cereale* L.) by sequencing 74 positive clones which have (GT/CA)n. Because rye chromosome 1R has many useful genes, many studies placed more efforts to develop 1R specific SSR markers. By 2004, only 9 SSR markers were available for 1R with the majority of them mapped to the distal end of chromosome 1R (Korzun et al. 2001, Ma et al. 2001, Khlestkina et al. 2004). In contrast, Kofler et al. (2008) reported the development of 74 polymorphic SSRs for short arm of rye chromosome 1R. The development of rye SSR markers started before 10 years ago (Saal and Wricke 1999). Since the genomic SSR development causes some problems on transferring among different genomes, labor intensive, new method of EST based SSR marker development was initiated. Hackauf and Wehling (2002) developed 528 EST- SSRs which have di, tri and tetra nucleotide

motifs. Recently, a significant amount of EST-SSR markers (1,385 SSRs) were developed for rye by Haseneyer et al. (2011). These SSR markers will be useful for genetic diversity assays, transferring SSR markers among related species such as wheat and triticale, etc.

Diversity array technology (DArT) markers

DArT is a high throughput marker development system and it mainly is based on microarray hybridization to detect the presence versus absence of thousands and thousands DNA fragments selected for representing a genome (Jaccoud et al. 2001, Wenzl et al. 2004). DArT markers were first developed for a variety of dicot plant species such as *Arabidopsis*, cassava (Wittenberg et al. 2005, Xia et al. 2005). Akbari et al. (2006) reported the development of 339 DArT markers for the hexaploid wheat genome and genetic linkage map assembled using these DArT markers covered all 21 chromosomes except 4D. Mantovani et al. (2008) developed 500 DArT markers using 56 durum accessions and incorporated the obtained DArT makers with 162 SSR markers in a genetic map of RIL population of durum wheat. Peleng et al. (2008) developed 493 DArT markers and mapped them in a tetraploid mapping population derived from a cross between durum wheat and emmer wheat. Bolibok-Bragoszewska et al. (2009) reported 1,818 DArT markers in rye using 16 rye varieties and 15 rye RIL lines of a RIL mapping population. DArT markers have been successfully used in genetic mapping of triticale populations. Badea et al. (2011) used three arrays composed of 25,720, 13,056 and 3,072 wheat, rye and newly synthesized triticale probes, respectively, leading to identification of 6,042 DArT markers.

SNP markers

SNP is defined as single nucleotide polymorphism which is the most abundant source for marker development in a given genome. For most species, SNP can be identified every as short as 100-200 bp and theoretically can provide unlimited number of markers for genetic mapping.

The development of SNP markers relies on the availability of corresponding DNA sequences from two or more genotypes to identify the nucleotide polymorphism between genotypes. In beginning, many SNP markers were developed from the large amount of EST sequences generated from different individuals. Recently, SNP marker has been developed by directly sequencing the whole genome as sequencing power is increasing and cost is reducing (see below). Compared to SSR markers, SNP analysis can be automated in high-throughput assay format without the need to do DNA separation by size (Raman et al. 2014, Fay and Bender 2005, Kumar et al. 2012). Genome wide SNP discovery and the corresponding microarray-based genotyping platform have been successfully developed in many crops such as maize, oilseed rape, rice, soybean, *Brassica*, and alfalfa and they have been successfully used in genetic mapping (Kumar et al. 2012). In wheat, the Illumina GoldenGate chips containing 9,000 genes and 90,000 genes have been developed and available for wheat genome mapping (Akhunov et al. 2009, Cavanagh et al. 2012, Wang et al. 2015). Wang et al. (2014) used 90k gene chip to map 46,977 SNPs in wheat genomes using eight different mapping populations, which provides an important reference for genetic mapping in tetraploid and hexaploid wheat. In rye, Haseneyer et al. (2011) developed 5,234 polymorphic SNP markers using RNA sequencing data.

Recently, genotyping based on next generation sequencing technology has become more popular to develop SNP markers because it is easier, has increased levels of high throughput, and very importantly, costs much less compared to array-based assay (Elshire et al. 2011). Genotyping-by-sequencing (GBS) usually involves the digestion of genomic DNA from different individuals with restriction enzymes which usually target gene-rich regions followed by library construction and high throughput genome sequencing. The sequence data has to be called using a SNP calling pipeline with the help of reference genome sequence (Huang et al. 2009, Kim et al.

2016).The application of GBS has been extended to population studies, germplasm characterization, genetic mapping and genomic-based breeding in almost important crops (Poland et al. 2012).

Genetic mapping of wheat, rye and triticale

The first effort to construct the genetic map of wheat started in 1990s by the organization of International Triticeae Mapping Initiative (ITMI). The genetic linkage map was reported for each chromosome group by the individual research group. Later, reports of wheat genetic maps contained all wheat chromosomes are available (Gupta 2008, Messmer et al. 1999, Lotti et al. 2000). The first generation of genetic maps were usually based on RFLP markers, but later, composite maps were constructed by using more than one type of molecular markers. The examples were the genetic map of Einkorn wheat, which was developed mainly by using RFLPs and SSR markers (Dubcovsky et al. 1996, Singh et al. 2007), and several durum wheat and bread wheat genetic maps with AFLPs, SSRs and RFLPs (Lotti et al. 2000, Nachit et al. 2001, Messmer et al. 1999, Gupta et al. 2008). From different genetic maps, consensus map can be constructed by using the common markers mapped and special mapping software. The first linkage genetic map specific for *Aegilops tauschii* was developed from AFLP markers (Gill et al. 1991, Boyko et al. 1999).

The first rye genetic map was developed in a DS2 x RXL10 F_2 population covered all the seven rye chromosomes with RFLP markers (Devos et al. 1993). Masojc et al. (2001) saturated this genetic map by adding 69 RAPD and 12 isozyme markers and the total genetic distance of the map was 1,140 cM. In 2003, the same genetic map was more saturated by 480 markers with 179 AFLPs, 200 RFLPs, 88 RAPDs and 12 isozymes and the total genetic distance was expanded from 1,140 cM to 1,386 cM by increasing the coverage of chromosomes 1R, 2R and

5R (Bednarek et al. 2003). Korzun et al. (1998) developed a genetic map of two F_2 populations derived from reciprocally crossing of two rye inbred lines, P83 and P105. The genetic map covers all the seven rye chromosomes and it consists of 91 loci including 88 RFLPs, 2 morphological markers and 1 isozyme marker. To map agronomical important traits in rye genome, Börner and Korzun (1998) developed a consensus map of rye covering all seven chromosomes with 413 markers including RFLPs, isozymes and the range of the markers per chromosome was from 41 to 83. The valuable genes for traits such as reduced plant height, self-fertility, male sterility, resistance for powdery mildew etc. have been mapped using this population and genetic maps. Philipp et al. (1994) constructed a genetic map of rye consisting 60 markers with RFLP, RADP, isozymes, morphological and physiological markers. Senft and Wricke (1996) developed an extended genetic map of F_2 population consisting 137 individuals using isozymes, RFLPs and RAPDs by integrating the genetic map developed in Philipp et al. (1994). Korzun et al. (2001) constructed another genetic map of pooled F_2 mapping population of 275 individuals with 139 RFLPs, 19 isozymes, 13 SSRs and 10 known function sequences. Ma et al. (2001) constructed a genetic map covering all 7 chromosomes, containing 184 markers including clones from wheat, rye, barley, oat and rice genomic and cDNA libraries and it spans 727.3 cM. Hackauf and Wehling (2002) constructed a genetic map using a back cross population including EST derived SSR markers, AFLP markers and the total genetic distance for all 7 rye chromosomes was 685 cM. Khlestkina et al. (2004) constructed a saturated genetic map of rye by using RFLPs and 99 SSR markers. They have used four mapping populations and the genetic distance was expanded 1,111 cM, 1,087 cM, 1,109 cM and 1,111 cM for P87 x P105, N6 x N2, N7 x N2, and N7 x N6 respectively. A F_2 population of rye consisting 94 individuals was used by Milczarski et al. (2007) to construct a genetic map. This map was constructed using 148 markers

with 99 RAPDs, 7 ISSRs, 41 STS, 14 RFLPs, 9 SCARs and 1 isozyme marker. The resulting 8 linkage groups were aligned with reference map of 7 rye chromosomes and the total genetic distance was obtained as 1,401.4 cM. Bolibok-Bragoszewska et al. (2009) constructed high resolution map of rye (L13 x L9) covering all the 7 chromosomes which consists 1,818 DArT loci. The total genetic distance of the map was 3,144.6 cM and the map density was 2.68 cM per marker. The shortest chromosome was 1R (301.9 cM) and the largest chromosome was 6R (578.7 cM). Milczarski et al. (2011) developed another high density consensus genetic map by using 9,703 segregating markers. This consensus map was constructed by five rye RIL populations. The total genetic distance of the genetic map was 1,593.0 cM and the total marker density was 1.1 marker per cM, which is much saturated with molecular markers compared to the previously published genetic maps.

Recently few research studies have been published on genetic mapping of triticale. Tyrka et al. (2015) constructed a genetic map of triticale using 50 microsatellite, 842 DArT and 16,888 SNP markers with 4,907 cM in total genetic distance and the mean distance between two bins was 3.0 cM per marker. They used 92 double haploid (DH) lines derived from two hexaploid winter triticale cultivars, ‘Hewo’ and ‘Magnat’. Another study constructed the genetic map of triticale (90 DH lines) deriving from two unrelated hexaploid triticale lines, ‘Saka3006’ and ‘Modus’ with the use of 155 SSRs, 28 RFLPs and 2397 DArT markers. The total genetic distance of the map was 2,397 cM and the mean distance between two markers was 4.1 cM (Tyrka et al. 2011).

Genomics of wheat and rye

Different draft and good quality of reference genome sequences have been available for wheat and rye which have large size of genome and high amount of repetitive DNA. In 2014,

individual chromosomal draft sequence of the hexaploid bread wheat, Chinese Spring was released. Very recently a pretty good reference genome sequence of Chinese Spring has released covering all 21 chromosomes (IWSC 2017). This released sequence contains the physical maps of all the 21 chromosomes, the sequenced BACs for 8 chromosomes including 1A, 1B, 3B, 3D, 6B, 7A, 7B and 7D and the partial sequence for chromosome arms 4AL and 5BS (IWSC 2017). Since rye has many useful genes on chromosome 1R, the sequencing of 1RS was first done with the construction of BAC libraries and shot-gun sequencing. This sequencing results represents 0.5% of 1RS arm and majority (84%) of the sequences represents the repetitive DNA (Bartos et al. 2008). The draft whole genome sequence of rye was obtained later by using whole genome shot gun sequencing, Illumina Hiseq2000 platform (Bauer et al. 2017). This genome was used to predict 27,784 rye genes. Moreover, the resequencing of 10 inbred lines of rye lines revealed more than 90 million single nucleotide variants. This rye draft genome sequence will be very useful in development of more markers to saturate the current genetic maps.

QTL mapping

In plants, many traits are controlled by multiple genes, located in different regions in the genome, and those genome loci are called quantitative trait loci (QTL). The genomic regions controlling quantitatively inherited traits can be identified by QTL mapping method, which is basically detection of the significant association of a trait with individual marker. QTL mapping consists of several steps, including development of the segregation population, genotyping of the population and construction of the genetic map of that population, phenotyping and statistical analysis of the marker data and phenotypic association (Young 1996, Doerge 2002, Sehgal et al. 2016). Currently, there are many software available to detect QTLs mainly including, MQTL (Tinker and Mather 1995), PLABQTL (Utz and Melchinger 1996), QTL Cartographer (Wang et

al. 2012), Qgene (Joehanes and Nelson 2008), etc. To detect QTLs, there are several mapping functions (algorithm). The traditional way to find the QTL is the single marker analysis (point analysis) where the trait data was analyzed with a single genetic marker one at a time. The most common method to detect QTLs is the interval mapping where the detection of QTL is conducted by testing a model at many positions between two marker loci (Lander and Bostein 1989). However, interval mapping cannot resolve two QTLs that are very close and also the confounding effect from the major QTL for other small QTLs. These problems were solved later by the introduction of composite interval mapping (CIM) function (Zeng 1994). In CIM, the effect of other QTLs are not present as residual variance, therefore CIM is more precise and powerful than simple interval mapping (Sehgal et al. 2016). Later, another method, multiple interval mapping (MIM) was developed which is more precise and powerful than CIM, where multiple intervals are used concurrently to fit numerous putative QTL directly in the model (Kao et al. 1999).

QTL mapping has been conducted in wheat to map disease resistance to Fusarium head blight, tan spot, and powdery mildew (Buerstmayr et al. 2009, Huang et al. 2004, Faris et al. 1999). Moreover, QTL mapping has also been used to map QTL controlling many wheat agronomical important traits such as yield, ear emergence time, plant height, grain filling rate (Kumar et al. 2007, Kato et al. 1999, Ramya et al. 2010). In triticale, QTL mapping has been used to identify regions associated with different traits such as biomass yield, plant height, Aluminum tolerance, biomass accumulation (Alheit et al. 2014, Niedziela et al. 2012, Wurschum et al. 2014, Busemeyer et al. 2013). Association mapping (AM) is another way to map QTL in eukaryotic organisms, which is based on linkage disequilibrium (LD). For AM, genetically diverse lines from a natural population are used in analysis compared to bi-parental mapping.

Because mapping lines are obtained from diverse backgrounds and from different time of history, it is more likely to detect marker and trait association, which is QTL (Abdurakhmonov and Abdukarimov 2008).

Identification and genetics of host resistance to BLS

To identify source of resistance, studies have been conducted to evaluate wheat cultivars and breeding lines, landraces for resistance to BLS under field or greenhouse conditions (Duveiller et al. 1993, El Attari et al. 1996, Milus et al. 1996, Tillman et al. 1996, Adhikari et al. 2011, Kandel et al. 2012, Adhikari et al. 2012). 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 a collection of 50 CIMMYT spring wheat genotypes and 24 local winter wheat cultivars, Milus et al. (1996) identified few lines, such as 'Magnum', 'Bayles', and 'Terral 101', as being resistant based on the size of water-soaking area developed around the inoculation sites. Only two lines were found to be partially resistant to BLS from a collection of 64 winter wheat breeding lines from France (El Attari et al. 1996). The field evaluation conducted by Tillman et al. (1996) on 5,000 accessions of worldwide bread wheat collection revealed only 26 resistant genotypes. The study also revealed a negative correlation between BLS resistance and plant maturity. Among the 605 winter wheat accessions, only 8.3% were found to be resistant or moderately resistant under greenhouse condition (Adhikari et al. 2011). Adhikari et al. (2012) conducted greenhouse disease evaluation on 566 accession of spring wheat landraces and identified relative higher percentage of lines with partial resistance in landraces. Kandel et al. (2012) evaluated 45 spring wheat cultivars and breeding lines from the upper Great Plain region under field conditions and identified only one genotype was less susceptible. Therefore, sources of resistance to BLS are

limited and only partial resistance is available in wheat adapted 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). It was also demonstrated that reaction to BLS and black chaff (head infection) is likely dependent from each other in wheat materials (Tillman et al. 1996).

By using wheat lines with partial resistance, few studies were carried out on heritability and genetic mapping of resistance to BLS (Duveiller et al. 1993, El Attari et al. 1996a, b, Tillman and Harrison, 1996, Adhikari et al. 2012, Kandel et al. 2015). Heritability of resistance to BLS in wheat varies from low to very high dependent on the lines used and both additive and dominance effect of different resistance genes are presented (El Attari et al. 1996a, b; Tillman and Harrison, 1996). The genotype x environment (G x E) interaction often occurs in the expression of resistance (Tillman and Harrison, 1996). Using diallelic crosses from three partially resistant (Mochis T88, Pavon 76 and Angostura F88) and two susceptible lines (Alondra, Turaco), Duveiller et al. (1993) identified five resistance genes (*Bls1*, *Bls2*, *Bls3*, *Bls4* and *Bls5*) in the resistant lines with each harboring two to three of these genes. Among them, *Bls1* has the highest effect on resistance.

Using RFLP marker map, El Attari et al. (1998) mapped QTLs in barley 'Morex' conditioning partial resistance to BLS on 3H and 7H chromosomes. Adhikari et al. (2012) employed association mapping and DArT markers for mapping BLS resistance in 566 spring wheat landraces leading to identification of five genomic regions on chromosome 1A, 4A, 4B, 6B and 7D. Gurung et al. (2014) conducted the association mapping of BLS resistance in spring wheat landraces again using the same phenotypic data as Adhikari et al. (2012), but with a large number of SNP markers. Four genomic regions on chromosomes 1A, 5A, 5D and 6B were

identified to significantly associate with BLS. Among 4 of them, two genomic regions were same as described in Adhikari et al. (2012). Kandel et al. (2015) identified two genomic locations, one on chromosome 2A (*Xwmc522*) and the other on 6B (*Xbarc134*), for BLS partial resistance in spring wheat breeding lines using the identity by descent mapping method.

In the evaluation of 35 triticale lines, Cunfer and Scolari (1982) reported that four of them, including UP 7th ITSN#20, UPT 72142, M2A-Bgc, and 'Siskiyou', possess high levels of resistance to BLS under both field and greenhouse conditions. Resistance in Siskiyou and M2A-Bgc has been also used to successfully develop elite resistant triticale lines in Georgia, US (Johnson et al. 1987). Classic genetic analysis on F_2 populations has indicated Siskiyou and M2A-Bgc as well as another resistant triticale OK 77842 carry single dominant resistance gene and the resistance genes in three lines are either the same or closely linked (Johnson et al. 1987). The genetic locus has been designated as *Xct1*, (Johnson et al. 1987). However, the gene locus has not been mapped. This gene will be very useful in breeding BLS resistant wheat germplasm in future. Our preliminary data has shown several triticale accessions including Siskiyou, are highly resistant to BLS caused by the strains from North Dakota (Sapkota et al. 2017).

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GENETIC MAPPING OF A MAJOR GENE IN TRITICALE CONFERRING RESISTANCE TO BACTERIAL LEAF STREAK

Abstract

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), is an important disease of wheat and triticale around the world. Although resistance to BLS is limited in wheat, several triticale accessions have high levels of resistance. To characterize the genetic basis of this resistance, we developed triticale mapping populations using resistant and a susceptible accession. Our initial mapping using results in a F_2 population derived from the cross of Siskiyou (R) × UC38 (S) suggested the resistance gene is likely located on rye chromosome 5R. In this study, we developed the cross of a $F_{2:5}$ recombinant inbred line (RIL) population from the cross of Siskiyou × Villax St. Jose for resistance QTL mapping. This population was genotyped by genotyping-by-sequencing (GBS) and a few 5R SSR markers and evaluated for reactions to BLS. QTL mapping revealed a single major QTL on chromosome 5R, which was underlined by the same SSR as in the Siskiyou × UC38 population and a GBS marker. The F_1 hybrids of the two crosses were highly resistant to BLS, indicating that resistance is largely dominant. This is the first study to map a major resistance for BLS and will facilitate introgression of this rye-derived BLS resistance gene into the wheat genome through molecular marker-mediated chromosome engineering.

Introduction

Bacterial leaf streak (BLS) can occur on a wide range of small grain crops as well as grass species. The diseases are caused by several *Xanthomonas* bacterial pathogens, which have been collectively designated as the 'translucens group' (Vauterin et al. 1992, 1995). Based on pathogenicity tests on small grains and bromegrass, three main pathovars have been defined,

including pv. *translucens*, which is pathogenic only on barley, pv. *undulosa*, pathogenic on wheat, barley, triticale and rye, and pv. *cerealis*, pathogenic on all species tested (Bragard et al. 1997). A recent phylogenetic analysis using whole genome sequence data also supported the existence of three clades related to host specificity (Langlois et al. 2017).

BLS, also known as black chaff when it occurs on floral spikes, caused by *X. translucens* pv. *undulosa* (*Xtu*), is the most common bacterial disease of wheat (*Triticum* spp.) and triticale (*X Triticosecale* Wittmack) (Cunfer and Scolari 1982, Duveiller et al. 1997). Outbreaks have been sporadic and often occur in warm and humid regions (Milus and Mirlohi 1995, Duveiller et al. 1997). In recent years, BLS has re-emerged as a threat to wheat production in many places, including the northern Great Plains of the United States (Adhikari et al. 2011, Kandel et al. 2012). The disease can cause yield losses of up to 40% by reducing kernel weight and/or number of kernels per spike (Waldron et al. 1929, Shane et al. 1987, Forster and Schaad 1988, Tillman et al. 1999). The disease also can affect protein content, degrading the grain quality (Shane et al. 1987). Seeds are believed to be the major source of primary inoculum, and planting clean seed can reduce disease incidence (Milus and Mirlohi 1995). However, no chemical or cultural practice is currently available to manage the disease in the field, and use of resistant cultivars is the only practical means of control.

A large assortment of wheat germplasm, including cultivars, breeding lines, and landraces, have been evaluated for reaction to BLS in the field and/or under greenhouse conditions (Milus and Mirlohi 1994, Tillman et al. 1996, Kandel et al. 2012, Adhikari et al. 2011, 2012). Although a wide range of genetic variation was observed among wheat accessions, only partial resistance has been observed. No immunity or high levels of resistance has been found in wheat. In some cases, partial resistance was shown to be environment-dependent and

associated with late maturity (Tillman et al. 1996, Kandel et al. 2012, El Attari et al. 1996, Tillman and Harrison 1996). Five genes were reported, namely *Bls1*, *Bls2*, *Bls3*, *Bls4*, and *Bls5*, to condition resistance in three wheat cultivars (Duveiller et al. 1993). In addition, Adhikari et al. (2012) identified five genomic regions on five chromosomes (1A, 4A, 4B, 6B, and 7D) associated with BLS resistance in spring wheat. Also, two genomic regions (2A and 6B) were identified to be associated with partial resistance in a collection of South Dakota spring wheat breeding lines (Kandel et al. 2015).

In the evaluation of 35 triticale lines, Cunfer and Scolari (1982) identified four lines, including UP 7th ITSN#20, UPT 72142, M2A-Bgc, and 'Siskiyou', with high levels of resistance to BLS under both field and greenhouse conditions. In addition, two elite triticale germplasm lines were developed in Georgia, USA with resistance derived from Siskiyou and M2A-Bgc (Johnson et al. 1989). Further genetic analyses suggested that resistance in the triticale accessions Siskiyou, M2A-Bgc, and OK 77842 is governed by a single dominant gene or tightly linked genes, designated *Xct1* (Johnson et al. 1987). Using *Xtu* strains from North Dakota, USA, we previously evaluated a worldwide triticale collection and also found several triticale lines, including Siskiyou, that were highly resistant (Sapkota et al. 2017). We have done an initial work to map the resistance gene using a F_2 population derived from the Siskiyou x UC38 cross. The bulked segregation analysis revealed that the resistant loci is likely located on the chromosome 5R because the tightly linked SSR marker, *XSCM138* is on this chromosome. In this work, we conducted QTL mapping of resistance in a recombinant inbred line population of Siskiyou x Villax St. Jose which has the same resistant parent as Siskiyou x UC38. The objectives of this study were to construct a high density genetic linkage map and to map QTL associated with BLS resistance in Siskiyou x Villax St. Jose RIL population. In addition,

molecular markers tightly linked to resistance QTL will be identified, which can be used in the assist in the transfer of this gene to wheat germplasm.

Materials and methods

Plant materials

The materials include a recombinant inbred line (RIL) triticale population, designated LTC0918, which was derived from the cross between highly resistant accession 'Siskiyou' (L12G09) and highly susceptible accession Villax St. Jose (L12G18). Siskiyou (CI 17603) was jointly developed by the International Maize and Wheat Improvement Center, Mexico, and the University of California, Davis, USA and released as a cultivar in California (Qualset et al. 1985). Villax St. Jose (PI 428848) is a cultivar from Morocco (see Kuleung et al. 2006). Our preliminary evaluation of over 500 triticale accessions in the greenhouse indicated Siskiyou is highly resistant to BLS while Villax St. Jose is highly susceptible (Sapkota et al. 2013). From the cross of Siskiyou/Villax St. Jose, we have developed a recombinant inbred line (RIL) population with a total of 141 $F_{2:5}$ individuals through single seed descent (SSD) during 2014 and 2015, and the $F_{2:5}$ recombinant inbred (RI) line population was used for genotyping by sequencing, disease evaluation and QTL mapping.

Disease evaluation

Disease evaluation was conducted in North Dakota State University (NDSU) Agricultural Experiment Station greenhouse which has a temperature setting from 15°C-21°C with a sixteen hours light. The plants were grown in racks which can hold 98 cones (4×13 cm). The cones were filled with Promix soil and two seeds were planted per cone with the border line ('Grandin'). The fertilizer which was used is Osmocotte (15-9-12). Then the plants were grown under greenhouse conditions for two weeks until three-leaf stage. Experiments were carried out by using

randomized complete block design (RCBD) with three replicates and two plants for each RIL were evaluated in one replicate.

The inoculum (*Xtu*) was applied to the three leaf stage plants by spray inoculation. The two bacterium strains, BLS-LB10 from North Dakota and Xt4699 (sequenced strain) from Kansas were used for disease evaluation and mapping. The bacteria were streaked from the stocks on WBA (Wilbrink's agar) plates and were incubated at 28°C for 48 h. Then, the bacterial cells were gently scratched and suspended in 1×PBS buffer (Phosphate buffer), and the solution was adjusted for the cell concentration to optical density of 0.4 at 600 nm followed by the adding of surfactant reagent tween 20 at two drops per 100 ml. The prepared inoculum was inoculated onto the plants by direct spraying at the rate of 100 ml per rack with an air-pressured (20 Psi) spray gun. The inoculated plants were kept in misting chambers for 48 h under 12 h photoperiod every day and with the humidity settings at every 2 minutes misting 10 seconds. Then, plants were moved to a greenhouse room for growth and disease development. The plants with the racks were placed in a water-filled pan to avoid being watered from the top. The disease was recorded at 5^{th} day after inoculation (DAI) by estimating the percentage of water-soaking area developed in the secondary leaf after five days of inoculation. The percentage of diseased area was directly used in the QTL mapping.

Genotyping by GBS (genotyping by sequencing) method

LTC0918 population was genotyped using genotyping by sequencing (GBS) method. Genotyping was carried out with the collaboration of Dr. Xuehui Li's laboratory, Department of Plant Sciences. The leaf tissues from each RIL were collected from one week old seedling plants and genomic DNA were extracted from the tissues with the Wizard Genomic DNA purification kit (A11125; Promega). The concentration of DNA sample was quantified with a Quant-iT

PicoGreen dsDNA assay kit. Then, the GBS library was constructed with a double digest using *PstI* and *MseI* enzymes following routine protocols and was sequenced on a single lane of illumina HiSeq2000. TASSEL-GBS software (Glaubitz et al. 2014) was used for SNP discovery and genotype calling.

Genotyping with simple sequence repeats (SSR) markers

In our preliminary study, bulked segregant analysis between the resistant parent, Siskiyou, susceptible parent, UC38 and the resistant and susceptible pools in F_2 population of Siskiyou x UC38 revealed that the resistance gene is likely located on chromosome 5R (work done by Dr. Aimin Wen). Thus, a total of 20 SSR markers on the chromosome 5R were also tested between Siskiyou and Villax St. Jose for polymorphism and the polymorphic primers were genotyped in the whole population LTC0918. In order to run SSR on 4300 DNA analyzer (LI-COR Bioscience, Lincoln, NE), the reverse complementary sequence of M13 (-21) primer (5′-TGTAAAACGACGGCCAGT-3') was added to the 5′ end of each forward primer. All SSR primers and DY682 or DY782 fluorescently labeled M13 (-21) primers were synthesized by Eurofins MWG Operon LLC (Louisville, KY). Each PCR reaction consisted of 1× buffer, 200 μM dNTPs, 1.5 mM $MgCl_2$, 0.05 μM of each SSR forward primer (tagged with M13 reverse complementary sequence) and reverse primers, 0.1 μM of DY682 or DY782 fluorescently-labeled M13 primer, 50 ng DNA, and 1 U Bulleseye Taq DNA polymerase (MidSci, Valley Park, MO) in a 10 μL volume. PCR program and gel electrophoresis were the same as the descriptions by Zhong et al. (2009). SSR bands were manually scored as 1 for Siskiyou allele, 0 for Villax St Jose and 3 for missing data.

Linkage map development

The SNPs and SSR markers were used to construct the linkage map of LTC0918 population. MapDisto software (version 1.7.7, Lorieux 2012) was used for constructing the linkage groups of the mapping population. The mapping function was chosen as Kosambi (1944) and the data was grouped in to separate linkage groups by using 'Find group' command with the logarithm of odds (LOD) as 3 and maximum recombination frequency were set as 0.3. Serriation II and sum of adjacent recombination frequencies (SARF) were used for ordering the loci. The linkage groups were assigned to specific chromosomes based on the physical locations of the GBS SNP markers, which were obtained by BLAST the tag sequences of GBS SNP markers against the wheat and rye reference genome sequences.

QTL analysis

QTL mapping was performed by using QGENE 4.3.10v (Joehanes and Nelson 2008) with the simple interval mapping (SIM), composite interval mapping (CIM) and multiple interval mapping (MIM) function. A permutation test consisting of 1000 permutations were used to determine an LOD threshold for CIM at an experiment-wise significance level of 0.05. The coefficient of determination (R^2) was determined for each QTL using Qgene software which provides an estimate of the amount of phenotypic variation explained by each QTL.

Results

Reaction of lines and the Siskiyou x Villax St. Jose recombinant inbred line (LTC0918) population to *Xtu* strains (BLS LB-10, Xt4699)

The parent, Siskiyou gave the resistant reaction (0-10% water-soaking) when it was inoculated with both *Xtu* strains whereas Villax St. Jose gave the susceptible reaction (>50% water soaking). UC38 is another susceptible parent for LTC0908 population which is derived by

crossing Siskiyou which is the resistant parent and UC38 which is the susceptible parent. Therefore UC38 also showed susceptible reaction (>50% water soaking) against both *Xtu* strains. The hard red winter wheat cultivar "RB07" also showed susceptible reaction by extensive water soaking similar to Villax St. Jose and UC38. The F_1 plants from both Siskiyou x Villax St. Jose and Siskiyou x UC38 were shown resistant reaction indicating that resistance is largely dominant (Figure 1).

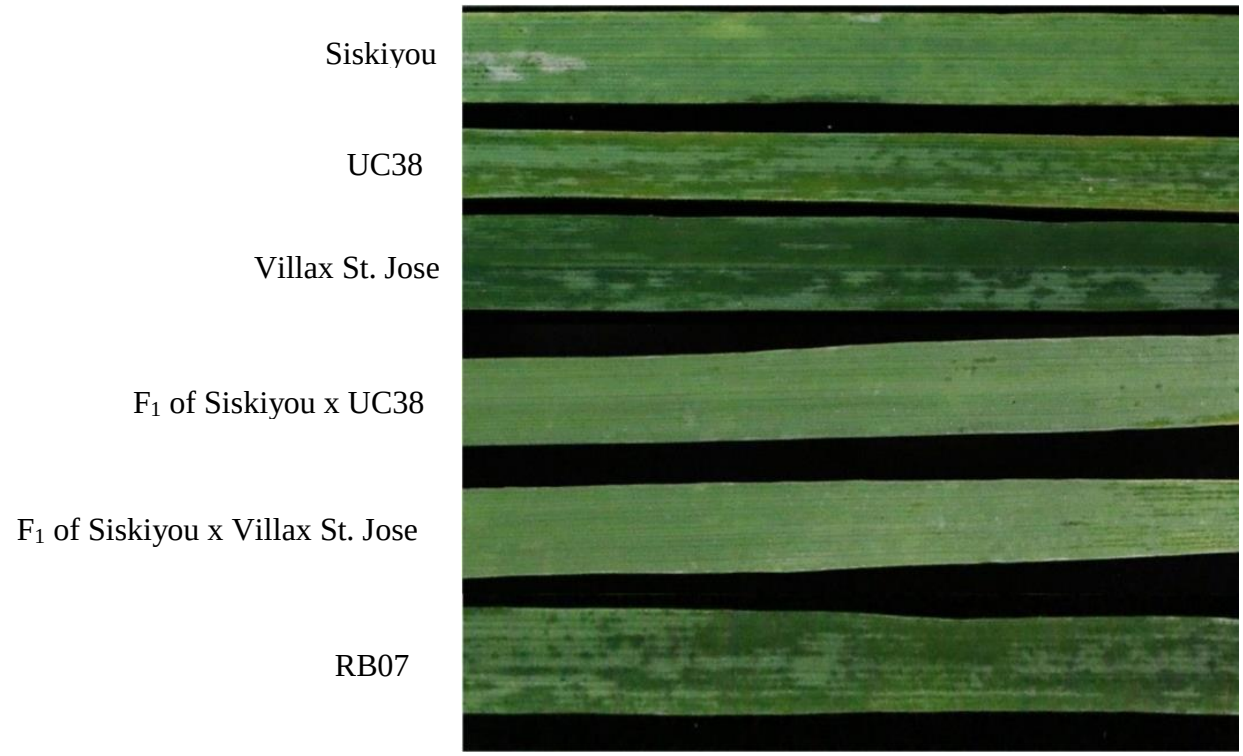

Figure 1. Reaction of the parental triticale lines and their F_1 progeny to bacterial leaf streak caused by *Xanthomonas translucens* pv. *undulosa* (BLS-LB10)

Development of SNP markers by genotyping by sequencing (GBS) in LTC0918 population

Library screening generated 120M raw sequencing reads and 10M were resulted as mapped reads. Universal Network Enabled Analysis Kit (UNEAK) identified 111,288 raw SNPs in the population of 143 individuals (2 parents + 141 RILs). Wells devoid of any genomic material were included to control for cross-contamination during library preparations. These blank wells resulted in only 37 total tag sequences and 28 genotype calls, suggesting high-

fidelity in sample preparation, and processing. After filtering the SNPs for presence of at least half the genotypes in the population, 14,099 remained. After filtering these subsequent SNPs for the presence of polymorphic genotype calls between Siskiyou and Villax St. Jose, 3,589 SNP remained.

Genotyping of SSR markers in LTC0918 population

Our preliminary results (Bulked segregant analysis) suggested that the resistance gene is located on rye chromosome 5R in Siskiyou (Figure 2); therefore, the 5R SSR primers were first tested on the parental lines of the LTC0918. Out of 20 primer pairs from the 5R chromosome, seven were found to be polymorphic between two parental lines, Siskiyou and Villax St. Jose (Figure 3), including SCM138, SCM151, REMS1174, REMS1205, REMS1237, REMS1264 and REMS1266. The rest of primers were either did not amplify any bands or amplified bands, but with no polymorphism. For these SSR markers, eight were polymorphic between Siskiyou and UC38, including SCM138, SCM151, SCM 159, Z2995, Z3057, REMS1218, REMS1237 and REMS1266 (work done by Dr. Aimin Wen). Thus, four SSR markers were common for both populations and they are SCM138, SCM151, REMS1237 and REMS1266 (Table 2). All the 7 polymorphic markers were then mapped in the Siskiyou x Villax St. Jose F_5 population using 141 individuals (Figure 4).

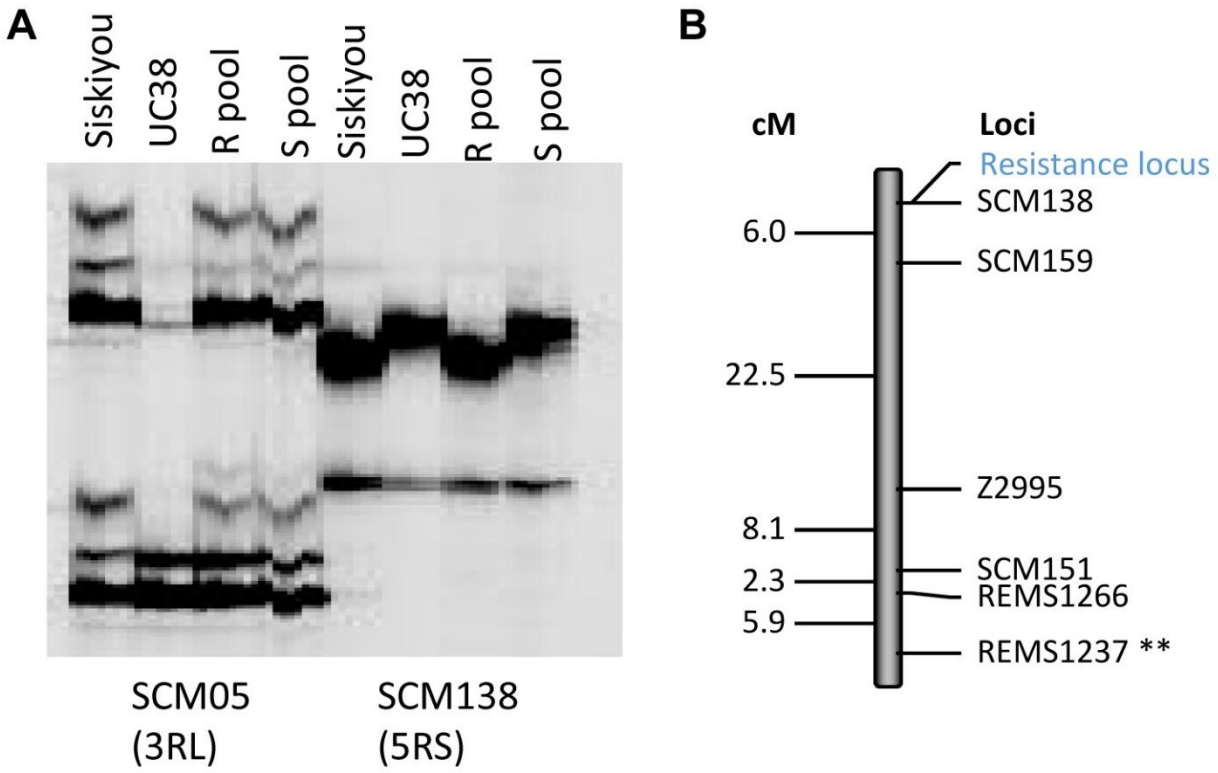

Figure 2. Bulked segregant analysis of BLS resistance in the Siskiyou / UC-38 F_2 population. **A.** Gel image of two SSR primers, SCM05 (3RL) and SCM138 (5RS) run on the parental lines and DNA pools of highly resistant and highly susceptible lines. SCM138 is polymorphic between resistant and susceptible pools. **B.** Linkage map of chromosome 5R developed in the Siskiyou / UC-38 F_2 population with 5R SSR markers. The BLS resistance locus co-segregates with the SSR marker *XSCM138*. Markers marked with stars indicate distorted segregation at $P < 0.01$. This work is done by Dr. Aimin Wen.

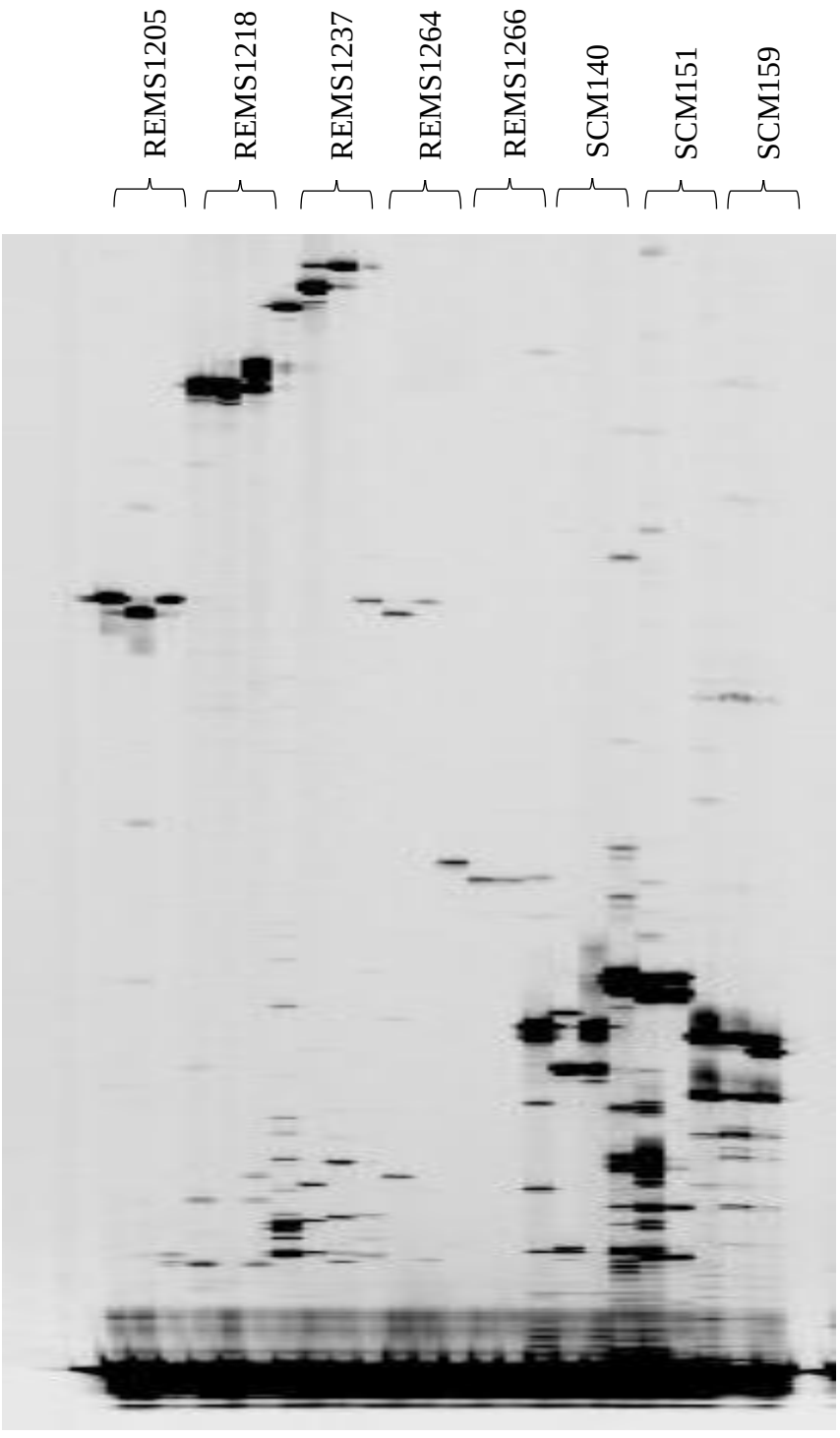

Figure 3. A LICOR gel image showing SSR Primer screenings in the three triticale parental lines, including Siskiyou, Villax St. Jose and UC38. In each primer, 1st lane indicates the Siskiyou allele, 2nd lane indicates Villax St. Jose and 3rd lane indicates UC38 allele.

Table 2. List of SSR markers on chromosome 5R that were tested in three triticale parental lines including Siskiyou, UC38 and Villax St. Jose

Primers	Sequences		Size[a] (bp)	Reference	Polymorphic[b]
SCM138	ATAGCCGCAGATGGTTGAGGAC	GAGAAGTCTACAAATCAAGGGGGC	188	Saal and Wricke 1999	A, B
SCM140	CCCCTCCGAAATCGTTC	GGAGGAGTTCTTCATCACACC	120	Hackauf and Wehling 2002	-
SCM141	ACTGCCGTGTGGTGAA	TGGGAAACATCAAACTAACTG	128	Hackauf and Wehling 2002	-
SCM151	CGGAAACTTAACAGGACACGA	GCAGGGGAAGGAAAAGAAGAG	148	Hackauf and Wehling 2002	A, B
SCM159	CGGGCCGGAAACACAAAA	GGCGGGAAGGAAAAACAGAAA	115	Hackauf and Wehling 2002	A
Z3512	AAATGCCTGCACACAAGCTA	GGAAAATAACCCCCTTGTTG	245	Sun et al. 2016	-
Z2064	TTTGCTCCACGTAGGGATCT	GTTGGGTGTGAGGCTTGTTT	153	Sun et al. 2016	-
Z2076	TGGATGCAAGTGACTCTTCG	GAATTCCTGCTTCAGCTTGC	109	Sun et al. 2016	-
Z2995	CATGCATGGGAGAGTGAGTG	GCAGCGAACTGACTGACTGA	230	Sun et al. 2016	A
Z3005	AGAATGTGTGCTCGACAACG	ATGGTGATGTTGTAGGGGGA	83	Sun et al. 2016	-
Z3010	TCCATCGAGGTGGAAGAATC	GCTCTCTCCTCTCCCCTCAT	158	Sun et al. 2016	-
Z3057	TCCCCAGGATTTCATTTCAC	AATATGACGACTGAAGCCGC	191	Sun et al. 2016	A
REMS1167	ATTGGAAGATCCGCCACC	ATCAGGCCACACAATCACCT	247	Khleskina et al. 2004	-
REMS1174	AGAACATCCAGGTGGTGGAC	TAACAATGCAGATGGCGAAC	302	Khleskina et al. 2004	B
REMS1186	CGTCTCGTCGCGTAAAAACT	ACCTACCCACCCACCGAT	221	Khleskina et al. 2004	-
REMS1205	TTGTTTTGCCAAAGAAGGCT	TCACATCATGGAGGAACCAA	281	Khleskina et al. 2004	B
REMS1218	CGCACAAACAAAAACACGAC	CAAACAAACCCATTGACACG	230	Khleskina et al. 2004	A
REMS1237	GCAATCTCAGATCCTACGGC	GCTTCTGACTGAGCGAACCT	288	Khleskina et al. 2004	A, B
REMS1264	AAAACCATCCACACATCCGT	GAACTCGCTCTTCATCCTCG	282	Khleskina et al. 2004	B
REMS1266	AAAGGAAAACCACCTCAGGG	GCATTTTGCAGGAGAAGCAT	202	Khleskina et al. 2004	A, B

[a] Information on the size is obtained from references.

[b] A indicates that the primers produce a polymorphic band between Siskiyou and UC38, B indicates the primers produce a polymorphic band between Siskiyou and Villax St. Jose, and "–" indicates the primers yield no product or a monomorphic band.

Construction of the genetic linkage map in LTC0918 population

The 3,589 SNP markers from GBS and seven chromosome 5R–specific SSR markers were used to construct linkage map in the Siskiyou × Villax St. Jose $F_{2:5}$ RIL population (Table 3). The map consisted of 21 major linkage groups corresponding to 14 wheat and 7 rye chromosomes covering 2,890.33 cM in genetic distance (Figure 4, Table 3). The identity of each linkage group was determined by searching sequences of each GBS tag from the wheat and rye reference genome sequence (https://wheat-urgi.versailles.inra.fr/Seq-Repository/ and http://pgsb.helmholtz-muenchen.de/plant/rye/gz/searchjsp/). For wheat and rye, the majority of mapped markers were found to have good hits on the designated A and B and R chromosomes. Some of the markers mapped on rye chromosomes had hits on the corresponding homoeologous wheat A and B chromosomes (Table 3). The genetic distance for each chromosome ranged from 79.95 (6A) to 206.56 cM (3A), and marker density for each chromosome ranged from 1.68 (4R) to 3.36 cM/marker (4B) (Table 3). Chromosome 5R contained 43 SNPs and 7 SSR markers spanning 136.72 cM (Table 3).

Table 3. Summary of genetic linkage maps of triticale developed in the recombinant inbred line population derived from the cross between Siskiyou and Villax St. Jose

Chromosome	Marker mapped[a]	Genetic distance (cM)	Marker density (cM/marker)
1A	73 (64)	152.68	2.09
2A	66 (64)	152.09	2.30
3A	87 (59)	206.56	2.37
4A	48 (38)	116.63	2.43
5A	52 (37)	166.92	3.21
6A	24 (21)	79.95	3.33
7A	72 (58)	194.43	2.70
1B	53 (45)	147.79	2.79
2B	84 (71)	150.92	1.80
3B	83 (72)	160.17	1.93
4B	34 (28)	114.44	3.36
5B	83 (72)	183.08	2.20
6B	66 (41)	140.49	2.13
7B	47 (38)	105.41	2.24
1R	43 (32+6)	90.24	2.10
2R	38 (29+3)	83.87	2.21
3R	57 (42+1)	110.73	1.94
4R	91 (67+3)	153.00	1.68
5R	**50 (41+1+7)**	**136.72**	**2.73**
6R	81 (40)	136.76	1.69
7R	43 (25+4)	107.45	2.50
A genome	422	1069.26	2.53
B genome	450	1002.30	2.23
R genome	403	818.77	2.03
Total	1275	2890.33	2.27

[a] Numbers in brackets, in order, indicate the number of markers having a strong hit to the corresponding chromosome from blast searching of the tag sequence against the wheat and rye reference genome sequences (wheat: https://wheat-urgi.versailles.inra.fr/Seq-Repository/; rye: (http://pgsb.helmholtz-muenchen.de/plant/rye/gz/searchjsp/), the numbers of markers mapping to an R genome chromosome that have hits to corresponding homoeologous wheat chromosomes, and (for 5R) the number of SSR markers mapped.

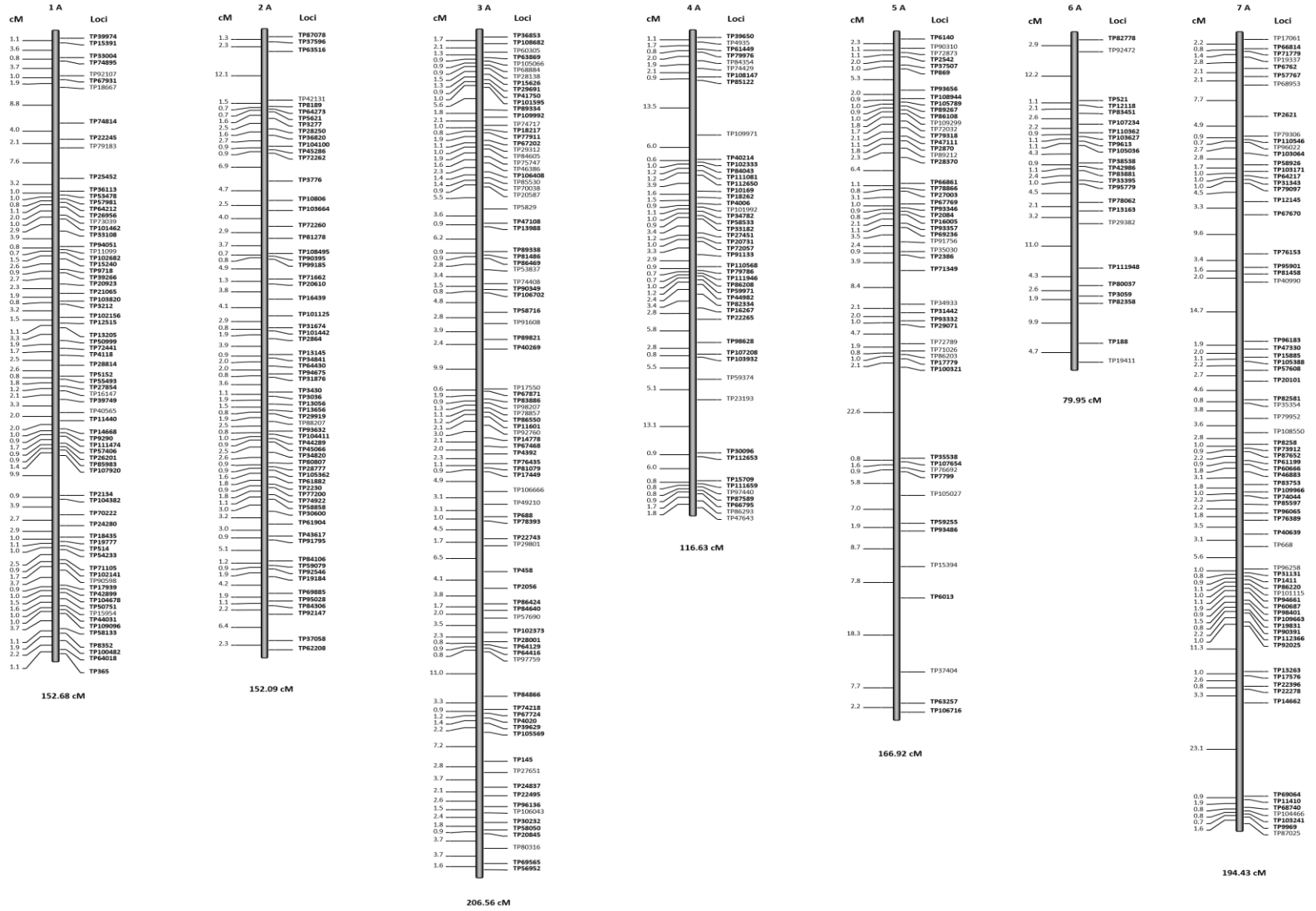

Figure 4. Genetic linkage maps (1A-7A) developed in a recombinant inbred line population derived from the cross between in Siskiyou and Villax St. Jose

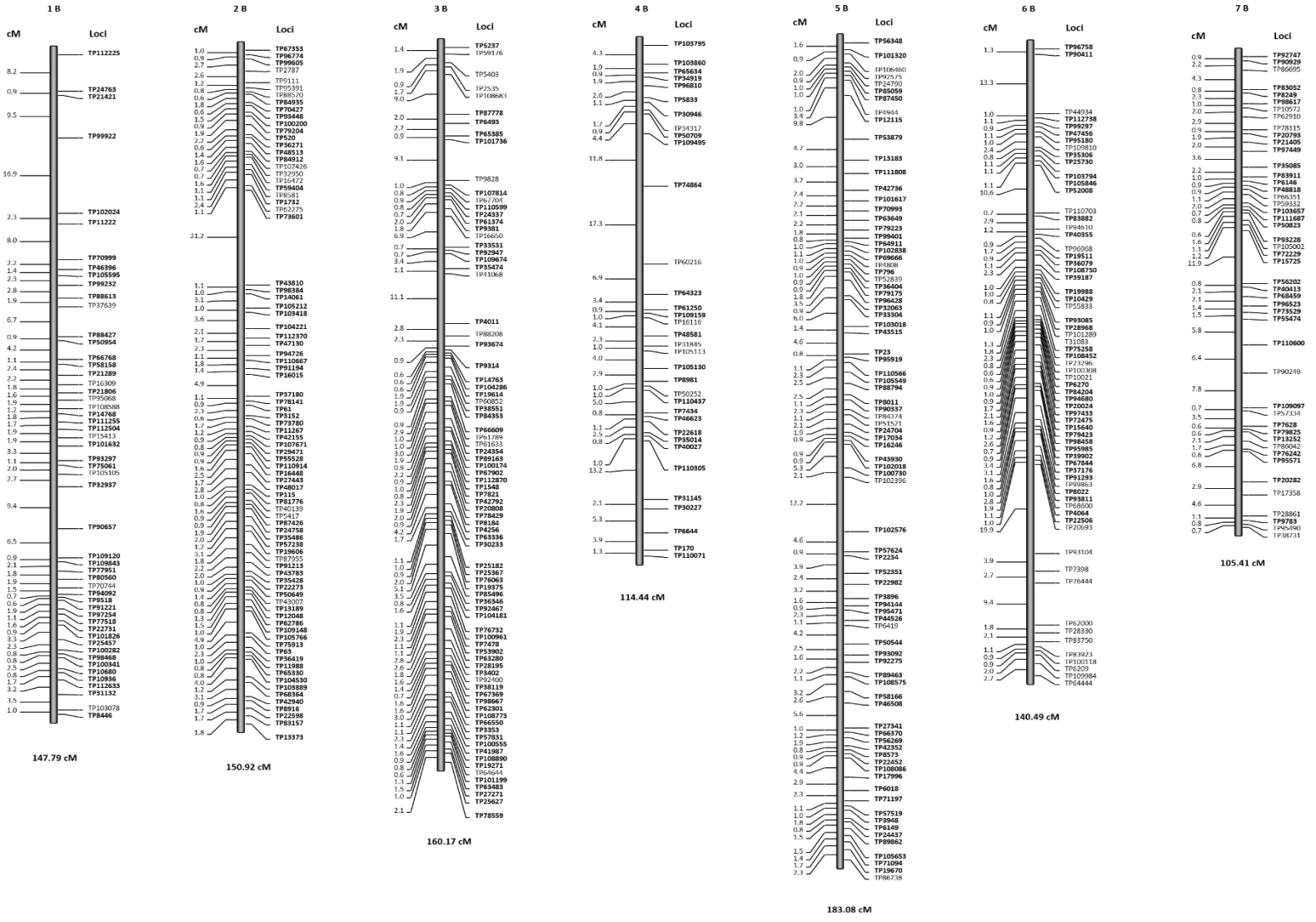

Figure 4. Genetic linkage maps (1B-7B) developed in a recombinant inbred line population derived from the cross between in Siskiyou and Villax St. Jose (continued)

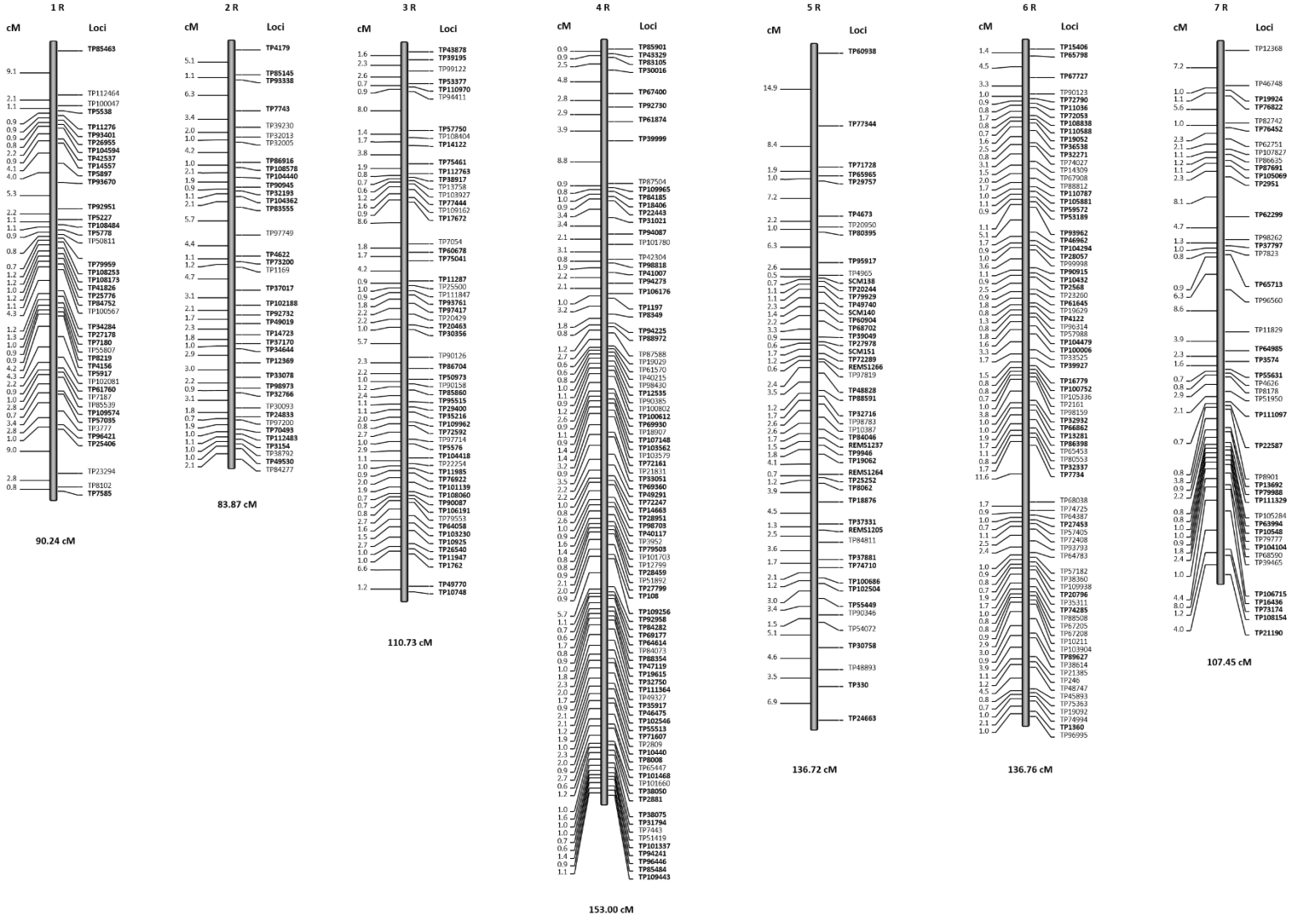

Figure 4. Genetic linkage maps (1R-7R) developed in a recombinant inbred line population derived from the cross between in Siskiyou and Villax St. Jose (continued)

QTL mapping of BLS resistance in the LTC0918 population

In the evaluations with either strains BLS-LB10 or XT4699, the Siskiyou × Villax St. Jose RIL population segregated for the reaction to BLS from resistant to susceptible as Villax St. Jose (Figure 5). Trait analysis with Qgene software indicated the deviation of the population from a normal distribution ($P<0.05$), suggesting the presence of a major gene (Figure 5). Using the composite interval mapping (CIM) function, a single and major QTL was identified on chromosome 5R for resistance to BLS for all three replicates with both strains. The QTL was delimited to a 4.9 cM genetic region by the SNP markers *TP95917* and *TP79929* (Table 4). The LOD cutoff value for significance was obtained as $\alpha_{0.01} = 4.322$. All QTLs had very higher LOD values ranging from 11.237 to 16.982 for BLS-LB10 and 10.22 to 12.399 for XT4699. The phenotypic variation for the QTLs ranged from 0.383 to 0.46, 0.331 to 0.391 for BLS-LB10 and XT4699 respectively (Table 4). The SSR marker *XSCM138* and SNP marker, *TP4965*, underlie the peak of all the QTL identified (Figure 6).

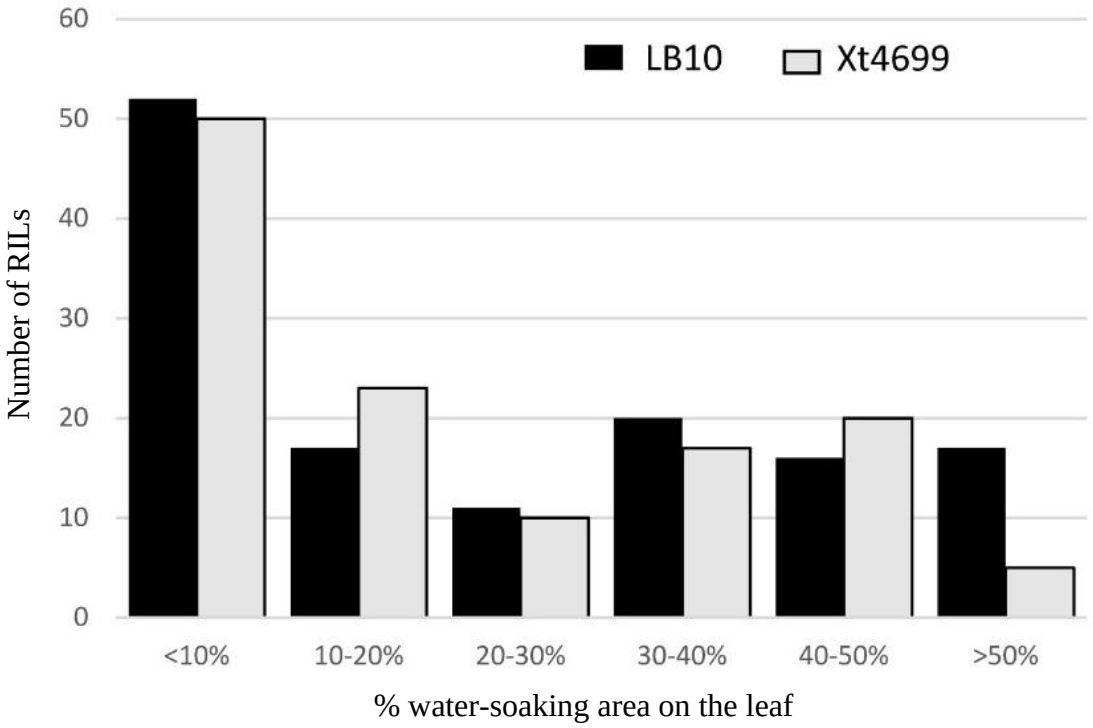

Figure 5. Histograms of the segregation of Siskiyou x Villax St. Jose $F_{2:5}$ recombinant inbred line population in reaction to two *Xanthomonas translucens* pv. *undulosa* strains BLS-LB10 and XT4699.

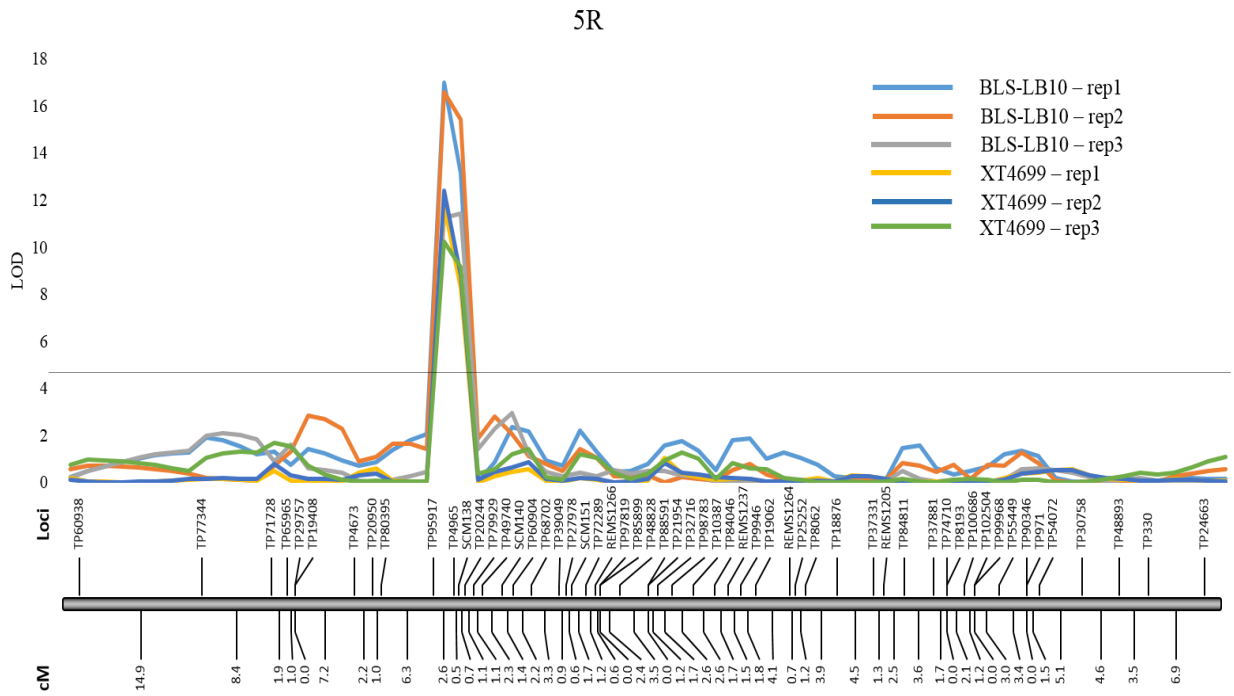

Figure 6. Composite interval mapping of the major QTLs for resistance to BLS on chromosome 5R in the Siskiyou x Villax St. Jose $F_{2:5}$ recombinant inbred line population. The marker loci and their genetic positions are shown below and above respectively, the chromosome. A line indicates a LOD cutoff of 4.322 for composite interval mapping.

Table 4. Summary of the significant quantitative trait locus associated with resistance to bacterial leaf streak caused by two *Xanthamonas translucens* strains

Strain	Flanking markers	*LOD*	R^2
BLS-LB10 – rep 1	*TP95917 – TP79929*	16.982	0.46
BLS-LB10 – rep 2	*TP95917 – TP79929*	16.568	0.45
BLS-LB10 – rep 3	*TP95917 – TP79929*	11.237	0.383
XT4699 – rep 1	*TP95917 – TP79929*	11.814	0.377
XT4699 – rep 2	*TP95917 – TP79929*	12.399	0.391
XT4699 – rep 3	*TP95917 – TP79929*	10.22	0.331

LOD means logarithm of the odds and R^2 means the phenotypic variation explains by each QTL

Discussion

Disease evaluations have shown that resistance to bacterial leaf streak (BLS) caused by *X. translucens* pv. *undulosa* (*Xtu*) in wheat is partial, whereas some triticale lines are highly

resistant (Cunfer and Scolar, 1982, Johnson et al. 1987). Triticale lines, or similar rye lines, therefore, could serve as a source of resistance for developing highly resistant wheat cultivars. However, resistance in triticale had not been further studied until now. From the evaluation of a worldwide collection of triticale accessions, one of the previous study done by our lab confirmed high levels of resistance in some triticale accessions and showed their resistance is also effective against North Dakota strains (Sapkota et al. 2017). Here, we developed segregating triticale populations to map the resistance locus by using Siskiyou (resistant) and two highly susceptible triticale accessions. In both populations, Siskiyou x UC38 which has been done by Dr. Aimin Wen and Siskiyou x Villax St. Jose resistance (my research) was shown to be conferred by a major gene (or tightly linked genes) on rye chromosome 5R. This locus was tightly linked to a SSR marker along with a few SNP markers (Fig. 2 and 6). To our knowledge, this is the first study to map a major locus conferring resistance to BLS caused by *Xtu*. Knowledge of the chromosomal location of the resistance locus and the identified molecular markers will be useful in the development of resistant triticale cultivars and, most importantly, in moving the resistance locus into wheat.

Previous studies suggested that partial resistance in wheat is complex and likely conferred by multiple genes, each one having a minor effect (Duveiller et al. 1993, Adhikari et al. 2012). In contrast, a single gene was suggested by Johnson et al. (1987) to condition complete or a high level of resistance in three triticale lines, which was designated as *Xct1* (Johnson et al. 1987). However, the location of *Xct1* was not determined. Siskiyou triticale was used in the study by Johnson et al. (1987). Therefore, the resistance locus mapped here is most likely *Xct1*, and, hereafter, we refer to the locus associated with *XSCM138* and SNP marker *TP4965* as *Xct1*. The fact that the resistance locus mapped to the rye genome, not the wheat genome, aligns with

expectations that complete or high levels of resistance to BLS have been identified only in triticale and rye lines and not wheat lines (El Attari et al. 1996, Kandel et al. 2012, Cunfer and Scolar, 1982, Charkhabi et al. 2015, Sapkota et al. 2017).

We hypothesize that *Xct1* in Siskiyou is a single dominant gene (or tightly linked genes) based on the reactions of F_1 individuals from both crosses (Siskiyou × UC-38 and Siskiyou × Villax St Jose), which were similar to that of Siskiyou parent. Second, our preliminary studies done by Dr. Wen obtained the ratio of homozygous to heterozygous for Siskiyou × UC-38 F_3 families also fits perfectly to 1:2:1, indicating the presence of a single gene. We did not calculate the goodness of fit to a 3:1 ratio in the Siskiyou × UC-38 F_2 population because we feel it is relatively arbitrary to define a cutoff value for the resistant vs. susceptible reaction. Third, we only detected one genomic region that was associated with BLS resistance with a major effect in the Siskiyou × Villax St. Jose RIL population using a high-density genetic linkage map. Nonetheless, we did observe that some lines had an intermediate reaction in the Siskiyou × Villax St. Jose RIL populations (Fig. 5). This may be due to low penetrance of the Siskiyou allele or poor expression of rye genes in triticale. It is also possible that small differences in environmental conditions or other minor genetic factors could modify the effect of *Xct1*.

The population of Siskiyou x Villax St. Jose and the F_2 population of Siskiyou x UC38, *Xct1* was shown to be tightly linked to the SSR marker *XSCM138*. This marker was mapped to chromosome 5R in several rye or triticale populations at positions ranging from 0 cM to 52.9 cM dependent on the coverage of the genetic map used (Saal and Wricke 1999, Korzun et al. 2001, Hackauf and Wehling 2003, Matos et al. 2007, Gustafson et al. 2009, Tenhola-Roininen et al. 2011). Two genetic maps indicate that the marker is probably close to the centromere (Korzun et al. 2001, Gustafson et al. 2009). In our map, developed from the Siskiyou × Villax St. Jose RIL

population, *XSCM138* mapped to the 46.1 cM position, which falls within the range of the genetic regions previously described. A rye consensus map, using several different rye populations, found that the genome structure of chromosome 5R is highly conserved in terms of the marker order between rye and triticale (Gustafson et al. 2009). Four of the SSRs are common in the two populations we developed, including *XSCM138*, *XSCM151*, *REMS1266*, *REMS1237* and had similar mapping orders on 5R (Fig. 2 and 6). The genetic order of the mapped SSR markers in both of two populations agrees well with the previously published maps (Saal and Wricke 1999, Khlestkina et al. 2004).

Rye is not only an important food and feed crops, but also serves as an important source of genes for improving wheat genetic diversity. Many useful rye genes have been transferred into wheat using triticale as a bridge followed by the development of wheat-rye chromosome translocation lines carrying a small rye chromosomal segment (Zellar and Hsam 1983). In particular, the wheat-rye 1A/1R or 1B/1R translocations have been incorporated into many wheat cultivars for resistance to leaf rust, stripe rust, powdery mildew and/or insects (Rabinovich 1998, Kumar et al. 2003).We have confirmed Siskiyou is a hexploid triticale, containing 14 pairs of wheat chromosomes and 7 pairs of rye chromosomes (Sapkota et al. 2017). Transfer of *Xct1* can be facilitated by using the Chinese Spring *ph1b* mutant (Sears 1982) along with the *Xct1*-associated molecular markers identified in this study. *XSCM138* can be directly used in the selection because of tight linkage with *Xct1*. The GBS marker *TP4965*, which is only 0.5 cM away from *XSCM138* is also under the peak of the QTL (Fig. 6) and may be useful after being converted into a semi-thermal asymmetric reverse PCR marker (Long et al. 2017).

The genetic linkage map we developed in the LTC0918 triticale population covered all 21 chromosomes with a total genetic distance of 2890.33 cM. The map contained a total 1,275

(SNP+SSR) markers with a marker density of 2.27 cM per marker. For the majority of SNP marker, the tag sequence had a strong hit to the corresponding chromosome where it mapped to when being blasted against the reference genome sequences of wheat and rye indicating the robustness of the genetic linkage map. The rest of the markers which do not have a blast hit are likely due to incomplete coverage of the reference genome, particularly for the rye genome. Our map had a similar total genetic distance and marker density as the one published by Tyrka et al. (2011). However, Tyrka et al. (2015) reported a genetic map of triticale which spanned 4,907.4 cM in genetic distance with a coverage of only 20 chromosomes. The significant longer genetic distance is likely due to the usage of much more DNA markers and an intervarietal population.

The host-pathogen interaction in BLS remains largely uncharacterized at the molecular level, but the genome sequences of several *X. translucens* strains have been published (Wichmann et al. 2013, Gardiner et al. 2014, Pesce et al. 2015, Jaenicke et al. 2016, Peng et al. 2016, Langlois et al. 2017), and these sequences have provided some insight into pathogen virulence and enabled the development of pathovar-specific markers. Many *Xanthomonas* pathogens, such as *X. oryzae* pv. *oryzae* (causal agent of rice bacterial leaf blight), *X. oryzae* pv. *oryzicola* (causal agent of rice bacterial leaf streak), and *X. citri* ssp. (the causal agents of citrus canker), inject transcription activator-like effectors (TALEs) into host cells through the bacterial type III secretion system. After entering plant cells, TALEs may act as virulence factors that activate host genes important for disease development, or in some host genotypes, as avirulence factors that activate a host "executor" resistance gene or that are recognized by a canonical NLR-type resistance protein (Bogdanove et al. 2010, White and Yang 2009). Several TALEs have been identified in the published *X. translucens* genomes, and some evidence has shown that some of these TALEs play a role as virulence factors in BLS of wheat (Peng et al. 2016, Falahi-

Charkhabi et al. 2017 in review). It is unknown whether any functions as an avirulence factor to trigger *Xct1*-mediated resistance. If a TALE acts as the avirulence factor by upregulating *Xct1* expression, identifying it could hasten the molecular cloning of *Xct1*.

In conclusion, we mapped a major gene conferring high levels of resistance to BLS in triticale and identified SSR and GBS markers that co-segregate with it. This work not only provides important information and tools for developing resistant triticale and transferring the resistance gene into wheat germplasm, but also provides a foundation for cloning the resistance gene and studying cereal-*X. translucens* interactions.

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APPENDIX: PHENOTYPIC DATA FOR THE BACTERIAL LEAF STREAK CAUSED BY XANTHOMONAS TRANSLUCENS PV. UNDULOSA STRAINS (BLS-LB10, XT4669) ON 'SISKIYOU × VILLAX ST. JOSE RECOMBINANT INBRED LINE POPULATION

RIL No	BLS LB10			Xt4699		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
1	0	-	5	-	2	5
2	30	0	0	0	0	2
3	0	2	50	0	0	0
4	50	20	20	20	20	30
6	15	5	5	20	5	2
7	5	0	0	2	10	2
8	-	-	-	-	-	-
9	-	50	-	50	10	30
10	80	40	50	40	60	60
11	5	0	0	2	0	5
12	15	10	10	50	5	50
13	60	50	5	40	40	40
14	15	30	15	20	2	30
15	15	0	0	0	0	0
16	70	40	40	5	-	40
17	-	-	-	-	-	-
18	40	5	60	30	5	5
19	-	40	-	-	-	-
21	0	2	0	2	0	5
22	40	50	70	30	30	30
23	50	10	20	-	2	40
24	5	2	40	2	0	15
25	-	0	0	0	0	5
26	10	5	0	0	0	0
27	0	0	0	2	0	0
28	30	10	40	2	20	60
30	5	0	0	0	0	20
32	0	0	0	0	0	5
33	15	0	0	0	0	2
34	5	2	0	0	0	0
36	15	2	0	0	0	20
37	-	50	50	30	2	0

RIL No	BLS LB10			Xt4699		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
38	40	40	20	40	-	-
39	20	0	2	1	0	10
40	5	5	10	5	0	2
41	-	30	30	5	20	30
42	40	5	5	0	10	0
43	30	5	5	10	15	40
44	80	30	20	30	50	50
45	5	0	0	15	20	5
46	1	0	0	0	0	2
47	20	2	10	10	5	5
48	50	40	40	20	50	70
49	1	0	0	0	5	0
50	70	20	20	50	40	40
52	80	40	60	30	50	60
53	20	0	0	0	2	0
54	-	-	-	-	-	-
56	5	0	-	5	-	20
57	30	10	20	30	5	40
58	80	10	20	50	10	50
59	60	20	30	20	20	10
60	60	-	-	-	-	-
61	-	-	-	-	-	-
62	40	40	30	-	30	60
63	-	-	-	-	-	-
64	10	2	0	10	1	20
66	80	50	-	-	-	-
67	15	0	0	0	2	2
69	5	30	0	40	40	50
70	40	-	-	-	-	-
71	60	40	30	60	20	50
72	5	5	0	20	0	40
73	-	-	-	-	-	-
74	-	0	5	-	-	0
75	5	10	20	15	5	15
76	1	0	0	2	0	0
78	0	0	0	0	0	0
80	-	-	30	-	40	-
81	15	20	5	1	20	40
82	5	0	5	-	-	-
83	5	0	0	-	-	-
84	-	-	-	-	-	15

RIL No	BLS LB10			Xt4699		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
86	5	0	0	1	0	0
87	30	40	60	50	40	40
88	70	5	20	20	40	40
89	100	30	40	30	30	60
90	60	30	20	20	30	5
91	0	0	-	-	5	0
92	80	10	20	5	2	2
93	-	-	-	-	-	-
95	10	5	5	0	-	-
96	60	2	30	2	2	40
100	20	-	0	0	2	2
101	-	5	-	-	-	30
102	70	10	10	10	0	20
104	-	-	20	-	-	-
105	60	50	30	50	30	50
106	-	0	-	1	-	0
107	20	2	50	30	5	30
108	50	10	20	5	20	30
109	90	40	30	-	10	50
110	80	2	20	5	10	40
111	60	5	40	20	30	30
112	5	2	-	5	2	5
113	80	50	15	60	40	60
114	10	10	30	20	10	20
115	-	20	40	-	2	60
116	80	5	40	80	40	20
117	0	0	-	0	0	0
118	2	0	0	30	0	5
121	50	50	50	50	30	30
122	20	30	20	50	20	60
125	5	0	0	0	0	0
126	5	0	0	0	0	0
127	-	2	0	0	0	5
128	5	0	0	0	0	0
129	90	60	60	50	60	60
132	0	0	0	0	0	0
134	80	20	40	40	20	50
135	5	50	40	10	20	60
136	50	20	70	5	40	60
137	0	2	0	10	0	0
138	-	0	0	0	0	5

RIL No	BLS LB10			Xt4699		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
139	40	20	60	40	10	50
140	5	5	2	0	5	15
141	5	20	5	5	10	15
142	80	50	40	40	20	50
143	80	20	50	30	10	0
144	90	50	60	-	60	70
145	70	15	60	50	50	-
146	-	-	5	-	-	-
147	5	0	5	10	1	5
148	50	20	2	70	2	60
149	30	5	20	20	10	20
150	40	20	20	20	40	80
151	0	0	0	2	0	30
152	50	30	50	30	50	60
153	-	5	0	2	0	10
154	30	10	30	50	0	50
155	-	5	30	0	0	20
157	70	40	50	30	10	50
158	70	30	20	10	40	60
159	2	10	30	-	-	-
160	50	20	20	20	10	20
163	5	50	2	40	20	60
164	0	0	0	0	0	0
167	0	0	10	0	10	10
168	80	50	50	40	30	60
169	0	0	0	1	0	0
170	0	0	0	0	0	0