GENETIC MAPPING AND CHARACTERIZATION OF NET BLOTCH DOMINANT RESISTANCE AND DOMINANT SUSCEPTIBILITY LOCI IN BARLEY

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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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Net blotch is an economically important foliar disease of barley that occurs in two distinct forms: Spot Form Net blotch (SFNB) and Net Form Net Blotch (NFNB) caused by the necrotrophic fungal pathogens *Pyrenophora teres* f. *maculata* (*Ptm*) and *Pyrenophora teres* f. *teres* (*Ptt*), respectively. The recent emergence and the identification of both *Ptm* and *Ptt* isolates virulent on popular ND malting barley varieties have warranted the identification of new resistance sources. Association mapping was conducted on 2,062 diverse barley accessions phenotyped at the seedling stage with four diverse *P. teres* f. *maculata* isolates and genotyped with the 9k Illumina barley iSelect chip. A total of 138 significant marker-trait associations (MTA; −log10*P* value > 3.0) corresponding to 27 resistance loci were identified of which 21 loci were novel and six corresponded to previously characterized SFNB resistance QTL. Further, two higly resistant lines PI67381 and PI84314 were crossed with the two susceptible cultivars Tradition and Pinnacle grown in the Upper Midwestern US to develop three bi-parental recombinant inbred line (RIL) mapping populations of Tradition x PI67381, Pinnacle x PI67381 and Pinnacle x PI84314. These RIL populations were phenotyped with six diverse *Ptm* isolates and genotyped using PCR-GBS. MapDisto and Qgene were used to analyze the data and a total of twelve QTL were identified on chromosome 2H, 3H, 4H, 6H and 7H, of which nine were previously reported and the remaining three are considered novel. These resistances and the markers delimiting the QTL are being utilized to develop prebreeding lines by introgressing SFNB resistance into the cultivars Pinnacle and Tradition utilizing marker assisted selection. The barley line CI5791 exhibits a high level of resistance to diverse *Ptt* isolates collected from around the world. A forward genetics approach and an exome capture-mediated mapping-by-sequencing identified a candidate *HvWRKY6* transcription factor gene required for NFNB

resistance on chr 3H. We hypothesize that the *HvWRKY6* gene function as a component of a conserved basal defense mechanism, which regulates the expression of other defense response genes that restrict lesion growth. The resistance/susceptibility loci identified in this study will facilitate the development of net blotch resistant cultivars.

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CHAPTER 1. LITERATURE REVIEW

Introduction

Barley (*Hordeum vulgare* L.) is one of the oldest and most important cultivated cereal crops worldwide, which falls in the family Poaceae (formerly called Gramineae), and tribe Triticeae of the grass family. It is mostly used for malting, animal feed, and human consumption (Poehlman, 1994). Among the cereals, it ranks fourth in terms of annual production after wheat, corn, and rice, with 145 million tonnes of production and 49.4 million hectares cultivated globally in 2014 (FAO, 2014). In the United States, barley is a major crop after wheat, corn and soybean with production mostly concentrated in the Northern Great Plains to the Pacific Northwest (Minnesota, North Dakota, Montana, Idaho, and Washington). North Dakota has consistently ranked in the top three positions in terms of both barley production and area harvested in the US. The US barley production in 2015 was 214.297 million bushels where North Dakota alone contributed 67.2 million bushels accounting for nearly one third of the US barley production (USDA 2017). Today, both six-rowed and two-rowed varieties are commonly grown in the US.

Barley is produced in areas where the growing season is relatively short and climatic conditions are cool and dry (Mathre, 1997). Based on row type, it is divided into two groups: two-rowed and six-rowed. The central floret is fertile, and the two laterals are sterile in two-rowed barley whereas all three florets are fertile in six-rowed type. Similarly, based on the growth habit, it is divided into spring and winter barley. Spring barley doesn't require vernalization and is usually sown in spring especially in the Northern Great Plains. Winter types are primarily planted in the fall in the southern states. Spring barley is mainly grown for malting

purposes and cultivated over larger areas in the US, whereas winter barley is grown mainly for feed/forage purposes.

Columbus brought barley to Central America in 1494 on his second voyage, but the warm climate of the region was not appropriate for barley growth (Thacher, 1903). Successful establishment of barley in North America occurred later, in the eastern colonies, and subsequently in the southwest US during the Spanish mission movement (Wendorf et al., 1979, and Wiebe, 1978). The first barley varieties grown in the early American settlements were the cultivars Chevalier and Thorpe which were two-rowed late maturing varieties commonly grown in England (Wiebe, 1978). Eventually, barley was introduced into all the colonies, where it was in demand as a grain for brewing and became an important crop by the middle of the 17th Century. In the 1850s, six-rowed varieties were reported as more common than two-rowed in New York. The University of Wisconsin experiment farm began distributing the six-rowed barley cultivar Manchuria around 1873, which spread rapidly from farm to farm.

North Dakota, Montana and Idaho have been the top three barley producing states in the US for the last 50 years (NASS, 2016), most of which is grown for malting, with a smaller portion grown for animal feed. In the past, six-rowed varieties were considered better for malting, but breeders have incorporated desirable malting characters in modern varieties of both types. Recently, there has been increased attention on hulless barley in the US to create a new market for feed barley as well as ethanol production (Thomason et al., 2005).

Evolution and Domestication of Barley

Cultivated barley (*Hordeum vulgare*) is one of the 32 *Hordeum* species and is the only species that is grown commercially. It is an annual diploid inbreeding species with chromosome numbers of 7 ($2n= 2x =14$) with a large genome of approximately 5,000 Mb (Bennett et al.,

1976, Wenzl et al., 2004). Cultivated barley and its close wild relative are morphologically similar and hybridize easily. As a result, it has been proposed that both the cultivated and wild types are subspecies of *H. vulgare*, with cultivated barley designated as *H. vulgare* subsp. *vulgare* and the wild type referred to as *H. vulgare* subsp. *spontaneum* (Bothmer et al., 1958).

Barley (*Hordeum vulgare* L. subsp. *vulgare*) was one of the first crops domesticated (Zohary et al., 2013). The actual series of events leading to barley domestication and the physical locations where barley was first domesticated, are not known with certainty, despite over 100 years of speculation and accumulation of a variety of data. Since modern barley is closely identical to present day *Hordeum vulgare* subsp. *spontaneum* C. Koch, it is speculated that cultivated barley is derived from this weedy relative. The presence of wild barley grains in several pre-agricultural pre-pottery Neolithic sites indicated that the cultivation of the wild relative *H. vulgare* subsp. *spontaneum* started long before modern barley (Harlan and Zohary, 1966; Tanno and Willcox, 2012; Weiss et al., 2006). Gathering ~23,000-year-old barley grain remnants in large quantities in Ohalo II, a location on the shore of the Sea of Galilee (Israel), provided evidence of the gathering and possible cultivation of wild barley at this early prehistoric time (Kislev et al., 1992; Weiss et al., 2008).

The Fertile Crescent spanning Israel, Jordan, Syria, Turkey, Iraq, and Iran is widely accepted as the primary region of barley domestication which occurred about 12,000 to 9,500 years ago, (Badr et. al., 2000, Weiss et al., 2006, Willcox, 2008 and 2013). However, an excavated site in southern Egypt where grains resembling both wild and cultivated barley were found was reportedly 17,000-18,300 years old (Wendorf et al., 1979). This finding predates other archaeological evidence where barley (*Hordeum vulgare*) remains were discovered about 10,000

years ago, about the same time that domesticated wheat is present in the Fertile Crescent (Harlan, 1979). Subsequently, barley was spread throughout Europe, Asia, and Africa.

Although, the Fertile Crescent is widely accepted as the origin of barley domestication, there are several controversies to this theory. Brown et al., (2009) and Abbo et al., (2010) hypothesized that multiple domestication events had occurred in more than one center based on the most recent genetic analysis and archeological record. Molina-Cano et al., (1987, 1999, and 2005) speculated Morocco as another domestication sites of barley based on RFLP, chloroplast DNA and morphology. The reason Morocco was considered as a domestication site may be due to a hybridization event with cultivated barley (Blattner and Badani Mendez, 2001). Similarly, Ethiopia has also been reported as the possible domestication sites of barley (Bekele,1983, Negassa, 1985 and Orabi et al., 2007). The huge phenotypic variation found in the Ethiopian barley collections is thought to be the reason that it might be another origin of barley domestication (Negassa, 1985). Morrell et al., (2007) has acclaimed the Himalayas (Tibet, Nepal and India) as the next domestication site because abundant evidence of early barley culture has been discovered in these areas. The Himalayan region was proposed as a possible domestication site because of the presence of six-rowed barley with the brittle rachis trait (Badr et al., 2000)

Important traits associated with the domestication process have been reviewed by Pourkheirandish and Komatsuda (2007). Brittle rachis types (*btr1* and *btr2*), row number (*vrs1*), hull status (*nud*), and dormancy (QTLs SD1 and SD2) are some of the important domestication traits in barley accumulated during the domestication process. All modern barley genotypes are either homozygous for the *btr1* or *btr2* alleles (most European and Western Asian barlies are *btr1*/*Btr2* and East Asian barlies are *Btr1*/*btr2*), and double homozygous *btr1* and *btr2* lines have not been identified (Pourkheirandish et al., 2015, Komatsuda, 2015). The cloning and

characterization of the barley *Btr* genes helps to study the evolution and domestication of different clade- and species-specific mechanisms of seed dispersal systems in grasses. These loci, and several others, are associated with the 'domestication syndrome', a set of characters shared among many domesticated crops. The 'domestication syndrome' was described by Harlan (1992) and reviewed by Gepts (2004). As compared with the wild barley, the cultivated barlies have relatively broader leaves, shorter stems and awns, tough ear rachis, shorter and thicker spikes, and larger grains (Yun et al., 2005).

In short, available evidence supports the idea that multiple domestication events have occurred, or barley is polyphyletic (Molina-Cano et al., 1999, Willcox, 2005, Azhaguvel and Komatsuda, 2007; Komatsuda et al., 2007) or in contrast, it may be monophyletic or has one domestication site (Abbo et al., 2010, Badr et al., 2000, and Blattner, 2001). This contradictory evidence found in different studies may likely be due to the method of analysis particularly when generating the phylogenetic trees, study materials, and different markers/traits. Still, these contradictions exemplify the complexity of the questions. Analyzing sequenced domestication genes may offer the best hope to clarify these confounding queries, and recent progress appears to be facilitating this goal.

Net Blotch of Barley

Net blotch is a foliar disease of barley caused by the necrotrophic fungal pathogen *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* [Sacc.] Shoemaker), that causes considerable barley yield and quality loss in growing regions around the world (Steffenson et al., 1991). This pathogen has a wide host range that includes all cultivated as well as wild species of barley and related species from the genera *Bromus*, *Avena*, and *Triticum* (Shipton et al., 1973, Liu et al., 2011). Net blotch occurs in two distinct forms: Spot Form Net blotch (SFNB) and Net

Form Net Blotch (NFNB) caused by *Pyrenophora teres* f. *maculata* (*Ptm*) and *Pyrenophora teres* f. *teres* (*Ptt*), respectively. These diseases are often favored by high humidity, precipitation and cool conditions, that are conducive to net blotch epidemics and can lead to significant yield loss (Mathre, 1997, Ma et al., 2004, McLean et al., 2009). Besides the leaves, these pathogens can also infect, leaf sheaths, stems and kernels, thus affecting the kernel size and malt quality (Grewal et al., 2008, Liu et al., 2011). The estimated yield loss due to this disease ranges from 10-40% on average and can be 100% when the host is susceptible, and the environment is favorable (Mathre, 1997, McLean et al., 2009). Jayasena et al., (2007) reported 23-44% yield loss due to SFNB and for every 10% increase in disease severity on the top three leaves, there was 0.4 t/ha yield loss on average. Kinzer et al., (2015) determined that for every 1% increase in SFNB severity, there is a 0.77% yield loss in the barley yield in North Dakota. Steffenson et al., (1991) observed significant reduction in 1000-kernel weight from 18.5-31.6% due to NFNB.

The symptoms of both SFNB and NFNB on resistance and susceptible hosts are different. Initially, both SFNB and NFNB appear as a small pinpoint dark brown necrotic spot on the leaf. These pinpoint dark brown necrotic lesions do not increase markedly in size in resistant genotypes for both SFNB and NFNB. In SFNB, the pinpoint spot increases in size to form circular or elliptical dark brown lesions surrounded by a chlorotic region and coalesce together later in the season (Smedegard-Petersen, 1971, Liu et al., 2011) on susceptible hosts. Therefore, the SFNB symptoms often get confused with another foliar disease spot blotch of barley caused by *Cochliobolus sativus*.

Although, the initial symptoms of NFNB on susceptible hosts is identical to SFNB, the later symptoms are entirely different and NFNB got its nomenclature based on the symptoms. The pinpoint spots increase in size to form net like patterned lesions with longitudinal and

transverse necrotic stripes surrounded with chlorosis that coalesce together (Liu et al., 2011). Lightfoot and Able (2010) have shown that *Ptm* is more localized within epidermal cells whereas *Ptt* can infect beyond the epidermal cell causing cell death relatively far away from the penetration sites.

Both *Ptm* and *Ptt* are morphologically identical (conidia and mycelium), but produce very distinct symptoms on the host and are genetically distinct (Smedegard-Peterson, 1971, McClean et al., 2009, Liu et al., 2011). Several molecular markers are available to correctly identify these two pathogens such as simple sequence repeats (SSRs) (Keiper et al., 2008, Liu et al., 2012), Amplified Fragment Length Polymorphism (AFLP) (Leisova et al., 2005 and 2006, Serenius et al., 2005 and 2007), randomly amplified polymorphic DNA (RAPD) (Campbell et al., 1999, Taylor et al., 2001, Williams et al., 2001), and SNP markers specific to the mating type genes (Lu et al., 2010). Williams et al., (2001) identified primer sets that can amplify a 411 bp size DNA fragment from *Ptm* and 378 bp size from *Ptt*. A real-time PCR based assay was developed to quantify *Ptm* from diseased barley tissue (Leisova et al., 2006). Recently, Poudel et al., (2017) developed a set of 12 sequence-specific PCR markers based on the expressed regions spread across the fungal genome that can precisely identify both *Ptt*, *Ptm* and their hybrids. Thus, molecular tools have been developed to precisely discriminate between the two diseases/pathogens. This is important when diagnosing the causal agent for disease management and when studying pathogen population genetics and diversity.

Life Cycle of *Pyrenophora teres* f. *teres* and *Pyrenophora teres* f. *maculata*

The life cycles of *Ptt* and *Ptm* are almost identical and involve both asexual and sexual stages. Both *Ptt* and *Ptm* are residual borne pathogens that can overwinter as pseudothecia (sexual fruiting bodies) or conidia on plant stubbles (Mathre, 1997). It takes up to six months to

develop a fertile pseudothecia under field condition when temperatures range between 10-15 ^{0}C (Shipton et al., 1973). However, it takes about two months under laboratory conditions to form pseudothecia. Following the growing season, pseudothecia actively release ascospores as far as 35 cm into the air, which act as a primary source of inoculum (Jordan, 1981, Deadman and Cooke, 1989). Alternatively, the mycelia and conidia that overwinter on plant stubbles or infected seed may also serve as a primary source of inoculum (Shipton et al., 1973). The ascospores germinate under 95-100% relative humidity, form appresoria, and produce penetration pegs that directly penetrate host epidermal cells to initiate intracellular growth and colonization (Hargreaves and Keon, 1983). After successfully infecting the host, *Pyrenophora teres* produces conidia throughout the growing season in multiple cycles (polycyclic), which serves as a source of secondary inoculum. Conidia are often disseminated via rain splash and wind to neighboring plants or fields (Mathre, 1997). Towards the end of the growing season, either pseudothecia are developed on the plant stubble, or conidia and mycelia overwinter on stubble or infected kernels, which serves as a primary source of inoculum for the next growing season. However, only *Ptt* has been shown to transfer across generations via infected seed to the subsequent growing seasons (Mathre, 1997, Leisova et al., 2005). Although it is not common, Smedegard-Peterson (1972) found *Ptt* pycnidia on the host and in culture.

Genetic Diversity and Mating Types of *Ptt* and *Ptm*

Pyrenophora teres is a heterothallic ascomycetes fungus that requires opposite mating types. Sexual reproduction is a major factor contributing to the vast genetic diversity present within *P. teres* natural populations (McDonald, 1963, Rau et al., 2007). Both forms, *Ptt* and *Ptm,* have two opposite mating types: *MAT1-1* or *MAT1-2* that are controlled by a single MAT locus (Rau et al., 2003, Liu et al., 2012). Genetic variation has been observed within *Ptt* and *Ptm* field

populations suggesting that sexual reproduction occurs frequently under natural conditions (Rau et al., 2003; Lehmensiek et al., 2010; Serenius et al., 2005, Liu et al., 2012, Poudel et al., 2017). The segregation of *MAT1-1* and *MAT1-2* in 1:1 ratios in field populations of *Ptt* and *Ptm* suggests that sexual reproduction frequently takes place in natural populations (Bogacki et al., 2010, Lu et al., 2010, Rau et al., 2007, Serenius et al., 2005 and 2007, Ksasia et al., 2015). Sexual reproduction has been reported in *Ptm* population in Australia, South Africa, and Finland and contributed to the rapid change in the diversity of the pathogen population leading to disease epidemics (Karki et al., 1986, Tekauz, 1990, Arabi et al., 2003, Jalli et al., 2011, McLean et al., 2014). Using AFLP markers, Rau et al., (2003) suggested that asexual reproduction is more prevalent in *Ptt* populations than *Ptm* in Sardinian, Italy. However, digenic and multilocus linkage disequilibrium analyses showed that significant levels of sexual reproduction also occur. Lehmensiek et al., (2010) also reported that asexual reproduction is common within *Ptm* and *Ptt* populations.

Sexual reproduction results in diverse genotypes, thus, facilitating high levels of genetic variability within populations by producing recombinant genotypes that have new combinations of virulence and or avirulence alleles that evade deployed resistances. It is essential to evaluate pathogen population in a given geographical region prior to the deployment of resistances as one can deploy effective resistance to the existing predominant pathotypes, but if virulence on the resistance exists in the pathogen population, even at low levels, the deployed resistance will only remain effective for a very short period of time due to selection pressure. As popular resistant barley cultivars are grown over vast acreage (monoculture), with time selection pressure exerted by these resistance genes or lack of susceptibility targets, select for the virulent isolates, which rapidly become prevalent, thus resistances are no longer effective and lead to disease epidemics.

Therefore, breeding programs must consider the pathogen genetics and virulence profiles present in the local pathogen population in order to deploy effective and durable resistances (Liu et al., 2011).

Molecular marker technology has aided in the identification of the two net forms, but has also allowed for the more precise characterization and tracking of pathogen populations, which can be correlated with virulence patterns and distribution. However, this requires the thorough characterization of virulence loci and the extensive study of pathogen populations. Genetic mapping studies to characterize virulence loci are currently best achieved through association mapping using natural pathogen populations (Dalman et al., 2013, LeBoldus et al., 2015). The understanding of virulence present in pathogen populations is essential when developing resistant cultivars (McLean et al., 2010, Williams et al., 2001, Lu et al., 2010).

Because of the very close relationship between *Ptt* and *Ptm* and their different virulence profiles on barley, the possible cross hybridization of the two pathogens and possible novel virulences in the hybrid isolates is a concern. The possibility of cross hybridization between *Ptm* and *Ptt* was first demonstrated under laboratory conditions (Campbell et al., 1999, Campbell and Crous, 2003, Rau et al., 2003, Jalli, 2011). The hybrid progeny of *Ptm* and *Ptt* produced under these laboratory conditions produce an intermediate jagged-type lesions on the host (Campbell and Crous, 2003; Jalli, 2011) and it was shown that the artificially produced hybrids of *Ptt* and *Ptm* have different virulence profiles than each of the parental isolates, and exhibited virulent reactions on barley lines to which both parental isolates were avirulent (Jalli, 2011). Cross hybridization between *Ptm* and *Ptt* isolates have been reported in the Southwestern Cape of South Africa using RAPD and AFLP markers (Campbell et al., 2002 and 2003). Similarly the naturally occurring hybrids, PTM-15 and PTM-16, were discovered in Tovacov, Czec Republic

(Leisova et al., 2005) and the hybrids SNB172 and WAC10721 were discovered in Western Australia (Lehmensiek et al., 2010; McLean et al., 2014). Although *Ptm* and *Ptt* can intercross it has been established that sexual reproduction between the two net forms is very rare and unlikely under natural conditions and the stability of the hybrids that do infrequently occur are still questionable; possibly due to fitness penalties on the hybrids (Smedegard-Peterson, 1971, McLean et al., 2014, Campbell et al., 2002 and 2003, Jayasena et al., 2004). Interestingly, Campbell et al., (1999 and 2002) demonstrated that a few hybrid progenies from two sensitive parental isolates were insensitive to commercial fungicides. Therefore, if hybrids are stable, theoretically, they could be more virulent than either of the parental isolates or more resistant to commercial fungicides, thus overcoming the existing host resistances or sensitivity to fungicides (Jalli, 2011, McLean et al., 2014). However, the appearance of these hybrids has not materialized as a threat and due to their very low frequency is not currently a major concern.

The variation in *P. teres* f. *teres* population was first reported in the United States by Pon (1949). Peever and Milgroom (1994) observed 46% genetic variation between populations of *Ptt* isolates collected from Germany, the US, and Canada using RAPD markers, which is most likely due to limited migration of the pathogen thereby limiting gene flow. However, two populations from Canada exhibited less genetic variation (5%), which was probably due to the collection of the populations from the same geographic region, not more than 20 km apart. Rau et al., (2003) collected 150 isolates from six geographically different locations in Sardinan, Italy, and based on the symptoms 55% of the isolates were *Ptt* and 45% were *Ptm* with one location predominantly containing only *Ptt* isolates. The AFLP analysis clustered these *Ptt* and *Ptm* isolates into two distinct groups, with no intermediates, suggesting no sexual reproduction occurring between the two forms in these areas. Jonsson et al., (2000) utilized RAPD markers to study the genetic

structure of two Swedish population of *Pyrenophora teres* each consisting of 64 isolates. They observed high levels of genetic variability within each population and low levels of gametic disequilibrium indicating that sexual recombination was prevalent. Consistent with the results of Peever and Milgroom (1994), similar results of low variation (only 5.3%) between fields was observed, which was most likely due to the proximity of the fields (~20 km). Serenius et al., (2007) studied the global genetic structure of 278 isolates collected from Northern Europe (Finland, Russia, Sweden, United Kingdom, Denmark, and the Czech Republic), North America (Canada and the United States) and Australia using AFLP markers. High genetic variation was observed between *Ptt* isolates (F_{CT} =0.238) originating from Northern Europe, North America and Australia which was also consisted with Peever and Milgroom's (1994) observations. The *P. teres* population from Australia clearly separated into two subgroups of *Ptt* and *Ptm*. The mating types MAT1-1 and MAT1-2 were also observed in equal proportion of 1:1 suggesting the prevalence of sexual reproduction. Liu et al., (2012) observed 40 distinct haplotypes using 13 SSR markers in the *Ptt* population consisting of 75 isolates collected in Fargo and Langdon, North Dakota. These 75 *Ptt* isolates were also evaluated on 22 barley differential lines identifying 49 pathotypes. These results indicated high genetic and phenotypic variation in the Eastern North Dakota *Ptt* population. However, clonal reproduction within populations were also observed in *Ptt* samples collected in three site-years: Fargo 2005, Langdon 2005, and Fargo 2007, showing that asexual reproduction was also common. The pairwise marker comparisons showed significant gametic disequilibrium and the presence of both mating types in the *Ptt* population, indicating the occurrence of sexual reproduction in the populations, but at a low rate. McLean et al., (2010) detected high genetic diversity among 44 *Ptm* isolates using sequenced-tagged microsatellite markers and the two mating types were found at a 1:1 ratio indicating the

prevalence of sexual reproduction. Although, they found very high genetic diversity in the *Ptm* population, the pathogenic diversity was low and did not correlate with genetic diversity. This result was consistent with Serenius et al., (2005) who reported low pathogenic diversity from a *Ptm* population with high genetic diversity. This could be due to the rapid evolution of whole genomes across a population as compared to the individual evolution of virulence/avirulence genes (Serenius et al., 2005, Liu et al., 2011). Similarly, Wu et al., (2003) observed a high degree of genetic diversity within each population of *Ptt* and *Ptm* using RFLP markers using a set of differential lines. The higher genetic diversity of *Ptm* (23%) and *Ptt* (40%) than previously reported was observed in South Australia using SSR markers (Bogacki et al., 2010).

Steffenson and Webster (1992) characterized 91 *Ptt* isolates collected from 1984-1986 in California inoculated on 22 barley differential lines identifying 13 distinct pathotypes with 28.6% of the isolates representing the most common pathotype (3-10-15-19-21) and less than 7% representing the least common pathotype '0', which were not virulent on any of the hosts. They speculated that the variation in the population was due to the occurrence of sexual reproduction in the California *Ptt* population. Five pathogenic groups were identified among 23 isolates of *Ptt* and *Ptm* based on their infection response on 11 barley genotypes and none of the barley lines were highly resistant to all isolates used in the study (Arabi et al., 2003). Wu et al., (2003) observed 15 pathotypes from the collection of 23 *Ptt* isolates based on their reactions on 25 barley differential lines. The barley lines originating from China exhibited resistance and the barley line Prato exhibited susceptible reactions to the *Ptt* isolates collected from California. However, the opposite reaction was observed where the Chinese barley lines were susceptible, and Prato was resistance to ND *Ptt* isolates (Steffenson and Webster, 1992, Liu et al., 2012). This showed that the genetic and phenotypic variation of the *Ptt* isolates collected from different

geographic regions harbor different virulent/avirulent genes because of different host-pathogen evolution and selection pressures exerted by distinct host genotypes.

The genetic variation of *P. teres* f. *maculata* populations have been studied using separate differential sets with few lines common between at least two studies in the USA, Canada, Australia, and Mediterranean region (Khan and Tekauz, 1982, Bockelman et al., 1983, Karki and Sharp, 1986, Arabi et al., 1992, and McLean et al., 2010). Twenty *Ptm* pathotypes in Western Canada were detected based on their reaction on 11 differential lines and none of the resistance present in these differentials was effective against all the isolates used in the study (Tekauz 1990). Khan and Tekauz (1982) also detected significant pathogenic variation between isolates collected from Western Australia and Canada based on their reaction on 15 breeding lines. Wu et al., (2003) reported four pathotypes from geographically diverse isolates collected from Denmark, New Zealand, Australia, Canada and Norway. Similarly, Grewal et al., (2008) observed variation in the virulence profile of both *Ptt* and *Ptm* isolates using 42 barley lines from Canada, Australia and some international barley (Korea, Ethiopia, Germany, United Kingdom) of which only 3 barley lines were resistance to all isolates. This variation in germplasm and the isolates suggested that the molecular markers linked with the resistance loci may not be applicable to all breeding programs for crop improvement. Gupta et al., (2001) reported that the virulence profile of *Ptt* has not changed through the last 19 years where as variability in the *Ptm* population over this span of time was observed. Gupta et al., (2012) grouped ninety-nine *Ptm* isolates into seven isolate groups (IGs) based on their infection responses on 26 differential barley lines in Western Australia. Interestingly, Tuohy et al., (2006) were unable to detect pathogenic variation among the *Ptt* and *Ptm* isolates collected within Ireland and Northern Europe. Recently, Akhavan et al., (2016) found 16 and 13 pathotype group among 39 *Ptt* and 27

Ptm isolates, respectively using barley differential sets in Canada. Observation of seven new pathotypes along with the absence of three previously reported pathotypes in the study indicated a shift in the virulence profile of the pathogen population. Wu et al., (2003) identified 15 pathotypes out of 23 *Ptt* isolates and 4 pathotypes out of 8 *Ptm* isolates using differential lines suggesting high genetic diversity in both the *Ptt* and *Ptm* populations. Consistence with other studies, Kinzer et al., (2015) identified 54 virulence group from a collection of 177 *Ptm* isolates collected in ND, USA, and reported 92% genetic variation with in the populations whereas low variation of about 8% among the population, suggesting the presence of high genetic variation within this local pathogen population.

The genetic structure of fungal population helps us to understand how rapidly a pathogen is evolving and provides important information for the intelligent deployment of resistance to the ever-changing pathogen population (Liu et al., 2011, Serenius et al., 2007). To date characterized resistances to SFNB and NFNB do not correlate, hence pathologists and breeders need to consider them as different disease when deploying resistance. Also, the high levels of pathogenic and genetic diversity in both *Ptm* and *Ptt* population across barley growing regions of the world suggests that it is crucial to pyramid resistances when deploying resistant barley cultivars.

Host Resistance and Susceptibility

Growing resistance cultivar is an effective strategy to reduce the yield and quality losses inflicted by this disease. However, historically Upper Midwestern barley breeding programs have devoted limited resources and time into deploying resistances to both SFNB and NFNB as compared to other diseases that have been considered a major threat to production like fusarium head blight, spot blotch and stem rust. Thus, the commercial cultivars grown in the region are

susceptible to moderately susceptible to both of these diseases, which are current threats to barley production in the Northern Great Plains and Canada.

Several studies have shown that some NFNB resistances are dominant in nature and are inherited in a Mendelian fashion (Geschele, 1928). NFNB resistance and/or susceptibility QTL have been identified across all seven barley chromosomes (reviewed in Liu et al., 2011) suggesting that resistance to NFNB is very complex, comprised of either dominant or recessive resistance (dominant susceptibility), incompletely dominant genes, and/or quantitative resistances or susceptibilities (Mode and Schaller, 1958, Bockelman et al., 1977, Ho et al., 1996, Friesen et al., 2006, Abu Qamar et al., 2008, Richards et al., 2016, Koladia et al., 2017a). Three incomplete dominant resistance genes *Pt1*, *Pt2* and *Pt3* were identified against *Ptt* isolates collected in California (Mode and Schaller, 1958). Bockelman et al., (1977) reported single dominant resistance genes to NFNB designated as *Rpt1a* (chr 3H), *Rpt3d* (chr 2H), *Rpt1b* (chr 3H), and *Rpt2c* (chr 5H) using trisomic analysis. *Rpt1a* was mapped from Tifang, *Rpt3d* and *Rpt1b* from CI7584, and *Rpt2c* from CI9819, respectively. Ho et al., (1996) reported a recessive resistance (dominant susceptibility) gene on chromosome 2H in barley line Leger that was effective against *Ptt* isolates WRS102 and WRS858 and was shown to be linked with the row type gene *Vrs1*. Steffenson et al., (1996) identified three major QTL at the seedling stage and seven QTL at the adult plant stage in a Steptoe x Morex DH population. Fetch et al., (2008) identified 18 spring barley lines from a collection of ~5000 barley accession that were resistant to both net form net blotch isolate ND89-19 and spot blotch (*C. sativus*) at adult stage. Out of these 18 accessions, none of which contained Mid-western US germplasm in their pedigree, they identified 8 barley lines (CI2291, CI7021, PI58228, PI83794, PI428626, PI434771, PI467387,

and Tx7934) that were highly resistance to both net blotch and spot blotch at both the seedling and adult plant stages.

A major QTL designated *QRpt6* from the barley line TR251 located at the centromeric region of barley 6H was shown to be effective against both net blotch forms at the seedling and adult plant stages (Grewal et al., 2008 and 2010). Besides *QRpt6*, they also identified QTL on 2H, 4H, 5H and 6H against *Ptt* isolate WRS858 effective at the seedling stage and on 3H, 5H, 6H, and 7H against *Ptt* isolate WRS1607 effective at adult stage. *QRpt6* was further validated in two RIL population of "MEH#486 x Harrington" and "McLeod X CDC Helgason" (Grewal et al., 2012). Ma et al., (2004) mapped one resistance gene *Rpt* in the cultivar Chevron against the *Ptt* isolate ND89-19 that explained 64% of phenotypic variation and was flanked by the RFLP markers *Xksua3b and Xwg719d*, which were 25.9 cM apart on the short arm of chromosome 6H. Manninen et al., (2006) identified *Rpt5* on chromosome 6H effective against *Ptt* isolates 84-28-01 (USA), 92-46/15 (Canada), 80-12 (UK) and 27-36 (Australia). One major QTL tightly linked with marker Bmag0173 was reported at the centromeric region of chromosome 6H against isolate 0-1, 15A and ND89-19 (Friesen et al., 2006). A single dominant resistance gene was also identified in the lines CIho 2291, CIho 5098, and Nomini against *Ptt* isolate ND89-19 (O'Boyle et al., 2011). The gene in CIho2291 designated as *Rpt-CIho2291* was mapped to a 34.3 cM interval region on chromosome 6H flanked by the SSR markers Bmag0173 and Bmag0500 whereas the gene in Nomini designated as *Rpt-Nomini* was mapped to a 9.2 cM region on chromosome 6H between the markers Bmag0344a and Bmag0103a (O'Boyle et al., 2014). Cakir et al., (2011) reported QTLs on 2H, 3H, 4H and 6H against isolate NB50 effective at the seedling stage and adult plant stage (adult plant resistance: APR) QTL on 3H, 5H and 6H against *Ptt* isolates NB324 and NB329.

Similarly, Wonnenberger et al., (2017b) also identified QTL on chromosome 3H, 4H, 5H, 6H, and 7H in Norwegian barley lines using the DH population of cultivar Arve and Lavrans. Recently, Koladia et al (2017a) mapped two dominant QTL in the barley line CI5791 on chromosome 3H and 6H in a CI5791 x Tifang RIL population using nine geographically distinct *Ptt* isolates. The CI5791 6H QTL was shown to be effective against all isolates used in the study whereas the CI5791 3H resistance was effective against only two Japanese isolates. Similarly, a dominant 3H QTL was also identified from Tifang in the same population and confered resistance to four *Ptt* isolates from Denmark, Brazil, and two California isolates, indicating that it might be allelic to CI5791 or these QTL represent two linked resistance genes (Koladia et al., 2017a). Besides these dominant resistances in barley against *Ptt*, recessive resistance genes (dominant susceptibility genes) have also been reported. Abu Qamar et al., (2008) mapped two major recessive resistance gene, *rpt.r* and *rpt.k,* to an ~5.9 cM interval at centromeric region of chromosome 6H in a Rika and Kombar population. The *rpt.r* and *rpt.k* genes were found in repulsion and were effective against *Ptt* isolates 6A and 15A, respectively. This region was further saturated to ~3.3 cM interval using EST-based marker (Liu et al., 2010b). Richards et al., (2016) further saturated this region to ~0.24 cM by utilizing synteny with *Brachypodium distachyon*. Allele analysis of the candidate genes in the region suggested that the major susceptibility locus conferring susceptibility to *Ptt* is probably conditioned by a single gene designated as *Spt1* and cvs Rika and Kombar harbor different alleles of *Spt1* conferring susceptibility to California *Ptt* isolate 6A and 15A. Additionally, Richards et al., (2017) identified 16 resistance/ susceptibility QTL loci effective against diverse isolates of NFNB using genome-wide association mapping on 957 barley lines from a worldwide barley collection, of which *QRptts-3HL, QRptts-4HS, QRptts-5HL.1, QRptts-5HL.2, and QRptts-7HL.1* were novel

QTL. Similarly, Wonnenberger et al., (2017a) identified 13 QTL on all seven chromosomes utilizing an association mapping approach using a collection of 209 Nordic spring barley lines. The QTL on chromosome 3H (58.31-61.29 cM) and 6H (54.10-59.33) were reported to be effective at both seedling and adult plant stages. The resistance barley cultivar Heartland is the current resistance source being utilized in Canada and Minnesota breeding program whose resistance has not been characterized (Steffenson and Smith, 2006).

Relatively, few SFNB resistance sources has been identified and mapped to date as compared with NFNB. Thus, resistance to SFNB is less understood as compared to NFNB. Williams et al., (1999) screened 96 barley lines with a mixture of 5 different isolates of *P. teres* f. *maculata* and identified only 4 lines: Galleon (Australia), WI2976 (Australia), OK82850 (USA), and Dairokkaku (Japan) that were resistance to SFNB. Similar results were obtained where limited sources of SFNB resistance were identified by McLean et al., (2012) by screening 95 barley lines at the seedling stage in Australia and Canada. They identified only 2 resistant lines Esperance Orge 289 and TR3189 that were resistant to all isolates at the seedling stage. However, 15 barley lines were resistance to two Canadian isolates and a mixture of Australian isolates at the adult stage. Neupane et al., (2015) identified only 15 resistant lines that were resistance to diverse *Ptm* isolates collected from the USA, Australia, New Zealand, and Denmark which accounted for less than 1% of a world barley core collection consisting of 2,062 accessions. All these results suggested that broad resistance to SFNB is rare.

Previous studies identified major and minor resistance QTL effective against specific *Ptm* isolates spread across all seven barley chromosomes. These QTL are identified either by using biparental mapping population including both RIL and Double Haploid (DH) population (Ho et al., 1996, Steffenson et al., 1996, William et al., 1999 and 2003, Molnar et al 2000, Friesen et al.,

2006, Grewal et al., 2008, Manninen et al., 2000, and 2006, Cakir et al., 2011) or association mapping approaches (Tamang et al., 2015, Wang et al., 2015, Burlakoti et al., 2017). The majority of which are different than the NFNB resistance loci (reviewed in Liu et al., 2011). The *Rpt4* QTL on chromosome 7H from the cultivar Galleon was the first SFNB dominant resistance reported and has been utilized in Australian barley breeding programs (Williams et al., 1999 and 2003). This *Rpt4* gene was further confirmed in the barley lines CI9214, Keel and Tilga (Williams et al., 2003). They also mapped an adult plant resistance QTL on chromosome 7H (140-170 cM) close to *Rpt4* and on chromosome 5H (20-40 cM) in barley line Galleon and VB9104. Another source that has been utilized in Australian breeding programs is the *Ha4* gene that confers resistance to the cereal cyst nematode (*Heterodera avenae*) and SFNB at adult stage (Arabi et al., 1992, Karakousis et al., 2003). Ho et al., (1996) reported a dominant gene from the barley line Leger to *Ptm* isolate WRS857, but didn't provide the location because of the low marker density, thus the gene wasn't linked with any of the markers used in their study. Grewal et al., (2008) identified three QTL: *QRpts4* on 4H, *QRpt7* on 7H, and *QRpt6* on 6H against SFNB isolate WRS857 in TR251 using a CDC Dolly x TR251 DH population. The *QRpt6* was shown to be effective against both net blotch forms as well as effective at both seedling and adult plant stages (Grewal et al., 2012). QTL *Rpt6* on chromosome 5H (R^2=65-84%) near the marker HVLEU was reported in line CI9819 against two Finnish isolates P1332 and P1333 (Manninen et al., 2006). One unnamed QTL on 4H was detected against SFNB isolate NZKF2 (New Zealand) explaining 64% of the phenotypic variation in a SM89010 x Q21861 DH population (Friesen et al., 2006). A new QTL in cultivar Baudin was also detected on the short arm of chromosome 6H (~23.9 cM) effective at both seedling and adult stages against *Ptm* isolates 95NB104, 95NB117, and WAC11160 (Cakir et al., 2011).

Recently, association mapping has been utilized to map SFNB resistance gene using different barley populations. Wang et al., (2015) conducted an association mapping study on elite breeding lines from the Northern region barley breeding programs of Australia and identified 29 QTL of which 22 confer resistance at both the seedling and adult plant stages, 2 QTL at the seedling stage only, and 5 QTL at adult plant stage only. In association mapping using 1,480 barley lines (worldwide barley collection), 27 distinct loci were identified of which 6 were consistent with previously reported loci and 21 were novel loci with QTL identified across all seven barley chromosomes (Tamang et al., 2015; Chapter 2 of this dissertation). Burlakoti et al., (2017) conducted an association study of SFNB with 376 advanced breeding lines from four barley-breeding programs in the Upper Midwest of United States. They reported 10 QTL effective against *Ptm* isolate SFNB-MT09 on chromosomes 2H, 3H, 5H, 6H, and 7H.

However, genes involved in SFNB resistance have not been cloned and characterized yet and none of these resistance loci have been utilized in barley breeding program in North Dakota, hence, the majority of the barley cultivars grown in this region are moderately susceptible to susceptible to SFNB. Similarly, barley lines CI5791, Heartland, and Algerian exhibiting high level resistance to all *Ptt* isolates collected from ND and would be excellent sources of resistance to NFNB in the Northern Great Plains. However, a few documented isolates that have overcome the remarkable resistance present in the line CI5791 have been reported. Arabi et al., (1992) reported that CI5791 is susceptible to R5 and S5 biotypes of *Ptt*. CI5791 resistance has also been compromised by Moroccan *Ptt* isolates as indicated by seedling virulence of the isolates SM25-2, SM25-3, and SM40-3 (Personal communication with Dr. Timothy Friesen). Recently, Akhavan et al., (2016) also reported one *Ptt* isolate that has overcome the CI5791 resistance in Western Canada. Due to the complex genetics resistance/ susceptibility and polygenic nature of

resistance in the host as well as availability of rare resistance sources, breeding for resistance to SFNB and NFNB is a challenge. Although there are a few SFNB and NFNB common resistance loci effective at both the seedling and adult plant stages to specific isolates, the majority of the resistance gene are different. This suggests that breeders have to treat these two forms of net blotch as different disease when deploying resistance.

Toxins and Host Selective Effectors

P. teres f. *teres* and *P. teres* f. *maculata* both induce necrotic lesions surrounded by chlorosis on the leaves of susceptible barley. These lesions, that are the result of pathogen colonization, facilitate nutrient extraction and ultimately sporulation by this necrotrophic pathogen. The chlorotic areas surrounding the necrotic lesions are typically free of hyphal growth and associated with diffusible toxins (Smedegard-Peterson, 1977) and proteinaceous effectors (Liu et al., 2011). These proteinaceous toxins and the effectors are the weapon of the pathogen to infect the host and play important roles in disease development.

Two phyto-toxins designated as toxin A and B were isolated and purified from culture filtrates of *Ptt* and *Ptm* isolates that alone produced symptoms on barley (Smedegard-Petersen, 1977). Toxin A was more effective than toxin B in producing symptoms on the host. However, the symptoms produced by the toxins were not as typical as the pathogen produced symptoms suggesting that the toxins don't necessarily determine pathogenicity, but contributed to the isolate virulence (Smedegard-Petersen, 1977). Later, Bach et al., (1979) isolated a new toxin called toxin C from the same isolates. These three toxins were found to be chemically and structurally similar to aspergillomarasmine A. Toxin A was identified as N-(2-amino-2-carboxyethyl) aspartic acid, toxin B was anydroaspergillomarasmine A, and toxin C as aspergillomarasmine A. Among these 3 toxins, toxin C was the most active and induced necrotic

lesions surrounded with light-yellow chlorosis, toxin A produced dark-yellow chlorotic symptoms with less necrosis and toxin B produced slight symptoms on susceptible barley (Weiergang et al., 2002). The author also showed that the toxin A can produce chlorosis between 48-72 hrs and necrosis began to appear after 120 hrs of post treatment. Friss et al., (1991) discovered that toxin A serves as the precursor of toxin C and toxin C can convert into toxin B without any enzymatic catalyst under low pH level in culture.

Another proteinaceous metabolite toxin isolated and purified from both *Ptt* and *Ptm* was able to induce necrotic spots identical to net blotch symptoms on susceptible barley (Sarpeleh et al., 2007). However, this toxin produced minimal symptoms on resistant line CI9214 and no symptom on non-host wheat, triticale, rye and faba bean, which suggested that this toxin is a host selective toxin (Sarpeleh et al., 2007). This toxin was highly heat stable and its activity was light and temperature dependent which was similar to SnTox1 produced by *Stagonospora nododrum* and PtrToxA by *Pyrenophora tritici-repentis* (Sarpeleh et al., 2007 and 2008, Manning et al., 2009, Liu et al., 2012). Additionally, another low molecular weight compound (LMWCs) that produced chlorosis on barley leaves was also identified by Sarpeleh et al., (2008). Recenlty, Liu et al., (2015) identified another proteinaceous effector designated as PttNE1 from *Ptt* isolate 0-1 by intracellular wash fluids (IWFs) from the susceptible barley cultivar Hector. PttNE1 was able to produce necrosis on Hector (susceptible) but no symptoms on the resistant cultivar NDB112. The sensitivity to these IWFs was mapped to the centromeric region of chromosome 6H using a Hector x NDB112 RIL population (Liu et al., 2015).

Necrotrophic fungal pathogens are shown to produce necrotrophic effectors (NE) also known as host-specific toxins (HSTs). HSTs are small secreted proteins or low molecular weight metabolites and are key pathogenicity/virulence factors of the pathogen. Dothideomycete

necrotrophic effectors were shown to predominantly interacting with corresponding dominant susceptibility proteins or gene products following an inverse gene-for-gene model resulting in susceptible reactions which is known as necrotrophic effector-triggered susceptibility (NETS) (Friesen et al., 2008, Liu et al., 2012 and 2015). In the inverse gene-for-gene model, necrotrophic pathogens hijack the plant resistance pathway by triggering programmed cell death (PCD), oxidative burst, accumulation of reactive oxygen species (ROS), and DNA laddering on sensitive hosts, which are hallmarks of typical biotrophic resistance reactions (Liu et al., 2015). However, the necrotrophs are able to utilize these innate host immunity responses to facilitate colonization, nutrients acquisition from the dying cells, and ultimately sporulation.

The identification and characterization of pathogen virulence/ avirulence or effector genes and functional characterization of their protein products will facilitate the understanding of the barley-*P. teres* interactions at the molecular level. The ability to cross *P. teres* and resulting genetic analyses have been utilized to clone pathogen virulence and avirulence genes which typically directly encode proteinatious effector proteins. Weiland et al., (1999) were the first to create a *P. teres* mapping population of the parental isolates 0-1 and 15A to study the genetics of their virulence/avirulence on the barley cultivar Harbin. They mapped the *AvrHar* locus utilizing this *P. teres* f. *teres* biparental population showing that it was a single host-parasite genetic interaction between a single dominant gene in the host and a single avirulence gene in the pathogen that resultsed in an incompatible interaction (resistance). Lai et al., (2007) identified two additional avirulence genes *AvrPra1* and *AvrPra2* using the same fungal population and AFLP markers. These avirulence genes control the virulence of *Ptt* isolate 0-1 on cultivar Prato. Interestingly, the *AvrPra2* mapped to the same linkage group of *AvrHar* but they segregated in repulsion suggesting that these might be the different allele at the same locus. Beattie et al.,

(2007) identified another avirulence gene designated as $Avr_{heartland}$, which determines avirulence on the cultivar Heartland using a biparental *Ptt* population of the two Canadian isolates WRS1607 and WRS1906. Afanasenko et al., (2007) studied the segregation pattern of host resistance genes and the pathogen virulence/avirulence gene using twelve F_2 barley population developed by crossing diverse sets of barley resistance lines with susceptible barley and two pathogen population (181-6 X A80 and H-22 X 92-178/9). They reported gene-for-gene interactions in the barley-*Ptt* pathosystem where resistance in the host is mostly isolate specific governed by one or two genes and the avirulence gene in *Ptt* is also governed by one or two genes.

Shjerve et al., (2014) used a bi-parental mapping population derived from a cross between isolate 15A and 6A and identified four virulence QTL (VK1, VK2, VR1, and VR2) associated with pathogen virulence. Susceptibility to progeny isolates that possessed single virulence genes mapped to the same centromeric regions of chromosome 6H in a Rika x Kombar DH population. This result suggested that 15A and 6A each produces two different unique necrotrophic effectors that have single or multiple targets at the same 6H region of the barley genome. Recently, Koladia et al., (2017b) identified 9 unique QTLs associated with virulence/avirulence factor of *P. teres* f. *teres* utilizing biparental population developed with the parental isolates BB25 (Denmark) and FGOH04Ptt-21 (Fargo, ND). Out of the 9 QTL identified, 3 major QTL contributed greater than 45% of the phenotypic variation, whereas the remaining 6 minor QTL contributed less than 20% of the phenotypic variation. They speculated that the variation in virulence of *Ptt* populations is associated with multiple loci with small effects resulting in the quantitative nature of virulence.

Further investigation or in-depth analyses of Mendalized interactions is required to determine if effector protein encoding genes acts in an inverse gene-for-gene relationship with corresponding sensitivity protein encoding genes in barley or if they function in a gene-for-gene interaction that results in avirulence. Inverse gene-for-gene action has been demonstrated in the wheat-*Stagonospora nodorum* and wheat-*P. tritici repentis* pathosystems (Friesen et al*.,* 2007; Faris et al., 2010; Tan et al., 2010, Liu et al., 2012). Recently, Richards et al., (2016) identified a candidate dominant *Ptt* susceptible gene *Spt1* in barley that follows the inverse gene-for-gene interaction model but the corresponding avirulence genes in *Ptt* have yet to be identified. Although, there are several studies reporting dominant susceptibility (recessive resistance) genes in barely against *P. teres* f. *teres*, there are currently no susceptibility genes reported in barley corresponding with *P. teres* f. *maculata* (Liu et al., 2011). But its logical to predict that this inverse gene-for-gene interaction may also exists in the barley-*P. teres* f. *maculata* pathosystem too.

Association Mapping

The ultimate goal of genetic mapping is to identify the actual gene that controls the phenotype of interest. However, at the resolution that we currently have in barley we typically map markers that are in close proximity to the genetic factors (genes) controlling the trait of interest. Historically, bi-parental genetic linkage mapping was the most commonly used approach to map genes or QTL where two parents with polymorphic phenotype were crossed to make the population. One advantage of bi-parental mapping is detection of rare alleles contributing to the phenotype. However, the drawbacks of bi-parental mapping are the time and cost of population development and the limited number of meiotic events (Zhu et al., 2008).

The association mapping (AM) approach is an alternative tool to genetically map genes or QTL at high resolution, which overcomes some of the limitations of biparental mapping (Zhu et al., 2008, Flint-Garcia et al., 2003). The advantages of AM include higher resolution which depends upon the linkage disequilibrium (LD) of the population utilized where a large pool of historical recombination events will provide the best resolution but to utilize this mapping power you must obtain a high level of marker density. Also, AM utilizes existing germplasm collections eliminating the need to develop mapping populations. Thus, AM is a powerful tool to identify marker-trait association (MTA) present in populations and has the potential to identify candidate genes very quickly compared to the tried and true positional cloning techniques that relied on generating very large bi-parental recombinant populations. However, there is a possibility of getting false positive MTAs when there is a failure to adequately account for population structure or kinship in the analyses and/or the optimal model/s are not selected for the analyses (Flint-Garcia et al., 2003, Yu et al., 2006).

Currently, AM is gaining popularity in plants and has been used to detect the markers associated with different complex traits in various plant species such as barley (Tamang et al., 2015, Burlakoti et al., 2017, Richards et al., 2017, Wang et al., 2015), wheat (*Triticum aestivum*) (Breseghello and Sorrels 2006, Tommasini et al., 2007), rice (*Oryza sativa*) (Agrama et al., 2007), Corn (*Zea mays*) (Kump et al., 2011), Soybean (*Glycine max*) (Wang 2008, Mamidi et al., 2011), *Arabidopsis* (Ehrenreich et al., 2009, Aranzana et al., 2005), Potato (*Solanum tubersum*) (Malosetti et al., 2007), and Canola (*Brassica napus*) (Honsdorf et al., 2010, Zou 2010). Recently, AM has been utilized to map both SFNB (Wang et al., 2015, Tamang et al., 2015, Burlakoti et al., 2017) and NFNB (Richards et al., 2017 and Wonnenberger et al., 2017a) resistance loci in different barley population with great success.

WRKY Transcription Factors

Transcription is the first step in gene expression, which is regulated by transcription factors (TFs). More than 1500 TFs were identified and clustered into 34 families that either activate or suppress the expression of genes (Reichmann et al., 2000). A typical TF contains a DNA binding domain (DBD) that recognizes a short motif in the target DNA sequence (typically 10 nucleotides) called transcription factor binding sites (TFBSs) which either positively or negatively regulate gene expression to achieve cellular homeostasis (Riechmann et al., 2000; Guo et al., 2005; Mitsuda and Ohme-Takagi, 2009). Eulgem (2006) observed various *Arabidopsis* TF family representatives that bind to promoter regions of defense-related genes either to activate or repress them. Based on their DNA binding domain, TFs are classified into several groups including the P2/ERF (APETALA 2/Ethylene-Responsive Element Binding Factor), NAC (No Apical Meristem, ATAF1/2, Cup-Shaped Cotyledon 2), SBP (Squamosa-Promoter Binding Protein) and WRKY superfamilies that are involved in diverse biotic/abiotic stress, developmental and physiological responses (Phukan et al., 2016).

WRKY TFs contains a highly conserved amino acid sequence WRKYGQK at their N-terminus and zinc-finger-motif (C-C-H-H/C) at their C-terminus (Eulgem et al., 2000, Robtzek et al., 2001). WRKY proteins bind to a specific W-box element (TTGACT), which can occur as hexamers (TTGAC/T), palindromes (TGACC/T-A/GTCA), or tandem repeats (TGACC/C-TGACC/T) in promotor regions of the target genes either to activate or repress gene function (Rushton et al., 2010, Agarwal et al., 2011, Eulgem et al., 2000, Yu et al., 2001). WRKY genes may have W-boxes in their own promoter regions suggesting that it is self-regulated, or it might be regulated by other WRKY transcription factors (Eulgem, 2005). In *Arabidopsis*, the WRKY superfamily consists of more than seventy WRKY TFs and is one of the largest TF families

(Robtzek et al., 2001 and 2002). WRKY TFs have been found to be involved in diverse plant physiological activities such as pathogen defense responses, biotic stress, senescence, root growth, etc. (Robtzek et al., 2001 and 2002, Skibbe et al., 2008) and to several abiotic stresses such as wound responses and nutrient deficiency (Kasajima et al., 2010, Chen et al., 2009, Li et al., 2017).

Several studies have provided evidence that WRKY TFs are the integral part of the plant immune system including PTI, ETI, and systemic acquired resistance (SAR) (Eulgem and Somssich, 2007; Rushton et al., 2010). Genetic studies have shown that *WRKYs* can either positively or negatively regulate the plant defense responses (Eulgem and Somssich, 2007, Robatzek et al., 2001 and 2002). *AtWRKY6* regulates both plant defense response against *Pseudomonas syringae* pv. *Tomato,* as well as senescence in *Arabidopsis* (Robatzek et al., 2002). This *AtWRKY6* regulates SIRK gene (Senescence-Induced Receptor like serine/threonine protein Kinase) that encodes a receptor-like kinase, which is exclusively localized to the plant cell nucleus (Robatzek et al., 2002). *AtWRKY6* has also been found to be involved in several abiotic stress responses including responses to boron deficiency, phosphorous deficiency, and acts as a positive regulator of Abscisic Acid Signaling (ABA) during seed germination and early seedling development in *Arabidopsis* (Kasajima et al., 2010, Li et al., 2017, Yun et al., 2016). *WRKY3* and *WRKY6* regulate defense response in tobacco (*Nicotiania attenuate*) against herbivore *Manduca sexta* larvae during feeding and their interaction also plays a role in defense responses (Skibbe et al, 2008). They observed that the host susceptibility to herbivore in the knockout plants are associated with impaired Jasnomate (JA) accumulation thereby interrupting the JA signaling pathway. However, the resistance to *M. sexta* in Nicotiania did not change with overexpression of either *WRKY3* and/or *WRKY6*.

In *Arabidopsis*, the bacterial effectors, *AvrRPS4* and *PopP2*, interacts with WRKY TFs interfering with the WRKY-dependent defenses (Sarris et al., 2015). The *WRKY52* binding domain has been integrated in *Arabidopsis RPS4*/*RRS1* NLR complex as a decoy forming an intramolecular guardee of the NLR that recognizes the bacterial effectors *AvrRPS4* and *PopP2*, and activate the defense response by direct interaction with the bacterial effectors and the NLR gene product. NLR-WRKY interactions were also observed in *AtWRKY16*/*TTR1*, and *AtWRKY19* suggesting involvement in ETI defense related responses (Rushton et al., 2010). This system has been characterized as an example of the integrated decoy model (Cesari et al., 2014) and has been discovered in different pathosystem such as rice- *Magnaporthe oryzae* (Zhai et al., 2014, Cesari et al., 2013), barley- *Puccinia graminis* (Wang et al., 2013), wheat- *Puccinia triticina* (Loutre et al., 2009), and Papaya-*Fusarium oxysporum* (Brotman et al., 2012) but with other integrated sensory domains that don't represent WRKY TFs.

Several WRKY TFs have been reported in other plant species that plays important role in defense response to pathogens. About 45 WRKYs has been reported in barley (Mangelsen et al., 2008). *HvWRKY1* and *HvWRKY2* in barley were found to repress PTI by interfering with the intracellular mildew A (MLA) protein (Shen et al., 2007; Chang et al., 2013). However, Meng and Wise (2012) identified *HvWRKY10*, *HvWRKY19*, and *HvWRKY28* as a positive regulator of ETI in barley against *Blumeria graminis*. Similarly, at least 109 WRKY TFs have been reported in rice (*Oryza sativa*) (Ross et al., 2007). The upregulation of *OsWRKY13*, *OsWRKY31*, *OsWRKY45*, *OsWRKY53* and *OsWRKY47* were found to be associated with the enhanced resistance in rice against *Magnaporthe oryzae* (Chujo et al., 2007; Wei et al., 2013). Wang et al., (2007) observed increase wax deposition on the leaf surface at infection sites of *Magnaporthe oryzae,* which were associate with overexpression of *OsWRKY89*. Similarly, in capsicum,

CaWRKY6 regulates *CaWRKY40* which activate resistance to *Ralstonia solanacearum* as well as adds tolerance to high-temperature and humidity (Cai et al., 2015)

In addition to positive regulators in *Arabidopsis* defense, WRKY TFs can also function as negative regulators. *WRKY53* has dual function depending upon the pathogen type: it positively regulated the plant response to *P. syringae* while negatively affected plant defense to *Ralstonia solanacearum* (Murray et al*.,* 2007; Hu et al., 2008). Similarly, Journot-Catalino et al., (2006) identified *WRKY11* and *WRKY17* as a negative regulator of basal defense responses in *Arabisopsis*. Li et al., (2004) observed similar result of enhanced resistance to the biotroph *Erysiphe cichoracearum* and increase in susceptibility to bacterial necrotroph *Erwinia carotovora* subsp *carotovora* with the upregulation of *WRKY70* in *Arabidopsis* and reported *WRKY70* as a positive regulator of SAR and JA. The TFs *WRKY38* and *WRKY62* were also found to be negative regulators of plant basal defense response to the bacterial pathogen *P. syringae* (Mao et al., 2007, Kim et al., 2008). They observed reduction in disease resistance with overexpression of *WRKY38* and/or *WRKY62*. Both *WRKY38* and *WRKY62* interact with Histone Deacetylase 19 (HDA19) and interfere with its resistance function. *WRKY38* and *WRKY62* can be induced in a NPR1 (Nonexpressor of Pathogenesis-related genes 1) dependent manner either by virulent *P. syringae* or SA (salicylic acid). Xing et al., (2008) observed *WRKY48* TF negatively influenced SAR by altering the expression of Pathogenesis-Related gene 1 (*PR1*) in *Arabidopsis* against *P. syringae*. They observed resistance reactions in loss-of-function *Arabidopsis* mutants, which was associated with increased SA regulation of PR1. The overexpression of *WRKY48* in transgenic *Arabidopsis* (gain-of-function) resulted in susceptibility, which was associated with reduced expression of PR1 genes. Grunewald et al., (2008) identified *WRKY23* as the negative regulator to plant defense response against cyst

nematode *Heterodera schachtii.* WRKY genes can also mediate cross talk between Jasmonic acid (JA) and SA signaling pathways (Li et al., 2004).

Summary

Net blotch of barley caused by the necrotrophic fungal pathogen *Pyrenophora teres* is a major foliar disease in major barley-growing regions throughout the US and the world (Liu et al., 2011). Net blotch occurs in two forms: SFNB caused by *P. teres* f. *maculata* and NFNB caused by *P. teres* f. *teres*. Although these two pathogens are closely related, their interactions with hosts are distinct. So, they should be treated separately while breeding and deploying resistance. Since the positive identification of SFNB in 2010, it has been found each year throughout the state of ND and is now considered a major threat in the Northern Great Plains because the isolates collected from this region are more virulent than any other isolates collected from other parts of the world (Liu and Friesen, 2010). Currently, both SFNB and NFNB are emerging as major barley disease in ND, Montana, and Eastern Idaho. Studies on SFNB resistance sources are behind relative to NFNB, thus resistances to SFNB are not well understood as compared to NFNB. Our objective was to identify and map the resistance sources of SFNB through association mapping and biparental-mapping approach; and to begin to identify and characterize an important NFNB dominant resistance gene from the line CI5791.

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CHAPTER 2. ASSOCIATION MAPPING OF SEEDLING RESISTANCE TO SPOT FORM NET BLOTCH IN A WORLDWIDE COLLECTION OF BARLEY

Abstract

Spot form net blotch (SFNB), caused by the necrotrophic fungal pathogen *Pyrenophora teres* f. *maculata*, is an important foliar disease of barley in major production regions around the world. Deployment of adequate host resistance is challenging because the virulence of *P. teres* f. *maculata* is highly variable and characterized minor-effect resistances are typically ineffective against the diverse pathogen populations. A world barley core collection consisting of 2,062 barley accessions of diverse origin and genotype were phenotyped at the seedling stage with four *P. teres* f. *maculata* isolates collected from the United States (FGO), New Zealand (NZKF2), Australia (SG1), and Denmark (DEN 2.6). Of the 2,062 barley accessions phenotyped, 1,480 were genotyped with the Illumina barley iSelect chip and passed the quality controls with 5,954 polymorphic markers used for further association mapping analysis. Genome-wide association mapping was utilized to identify and map resistance loci from the seedling disease response data and the single nucleotide polymorphism (SNP) marker data. The best among six different regression models was identified for each isolate and association analysis was performed separately for each. A total of 138 significant (−log10*P* value > 3.0) marker-trait associations (MTA) were detected. Using a 5 cM cutoff, a total of 10, 8, 13, and 10 quantitative

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trait loci (QTL) associated with SFNB resistance were identified for the FGO, SG1, NZKF2, and DEN 2.6 isolates, respectively. Loci containing from 1 to 34 MTA were identified on all seven barley chromosomes with one locus at 66 to 69 cM on chromosome 2H common to all four isolates. Six distinct loci were identified by the association mapping (AM) analysis that corresponded to previously characterized SFNB resistance QTL identified by biparental population analysis (*QRpt4*, *QRpt6*, *Rpt4, Rpt6*, *Rpt7*, and a QTL on 4H that was not given a provisional gene or QTL nomenclature). The 21 putative novel loci identified may represent a broad spectrum of resistance and or susceptibility loci. This is the first comprehensive AM study to characterize SFNB resistance loci underlying broad populations of the barley host and *P. teres* f. *maculata* pathogen.

Introduction

Net blotch of barley (*Hordeum vulgare* L.) caused by the necrotrophic fungal pathogen *Pyrenophora teres* Drechsler (anamorph: *Drechslera teres* [Sacc.] Shoem.) is a destructive pathogen in many barley-growing regions throughout the world (Liu et al., 2011). This pathogen is separated into the net and spot forms based on predominant disease symptoms with the causal agents being *P. teres* f. *teres* and *P. teres* f. *maculata*, respectively (Smedegård-Petersen, 1971). Spot form net blotch (SFNB) has emerged as a major leaf spot disease in several barley-growing regions of the world including regions of Australia, Canada, Europe, South Africa, and recently the United States (Karki et al., 1986, Khan and Tekauz, 1982, Liu et al., 2011, Mathre, 1997, McLean et al., 2009, Tekauz, 1990). Although SFNB had previously been reported in the Upper Midwestern United States, it was not considered a problem (Liu and Friesen, 2010). However, since it was identified in North Dakota in 2006, it has become a major concern for growers in the Northern Great Plains due to what may be increased virulence as

indicated by comparison with isolates collected in other parts of the world (Neupane et al., 2015).

The prevalence and increase of SFNB epidemics may be due to a combination of different factors including minimum or no-till practices, high-density cropping, cultivation of predominantly susceptible varieties, and shifts of virulence in the pathogen populations due to selection by popularly grown varieties (Liu et al., 2011, McLean et al., 2009). In years when environmental conditions are conducive to disease and susceptible varieties are grown, SFNB can cause 10 to 40% yield losses in barley (Jayasena et al., 2007, Khan and Tekauz, 1982, Mathre, 1997, Murray and Brennan, 2010). Chemical control, cultural practices, and host resistance are all commonly used to manage SFNB (Brown et al., 1993, Jordan and Allen, 1984, McLean et al., 2010, Youcef-Benkada et al., 1994), yet deployment of high-quality resistant cultivars would be the most efficient and environmentally friendly means of disease management.

Wide ranges of pathogenic variability in *P. teres* f. *maculata* populations have been reported within and across distinct geographic regions (Gupta and Loughman, 2001, Steffenson and Webster, 1992, Tekauz, 1990, Tuohy et al., 2006). Based on what is known in other well-characterized cereal host−Dothidiomycete necrotrophic pathosystems (Friesen et al., 2008, Friesen et al., 2010, Oliver et al., 2012), it is expected that *P. teres* produces a diversity of necrotrophic effectors (previously known as host specific/selective toxins) that target distinct susceptibility genes in barley resulting in quantitative susceptibility (Liu and Friesen., 2010, McLean et al., 2009). Resistant varieties lacking these multiple sensitivity genes are uncommon, thus sources of resistance that are effective against diverse SFNB populations are rare.

Quantitative trait loci (QTL) analysis of SFNB infection types on biparental barley mapping populations identified resistance/susceptibility genes on barley chromosomes 2H (Molnar et al., 2000), 4H (Friesen et al., 2006, Grewal et al., 2008), 5H (Manninen et al., 2006), 6H (Grewal et al., 2008), and 7H (Grewal et al., 2008, Williams et al., 1999, Williams et al., 2003). These QTL and the markers linked with resistance may be specific to these genotypes and may represent interactions specific to narrow populations of both the host and pathogen.

Association mapping (AM) presents an alternative approach to biparental mapping that can efficiently capture diverse marker-trait associations (MTA) (Flint-Garcia et al., 2003, Myles et al., 2009, Zhu et al., 2008). This approach has been used effectively in diverse crop plants for many traits including complex yield traits in rice (Agrama et al., 2007), resistance genes/QTL in maize (Kump et al., 2011), wheat (Breseghello and Sorrells, 2006, Ghavami et al., 2011, Tommasini et al., 2007), potato (Malosetti et al., 2007) and iron deficiency chlorosis (IDC) in soybean (Mamidi et al., 2014, Wang et al., 2008), flowering time in Arabidopsis (Aranzan et al., 2005, Ehrenreich et al., 2009), and quality traits in canola (Honsdorf et al., 2010, Zou et al., 2010). In barley, different marker types have been identified that are associated with phenotypes including resistance to the diseases spot blotch, Fusarium head blight, powdery mildew, and leaf rust (Berger et al., 2013, Massman et al., 2011, Roy et al., 2010). Loci contributing to other important yet complexly inherited agronomic traits including malting quality (Beattie et al., 2010, Gutierrez et al., 2010), yield and yield stability (Kraakman et al., 2004), flowering time (Ivandic et al., 2002), water-stress resistance (Ivandic et al., 2003), salt tolerance (Pakniyat et al., 1997), and winter-hardiness (Von Zitzewitz et al., 2011) have also been identified utilizing AM.

The objective of this study was to use the AM approach on a large population of barley genotypes from around the world to identify loci that contribute to resistance against diverse

SFNB isolates collected from four important barley-producing regions. The identification of single nucleotide polymorphism (SNP) markers associated with SFNB resistance loci and/or lacking susceptibility genes from resistant lines identified from the world barley core collection (BCC; USDA-ARS National Small Grains Collection) will facilitate the deployment of effective and durable SFNB resistance via marker-assisted selection or genome-wide selection strategies.

Materials and Methods

Plant Materials and Phenotyping

A geographically diverse sample of 2,062 barley core collection (BCC) accessions comprising cultivars, breeding lines, landraces, and genetic stocks were obtained from the National Small Grain Collection, Aberdeen, Idaho (Supplementary Table 2.1). The lines were grown in the greenhouse at the USDA, Fargo, North Dakota, USA during 2011 and 2012. Three seeds of each barley line were planted in single cones (3.8 cm diameter and 20 cm long) and were placed into cone racks bordered with the susceptible barley cultivar (cv) Robust. Each cone contained 3 seedlings and was evaluated collectively as a single replicate. Barley lines CIho-14219 and PI 67381 were used as resistant checks and the cultivar Robust was used as the susceptible check.

Four geographically diverse isolates of *P. teres* f. *maculata* FGOB10Ptm-1 (FGO), SG1, NZKF2, and DEN2.6 collected in the USA, Australia, New Zealand, and Denmark, respectively, were used to evaluate the barley lines for SFNB disease reaction. Fungal inoculum preparation, inoculation, and incubation used for the AM analyses described in this manuscript were performed and described in Neupane et al., 2015, which is published as an adjoining manuscript. Three independent replicates were performed for each isolate and the mean of the three replicates

was considered for further analysis. Disease was evaluated 7 days after inoculation using the 1 to 5 rating scale as described in Neupane et al. (2015).

Genotyping

Of the 2,417 barley accessions genotyped with the barley 9k Illumina Infinium iSELECT assay through the triticeae coordinated agriculture project (T-CAP), 2,062 of the barley accessions were evaluated for their reaction to the *P. teres* f *maculata* isolates (Neupane et al., 2015). The genotyping was performed and described by Muñoz-Amatriaín et al. (2014) and the data were obtained from The Triticeae Toolbox (T3) website (http://malt.pw.usda.gov/t3/sandbox/barley/). For this AM analysis we utilized the 6,244 quality SNPs that passed the quality control criteria and 1,480 unique iCore spring barley lines that were previously described (Munoz-Amatrain et al., 2014). Throughout the manuscript the analyses relied on the marker positions, which were previously determined and published as the iSelect consensus map (Munoz-Amatrain et al., 2014).

Association Mapping

Imputation and Marker Properties

All missing genotypic data were imputed using a "likelihood" based imputation with default settings in fastPhase 1.3 (Scheet et al., 2006). The minor allele frequency (MAF) for markers was estimated using the FREQ procedure in SAS 9.3 and the markers that had MAF < 5% were removed from further analysis. The structure, kinship, and AM were also performed separately utilizing all the markers, the markers that have MAF > 1% and the markers > 5% MAF which are reported in the manuscript. From the results, there were very minor changes between the analyses in terms of significant markers or the number of QTL identified.

Linkage Disequilibrium and LD Decay

Linkage disequilibrium (LD) was estimated as the squared allele frequencies correlation (R^2) for each of the pair-wise comparison of the markers using the Corr procedure in SAS 9.3. Linkage disequilibrium decay graphs were plotted with genetic (cM) distance versus R^2 for all inter-chromosomal marker pairs using nonlinear regression (Remington et al., 2001). The expected decay of LD was estimated using the formula described by Mamidi et al. (2011)

Population Structure, Principal Component Analysis, and Relationship Matrix

To prevent linkage bias in estimation of population structure (Q), principal component analysis (PCA) and relationship matrix (K-matrix), a subset of markers that had an LD <0.5 with every other marker combination were used (Weber et al., 2008). STRUCTURE 2.3 was used to estimate the number of sub-populations. The admixture model was used with a burn-in of 20,000 and 50,000 iterations for subpopulation numbers (k) ranging from 1 to 15 considering the allele frequencies to be independent. Five runs for each k value were performed and the posterior probability was determined for each run. The optimum number of subpopulations was determined using the Δk approach (Evanno et al., 2005) implemented in Structure harvester (Earl and Von Holdt., 2011).

Principal component analysis (PCA) was performed in SAS 9.3 to estimate PCs that controlled the population structure in the regression model. The number of principal components that explained ~25% of the cumulative variation in the population were selected to be included as cofactors in the association analysis (Mamidi et al., 2014, Stich and Melchinger, 2009). An identity by state (IBS) matrix (Zhao et al., 2007) to control the population relatedness was estimated as a Gower similarity ratio implemented in the distance procedure in SAS 9.3.

Marker-Trait Association Model Testing

Six different linear regression models were tested for marker-trait associations - Naive, PC, Q, kin, PC+kinship, Q+kinship (Mamidi et al., 2014). Three models that did not have kinship were estimated in SAS 9.3 and three models that had the kinship matrix as a random effect were estimated using GEMMA 0.90 (Zhou and Stephen, 2012). Three general-linear models (GLMs) considered only the fixed effects while the remaining three mixed-linear models (MLMs) considered both the fixed and random effects. The underlying linear equation for the sixth model was

$$Y = X\alpha + Q\beta + Kv + \mathcal{E}$$

In this model, Y was a vector for phenotypic observation, X was a matrix of alleles of the markers, α was the fixed effects related to the SNP markers, Q was the population structure, β was a vector of the fixed effects related to population structure, K was the subpopulation numbers, v was a vector of the random effects related to the relatedness among the individuals, and Ɛ was a vector of the residual effects. For each model, the positive false discovery rate (pFDR) was estimated for all markers using the PROC MULTTEST in SAS 9.3 to correct for multiple marker-trait association (Storey, 2002). For the selection of best model, a rank based mean square difference (MSD) was used (Mamidi et al., 2014) based on the suggestion of Yu et al. (2006) of random errors. MSD values were calculated between the observed p-values and expected p-values. Expected p-values were estimated by dividing the rank of observed p-value with number of makers used in this study. The model with the lowest MSD value was considered the best model. Markers were considered significant if the *P* -value was less than 0.001. The amount of phenotypic variation (R^2) was estimated for each significant marker using a simple regression using the REG procedure in SAS 9.3. Multiple R^2 values for significant markers were

calculated using stepwise regression implemented in the Reg procedure in 9.3 (Mamidi et al., 2014). The allelic means of the significant markers were estimated in SAS Brown et al., 1993.3 using the Means procedure. Further, a QTL was defined as the region that harbors multiple significant markers ($p < 0.001$) within a distance at which the whole genome LD decay stabilizes. The position of the significant markers was obtained from a core consensus map developed by Muñoz-Amatriaín et al. (2014).

Results

Phenotypic Analysis Across Barley Accession

A total of 2,062 spring BCC accessions were evaluated for their reaction to the four *P. teres* f *maculata* isolates, (FGO, NZKF2, SG1 and DEN2.6) by Neupane et al. (2015). The manuscript describing the phenotypic analyses and results is published as an adjoining manuscript (Neupane et al., 2015). Of the 2,062 accessions reported in Neupane et al., 2015, only 1480 accessions were utilized in our analysis due to marker data not passing QC and redundant genotypes being eliminated as determined by Muñoz-Amatriaín et al. (2014).

Marker Properties

Of the 2,062 barley accessions evaluated for their reaction to the *P. teres* f. *maculata* isolates (Neupane et al., 2015), 1,947 barley accessions were genotyped with a total of 6,244 SNP markers. Of the 1,947 spring barley accessions genotyped, 467 were removed due to genetic redundancy or inconsistent passport data, leaving 1,480 spring barley accessions in the AM analysis (Munoz-amatriain et al, 2014). Approximately 0.43% of the missing genotypic data were imputed. Out of 6,244 SNPs, only 5,954 markers that had an MAF > 0.05 were used for further analysis. Based on the LD coefficient (r^2; correlation squared between markers), 4,402

markers that had LD > 0.5 with at least one other marker were removed for estimating the confounding factors for population structure and relatedness.

Linkage Disequilibrium

A non-linear regression model estimates the average genome wide LD decay across the genome using the inter-chromosomal comparison of LD. The whole genome wide LD decay was extended up to 5 cM at $r^2 \geq 0.1$ (Fig 2.1).

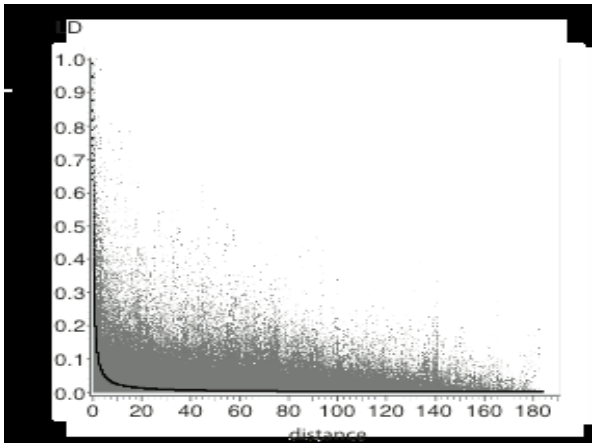

Fig 2.1. Pattern of linkage disequilibrium (LD) decay created by plotting r^2 values against the genetic distance (cM) for the whole genome. The curve shows nonlinear regression of r^2 on a weighted genetic distance.

Population Structure, Principal Component Analysis, and Relationship Matrix

A subset of 1,842 markers that had a LD < 0.5 with any other marker in the subset was used to analyze the population structure, PCA, and relationship matrix. The Bayesian-based clustering approach in STRUCTURE revealed 8 subpopulations by the Δk approach (Fig 2.2). The number of barley accessions in each subpopulation varied between 94 (subpopulation 4) and 238 (subpopulation 2). Accounting for this population structure in the AM analysis reduces the number of false-positive QTL identified.

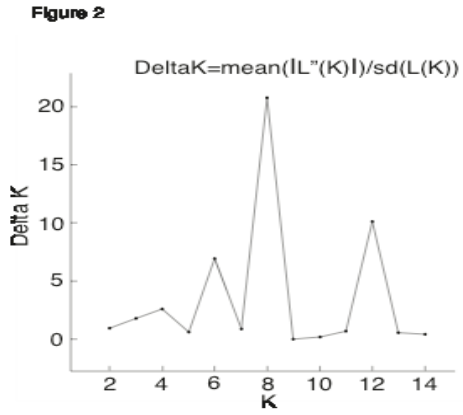

Fig 2.2. A graph with Δ*k* and number of subpopulations. The Bayesian-based clustering approach in STRUCTURE revealed eight subpopulations by the Δ*k* approach (Evanno et al. 2005). The peak represents the appropriate number of subpopulation.

Six PCs that explained about 25% of the variation were used as cofactors in the mixed model. The first PC explained 7.92% of the variation followed by PC2 that explained 6.12%.

AM Analyses

FGO Isolate

For the AM analyses, the four isolates were analyzed separately and the average score of the three replicates for each isolate was used for the phenotypic data. Out of the six models tested for the FGO isolate, the model with only kinship (IBS) was the best with an MSD of 0.001. A total of 29 SNP markers were found to be significantly associated with resistance against isolate FGO at a cutoff of $P < 0.001$. The 29 markers were located on all seven barley chromosomes, with one marker on chromosome 1H, two on 2H, six on 3H, one on 4H, two on 5H, four on 6H, and four on 7H (Table 2.1). There was no marker position information for the remaining nine markers. The *P* values of significant markers had a range of 4.37E-09 to 3.43E-04. The MAF for the significant markers ranged from 7.91 to 49.80. The R^2 (phenotypic variation) for all significant markers was up to 4.5% (Table 2.1). Based on a cutoff of 5 cM, a total of 10 loci associated with resistance were identified. Of the loci associated with resistance, one was

identified on chromosome 1H (114.3 cM), two on chromosome 2H (69.55 and 137.44 cM), one on 3H (99.26 to 99.66 cM), one on 4H (99.68 cM), one on 5H (31.86 cM), one on 6H (59.01 to 60.21 cM), and three on 7H (26.35 to 26.92, 109.0, and 133.84 cM) (Fig. 3D). From the stepwise regression, 17 markers were included in the model and cumulatively explained 18.78% of the phenotypic variation. All 10 loci associated with resistance contained markers that were included in the stepwise regression.

Table 2.1. SNP markers significantly associated with resistance/susceptibility to P. teres f. maculata isolate FGO.

Marker	Chr[a]	cM[a,b]	R^2 (%)[c]	MAF[d]	*P*-value[e]	pFDR[f]
11_21392	1H	114.3	0.0330	13.446	3.09	0.152
12_11504	2H	69.55	0.0185	9.392	3.02	0.170
SCRI_RS_155161	2H	137.44	0.0003	31.62	3.75	0.069
SCRI_RS_221787	3H	99.26	0.0006	26.55	4.24	0.039
SCRI_RS_164704	3H	99.26	0.0004	26.42	3.73	0.069
SCRI_RS_133339	3H	99.56	0.0002	27.64	3.67	0.069
SCRI_RS_211929	3H	99.66	0.0007	27.43	4.25	0.039
SCRI_RS_235791	3H	99.66	0.0006	27.5	4.13	0.042
12_30423	3H	99.66	0.0013	32.43	3.56	0.080
SCRI_RS_131671	4H	99.68	0.0044	45.473	3.12	0.152
SCRI_RS_108416	5H	31.86	0.0033	39.26	4.66	0.038
SCRI_RS_205100	5H	31.86	0.0033	39.39	4.27	0.039
SCRI_RS_213566	6H	59.01	0.0316	26.892	3.11	0.152
SCRI_RS_188243	6H	59.21	0.0279	8.24	8.36	0.000
SCRI_RS_186193	6H	59.21	0.0252	10.27	6.25	0.001
12_30144	6H	60.21	0.0417	32.64	4.22	0.039
12_30530	7H	26.35	0.0192	36.01	3.55	0.080
SCRI_RS_179528	7H	26.92	0.0091	41.689	3.08	0.152
SCRI_RS_112204	7H	109	0.0086	49.8	3.76	0.069
SCRI_RS_202130	7H	133.84	0.0191	35.81	4.44	0.039
SCRI_RS_195914	N/A	N/A	0.0258	11.28	4.04	0.047
SCRI_RS_136604	N/A	N/A	0.0372	8.72	3.77	0.069
SCRI_RS_45644	N/A	N/A	0.0450	7.91	3.67	0.069
SCRI_RS_237419	N/A	N/A	0.0183	33.85	3.51	0.083
SCRI_RS_224297	N/A	N/A	0.0185	33.78	3.46	0.088
SCRI_RS_158011	N/A	N/A	0.0185	33.716	3.37	0.105
SCRI_RS_188305	N/A	N/A	0.0361	8.784	3.34	0.107
SCRI_RS_209824	N/A	N/A	0.0418	32.973	3.28	0.118
SCRI_RS_156620	N/A	N/A	0.0000	41.959	3.16	0.149

[a] N/A indicates that the marker was not anchored to the consensus map
[b] Genetic distances in centimorgans (cM)
[c] Phenotypic variation explained by individual markers
[d] Minor allele frequency (MAF)
[d] Minor allele frequency (MAF)
[f] False discovery rate (FDR)

SG1 Isolate

For the SG1 isolate, the model with structure and kinship (IBS) was the best model with MSD = 0.000668. A total of 17 markers were significant of which two are on 1H, five on 2H, one on 3H, two on 4H, two on 5H, and one on 6H. The remaining four markers did not have any position information (Table 2.2). The MAF values for all significant markers had a range of 6.08 to 32.97. The *P* values for the significant markers ranged between 1.60E-05 and 3.76E-05. The R^2 (phenotypic variation) for all significant markers was up to 5.68% (Table 2.2). There were eight loci identified that contained markers significantly associated with resistance with two being located on chromosome 1H (50 and 98.45 cM), one on 2H (65.71 to 66.11 cM), one on 3H (88.17 cM), two on 4H (32.43 and 47.17 cM), one on 5H (31.86 cM) and one on 6H (101.83 cM) (Fig. 3C). From the stepwise regression, seven markers were included in the model and cumulatively explained 7.74% of the phenotypic variation.

Table 2.2. SNP markers significantly associated with resistance/susceptibility to P. teres f. maculata isolate SG1.

Marker	Chr[a]	cM[a,b]	R^2 (%)[c]	MAF[d]	*P*-value[e]	pFDR[f]
11_20810	1H	50	0.001	38.649	3.17	0.271
SCRI_RS_188909	1H	98.45	0.008	27.365	3.22	0.262
SCRI_RS_151535	2H	65.71	0.000	27.905	3.98	0.147
SCRI_RS_175065	2H	65.81	0.000	24.73	3.76	0.157
SCRI_RS_154617	2H	66.11	0.000	23.58	4.8	0.064
11_10325	2H	66.11	0.000	24.73	3.52	0.181
11_10733	2H	66.11	0.000	24.865	3.36	0.207
SCRI_RS_159340	3H	88.17	0.042	10.068	3.65	0.157
12_30907	4H	32.43	0.005	11.351	3.11	0.287
SCRI_RS_9296	4H	47.17	0.003	20.338	3.09	0.287
SCRI_RS_108416	5H	31.86	0.008	39.257	3.71	0.157
SCRI_RS_205100	5H	31.86	0.008	39.392	3.48	0.181
SCRI_RS_151574	6H	101.83	0.000	30.946	3.04	0.300
SCRI_RS_209824	N/A	N/A	0.057	32.97	4.65	0.064
SCRI_RS_139690	N/A	N/A	0.020	6.08	4.42	0.071
11_20336	N/A	N/A	0.000	49.662	3.82	0.157
SCRI_RS_8401	N/A	N/A	0.001	47.568	3.45	0.181

[a] N/A indicates that the marker was not anchored to the consensus map
[b] Genetic distances in centimorgans (cM)
[c] Phenotypic variation explained by individual markers
[d] Minor allele frequency (MAF)
[d] Minor allele frequency (MAF)
[f] False discovery rate (FDR)

NZKF2 Isolate

Out of the six models tested, the best model for the isolate NZKF2 data were structure and kinship (IBS) with an MSD = 0.001829. From the 54 markers identified as significant, two were located on 2H, 12 on 3H, 21 on 4H, one on 5H, three on 6H, and seven on 7H (Table 2.3). The remaining eight markers did not have marker position information. The *P* values of the markers had a range of 3.75E-12 to 9.77E-04. The MAF values for all significant markers ranged from 5.14 to 49.19. The R^2 (phenotypic variation) for all significant markers was up to 15.97% (Table 2.3). A total of 13 loci associated with resistance to NZKF2 were identified, two on 2H (23.76, 69 cM), three on 3H (53.4, 99.26 to 103.86, and 153.39 cM), three on 4H (53.67 to 57.32, 103.48 to 103.58, and 117.13 cM), one on 5H (111.56 cM), one on 6H (59.21 cM), and three on 7H (78.07, 133.84, and 145.68 to 150.36 cM) (Fig. 3B). From the stepwise regression, 23 markers were included in the model and together explained 44.35% of the phenotypic variation. All 113 loci containing markers associated with resistance were included in the stepwise regression.

Table 2.3. SNP markers significantly associated with resistance/susceptibility to *P. teres* f. *maculata* isolate NZKF2

Marker	Chr[a]	cM[a,b]	R^2 (%)[c]	MAF[d]	*P*-value[e]	pFDR[f]
SCRI_RS_152744	2H	23.76	0.004	39.46	3.03	0.095
12_20878	2H	69	0.071	5.14	3.12	0.084
SCRI_RS_127994	3H	53.42	0.083	25.2	5.03	0.005
12_30785	3H	53.42	0.084	24.19	4.21	0.019
SCRI_RS_221787	3H	99.26	0.001	26.55	3.41	0.061
SCRI_RS_164704	3H	99.26	0.001	26.42	3.25	0.071
SCRI_RS_225641	3H	99.46	0.002	27.64	3.01	0.098
SCRI_RS_133339	3H	99.56	0.001	27.64	3.84	0.03
SCRI_RS_235791	3H	99.66	0.001	27.5	4.11	0.02
SCRI_RS_211929	3H	99.66	0.001	27.43	3.95	0.025
12_30423	3H	99.66	0.001	32.43	3.56	0.052
SCRI_RS_167825	3H	103.46	0.048	29.46	4.14	0.02
SCRI_RS_163092	3H	103.86	0.048	28.04	4.05	0.021
SCRI_RS_156315	3H	153.39	0.030	42.7	3.07	0.091
SCRI_RS_157310	4H	53.67	0.011	37.16	3.38	0.061
SCRI_RS_128723	4H	54.66	0.139	30.95	5.93	0.001
SCRI_RS_155554	4H	54.66	0.119	30.41	5.56	0.002
SCRI_RS_208828	4H	54.66	0.154	37.43	5.53	0.002
SCRI_RS_221172	4H	54.66	0.137	35.2	4.99	0.005
11_20135	4H	54.95	0.087	22.3	11.43	0

Table 2.3. SNP markers significantly associated with resistance/susceptibility to *P. teres* f. *maculata* isolate NZKF2 (continued).

Marker	Chr[a]	cM[a,b]	R^2 (%)[c]	MAF[d]	*P*-value[e]	pFDR[f]
11_10262	4H	54.95	0.128	43.31	9.57	0
12_31462	4H	54.95	0.132	28.24	7.24	0
11_20450	4H	54.95	0.055	17.91	4.37	0.016
11_20412	4H	54.95	0.077	46.22	4.28	0.017
SCRI_RS_168496	4H	54.95	0.134	27.36	4.14	0.02
SCRI_RS_141730	4H	54.95	0.160	33.58	4.09	0.02
SCRI_RS_228477	4H	54.95	0.132	26.89	3.72	0.039
11_10568	4H	54.95	0.138	41.22	3.69	0.039
12_30839	4H	54.95	0.037	28.11	3.5	0.053
11_20020	4H	54.95	0.106	37.84	3.26	0.071
11_10509	4H	55.64	0.065	40.47	3.41	0.061
SCRI_RS_189180	4H	57.32	0.136	47.09	3.49	0.054
SCRI_RS_148330	4H	103.48	0.090	18.65	3.24	0.071
SCRI_RS_192689	4H	103.58	0.017	40.27	4.34	0.016
11_21035	4H	117.13	0.119	47.23	3.04	0.094
12_11298	5H	111.56	0.003	11.69	5.18	0.004
SCRI_RS_188243	6H	59.21	0.018	8.24	7.49	0
SCRI_RS_176650	6H	59.21	0.010	20.41	4.56	0.012
SCRI_RS_186193	6H	59.21	0.014	10.27	3.21	0.073
12_11477	7H	78.07	0.003	10.07	3.23	0.071
12_11536	7H	78.07	0.003	10.07	3.23	0.071
12_31000	7H	78.07	0.003	10.07	3.23	0.071
SCRI_RS_202130	7H	133.84	0.006	35.81	4.47	0.013
11_20847	7H	145.68	0.009	40.95	3.55	0.052
11_10687	7H	146.03	0.012	40.54	3.4	0.061
12_10677	7H	150.36	0.019	10.81	3.07	0.091
SCRI_RS_208732	N/A	N/A	0.128	43.51	9.93	0
SCRI_RS_147636	N/A	N/A	0.124	44.12	8.74	0
11_21017	N/A	N/A	0.062	16.82	4.01	0.022
12_20803	N/A	N/A	0.002	14.26	3.51	0.053
SCRI_RS_156237	N/A	N/A	0.039	31.62	3.35	0.064
SCRI_RS_133327	N/A	N/A	0.120	24.32	3.26	0.071
SCRI_RS_145381	N/A	N/A	0.002	48.78	3.19	0.074
SCRI_RS_146785	N/A	N/A	0.002	49.19	3.17	0.077

[a] N/A indicates that the marker was not anchored to the consensus map
[b] Genetic distances in centimorgans (cM)
[c] Phenotypic variation explained by individual markers
[d] Minor allele frequency (MAF)
[d] Minor allele frequency (MAF)
[f] False discovery rate (FDR)

DEN2.6 Isolate

Out of the six models tested for the DEN 2.6 isolate, the best model identified was structure and kinship (IBS) with an MSD = 0.000577. A total of 38 markers were highly significant with one marker on 1H, one marker on 2H, four markers on 3H, 20 on 4H, one on 5H, three on 6H, and one on 7H (Table 2.4). The remaining seven markers did not have any marker position information.

The *P* values ranged between 9.65E-15 and 4.99E-04. The MAF values for all significant markers ranged from 5.16 to 46.22. The R^2 (phenotypic variation) for all significant markers was up to 14.78% (Table 2.4). Analysis of the isolate DEN 2.6 data identified a total of six loci with markers associated with resistance with one locus being on 1H (88.25 cM), one on 2H (69 cM), three on 3H (53.42, 65.16, and 150.19 to 154.47cM), three on 4H (53.77 to 54.95, 59.22, and 96.6 to 96.99 cM), one on 5H (111.56 cM), one on 6H (59.21 cM), and one on 7H (0 cM) (Fig. 3A). From the stepwise regression, 20 markers were included in the stepwise regression model. The significant markers included in the stepwise regression together explained 37.1% of the phenotypic variation for this isolate and markers from all 10 loci were included. 2H, 3H, 4H, 6H, and 7H in this study.

Table 2.4. SNP markers significantly associated with resistance/susceptibility to *P. teres* f. *maculata* isolate DEN2.6.

Marker	Chr[a]	cM[a,b]	R^2 (%)[c]	MAF[d]	*P*-value[e]	pFDR[f]
11_20792	1H	88.25	0.015	31.689	3.02	0.141
12_20878	2H	69	0.086	5.135	3.11	0.127
SCRI_RS_127994	3H	53.42	0.077	25.203	3.07	0.131
11_21305	3H	65.16	0.052	37.7	3.95	0.033
SCRI_RS_229623	3H	150.19	0.029	16.55	3.38	0.088
12_10014	3H	154.47	0.053	22.03	3.47	0.086
SCRI_RS_168580	4H	53.77	0.031	16.22	3.39	0.088
SCRI_RS_184107	4H	53.77	0.031	16.22	3.39	0.088
12_30878	4H	53.87	0.024	14.257	3.21	0.111
SCRI_RS_128723	4H	54.66	0.147	30.95	7.51	0.000
SCRI_RS_155554	4H	54.66	0.124	30.41	5.85	0.001
SCRI_RS_208828	4H	54.66	0.148	37.43	5.49	0.002
SCRI_RS_221172	4H	54.66	0.141	35.2	5.47	0.002
11_20135	4H	54.95	0.109	22.3	14.02	0.000
11_10262	4H	54.95	0.145	43.31	10.33	0.000
11_20412	4H	54.95	0.094	46.22	5.76	0.001
11_20450	4H	54.95	0.069	17.91	5.62	0.001
12_31462	4H	54.95	0.124	28.24	5.34	0.002
11_20472	4H	54.95	0.057	20.34	4.46	0.013
12_30839	4H	54.95	0.039	28.11	3.71	0.054
SCRI_RS_168496	4H	54.95	0.130	27.36	3.42	0.088
SCRI_RS_141730	4H	54.95	0.149	33.581	3.08	0.131
SCRI_RS_147712	4H	59.22	0.097	17.43	3.97	0.033
SCRI_RS_163033	4H	59.22	0.095	17.3	3.35	0.092
11_20762	4H	96.6	0.075	37.432	3.22	0.111
12_10666	4H	96.99	0.075	38.51	3.3	0.099
12_11298	5H	111.56	0.001	11.69	4.19	0.021
SCRI_RS_188243	6H	59.21	0.024	8.24	8.77	0.000
SCRI_RS_176650	6H	59.21	0.014	20.41	5.32	0.002
SCRI_RS_186193	6H	59.21	0.020	10.27	4.27	0.018

Table 2.4. SNP markers significantly associated with resistance/susceptibility *P. teres* f. *maculata* isolate DEN2.6 (continued).

Marker	Chr[a]	cM[a,b]	R^2 (%)[c]	MAF[d]	*P*-value[e]	pFDR[f]
11_21419	7H	0	0.013	41.284	3.14	0.121
SCRI_RS_208732	N/A	N/A	0.144	43.51	10.17	0.000
SCRI_RS_147636	N/A	N/A	0.139	44.12	9.28	0.000
11_21017	N/A	N/A	0.082	16.82	6.1	0.001
SCRI_RS_161627	N/A	N/A	0.015	9.12	3.62	0.063
SCRI_RS_114164	N/A	N/A	0.082	26.351	3.28	0.100
SCRI_RS_156237	N/A	N/A	0.041	31.622	3.18	0.113
SCRI_RS_168610	N/A	N/A	0.098	18.311	3.01	0.142

[a] N/A indicates that the marker was not anchored to the consensus map
[b] Genetic distances in centimorgans (cM)
[c] Phenotypic variation explained by individual markers
[d] Minor allele frequency (MAF)
[d] Minor allele frequency (MAF)
[f] False discovery rate (FDR)

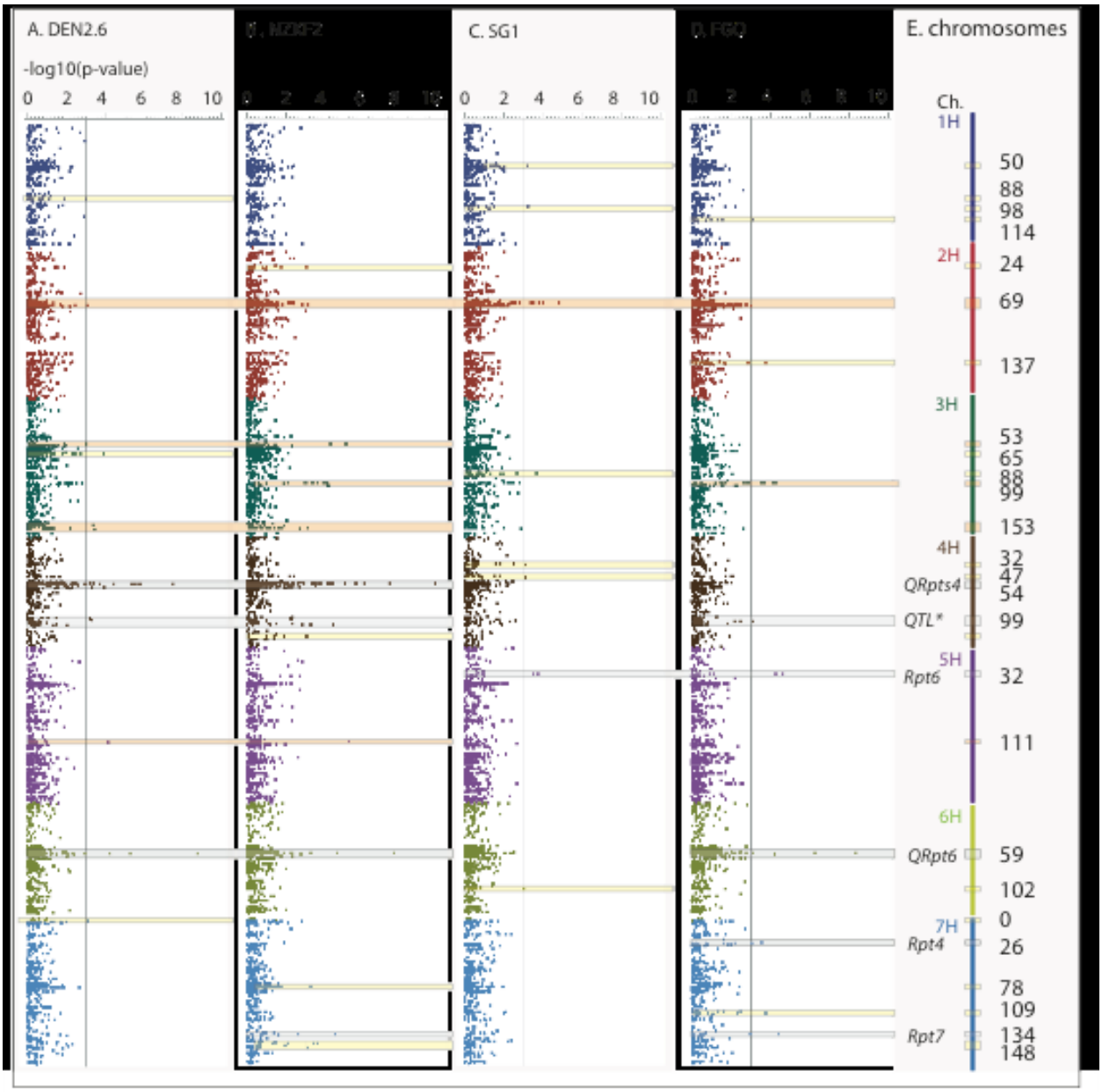

Fig 2.3. Association mapping of spot form net blotch (SFNB) isolates on the world barley core collection (BCC). Panels **A to D** show Manhattan plots generated from the disease reaction to four isolates of Pyrenophora teres f. maculata: **A**, isolate DEN 2.6 from Denmark; **B**, isolate NZKF2 from New Zealand; **C**, isolate SG1 from Australia; and **D**, isolate FGO from Fargo, ND (United States). All colored pixels represent single nucleotide polymorphism markers from the 9K Illumina genotyping on the seven barley chromosomes; from blue at the top as chromosome 1H and light blue at the bottom as chromosome 7H. All markers above the LOD threshold of 3.0 (P value(-log10)) were significantly associated with SFNB resistance/susceptibility. Resistance/susceptibility loci are designated as colored bars. Orange bars are loci common to several isolates, yellow are specific to a single isolate, and gray are loci corresponding to quantitative trait loci (QTL) regions previously identified by biparental mapping. **E**, A map of the seven barley chromosomes is shown as colored vertical bars with chromosome designations given. The 27 resistance loci detected in these analyses are shown as horizontal bars with approximate centimorgan values given to the left and known SFNB resistance genes/QTL nomenclatures provided on the right.

Discussion

The BCC represents a worldwide base of diversity allowing for the use of historical recombination for association mapping of variation in responses to isolates of *P. teres* f. *maculata*; the cause of SFNB. The distribution of SFNB infection types across the BCC accessions analyzed indicated that susceptibility or resistance is polygenic in nature consistent with previous studies (Liu et al., 2011, McClean et al., 2012, Williams et al., 1999, Williams et al., 2003). The phenotyping data coupled with robust genotyping of the BCC accessions challenged with diverse *P. teres* f. *teres* isolates allowed for the capture of multiple MTAs. This AM analysis is the first report of AM of SFNB resistance in barley and includes worldwide diversity of the host challenged with isolates from regions around the globe.

The power of a mixed model to harness historical recombination events using AM is dependent on phenotyping, markers, population structure, and relatedness, thus requiring the testing of multiple models to determine which ones performed best for the specific isolate being analyzed (Atwell et al., 2010, Flint and Mackay, 2009, Mamidi et al., 2014). The mixed model which accounted for both population structure (Q) and kinship (K) was the best model for the Australian isolate SG1, the New Zealand isolate NZKF2, and the Denmark isolate DEN 2.6, whereas the model which only accounted for kinship was the best model for the U.S. isolate FGO. The AM analyses utilizing these models identified a total of 138 significant ($-\log10(P)$value > 3.0) MTAs. Using a 5 cM cutoff to define a QTL, a total of 10, 8, 13, and 10 loci associated with SFNB resistance/susceptibility were identified for FGO, SG1, NZKF2, and DEN 2.6, respectively. A total of 10 of the 41 loci identified were common to two or more of the isolates, resulting in a total of 27 distinct loci with six consistent to previously identified QTL and the remaining 21 representing putative novel SFNB resistance loci.

The analysis for MTAs was also conducted utilizing the more stringent positive false discovery rate (pFDR) < 0.1 (Tables 1 to 4). These analyses resulted in a reduction from the 138 significant markers using *P* value down to 105 significant markers using pFDR. The more stringent pFDR analysis reduced the number of significant loci identified to 7, 1, 13, and 7 loci for FGO, SG1, NZKF2, and DEN 2.6, respectively. The reduction of the FGO, SG1, and DEN 2.6 isolates from 10, 8 and 10 to 7, 1, 7 significant loci, respectively, is due to the higher stringency of pFDR analysis. The MTAs defining the locus on Ch 2H at ~69 cM (Fig. 3) was the one locus that was common to all four isolates and remained significant for SG1 using both *P* value and pFDR. Two other markers at one of the seven remaining loci that was significant using *P* value and were not significant with pFDR corresponded to the previously identified *Rpt6* QTL (Fig. 3). Since these two independent phenotyping data sets both identified these specific MTAs corresponding to a known resistance locus, it is unlikely that the loss of this locus using pFDR was due to a false association when using the *P* value. Thus, the *P* value analysis that identified two significant common markers between isolates SG1 and FGO indicates that these two isolates may carry effectors that target common host susceptibility genes underlying this locus, but these MTAs were eliminated when the pFDR was used. This suggested that when running association analyses on a diverse host population for complex quantitative traits the stringent pFDR may eliminate some important MTAs or loci so here we reported all loci identified using the less stringent *P* value analyses. However, the pFDR values were left in the tables for comparison.

We detected MTAs that corresponded to six loci on four chromosomes that were previously identified and mapped at low resolution by biparental QTL analyses. A common locus for isolates NZKF2 and DEN 2.6 was identified on chromosome 4H at position 53.67 to

59.22 cM explaining up to 16% of the phenotypic variation. There are 36 significant markers for the NZKF2 and DEN 2.6 isolates at this locus with the markers for NZKF2 having the highest significance of any MTAs in the analysis. Neither of the genes underlying these SNP markers are predicted to encode susceptibility target/biotrophic resistance-like proteins, as characterized in the *Stagonospora nodorum*−wheat pathosystem (Farris et al., 2010). However, there are other significant SNP markers in the region in resistance-like genes such as leucine-rich receptor-like protein kinases. This region resides in the same region as *QRpts4* as reported by Grewal et al. (2008), which explained 21% of the phenotypic variation in a doubled haploid (DH) population of CDC Dolly (susceptible) and TR251 (resistant). The QTL region reported by Friesen et al. (2006) on chromosome 4H using the NZKF2 isolate on the DH population of SM89010/Q21861 is proximal to this region, but is close to the NZKF2, DEN 2.6, and FGO common locus at 99.68 to 103.58 cM that explained 8.3% of the phenotypic variation and the NZKF2 specific locus at 117.13 cM explaining 11.7% of the phenotypic variation.

Using the FGO and SG1 isolates, two identical markers were identified (SCRI_RS_108416 and SCRI_RS_205100) on chromosome 5H at position 31.86 that explained 0.1% of the phenotypic variation. This locus is probably the *Rpt6* QTL reported by Manninen et al. (2006) that explained up to 84% of the phenotypic variation in a DH population derived from Rolfi (susceptible) and CI 9819 (resistant) using Finnish isolates. The reason for low phenotypic variation in our study might be due to the different populations and different isolates.

The MTAs detected on chromosome 6H at position 58.9 to 60.2 cM were common for DEN 2.6, NZKF2, and FGO and the phenotypic variation explained by this common locus was as high as 4%. This locus is located at a similar genetic interval as the previously described *QRpt6* QTL reported by Grewal et al. (2008) in the Canadian breeding line TR251.

This locus from line TR251 also exhibited resistance to net form net blotch (NFNB). Qamar et al. (2008) also identified an NFNB resistance locus at the *QRpt6* region on chromosome 6H, cosegregating with microsatellite marker Bmag0173 and Bmag0807 (Qamar et al., 2008). The highly significant markers SCRI_RS_188243 (gene *MLOC_73854.4*) and SCRI-RS-186193 (gene *MLOC_16471.1*) for the FGO, NZKF2, and DEN 2.6 isolates were identified on chromosome 6H at 59.2 cM. These two common markers, with the gene designations *MLOC_73854.4*and *MLOC_16471.1*, are predicted to encode an unknown protein and an SPla/RYanodine receptor (SPRY) domain-containing protein, respectively. These two markers explained up to 2.8 and 2.3% of the phenotypic variation, respectively. From this AM panel data, it is premature to consider the genes containing these SNP markers as candidate host susceptibility or resistance genes; however, there are other markers and genes annotated in the region that code for R-like genes including NBS-LRR and serine/threonine protein kinase genes.

The *Rpt4* gene located on the long arm of chromosome 7H was the first SFNB resistance gene described in the cultivar Galleon. Later, *Rpt4* was mapped in several breeding lines and varieties such as CI9214, Keel, Chebec, and Tilga (Williams et al., 1999, and 2003); however, this gene was not utilized in Australian breeding programs due to its lack of effectiveness at the adult plant stage (Williams et al., 1999). We identified one locus at the *Rpt4* region (26.4 to 26.9 cM) that explained up to 3% of the phenotypic variation with the U.S. isolate FGO, but interestingly was not detected using the Australian isolate SG1. This QTL may represent the seedling resistance gene *Rpt4* identified on the long arm of chromosome 7H (position 6.9 to 25.6 cM) flanked by the restriction fragment length polymorphic markers Xpsr117(D) and Xcdo673 (Williams et al., 1999). A minor effect locus at position 0.7 cM was also detected on chromosome 7H against isolate DEN 2.6 and a locus common to isolates FGO and NZKF2 was

identified at position 132.6 to 133.8 cM explaining up to 5.1% of the phenotypic variation. We also identified two other loci at 109 cM and 145.68 to 150.36 cM close to this region that were identified using the FGO and NZKF2 isolates, respectively. Any of these loci could be the same as the *QRpt7*gene reported by Williams et al. (Williams et al., 2003) on chromosome 7H at position 116 to 134 cM. The identification of all six well-characterized SFNB resistance loci suggests that these AM analyses were robust and that the remaining 21 novel resistance loci may represent putative new sources of SFNB resistance or lack of susceptibility that could also be utilized in resistance breeding efforts.

The last remaining SFNB QTL previously reported by Molnar et al. (2000) was reported as a major resistance gene on chromosome 2H. However, the location was not precise so it is difficult to determine if any of the 2H loci identified in this AM analysis are the same as that reported by Molnar et al. (2000). We detected MTAs on chromosome 2H at ~24, 69, and 137 cM (Fig. 3). The locus identified at ~69 cM was the only locus identified with all four isolates tested (Fig. 3), and explained up to 5.6% of the phenotypic variation. Thus, this may be the best candidate for the major resistance reported by Molnar et al. (Molnar et al. 2000), but due to the low resolution of the mapping in the Leger × CI 9831 DHL population this is only speculation.

We have possibly detected all the major and minor SFNB resistance QTL previously reported on barley chromosomes 2H, 4H, 5H, 6H, and 7H (Friesen et al., 2006, Grewal et al., 2008, Ho et al., 1996, Manninen et al., 2006, Molnar et al., 2000, Williams et al., 1999, Williams et al., 2003) and new QTL present on all seven barley chromosomes. Similarly, NFNB resistance QTL have been identified throughout the genome (Liu and Friesen, 2010); however, resistance to both forms of the net blotch pathogen were rarely identified in similar locations (Grewal et al., 2008, Manninen et al., 2006), suggesting that the majority of resistances are distinct for the

different forms of *P. teres* and in regard to breeding, the two need to be treated as separate diseases. Growing barley cultivars with genetic resistance to SFNB and NFNB is the most sustainable strategy of disease management (Liu et al., 2011, McLean et al., 2014). However, the diversity of virulence/avirulence genes within the pathogen populations and complexity of the interaction with host susceptibility targets and resistance genes requires robust genetic characterization of both the host and pathogen to understand the underlying genetic interactions involved. To date, only 11 different genotypes from the primary barley germplasm pool had been genetically characterized for SFNB resistance, thus deeper screening was required to identify diverse resistances or lack of functional susceptibility targets. This was accomplished in this study utilizing AM to capture diverse loci associated with SFNB resistance/susceptibility.

A recent review of host specific toxins (also referred to as necrotrophic effectors) produced by the Pleosporales necrotrophic pathogens in the Dothideomycete class suggested that necrotrophic effectors (NE) predominantly interact with dominant host susceptibility gene products (Stergiopoulos et al., 2013). These interactions followed the inverse-gene-for-gene model (Friesen et al., 2010) or NE triggered susceptibility (NETS) model (Liu et al., 2014, Sjherve et al., 2014), resulting in quantitative susceptibility to these pathogens which can also be viewed as quantitative recessive resistance. The reports of proteinaceous and nonproteinaceous toxins being produced by *P. teres* f. *maculata* and *P. teres* f. *teres* (reviewed in Liu et al. 2011) as well as mapping of distinct *P. teres* f. *teres* virulence QTL targeting regions of dominant susceptibility in barley (Liu et al., 2014, Sjherve et al., 2014), suggests that the NETS model may be at least partially responsible for virulence in *P. teres* f. *maculata*. Thus, the accumulation of distinct NE genes spread throughout the genome of the pathogen that theoretically interact with host susceptibility genes, facilitates the pathogens ability to induce disease. The diversity of NEs

within pathogen populations explains the variability in virulence of *P. teres* f. *maculata* isolates collected from around the world and may explain why Tekauz (1990) was unable to identify effective resistance sources against isolates collected in western Canada.

P. teres f. *maculata* collections from local and global fungal populations show wide variations for virulence on differential barley lines (reviewed in Liu et al. 2011). This indicates that the variability in virulence gives the pathogen the potential to shift the population toward the prevalence of NEs that target susceptibility factors within popular cultivars resulting in what appears to be virulence on the limited sources of resistance present (Arabi et al., 2003, Bockelman et al., 1983, Khan and Tekauz, 1982, Liu et al., 2011, McClean et al., 2009, Sjherve et al., 2014, Tekauz, 1990, Wu et al., 2003). The frequency of virulent genotypes in pathogen populations can rapidly increase as popular cultivars with incomplete resistance or lack of some susceptibility exert selection pressure. Also, these sexual pathogen populations allow for recombination of virulence genes giving rise to new possibly more virulent isolates containing several effectors that were selected for and brought together by recombination. Because of these complex interactions, breeding strategies focused on developing highly resistant lines must combine multiple loci, with some lacking host susceptibility targets and possibly others harboring active resistance loci. Knowledge of these host−pathogen genetic interactions and the genes/loci determining both lack of susceptibility and resistance is critical for intelligent deployment of SFNB resistant lines. These AM analyses provide very important information on these susceptibility/resistance loci but to effectively utilize new loci containing nonfunctional SFNB susceptibility targets and/or resistance from the BCC, further genetic characterization of the host−pathogen genetic interactions through targeted biparental populations of both the host and pathogen is needed. We are further validating the distinct QTL identified by preparing DH

and recombinant inbred line populations by crossing susceptible malting cultivars with highly resistant BCC accessions that we determined are missing the susceptibility targets or containing the major resistances. These populations will expedite the development of elite breeding lines that can be utilized in the development of resistant cultivars that can be grown in the upper Midwestern region of the United States and elsewhere, whether the mechanism is through the removal of susceptibility factors or the incorporation of resistance.

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CHAPTER 3. QTL MAPPING OF SPOT FORM NET BLOTCH SUSCEPTIBILITY/ RESISTANCE LOCI IN UPPER MIDWESTERN US TWO- AND SIX-ROWED BARLEY CULTIVARS

Abstract

Spot form net blotch (NFNB), caused by the necrotrophic fungal pathogen *Pyrenophora teres* f. *maculata* (*Ptm*) is a devastating foliar disease of barley that has the potential to cause significant yield and quality losses. The disease has recently emerged as a major concern in the Northern Great Plains of the US and is a major problem in many barley growing regions across the world, including Africa, Australia, Canada and Europe. Identifying and deploying resistance or the removal of susceptibility loci from elite germplasm is an economic and effective way to manage the disease. Three recombinant inbred line (RIL) populations were developed from crosses of the two popular upper Midwestern malting barley cultivars, Tradition and Pinnacle, with the two resistant barley lines, PI67381 and PI84314, that were shown to have broad resistances to diverse isolates collected from across the globe. The three RIL populations, Tradition X PI67381 (T67381 consisting of 120 individuals), Pinnacle X PI67381 (P67381 consisting of 117 individuals), and Pinnacle X PI84314 (P84314 consisting of 115 individuals) were phenotyped at the seedling stage in the greenhouse with six geographically distinct *Ptm* isolates: FGO (Fargo, USA), PA14 (Montana, USA), CA17 (Montana, USA), SG1 (Australia), NZKF2 (New Zealand) and DEN2.6 (Denmark). Population specific PCR-GBS panels were developed from Illumina 9K SNP array data and used to genotype all three populations. MapDisto and Qgene were used to analyze the data and quantitative trait loci (QTL) were identified on chromosome 2H, 3H, 4H, 6H and 7H. A common QTL among all three RIL population were detected on chr 2H (R^2=14-40%) and 7H (R^2=24-80%). A total of 12 QTLs

were identified of which nine were previously reported and the remaining three QTL: *QRptm-2H-77-83, QRptm-2H-141-152*, and *QRptm-7H-92-95* are considered novel. These novel resistances and the markers delimiting these QTLs can be utilized in barley breeding programs to develop SFNB resistant cultivars utilizing marker assisted selection.

Introduction

Barley, *Hordeum vulgare*, the oldest known domesticated cereal crop is still very important because of its use in malting and the production of beer and spirits, which effects economies worldwide. It is also important in specific regions of the world due to its application as human food and animal feed. Currently there are several diseases that threaten barley production, and net blotch caused by the necrotrophic fungal pathogen *Pyrenophora teres* is an economically important foliar disease in barley growing regions around the world. The disease net blotch occurs in two distinct forms: spot form net blotch (SFNB) caused by *P. teres* f. *maculata* (*Ptm*) and net form net blotch (NFNB) caused by *P. teres* f. *teres* (*Ptt*). Both *Ptm* and *Ptt* are morphologically identical (conidia, mycelium), but genetically separate into different species that produce distinct symptoms on susceptible host genotypes (Smedegard-Peterson, 1971, McClean et al., 2009, Liu et al., 2011). Therefore, the host-parasite genetic interactions occurring in these two pathosystem are considered distinct, thus must be considered as separate diseases when deploying genetic resistances.

Spot form net blotch is favored by cool and moist conditions which occur during the barley growing season in the Northern Great Plain of the United States. It is an emerging foliar disease in major barley growing regions across the world, which includes Australia, Canada, Europe, South Africa, and the United States (Khan and Tekauz, 1982, Liu et al., 2010 and 2011). Karki and Sharp (1986) reported SFNB in the upper Midwestern United States as early as 1981

but at the time the low incidence of disease was not a concern and therefore limited studies were conducted on the disease in the region. However, after SFNB was reported in 2010 by Liu et al. (2010), in North Dakota, and Lartey et al., (2013) at the Montana and North Dakota border, and reports of minor field epidemics causing ~75% reductions in yield on popular six- and two-rowed malting varieties, barley pathologists, breeders, growers and end users are becoming more concerned by the threat of SFNB. This concern is certainly warranted in the region as the presence of highly virulent isolates are being detected that are more virulent than foreign isolates collected around the world where SFNB has caused major problems for the barley industry (Neupane et al., 2015).

The SFNB pathogen can overwinter in plant stubble, soil, and seed. Under favorable conditions, SFNB can cause yield losses of 10-40% (Mathre, 1997), but there have been reports of losses nearing 75% on the barley cultivar Tradition under irrigation near the North Dakota and Montana border. Chemical fungicides and cultural practices can help manage the disease; however, host resistance is the most economic method of reducing yield and quality losses. Resistances or susceptibilities to SFNB are quantitative in nature and the highly diverse population of *Ptm* have made understanding resistance or susceptibility mechanism difficult as the underlying mechanisms of resistance appear to be quite complex (Liu et al., 2011). This pathogen can reproduce sexually, and virulence profiles of populations can shift and change rapidly (Arabi et al., 2003, Karki et al., 1986, Tekauz, 1990., McLean et al., 2014). The rapid changes in virulence and shifts in profile of *Ptm* populations and the complex resistance mechanism has posed a major challenge to deploying effective and durable resistances (Khan and Tekauz, 1982, Arabi et al., 1992, Gupta et al., 2001, McLean et al., 2009, 2012, Liu et al., 2010, 2011). Relatively, few SFNB resistance sources have been identified and mapped to date

compared to NFNB, thus breeding for SFNB and understanding resistance mechanisms is lagging in comparison. However, since both diseases seem to occupy and possibly compete in the same environmental niches there have been reports of "ebb and flow" between the predominate forms of the disease in the same regions across time (Louw et al., 1996, Arabi et al., 1992, McLean et al., 2009, Liu et al., 2010), thus resistance to both forms must be considered in most barley growing region.

Several previous studies had already reported major and minor quantitative trait loci (QTL) resistances against SFNB located across all seven barley chromosomes. These studies primarily relied on bi-parental mapping population including both Recombinant Inbred Line (RIL) and Double Haploid (DH) populations (Ho et al., 1996, Steffenson et al., 1996, William et al., 1999 and 2003, Molnar et al., 2000, Friesen et al., 2006, Grewal et al., 2008, Manninen et al., 2000 and 2006, Cakir et al., 2011). Recently, a few studies have utilized the association mapping approach (Tamang et al., 2015, Chapter 2, Wang et al., 2015, Burlakoti et al., 2017).

Williams et al., (1999 and 2003) first reported the *Rpt4* gene/ QTL as a dominant resistance gene on chromosome 7H in the cultivar Galleon that was effective at the seedling stage. Similarly, other QTL have been mapped to chromosome 1H (Tamang et al., 2015), 2H (Ho et al., 1996, Molnar et al., 2000, Cakir 2011, Burlakoti et al., 2017, Tamang et al. 2015); 3H (Wang et al., 2015, Burlakoti et al., 2017); 4H (Steffenson et al 1996, Friesen et al., 2006, Grewal 2008, Wang et al., 2015), 5H (Manninen et al., 2006, Burlakoti et al., 2017), 6H (Grewal 2008, Manninen et al 2000, Cakir 2011, Burlakoti et al., 2017, Tamang et al., 2015), and 7H (Wang et al., 2015, Tamang et al., 2015, Burlakoti et al., 2017). Therefore, the resistance to SFNB is complex and polygenic in nature. However, utilization of these identified resistances has only recently been selected for in the North Dakota barley breeding program, hence, the

majority of the barley cultivars grown in the region are moderately susceptible to susceptible to the regional population of SFNB (Ransom et al., 2014).

In our previous association mapping study utilizing a world barley core collection, we identified 27 distinct loci on all seven barley chromosomes with 6 consistent to previous studies and the remaining 21 representing novel loci which may be resistance or susceptibility loci to *P. tere* f. *maculata* isolates (Tamang et al., 2015, chapter 2). Two barley lines, PI67381 and PI84314, showed broad resistance with low infection types (resistance) to four diverse isolates collected from geographically distinct regions of the world (Neupane et al., 2015). These two barley accessions were selected to develop RIL populations by crossing with popular six- and two-rowed barley cultivars (Tradition and Pinnacle) grown in North Dakota, which are susceptible to many of the regional SFNB isolates. Here, we report on the QTL mapping using three RIL populations developed from these crosses.

Materials and Methods

Plant Materials

Three recombinant inbred line (RIL) populations consisting of 120, 117, and 115 $F_{2:7}$ individuals of Tradition X PI67381 (T67381), Pinnacle X PI67381 (PI67381), and Pinnacle X PI84314 (PI84314), respectively, were developed by single-seed descent. Both PI67381, an advanced breeding line from Turkistan and PI84314, a landrace from Uzbekistan were highly resistance to four diverse *P. teres* f. *maculata* isolates collected from geographically distinct regions of the world (Neupane et al., 2015) and were used as the resistant parents in the crosses. The 6-rowed cultivar Tradition released by Anhauser-Busch in 2003 and the 2-rowed cultivar Pinnacle released by North Dakota State University in 2006 were used as the SFNB susceptible parents in the crosses.

Disease Phenotyping

Six geographically diverse isolates of *P. teres* f. *maculata,* FGOB10Ptm-1 (FGO), CA17, PA14, SG1, NZKF2, and DEN2.6, were used to phenotype all three RIL populations. The isolate FGO was collected in North Dakota, USA (Carlsen et al., 2017); CA17 and PA14 were collected in Montana, USA; SG1 was collected in Australia (Carlsen et al., 2017); NZKF2 was collected in New Zealand and DEN2.6 in Denmark (All isolates were obtained from Dr. Timothy Friesen, USDA). Four *Ptm* isolates FGO, SG1, DEN2.6 and NZKF2 were previously utilized in an association mapping study (Tamang et al., 2015; chapter 2). The *Ptm* isolates PA14 and CA17 were recently collected from Montana, USA, and are relatively new isolates from a highly virulent population of *P. teres* f. *maculata* (Personal communication with Dr. Timothy Friesen). The three RIL populations were phenotyped with each isolate at the seedling stage in the greenhouse. The experimental design, inoculum preparation, inoculation, disease assessment and rating scale were performed as described in Neupane et al., 2015. Briefly, inoculum was prepared by growing fungal mycelium plugs on V8-PDA media (150 ml V8 juice, 10 g Difco PDA, 3 g $CaCo_3$, 10 g agar, and 850 ml H_2O) and spores collected as previously described (Neupane et al., 2015). Three seeds of each RIL were planted in single cones (3.8 cm diameter and 20 cm long) and placed in cone racks bordered with the susceptible parent cvs Tradition and Pinnacle to minimize edge effect. The resistant parents PI67381 and PI84314 were used as the resistant checks whereas Tradition and Pinnacle were used as susceptible checks. Inoculation was performed when the seedlings were at the 2 leaf stage (~2 weeks old). Disease severity was evaluated 7 days after inoculation (DAI) using the 1-5 rating scale described in Neupane et al., (2015). Each cone containing three seedlings was scored collectively as a single replicate. At

least three independent replicates were assessed for each isolate and the average values of the three replicates were utilized for further analyses.

Segregation Analysis

Tradition X PI67381 F_2s were phenotyped at the seedling stage in green house with the FGO isolate. Single F_2 individuals were planted in a single container similar to the RIL population. A chi-square test was performed to identify if resistance/susceptibility was predominantly governed by single dominant resistance or susceptibility gene following a 3:1 segregation ratio.

Genotyping by Sequencing

The T3 database (www.triticeaetoolbox.org/barley) 9k Illumina *Infinium* iSELECT chip SNP data was mined for polymorphic markers between the parental genotypes and utilized to develop population specific PCR genotyping-by-sequencing (PCR-GBS) SNP marker panels. The population specific polymorphic marker panels developed contained a total of 365, 351, and 328 markers for the T67381, PI67381, and PI84314 populations, respectively. The markers were developed ensuring to cover all 7 barley chromosomes with an average density of ~5 cM per marker. Primer development, DNA extraction, PCR cycle parameters, library preparation, and sequencing on the Ion Torrent PGM were performed as previously described in Richards et al., (2016). Briefly, the 22 base pair (bp) CS1 adaptor (5'-ACACTGACGACATGGTTCTACA-3') (Fluidigm) was added to the 5' end of all the SNP specific forward primers and the CS2 adaptor (5'-TACGGTAGCAGAGACTTGGTCT-3') was attached to the 5' end of all the reverse primers. Barcoded adaptor primers were designed that contained Ion Torrent A-adaptor sequence (a unique 12 nucleotide barcode) and the CS1 adaptor sequence at the 3' end (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG(NNNNNNNNCGAT)ACACTGACGACATG

GTTCTACA-3'). A universal reverse primer was designed containing the Ion Torrent P1 adaptor sequence and the CS2 adaptor sequence at the 3' end (5'- CCACTACGCCTCCGCT TTCCTCTCTATGGGCAGTCGGTGATTACGGTAGCAGAGACTTGGTCT-3'). Primers were then multiplexed by adding 5 ml of each forward and reverse primer (100 mM) into a sterile 1.5 ml tube with nuclease-free water to a final volume of 1 ml. Each single PCR reaction contained 1.5 µl genomic DNA, 1 µl of primer pool (100 nM each primer), and 2.5 µl Platinum Multiplex PCR Master Mix (Life Technologies) for a total reaction volume of 5 µl in a 96-well PCR plate. The primary PCR program was set as: denaturation at 94^{o} for 10min, 10 cycles of 94^{o} for 20 secs and 64^{o}-56^{o} touchdown decrease by 0.8^{o} per each cycle for 1 min, followed by 20 cycles of 94^{o} for 20 secs, 57^{o} for 1 min, and 68^{o} for 30 secs, and final extension of 72^{o} for 3 min. The PCR plate was centrifuged briefly and 15 µl nuclease free H_2O was added to each well. To ensure successful amplification 2 µl of each sample was separated on a 1% agarose gel. After varification of the amplification products the genotype specific samples were barcoded. The barcoding PCR reactions consisted of 11.6 µl of H2O, 4 µl Promega GoTaq Buffer (1X), 0.3 µl dNTPs (500 µM), 1 µl universal reverse primer (100 nM), 1 µl barcode adaptor primer (0.4 µM), 0.1 µl Promega Taq polymerase (1 unit), and 2 µl DNA from each diluted primary PCR reaction, in 96-well PCR plates. The barcoding PCR parameters were the same as the primary PCR amplification. The PCR plates were centrifuged briefly, and each sample pooled by aliquoting 5 µl from each well into 1.5 ml tubes then purified using the E.Z.N.A Cycle Pure Kit. The purified sequencing libraries were amplified in a reaction consisted of 19.3 µl H_2O, 6 µl GoTaq Buffer (5X), 1 µl Ion Torrent ABC1 primer (10 pM), 1 µl Ion Torrent P1 primer (10 pM), 0.5 µl dNTPs (500 µM), and 0.2 µl Promega GoTaq polymerase (1 unit). To ensure library amplification two PCR reactions of each library were run, one with taq polymerase and the second without taq

polymerase, concurrently with the PCR parameters of initial denaturation at 95^{o} for 5 min, 8 cycles of 94^{o} for 30 secs, 62^{o} for 30 secs, and 72^{o} for 30 secs, and final extension with 72^{o} for 7 min. The amplified libraries were then quantified using the Qubit Fluorometer (Life Technologies) with the Qubit dsDNA High Sensitivity quantification kit. The final libraries were diluted to ~3 pg/µl and sequenced on the Ion Torrent PGM utilizing the Ion PGM Template OT2 200 Kit, Ion PGM Sequencing 200 Kit v2, and an Ion 318 Chip (Life Technologies).

Linkage Mapping and QTL Analysis

The average disease severities from three independent replicates of the phenotyping assays were used for SFNB resistance/susceptibility QTL mapping. A total of 365, 351, and 328 markers were used to genotype the T67381, PI67381, and PI84314 populations, respectively. We filtered individual SNP calls for a minimum genotype quality of 10, minimum read depth of 3. The markers with more than 30% missing data and MAF < 25% were removed from further analysis. Linkage maps were developed for T67381, PI67381, and PI84314 populations, respectively using MapDisto 2.0 (Lorieux, 2012). A command 'find groups' was used to make marker linkage groups with a logarithm of the odds (LOD) value of 3.0 and rmax of 0.3. The 'AutoOrder', 'AutoCheckInversions', and 'AutoRipple' commands were used to develop a linkage map at a logarithm of the odds (LOD) of 3.0 and Kosambi mapping function was used to calculate the genetic distances. Final linkage maps were developed using 'Automap' command for QTL analysis. QTL analysis was conducted in QGene 4.0 (Johanes and Nelson 2008) using composite interval mapping (CIM) to identify resistance/ susceptibility QTL to SFNB. A permutation test with 1,000 iterations was performed to find LOD threshold at significance level α=0.05 and 0.01.

Results

Trait Evaluations

The average disease reactions of all three RIL populations to all six *Ptm* isolates ranged between 1.0-4.0 using the 1-5 rating scale described by Neupane et al. (2015). Barley line PI67381 and PI84314 exhibited average reactions of 1.4 and 1.3, respectively, and were consistently resistant to all the *Ptm* isolates assayed (Table A1, A2, and A3). Tradition and Pinnacle exhibited average reactions of 2.7 and 2.8, respectively. The average disease reactions in all three population ranges between 1.3-4.0, 1.2-3.8, 1.0-3.5, 1.2-3.5, 1.0-2.3, and 1.0-2.5 for the FGO, PA14, CA17, SG1, DEN2.6, and NZKF2 isolates, respectively, (Table A1, A2, and A3). Most of the RIL progenies exhibited intermediate infection types (ITs), but some of the progeny exhibited lower ITs than the resistant parents (PI67381 and PI84314) and higher ITs than the susceptible parents (Tradition and Pinnacle) indicating transgressive segregation (Table A1, A2, and A3; Fig 3.1). The US isolate FGO was the most virulent showing the highest ITs on the resistant and susceptible parents as well as progeny and the Denmark isolate DEN2.6 was the least virulent showing the lowest average ITs (Table A1, A2, and A3).

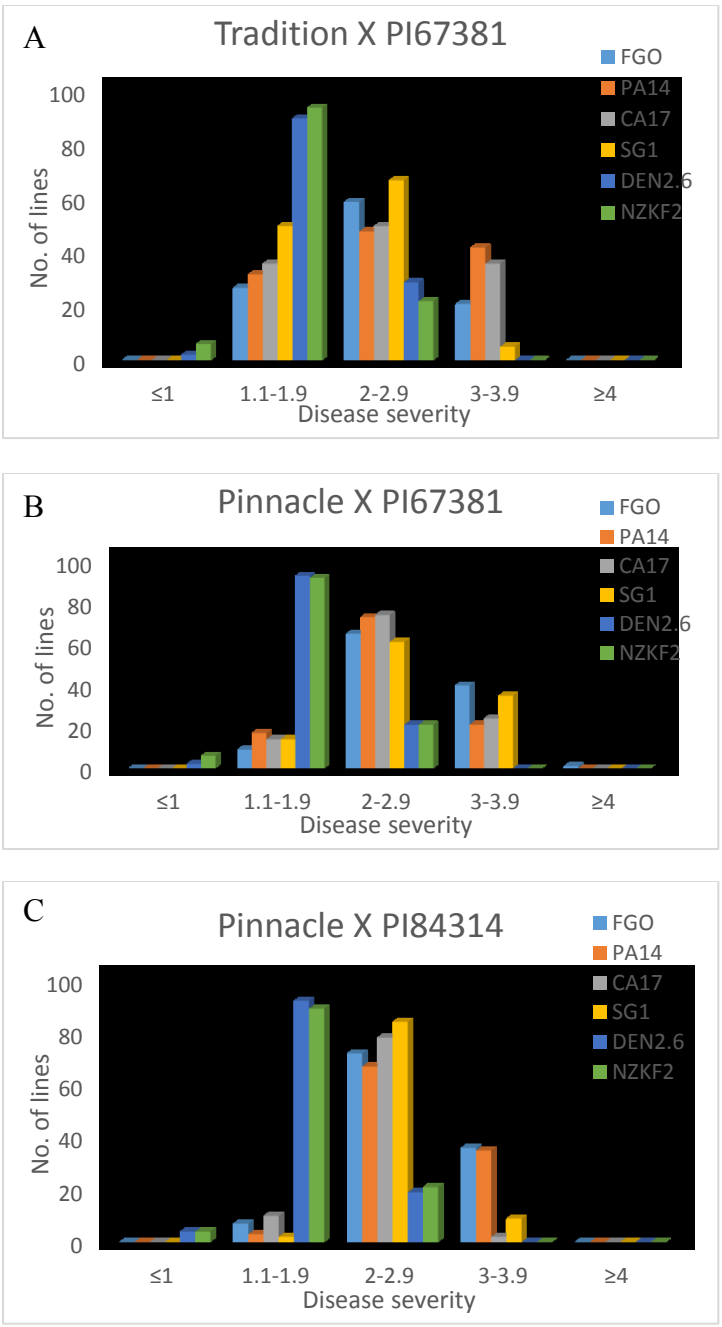

Fig 3.1. The seedling disease reaction type (IT) distributions of three $F_{2:7}$ RIL populations **A.** Tradition X PI67381, **B.** Pinnacle X PI67381, and **C.** Pinnacle X PI84314 phenotyped with six *P. teres* f. *maculata* isolates FGO, PA14, CA17, SG1, DEN2.6 and NZKF2. The disease was scored based on 1-5 rating scale with 1 being highly resistant and 5 being highly susceptible. Most of the RIL progenies exhibited intermediate ITs, but some progenies exhibited extreme phenotypes compared to the resistant and susceptible parents indicating transgressive segregation. The parental disease reactions are indicated by arrows in the histograms (PI67381=1.4, PI84314=1.3, Tradition=2.7, and Pinnacle=2.8). The X-axis represents the ITs and the Y-axis represents the number of barley lines.

Segregation Analysis Results

A χ^2 analysis of the T67381 F_2 phenotypic data based on 113 F_2 individuals indicated that the ratio did not significantly deviate from a 3:1 single gene segregation ratio, which segregated in 1 resistance to 3 susceptible (χ^2=1.30, p=0.05, Fig 3.2, Table 3.1). This suggested that the phenotype is predominately governed by one single dominant susceptibility gene contributed by the susceptible parental line Tradition.

Table 3.1. Segregation analyses for resistance/susceptibility to *P. teres* f. *maculata* isolate FGO in an F_2 population of 113 individuals derived from a Tradition X CI67381 cross.

Resistant F_2	Susceptible F_2	χ^2 (1:3)
23	90	1.30*

*Non-significant at p=0.05 level.

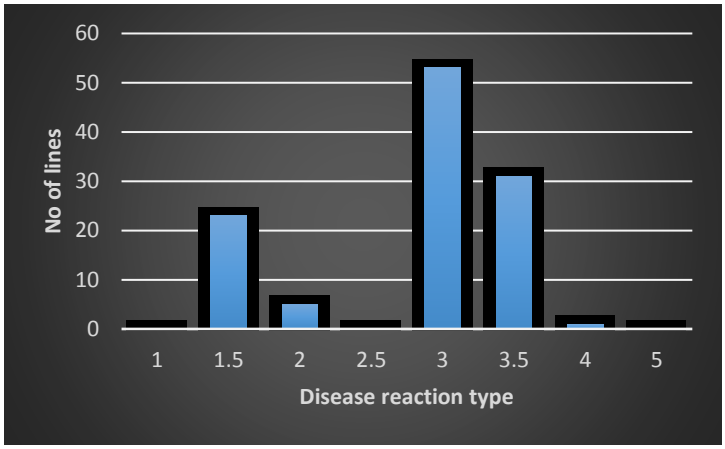

Fig 3.2. Histograms showing the seedling infection type distribution of Tradition X PI67381 F_2 individuals inoculated with the *P. teres* f. *maculata* isolate FGO. The histogram resembles bimodal distribution of the disease reaction at a cutoff of 2 (resistant=<2 and susceptible=>2). The disease was scored based on 1-5 rating scale with 1 being highly resistant and 5 being highly susceptible. The X-axis represents the disease reaction type and the Y-axis represents the number of lines.

QTL Mapping

From the total 365, 351, and 328 polymorphic markers utilized in the population specific PCR-GBS panels designed for the T67381, P67381, and P84314 populations, 234, 200, and 215 markers produced quality calls, respectively, and were used to develop the genetic linkage maps.

The resulting maps contained 7 linkage groups corresponding to the 7 barley chromosomes (Fig 3.3, 3.4, and 3.5). These maps accounted for a total genetic map length of 1088.5, 1093.98, and 1079.7 cM for the T67381, P67381, and P84314 populations, respectively. This resulted in an average marker density of 1 marker/ 5.04 cM across all three populations.

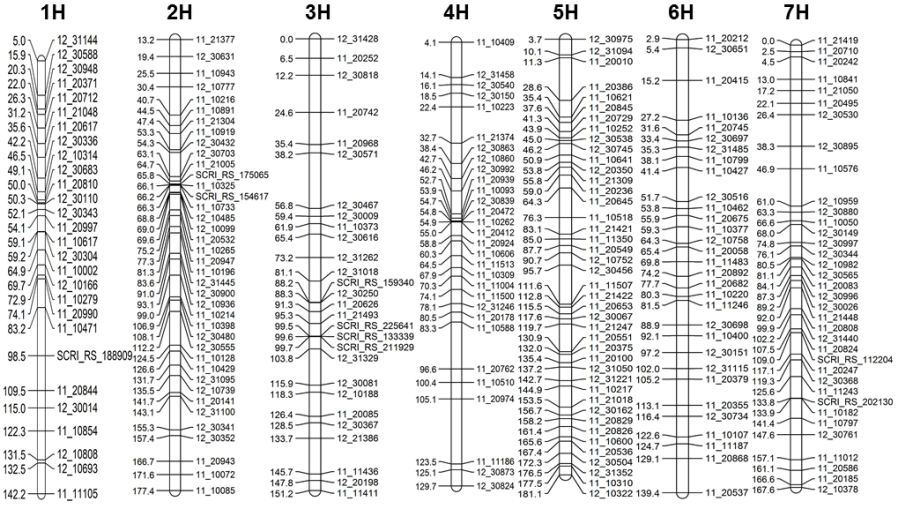

Fig 3.3. Linkage map of the Tradition X PI67381 RIL population developed using Qgene software with 234 SNP markers showing the seven barley chromosomes. The left side of each chromosome shows the cM position based on the barley POPSEQ map (Mascher, et al., 2013) and the right side represents the markers.

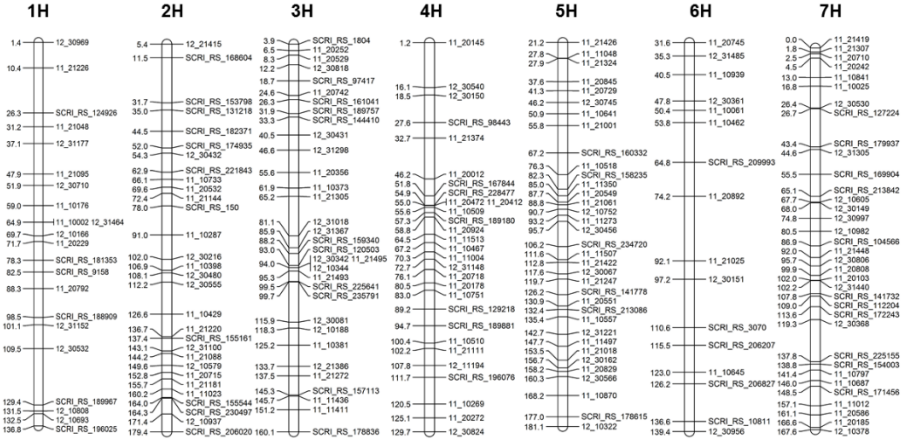

Fig 3.4. Linkage map of the Pinnacle X PI67381 RIL population developed using Qgene software with 200 SNP markers showing the seven barley chromosomes. The left side of each chromosome shows the cM position based on the barley POPSEQ map (Mascher, et al., 2013) and the right side represents the markers.

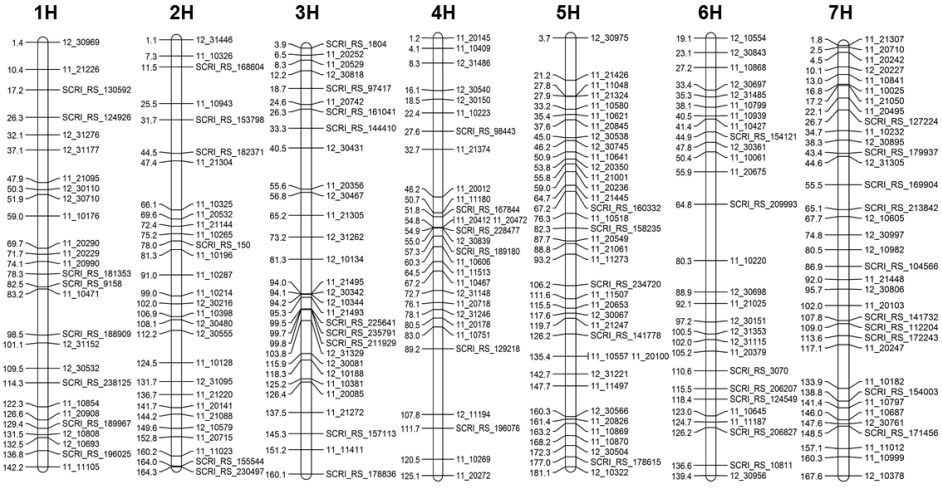

Fig 3.5. Linkage map of the Pinnacle X PI84314 RIL population developed using Qgene software with 215 SNP markers showing the seven barley chromosomes. The left side of each chromosome shows the cM position based on the barley POPSEQ map (Mascher, et al., 2013) and the right side represents the markers.

The average number of markers per chromosome was 33.4, 28.6, and 30.7 for the T67381, P67381, and P84314 populations, respectively. The number of markers ranged from 28 on chromosome 1H and 3H to 42 on 5H in T67381 while it was 16 markers on 6H to 35 markers on 5H in the P67381 population and 27 on 1H to 35 on 5H for the P84314 population.

The LOD thresholds at a significance level of 0.05 and 0.01, were calculated by 1000 permutations, and are shown in Table 3.2. The LOD threshold values (α=0.05) were used to determine significant QTL against each of the 6 *Ptm* isolates in the T67381, P67381, and P84314 populations, respectively. Quantitative trait loci (QTLs) were mapped to barley chromosomes 2H, 3H, 4H, 6H, and 7H in this study.

Table 3.2. LOD threshold level at a significance level of α=0.05 and 0.01 calculated by 1000 permutations for three RIL populations.

	Tradition X PI67381		Pinnacle X PI67381		Pinnacle X PI84314	
Isolates	**0.05**	**0.01**	**0.05**	**0.01**	**0.05**	**0.01**
FGO	3.75	5.04	3.50	4.52	3.66	5.00
PA14	3.85	5.00	3.54	4.61	3.69	4.99
CA17	3.77	4.79	3.57	4.85	3.65	4.83
SG1	3.70	4.83	3.62	4.67	3.85	5.19
DEN2.6	3.81	5.10	3.54	4.90	3.78	4.98
NZKF2	3.73	4.80	3.60	4.62	3.84	4.69

Chromosome 2H QTL

A common QTL *QRptm-2H-1-31* was mapped on chr 2H at 1.08 to 31.74 cM with LOD values ranging from 3.92-6.49, 3.85-7.61, and 3.70-12.38 in all three populations against all isolates assayed except SG1 and DEN2.6 in T67381; CA17, SG1 and NZKF2 in P67381; and FGO in P84314. The flanking SNP markers were 11_21377 (13.19 cM) and 12_30631 (19.42 cM) in the T67381; SCRI_RS_168604 (11.49 cM) and SCRI_RS_153798 (31.74 cM) in the P67381; and 12_31446 (1.08 cM) and SCRI_RS_168604 (11.49 cM) in the P84314 populations. The R^2 value for these QTL ranged between 14-22%, 15-27%, and 15-40% in the T67381, P67381, and P67381 population, respectively (Fig 3.6A, 3.6B, 3.6C, 3.11 and Table 3.3, 3.4 and 3.5).

Another QTL *QRptm-2H-77-83* was identified between SNP markers 11_20947 (77.34 cM) and 12_31445 (83.59) with a LOD value of 6.07 for the DEN2.6 isolate in the T67381 population (R^2 =20%) (Fig 3.6A, 3.11, Table 3.3 and 3.6). Similarly, in the P67381 population, a QTL *QRptm-2H-126-137* was mapped on chr 2H between markers 11_10429 (126.63 cM) and SCRI_RS_155161 (137.44) for FGO isolate with a LOD value of 5.1 and R^2 =18% (Fig 3.6B, 3.11, Table 3.4 and 3.6).

In the P84314 population, one QTL was mapped between the SNP markers 12_10579 (149.60 cM) and 11_20715 (152.83 cM) with a LOD value of 4.50 for the FGO isolate (R^2 =17%) and another QTL between markers 11_20141 (141.70 cM) and 11_21088 (144.2 cM) with a LOD value of 6.29 for the PA14 (R^2 =25%) (Fig 3.6C, 3.11, Table 3.5 and 3.6). This QTL was designated *QRptm-2H-141-152*.

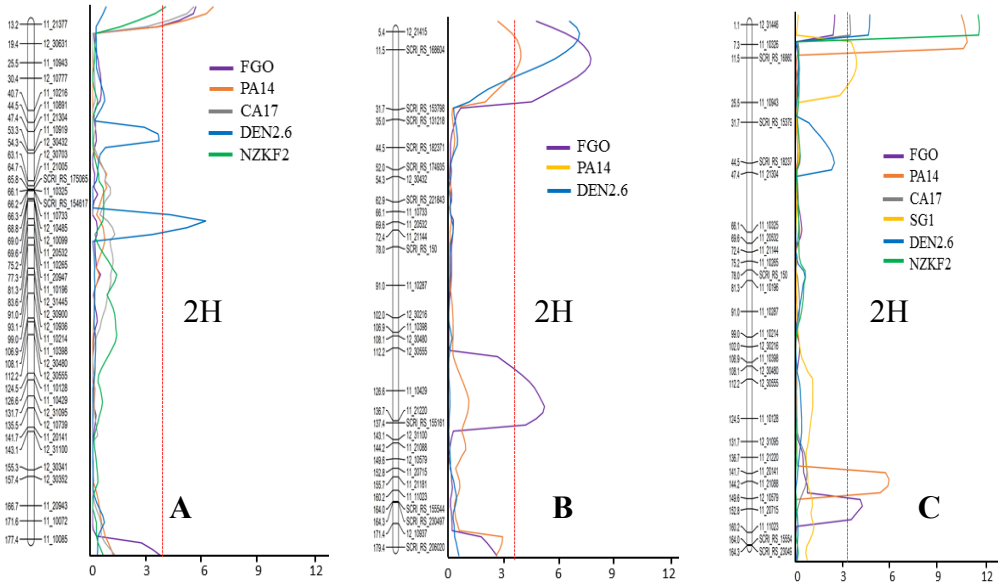

Fig 3.6. Composite interval mapping of ch. 2H showing significant SFNB resistance/ susceptibility QTL in three RIL populations; **A**. Tradition X PI67381, **B**. Pinnacle X PI67381, and **C**. Pinnacle X PI84314. The QTL analyses were performed with a global collection of *P. teres* f. *maculata* isolates FGO (purple), PA14 (orange), CA17 (gray), SG1 (yellow), DEN2.6 (blue), and NZKF2 (green). Chromosome 2H is shown with the markers on y-axis and LOD values on x-axis. The red dotted line represents the LOD threshold calculated by 1000 permutations at a 0.05 significance level. The LOD and R^2 values for each QTL are presented in Tables 3.3, 3.4, and 3.5.

Chromosome 3H QTL

In the T67381 population, a QTL *QRptm-3H-81-88* was identified against CA17 isolate on chr 3H with a LOD value of 4.52 and (R^2=16%) between the flanking markers 12_31018 (81.11 cM) and SCRI_RS_159340 (88.17 cM) (Fig 3.7A, 3.11, Table 3.3 and 3.6). Similarly, in the P84314 population, a QTL *QRptm-3H-56-65* on chr 3H was identified between markers

12_30467 and 11_21305 positioned at 56.79–65.16 cM. *QRptm-3H-56-65* had a LOD value of 4.47 with the SG1 isolate (R^2 =20%) (Fig 3.7B, 3.11, Table 3.5 and 3.6).

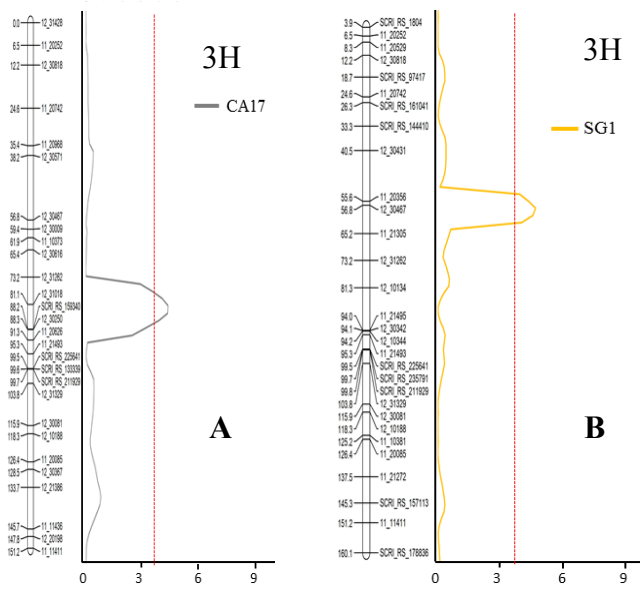

Fig 3.7. Composite interval mapping of chr 3H showing significant SFNB resistance/ susceptibility QTL in two RIL populations; (**A**) Tradition X PI67381 and (**B**) Pinnacle X PI84314, with the *P. teres* f. *maculata* isolates CA17 (gray) and SG1 (yellow), respectively. Chromosome 3H is shown with the markers on the y-axis and LOD values on x-axis. The red dotted line represents the LOD threshold calculated by 1000 permutations at a 0.05 significance level. The LOD and R^2 values for each QTL are presented in Tables 3.3, and 3.5.

Chromosome 4H QTL

The QTL, *QRptm-4H-58-64,* on chr 4H was identified at ~58.1-64.45 cM in the T67381 population with *Ptm* isolates DEN2.6 and NZKF2 with LOD scores of 7.84 and 10.88, respectively. The *QRptm-4H-58 -64* QTL mapped to an interval between SNP markers 11_20924 (58.82 cM) and 11_11513 (64.45 cM) (Fig 3.8A, 3.11, Table 3.3 and 3.6) and R^2=26-34%.

In the P67381 population, a second QTL was mapped between the SNP markers 11_10269 (120.53 cM) and 11_20272 (125.11 cM) with LOD values of 3.74 and 11.80 (R^2=14-38%) (Fig 3.8B, Table 3.4 and 3.6) with *Ptm* isolates DEN2.6 and NZKF2, respectively. Similarly, the same QTL was detected in the P84314 population with LOD values of 4.54 and

7.77 with the same two isolates, DEN2.6 and NZKF2 (R^2=17-28%) (Fig 3.8C, 3.11, Table 3.5 and 3.6), respectively. This QTL at 120.53-125.11 cM was given the nomenclature *QRptm-4H-120-125.*

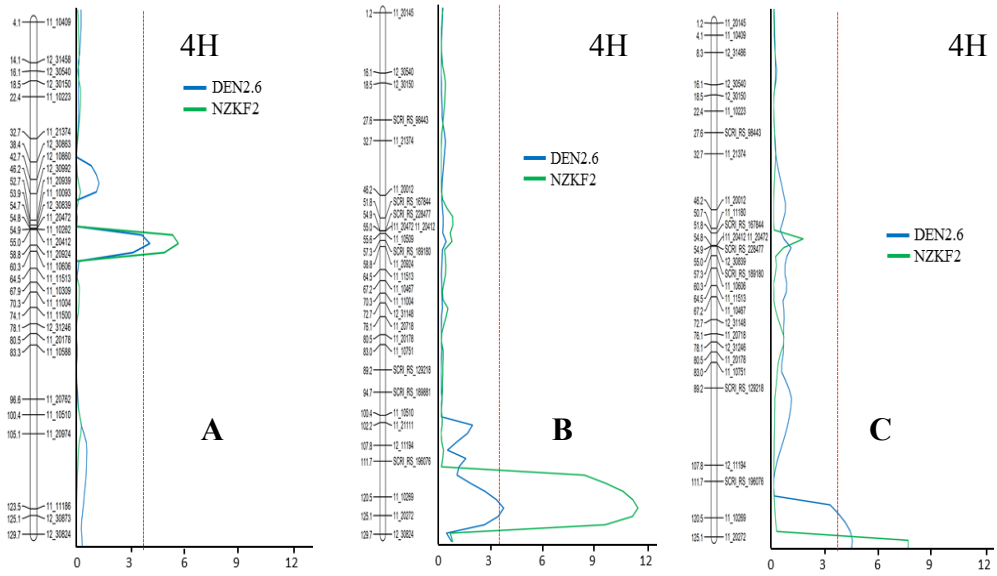

Fig 3.8. Composite interval mapping of chr 4H showing significant SFNB resistance/ susceptibility QTL in three RIL populations; **A.** Tradition X PI67381, **B.** Pinnacle X PI67381, **C.** Pinnacle X PI84314. The QTL analyses were performed with *P. teres* f. *maculata* isolates DEN2.6 (blue) and NZKF2 (green). Chromosome 4H is shown with the markers on y-axis and LOD values on x-axis. The red dotted line represents the LOD threshold calculated by 1000 permutations at a 0.05 significance level. The LOD and R^2 values for each QTL are presented in Tables 3.3, 3.4 and 3.5.

Chromosome 6H QTL

A QTL in the T67381 population was identified at 59.33 to 64.29 cM having the flanking markers 11_10377 and 12_10758 with a LOD value of 4.34 and a R^2=15% against *Ptm* isolate NZKF2 (Fig 3.9A, 3.11, Table 3.3, and 3.6). Similarly, a QTL was identified at the 55.9-64.78 cM region between the markers 11_20675 and 11_10220 in the P84314 population with a LOD value of 12.2 (FGO), 8.52 (PA14), and 4.30 (CA17) (Fig 3.9B, 3.11, Table 3.5 and 3.6). The R^2 values for this QTL against FGO, PA14, and DEN2.6 were 39%, 32% and 17%, respectively. This QTL in T67381 and P84314 on chr 6H was designated as *QRptm-6H-55-64* (Table 3.6).

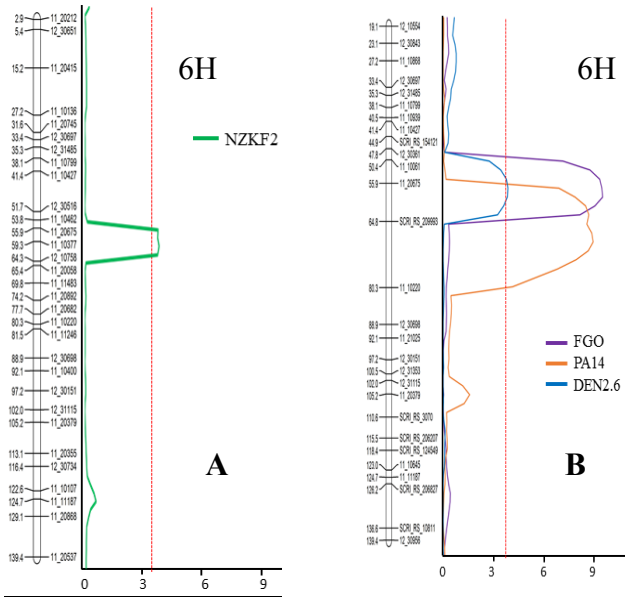

Fig 3.9. Composite interval mapping of chr 6H showing significant SFNB resistance/ susceptibility QTL in two RIL populations; **A**. Tradition X PI67381, and **B**. Pinnacle X PI84314. The QTL analyses were performed with the *P. teres* f. *maculata* isolates FGO (purple), PA14 (orange), DEN2.6 (blue), and NZKF2 (green). Chromosome 6H is shown with the markers on the y-axis and LOD values on the x-axis. The red dotted line represents the LOD threshold calculated by 1000 permutations at a 0.05 significance level. The LOD and R^2 values for each QTL are presented in Tables 3.3, and 3.5.

Chromosome 7H QTL

A major QTL on chr 7H was identified to isolates FGO, CA17, PA14 and SG1 in the T67381, P67381, and P84314 populations. This QTL was mapped at 119.33 -125.55 cM between SNP markers 12_30368 and 11_11243 with LOD values of 18.67 to 41.77 (R^2=51-80%) in the T67381 population (Fig 3.10A, 3.11, Table 3.3 and 3.6). For the P67381 population the QTL was positioned between the SNP markers 12_30368 (119.33 cM) and SCRI_RS_225155 (137.76 cM) with LOD values of 11.80 to 19.63 (R^2=43-56%) (Fig 3.10B, 3.11 Table 3.4). In the P84314 population, this QTL was detected between markers 11_20247 (117.1 cM) and 11_10182 (133.92 cM) against isolate PA14 with a LOD value of 6.24 (R^2=24%) (Fig 3.10C, 3.11 and Table 3.5). This QTL was designated as *QRptm-7H-119-137*.

Similarly, in the P84314 population, a QTL close to *QRptm-7H-119-137* was detected on chr 7H between markers SCRI_RS_154003 (138.76 cM) and 11_10797 (141.37 cM) against FGO (R^2=15%), between 11_10687 (146.03) and 12_30761 (147.63) against SG1 (R^2=28%), and between 11_11012 (157.08 cM) and 11_10999 (160.25 cM) against NZKF2 (R^2=26%) with LOD value of 3.82, 6.55, and 7.17, respectively (Fig 3.10C, 3.11, Table 3.6). This QTL was given nomenclature as *QRptm-7H-138-160.*

With the DEN2.6 isolate, another QTL designated *QRptm-7H-92-95* was detected between the markers 11_21448 (92.0 cM) and 12_30806 (95.7 cM) with a LOD value of 4.27 (R^2=15%) in the P67381 population (Fig 3.10B, 3.11, Table 3.6)

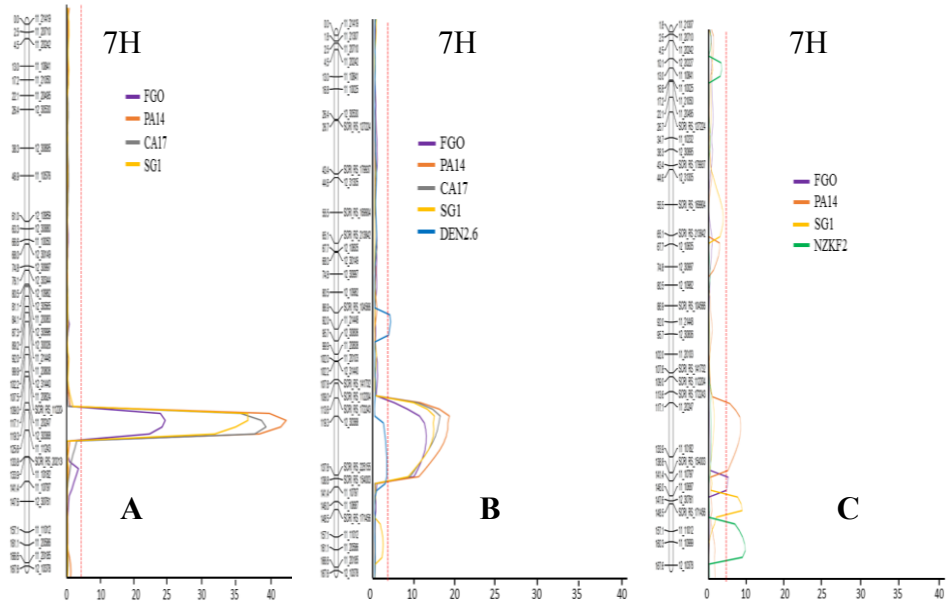

Fig 3.10. Composite interval mapping of chr 7H showing significant SFNB resistance/ susceptibility QTL in the three RIL populations; **A**. Tradition X PI67381, **B.** Pinnacle X PI67381, and **C**. Pinnacle X PI84314. The QTL analyses were performed with a global collection of *P. teres* f. *maculata* isolates FGO (purple), PA14 (orange), CA17 (gray), SG1 (yellow), DEN2.6 (blue), and NZKF2 (green). Chromosome 7H is shown with the markers on the y-axis and LOD values on the x-axis. The red dotted line represents the LOD threshold calculated by 1000 permutations at a 0.05 significance level. The LOD and R^2 values for each QTL are presented in Tables 3.3, 3.4 and 3.5.

Table 3.3. Quantitative trait loci (QTL) associated with resistance/susceptibility to diverse *P. teres* f. *maculata* isolates in the Tradition X PI67381 population, showing genetic positions (cM), flanking markers, phenotypic variation explained by the QTL, and the isolates for which the source of resistance/susceptibility were detected.

Chromosome	Isolate	Flanking Markers	Interval (cM)	LOD	R^2
2H	FGO	11_21377- 12_30631	13.19-19.42	5.54[a]	19
	PA14	11_21377- 12_30631	13.19-19.42	6.49[a]	22
	CA17	11_21377- 12_30631	13.19-19.42	5.44[a]	19
	NZKF2	11_21377 - 12_30631	13.19-19.42	3.92	14
	DEN2.6	11_20947- 12_31445	77.1-83.9	6.07[a]	21
3H	CA17	12_31018 - SCRI_RS_159340	81.11-88.17	4.52	16
4H	DEN2.6	11_20924 - 11_11513	58.1-64.45	7.84[a]	26
	NZKF2	11_20924 - 11_11513	58.1-64.45	10.88[a]	34
6H	NZKF2	11_10377 -12_10758	59.33-64.29	4.34	15
7H	FGO	12_30368 - 11_11243	119.33-125.55	18.67[a]	51
	PA14	12_30368 - 11_11243	119.33-125.55	41.77[a]	80
	CA17	12_30368 - 11_11243	119.33-125.55	37.89[a]	77
	SG1	12_30368 - 11_11243	119.33-125.55	34.44[a]	73

[a] Significant LOD with threshold set at a significance level α=0.01
R^2 Phenotypic variation explained by each QTL against the respective *P. teres* f. *maculata* isolates.

Table 3.4. Quantitative trait loci (QTL) associated with resistance/susceptibility to diverse *P. teres* f. *maculata* isolates in the Pinnacle x PI67381 population, showing genetic positions (cM), flanking markers, phenotypic variation explained by the QTL, and the isolates for which the source of resistance/susceptibility were detected.

Chromosome	Isolate	Flanking Markers	Interval (cM)	LOD	R^2
2H	FGO	SCRI_RS_168604 - SCRI_RS_153798	11.49- 31.74	7.61[a]	27
	PA14	SCRI_RS_168604 - SCRI_RS_153798	11.49- 31.74	3.85[a]	15
	DEN2.6	SCRI_RS_168604 - SCRI_RS_153798	11.49- 31.74	7.00[a]	24
	FGO	11_10429 - SCRI_RS_155161	126.63- 137.44	5.11[a]	19
4H	DEN2.6	11_20269 - 11_20272	120.53-125.11	3.74	14
	NZKF2	11_20269 - 11_20272	120.53-125.11	11.80[a]	38
7H	FGO	12_30368 - SCRI_RS_225155	119.33-137.76	13.84[a]	43
	PA14	12_30368 - SCRI_RS_225155	119.33-137.76	19.63[a]	56
	CA17	12_30368 - SCRI_RS_225155	119.33-137.76	17.54[a]	52
	SG1	12_30368 - SCRI_RS_225155	119.33-137.76	16.00[a]	49
	DEN2.6	11_21448- 12_30806	92.00- 95.7	4.27	15

[a] Significant LOD with threshold set at a significance level α=0.01
R^2 Phenotypic variation explained by each QTL against respective *P. teres* f. *maculata* isolates.

Table 3.5. Quantitative trait loci (QTL) associated with resistance/susceptibility to diverse *P. teres* f. *maculata* isolates in the Pinnacle x PI84314 population, showing genetic positions (cM), flanking markers, phenotypic variation explained by the QTL, and the isolates for which the source of resistance/susceptibility are effective against.

Chromosome	Isolate	Flanking Markers	Interval (cM)	LOD	R^2
2H	PA14	12_31446 - SCRI_RS_168604	1.08- 11.49	11.57[a]	40
	CA17	12_31446 - SCRI_RS_168604	1.08- 11.49	3.70	15
	SG1	12_31446 - SCRI_RS_168604	1.08- 11.49	5.00[a]	19
	DEN2.6	12_31446 - SCRI_RS_168604	1.08- 11.49	4.11	18
	NZKF2	12_31446 - SCRI_RS_168604	1.08- 11.49	12.38[a]	40
2H	FGO	12_10579 - 11_20715	149.6- 152.83	4.50	17
	PA14	12_10579- 11_20715	149.6- 152.83	6.29[a]	25
3H	SG1	12_30467- 11_21305	56.79-65.16	4.47	20
4H	DEN2.6	11_10269 - 11_20272	120.53-125.11	4.54	17
	NZKF2	11_10269 - 11_20272	120.53-125.11	7.77[a]	28
6H	FGO	11_20675 - SCRI_RS_209933	55.9- 64.78	12.2[a]	39
	PA14	11_20675 - SCRI_RS_209933	55.9- 64.78	8.52[a]	32
	DEN2.6	11_20675 - SCRI_RS_209933	55.9- 64.78	4.30	17
7H	FGO	SCRI_RS_154003 -11_10797	138.76-141.37	3.82	15
	PA14	11_20247-11 _10182	117.10-133.92	6.24[a]	24
	SG1	11_10687-12 _30761	146.03-147.63	6.55[a]	28
	NZKF2	11_11012 -11_10999	157.08-160.25	7.17[a]	26

[a] Significant LOD with threshold set at a significance level α=0.01

R^2 Phenotypic variation explained by each QTL against respective *P. teres* f. *maculata* isolates.

Table 3.6. QTLs detected in all three populations, with chromosome, their position relative to the POPseq positions, and phenotypic variation explained by the QTL against the global set of *P. teres* f. *maculata* isolates.

QTLs	Population	Isolate	Chr	Interval (cM)[a]	R^2 (%)[b]	References[c]
QRptm-2H-1-31	T67381, P67381, P84314	FGO, PA14, CA17, DEN2.6, NZKF2	2H	1.08-31.74	14-40	Burlakoti et al., 2017 (*SFNB-2H-8-10)*
QRptm-2H-77-83	T67381	DEN2.6	2H	77.34-83.89	21	Novel
QRptm-2H-126-137	P67381	FGO	2H	126.63-137.44	19	Tamang et al., 2015
QRptm-2H-141-152	P84314	FGO, PA14	2H	141.6-152.83	17-25	Novel
QRptm-3H-56-65	P84314	SG1	3H	56.79-65.16	20	Tamang et al., 2015 Wang et al., 2015 (*QRptm3-2*) Burlakoti et al., 2017 (*SFNB-3H-58.64)*
QRptm-3H-81-88	T67381	CA17	3H	81.11-88.17	16	Tamang et al., 2015
QRptm-4H-58-64	T67381	DEN2.6, NZKF2	4H	58.10-64.45	26-34	Grewal et al., 2008 (*QRpts4)* Tamang et al., (2015)
QRptm-4H-120-125	P67381, P84314	DEN2.6, NZKF2	4H	120.53-125.11	14-38	Wang et al., 2015 (*QRptm4-2*)
QRptm-6H-55-64	T67381, P84314	FGO, PA14, DEN2.6	6H	55.9- 64.78	15-33	Tamang et al., 2015
QRptm-7H-92-95	P67381	DEN2.6	7H	92.00-95.7	15	Novel
QRptm-7H-119-137	T67381, P67381, P84314	FGO, PA14, CA17, SG1	7H	119.33-137.55	24-80	William et al., 1999 and 2003 (*Rpt4*) Grewal et al., 2008 (*QRpts7)* Wang et al., 2015 (*QRptm7-3*) Tamang et al., 2015
QRptm-7H-138-160	P84314	FGO, SG1, NZKF2	7H	138.76-160.25	15-28	Tamang et al., 2015 Wang et al., 2015

[a]Interval position (cM) of QTL.
[b]Phenotypic variation explained by each QTL.
[c]Previously reported QTL associated with SFNB resistance/susceptibility.
R^2 Phenotypic variation explained by each QTL against respective *P. teres* f. *maculata* isolates.

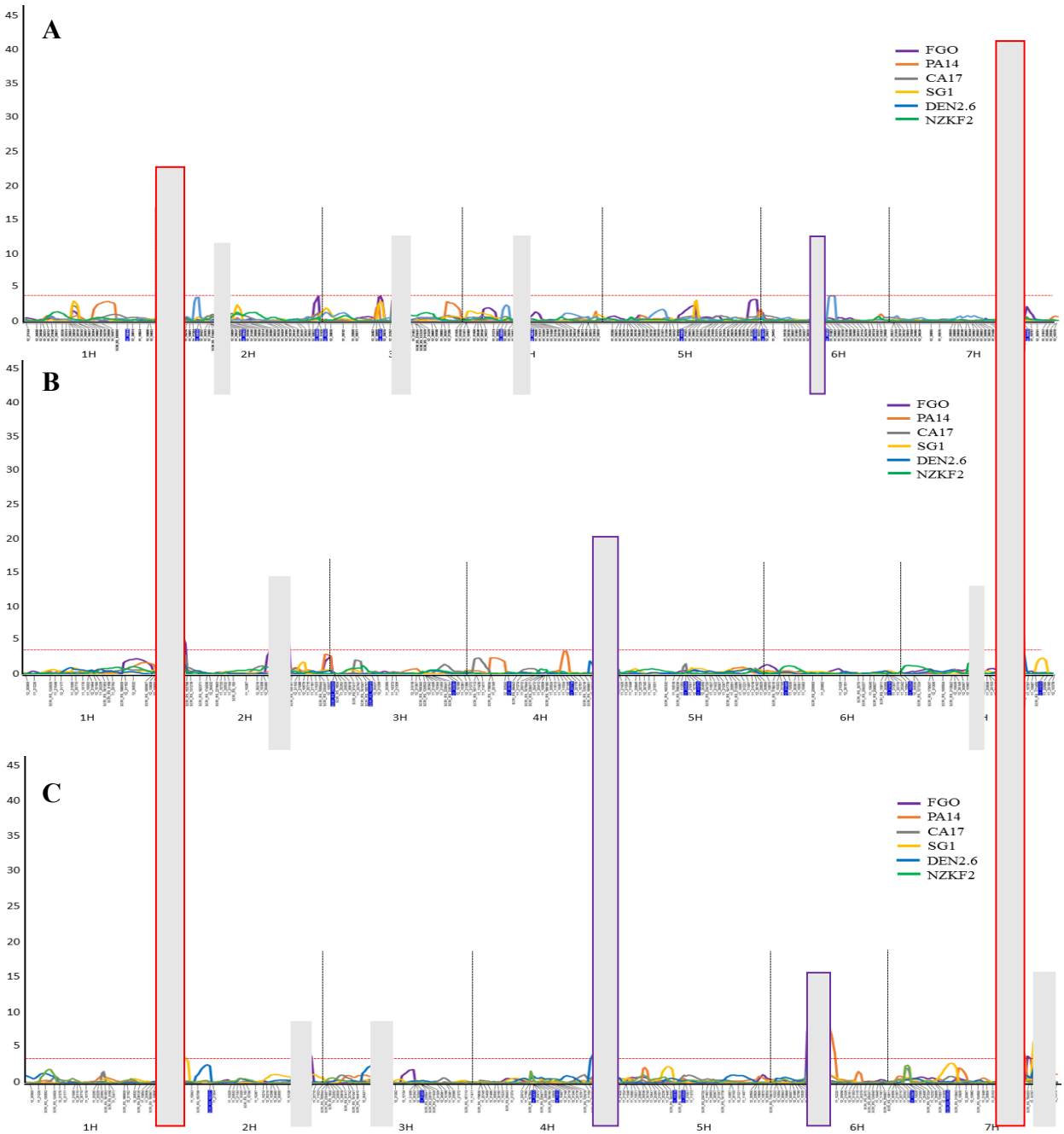

Fig 3.11. Composite interval QTL mapping of spot form net blotch (SFNB) resistance/ susceptibility on three; A. Tradition X PI67381, B. Pinnacle X PI67381, and C. Pinnacle X PI84314, RIL populations against the collection of *P. teres* f. *maculata* isolates FGO (purple), PA14 (orange), CA17 (gray), SG1 (yellow), DEN2.6 (blue), and NZKF2 (green). The disease was scored based on 1-5 rating scale with 1 being highly resistant and 5 being highly susceptible. The position of the markers is shown on the x-axis with the seven barley chromosomes (divided with lines) and the y-axis representing the LOD values. The red dotted line represents the LOD threshold calculated by 1000 permutations at a 0.05 significance level. The red box represents the loci common between all three RIL population. The Blue box represents the loci common between at least two population. The gray boxes represent the loci specific to *Ptm* isolates and the population. A total of 12 QTL were identified in this study.

Discussion

QTL mapping is efficient at identifying major and minor loci/genes that contribute to phenotypic variation in complex or quantitative host-parasite genetic interactions resulting in compatibility (susceptibility) or incompatibility (resistance) when robust phenotyping data is available. The QTL analyses of the complex barley-*P. teres* f. *maculata* interactions are the first steps toward identifying resistance/susceptibility loci present within specific bi-parental populations, that can be utilized in SFNB resistance deployment. Also, from a more basic research perspective, these analyses are also a first step in the identification and cloning of the genes underlying the QTL. However, to achieve this goal in quantitative resistance mechanisms it is important to be able to Mendalize interactions, which requires QTL or genetic analyses from both the host resistance/susceptibilitys and the corresponding pathogen virulence loci.

This study was conducted to identify QTL conferring seedling resistance/susceptibility to *P. teres* f. *maculata* in the three bi-parental mapping populations: Tradition X PI67381, Pinnacle X PI67381, and Pinnacle X PI84314. Two susceptible cultivars Tradition and Pinnacle were used to develop RIL population with a common resistance source in order to determine if these two susceptible cultivars possess the same or different susceptibility gene. Diverse isolates from different geographic regions were utilized because geographically distinct pathogen populations evolved separately gaining and/or conserving different virulence gene profiles. Thus, identifying broader spectrum resistance QTL/genes that can be incorporated into elite upper Midwestern US malting barley lines is important especially since SFNB has become a major problem in the Northern Great Plains. This has been exemplified by the apparent increased virulence in the pathogen population of Western North Dakota and Eastern Montana as compared to isolates collected in other parts of the US and world.

To date, relatively, few SFNB resistance sources have been identified and mapped as compared with NFNB. Therefore, resistance or susceptibility to SFNB is not well characterized and represents a significant knowledge gap in a very important barley-SFNB pathosystem. The SFNB resistance is quantitative in nature and the high diversity in the *Ptm* population presents a major challenge in genetically characterizing these complex interactions (Liu et al., 2011, Wang et al., 2015). This complexity along with the rapid change and diversity in *Ptm* virulence profiles poses a major challenge in breeding for resistance (Khan and Tekauz 1982, Arabi et al 1992, Gupta et al 2001, McLean et al 2009 and 2012, Liu et al 2010 and 2011, Wang et al., 2015). Although, comparatively many more SFNB resistance studies have been performed at the seedling stage in the past, as compared to adult plant resistance (APR) analyses, some QTL appear to be common to both growth stages (Grewal et al., 2008, Cakir et al., 2003, Wang et al., 2015). Thus, some markers targeting QTL identified at the seedling stage should also incorporate resistance that is effective in the field at the adult stages. However, further field studies are warranted to learn more about APR resistance mechanisms and to determine which QTL or genes provide effective resistance at both the seedling and APR stages. However, seedling resistance is important as adult plant resistance because seedling resistance plays an important role in minimizing the disease epidemics in the field as net blotch is polycylic in nature.

Recently, Burlakoti et al., (2017) reported a QTL located on chr 2H designated *SFNB-2H-8-10* (R^2=1.5-4.3%) in an association mapping panel consisting mostly of advanced breeding lines from Upper Midwest breeding programs. The QTL was identified using a local isolate collected from Montana designated SFNB-MT09. We also identified a common QTL *QRptm-2H-1-31* localized to the same region as *SFNB-2H-8-10* on 2H (1.08-31.74 cM; R^2=14-40%) in all three bi-parental populations. This QTL provides resistance against several of the isolates in

all three populations, including the majority of the US isolates suggesting that *QRptm-2H-1-31/ SFNB-2H-8-10* may represent a broad-spectrum resistance effective against a wide range of *Ptm* isolates. Interestingly, it also appears to be present in some of the elite upper midwestern adapted lines thus may not need to be introgressed from the unadapted resistant materials investigated here.

Three other QTL were identified on 2H in different positions. In T67381, the QTL *QRptm-2H-77-83* was identified against isolate DEN2.6 (R^2=21%) and is 8 cM away from the QTL (69.0 cM) identified by Tamang et al., (2015) with the same isolate DEN2.6. Thus, these two QTL may be distinct and it could be novel. Similarly, in P67381, QTL *QRptm-2H-126-137* (R^2=19%) was identified at 126.63-137.44 cM on chr 2H against isolate FGO. Tamang et al., (2015) also detected a QTL at 137.44 cM with the same isolate FGO, so this QTL is probably the same QTL identified in the AM analysis (Tamang et al., 2015, chapter 2). Similarly, in P84314, a QTL *QRptm-2H-141-152* was mapped to 141.6-152.83 cM against FGO and PA14 (R^2=17-25 %). Ho et al., (1996) and Molnar et al., (2000) reported QTL on chr 2H but did not provided any approximate position due to the lack of marker density and information in the Leger X CI9831 DH population. The 2H QTL *QRptm-2H-141-152* identified in this study could be any of the QTL reported by Ho et al., (1996), and Molnar et al., (2000) or could also be a novel locus.

One QTL *QRptm-3H-81-88* was identified on chr 3H in the T67381 population with *Ptm* isolate CA17 at 81.11-88.17 cM and explained 16% phenotypic variation. Tamang et al. (2015) reported a QTL in the same region on chr 3H at 88.17 cM with the SG1 isolate. So, the QTL *QRptm-3H-81-88* identified here could be the same QTL identified by Tamang et al., (2015). However, the QTL *SFNB-3H-91.88* reported by Burlakoti et al. (2017) may not be the same loci as *QRptm-3H-81-88* that was identified in this study, as they are 3 cM apart but they are

probably the same QTL. Another QTL *QRptm-3H-56-65* was detected on 3H only in the P84314 population at 56.79-65.16 cM against the SG1 isolate and explains 20% of the phenotypic variation. Tamang et al., (2015) reported QTL at 53.42 cM against the isolate NZKF2 and at 53.42 and 65.16 cM against the isolate DEN2.6. Wang et al., 2015 reported *QRptm3-2* at 57.16 cM against the Australian isolate SNB331; and Burlakoti et al., (2017) reported *SFNB-3H-58.64* at 58.64 cM. Thus, *QRptm-3H-56-65* is probably the same as one of these previously reported QTL. However, it is probably not the same gene previously reported by Tamang et al., (2015) associated with NZKF2 in the association mapping panel as it is ~3 cM apart from QTL *QRptm-3H-56-65*. In a separate study, we identified a *HvWRKY6* transcription factor gene via mutant analysis and exome capture that maps close to this QTL at 50.7 cM in the NFNB highly resistant line CI5791. The *HvWRKY6* mutation results in expansion of the typical pinpoint necrotic fleck lesions exhibited on the highly resistant line CI5791 resulting in NFNB isolates forming SFNB susceptible type lesions on the otherwise highly resistant genotype (Tamang et al., unpublished; chapter 4). Thus, the 3H QTL may correspond with the *HvWRKY6* transcription factor that is required for NFNB resistance but also functions similarly in SFNB resistance/susceptibility signaling but has some functional or transcriptional regulation polymorphism against both SFNB and NFNB. Interestingly, a NFNB resistance QTL also maps directly on top of the *WRKY6* transcription factor gene as well (Koladia et al., 2017).

We detected the QTL *QRptm-4H-58-64* at ~58.10-64.45 cM on chr 4H in the T67381 population (R^2=26-34%) with the isolates DEN2.6 and NZKF2. This same QTL was also reported in an association mapping study (Tamang et al., 2015), reported on 4H at 53.67-59.22 cM using the same isolates, DEN2.6 and NZKF2.. Thus, it is likely that this is the same QTL and is specific to DEN2.6 and NZKF2. This QTL is ~ 4 cM away from *QRpts4*, (50-54 cM)

identified by Grewal et al., (2008) in a different mapping population which was effective against both SFNB and NFNB. So, it is probably the same gene previously reported by Grewal et al., (2008). Another QTL *QRptm-4H-120-125* (R^2=14-38%) on chr 4H was identified in the P67381 and P84314 populations with DEN2.6 and NZKF2. This QTL resides very close (~3 cM) to the *QRptm4-2* QTL previously reported at 128.9 cM (Wang et al., 2015), which is effective at both the seedling and adult plant stages. Therefore, this QTL *QRptm-4H-120-125* may correspond with *QRptm-4-2*. Friesen et al., (2006) also reported a QTL on 4H against NZKF2 in a double haploid population derived from a SM89010 x Q21861 population that could correspond with either of the two QTLs identified in this study.

Barley chromosome 6H harbors a very complex region that confers resistance/ susceptibility to both SFNB and NFNB (Gupta et al., 2001, Friesen et al., 2006, Liu et al., 2011, Wang et al., 2015, Richards et al., 2016, Koladia et al., 2017). The QTL *QRptm-6H-55-64* on 6H was detected in T67381 (59.33-64.29 cM) with *Ptm* isolate NZKF2 and in P84314 (55.9- 64.78 cM) using the FGO, PA14, and DEN2.6 isolates and explained 15-33% of the phenotypic variation. This QTL might be the same loci identified by Tamang et al., (2015, chapter 2) at 59.01-60.21 against *Ptm* isolates FGO, DEN2.6, and NZKF2. This QTL is at least 11 cM away from *QRpt6* (75-78 cM), which is reported to be effective against both SFNB and NFNB isolates (Grewal et al., 2008) and Manninen et al., (2000) also reported a QTL on 6H but did not provide any specific position, thus it is difficult to determine if they are the same QTL.

QTL *Rpt4* was the first reported dominant resistance gene against *Ptm* on chromosome 7H in the cultivar Galleon effective at the seedling stage (William et al., 1999 and 2003). *QRpt7* (116-134 cM) reported by Grewal et al., (2008), *QRptm7-3* (115.75 cM) by Wang et al., (2015), and QTL at 133.84 cM (marker SCRI_RS_202130) against NZKF2 by Tamang et al., (2015) are

close to *Rpt4*. We also identified a major QTL explaining a high level of the phenotypic variation (R^2=24-80%) on 7H in the T67381, P67381, and P84314 populations that is effective against isolates FGO, PA14, CA17, and SG1 that lies in the same region as *Rpt4*, *QRpt7*, and *QRptm7-3*. We believe that this 7H QTL designated *QRptm-7H-119-137* is the same as those previously reported. However, analysis of T67381 F_2 individuals segregated in 3 susceptible to 1 resistant when phenotyped with the most virulent local *Ptm* isolate FGO suggesting that the gene conferring resistance is recessive or more appropriately conferring dominant susceptibility. Thus, we hypothesize that *QRptm-7H-119-137* is probably a dominant susceptibility locus (recessive resistance) expressed in Pinnacle and Tradition, which was not reported for *Rpt4*, *QRpt7*, or *QRptm7-3*. Thus, if these QTL/genes truly conferred dominant resistance then *QRptm-7H-119-137* could be considered a novel dominant susceptibility gene or recessive resistance gene.

Similarly, we identified another QTL *QRptm-7H-138-160* on chr 7H at 138.76-160.25 cM against *Ptm* isolate FGO, SG1 and NZKF2 adjacent to *QRptm-7H-119-137* explaining phenotypic variation of 15-28%. This QTL *7H-138-160* could be the same QTL as *Rpt4* (William et al., 1999 and 2003) or *QRptm7-4* (152.90 cM) and *QRptm7-5* (159.30 cM) on chr 7H (Wang et al., 2015) or the same as QTL identified by Tamang et al., (2015) at 145.68-150.36 cM with SNP markers 11_20847, 11_10687, and 12_10677 against isolate NZKF2. Since, several previous studies have repeatedly reported resistance/susceptibility loci against *Ptm* isolates collected around the world on barley chromosome 7H, we speculate that 7H harbors multiple loci effective against SFNB (Williams et al., 1999, and 2003, Grewal et al., 2008, Wang et al., 2015, Tamang et al., 2015). Another QTL *QRptm-7H-92-95* on 7H was detected at 92.0-95.7 cM with the isolate DEN2.6 (R^2=15%) in the P67381 population which is ~8 cM away from the QTL (100-112 cM) effective in APR reported by Williams et al., (2003). This is probably the same

QTL previously reported, but since it is ~8 cM distal to the previously reported QTL, it could represent a novel locus.

From the three RIL populations and six isolates used a total of 12 QTL were identified on chromosomes 2H, 3H, 4H, 6H, and 7H. Common QTL were mapped on chr 2H and 7H in all three population, on chr 4H in the P67381 and P84314 population, and on chr 6H in T67381 and P84314 populations. Distinct QTL were mapped to 2H, 3H, 4H, and 7H in all three populations (Table 3.3, 3.4, 3.5, and 3.6, Fig 3.11). Some isolate specific QTL in one population are not detected on the other which could be due to the diversity of *Ptm* virulence or avirulence genes present in the isolates, which probably produce several host selective necrotrophic effectors that could have multiple susceptibility targets in the host to facilitate colonization and disease (Shjerve et al., 2014, Liu et al., 2011). In some populations, there may not be polymorphism in the effector targets or the isolate is missing the effector/s.

Our results confirmed previously identified QTL for SFNB (William et al., 1999 and 2003, Molnar et al., 2000, Friesen et al., 2006, Grewal et al., 2008, Manninen et al., 2000, and 2006, Cakir et al., 2011, Tamang et al., 2015, Wang et al., 2015, and Burlakoti et al., 2017). Besides these previously described QTL, we identified 3 novel seedling resistance loci designated: *QRptm-2H-77-83, QRptm-2H-141-152*, and *QRptm-7H-92-95*. These resistances and the SNP markers delimiting the QTL are being utilized to develop prebreeding lines by introgressing SFNB resistance into the cultivars Pinnacle and Tradition via marker assisted selection. Further studies are required to determine if any of these seedling QTL are effective at the adult plant stage. Also, we are utilizing gamma irradiation to create mutants of cultivar Tradition to clone and study the gene/loci responsible for resistance/ susceptibility to SFNB.

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CHAPTER 4. EXOME CAPTURE-MEDIATED MAPPING-BY-SEQUENCING IDENTIFIES THE *HvWRKY6* GENE AS REQUIRED FOR NET FORM NET BLOTCH RESISTANCE IN BARLEY LINE CI5791

Abstract

Host resistance is a desirable and effective means of controlling disease to maximize crop yield and quality. The barley line CI5791 exhibits a high level of resistance to diverse *Pyrenophora teres* f. *teres* (*Ptt*) isolates collected from around the world; the causal agent of the disease net form net blotch. To identify genes involved in this resistance mechanism, CI5791 seed was γ-irradiated and two mutants were identified at the M_2 generation and designated as CI5791-γ3 and CI5791-γ8. The phenotyping and genotyping of CI5791-γ3 x -γ8 and CI5791-γ8 x -γ3 F_1s suggested that these two mutations are allelic to each other. Analysis of CI5791-γ3 x Heartland and CI5791-γ8 x Heartland F_2 populations segregated in 3 resistances to 1 susceptible ratio showing that the susceptibility was due to the mutation in a single dominant gene. Genotyping via PCR-GBS and phenotyping of the 34 homozygous susceptible mutants from the CI5791-γ3 x Heartland F_2 population with *Ptt* isolate 0-1 followed by genetic mapping delimited the mutation to an ~75 cM interval flanked by the SNP markers 11_20742 and 11_21493 on chromosome 3H. Exome capture mediated by mapping-by-sequencing identified a single mutated gene on chr 3H at ~50.7 cM which is the ortholog of the *Arabidopsis WRKY6* transcription factor, designated as *HvWRKY6.* Analysis of the full length *HvWRKY6* gene sequences from the resistant line CI5791, the moderately susceptible line Morex, and susceptible line Tifang revealed that they are identical, thus, *HvWRKY6* appears to be conserved. BSMV-VIGS experiment showed that the specific silencing of the candidate *HvWRKY6* gene results in a susceptible phenotype when inoculated with *Ptt* isolate 0-1. Thus, it appears the *HvWRKY6* gene

functions as a component of a conserved basal defense mechanism, which regulates the exression of other defense response genes that restrict lesion growth and pathogen spread in CI5791.

Introduction

Net blotch, caused by *Pyrenophora teres* is an economically important foliar disease of barley (*Hordeum vulgare* L.) in major growing regions worldwide. This disease exists in two forms; net form net blotch (NFNB) caused by *Pyrenophora teres* f. *teres* (*Ptt*) and spot form net blotch (SFNB) caused by *Pyrenophora teres* f. *maculata* (*Ptm*) (Steffenson and Webster 1992, Smedegard-Peterson 1971). Although, these two pathogens are morphologically identical (conidia and mycelium), their genetics as well as host-pathogen interactions are quite distinct (Liu et al., 2011), thus they are considered as different diseases. The symptoms of NFNB first appear as a small dark brown necrotic lesion that expand over time forming longitudinal and transverse striated necrotic lesions, net like in appearance, that are commonly surrounded by chlorosis on susceptible host genotypes. Whereas the symptoms of SFNB also first appear as a small dark brown necrotic lesion that expand over time producing elliptical necrotic lesions that are also typically surrounded by chlorosis.

The barley line CI5791, is an Ethiopian breeding line, that is highly resistant to most *Ptt* isolates collected from North America and isolates tested from barley growing regions worldwide (Mode and Schaller 1958; Steffenson and Webster 1992; Wu et al., 2003; Koladia et al., 2017, Richards et al., 2016). However, CI5791 resistance has been compromised by a few Canadian and French isolates (Akhavan et al., 2016; Arabi et al., 1992), and some recently collected Moroccan isolates that are moderately virulent on CI5791 (Personal communication with Dr. Timothy Friesen). Although, CI5791 resistance is remarkably broad and effective, it is apparent that the pathogen has the molecular repertoire to overcome the resistance. Yet, CI5791

can still be considered an excellent source of resistance to be utilized in breeding programs, but should be complemented with other genes to ensure its durability and effectiveness against the diverse *Ptt* populations.

Several previous studies mapped NFNB resistance genes with different resistance specificities to the centromeric region of barley chromosome 6H, thus, this locus has been considered a complex region hypothesized to harbor multiple dominant resistance genes (Steffenson et al., 1996, Cakir et al, 2003, Wu et al., 2003, Friesen et al., 2006, Koladia et al., 2017). In contrast, several other studies had reported recessive resistance genes, or more appropriately dominant susceptibility genes, in the same region of 6H (Abu Qamar et al., 2008, Liu et al., 2011 and 2015, Richards et al., 2016). Koladia et al., (2017) recently mapped two dominant resistance QTL contributed by CI5791 on chromosome 3H and 6H using a CI5791 x Tifang recombinant inbred line (RIL) population when mapping resistance to nine geographically distinct *Ptt* isolates, individually. The major CI5791 6H QTL was shown to be effective against all isolates used in the study whereas the CI5791 3H resistance was effective against only two Japanese isolates. Interestingly, a 3H QTL was also contributed by Tifang and shown to provide resistance against the *Ptt* isolates Br. Pteres (Brazil), BB06 (Denmark), 6A and 15A (California, USA). Bockelman et al., (1977) were the first to report dominant resistance on chromosome 3H contributed by the barley line Tifang using trisomic analysis which is probably the same dominant resistance QTL detected by Koladia et al., (2017).

Exome capture is a cost-effective yet powerful molecular tool that targets the coding regions (exons) of a specific species and is useful for reducing the complexity of genomes to identify polymorphism within expressed genes across populations. This tool has facilitated the efficient identification of polymorphism within coding regions that contribute to disease in

humans, other animals and plants (Choi et al., 2009, Wang et al., 2010, Raca et al., 2011, Bamshad et al., 2011, Cosart et al., 2011, Mascher et al., 2013, 2014 and 2016, Warr et al., 2015, Russell et al., 2016). An exome capture array specific to barley (*Hordeum vulagre*) has been developed based on RNAseq, cDNA, and EST sequences available at the time of development. The array represents 61.6 mega base pairs of coding sequence of the complex ~5.6 Gb barley genome (Mascher et al., 2013). Since its development, it has been used to study the domestication and evolution of barley by resequencing and identifying the variants in the coding regions of wild barley (*Hordeum sponteneum*), and land races (*Hordeum vulgare*) including ancient barley germplasm (Russell et al., 2016, Mascher et al., 2016). Richards et al., (2016) identified the *Rpr2* gene in cv Morex utilizing the exome capture which was hypothesized as a key component of a basal resistance pathway that recognizes a spore coat protein of two diverse pathogens, *Puccinia graminis* f. sp. *tritici* and *Ptt*. Mascher et al., (2014) identified the *HvMND* gene that governs increased tiller numbers utilizing mapping-by-sequencing and exome capture in barley. It was demonstrated that the method could easily be applied to analyse and discover genes underlying mutant phenotypes that were generated via irradiation or chemical mutagenesis. Resistance gene enrichment sequencing (RenSeq) technology and single-molecule real-time (SMRT) sequencing (SMRT RenSeq) are another form of exome capture that were specifically designed to rapidly identify disease resistance gene (*R* gene) in plants that fell into the nucleotide binding site-leucine rich repeat (NLR) class of immunity receptors (Jupe et al., 2013, Witek et al., 2014, Steuernagel et al., 2016). A cDNA RenSeq method was also utilized to reduce the number of candidate *R*-genes to be analyzed accelerating the identification of *R* genes in tomato (Andolfo et al., 2014).

The WRKY transcription factors are one of the largest groups of transcription regulators consisting of a highly conserved amino acid sequence WRKYGQK at their N-terminus and a zinc-finger-motif (C-C-H-H/C) at their C-terminus (Eulgem et al., 2000, Bakshi et al., 2014). WRKY proteins bind to specific W-box elements (TTGAC/T) in the promotor regions of their targeted genes, thereby, either activating or repressing transcription (Rushton et al., 2010, Agarwal et al., 2011, Eulgem et al., 2000, Yu et al., 2001). WRKY TFs are known to be important in diverse plant physiological activities such as pathogen defense responses, and abiotic stress responses such as wounding, nutrient deficiency, salt stress (Kasajima et al., 2010, Chen et al., 2009, Li et al., 2017, Cai et al., 2017, Hichri et al., 2017), and developmental processes including senescence, and root growth (Robtzek et al., 2001 and 2002, Skibbe et al., 2008). Studies have shown that *WRKYs* can either positively or negatively regulate plant defense responses (Eulgem and Somssich, 2007, Robatzek et al., 2002). In wheat, the *TaWRKY70* TF identified within the fusarium head blight *QTL-2DL* governs resistance against *Fusarium graminarium* by regulating the three downstream resistance genes *TaACT*, *TaDGK*, and *TaGLI* (Kage et al., 2017). *AtWRKY6* regulates both plant defense responses against *Pseudomonas syringae* pv. *tomato* as well as senescence in *Arabidopsis* (Robatzek et al., 2002). *WRKY3* and *WRKY6* regulate defense response in *Nicotiania attenuate* against the herbivore *Manduca sexta* larvae (Skibbe et al, 2008). In *Arabidopsis*, the *RPS4*/*RRS1* dual NLR mechanism has an integrated WRKY domain on the *RRS1* NLR that is hypothesized to function as a decoy that detects the bacterial effectors *AvrRPS4* and *PopP2* to stop disease development (Sarris et al., 2015). Other WRKY gene families have also been reported to play vital roles in defense responses in rice against *Magnaporthe grisea* (rice blast) and *Xanthomonas oryzea* (bacterial leaf blight) (Liu et al., 2007, Shimono et al., 2007, Wang et al., 2007). In contrast, *WRKY11* and *WRKY17* function as negative regulators of basal defense responses in *Arabisopsis* (Journot-Catalino et al., 2006). Li

et al., (2004) reported enhanced resistance to the biotroph *Erysiphe cichoracearum* whereas there was an increase in susceptibility to the bacterial necrotroph *Erwinia carotovora* subsp *carotovora* following the upregulation of *WRKY70* in *Arabidopsis*. The TFs *WRKY38* and *WRKY62* were also found to be negative regulators of plant basal defense responses to the bacterial pathogen *P. syringae* and the compromised immunity was associated with overexpression of either TF (Mao et al., 2007, Kim et al., 2008). Grunewald et al., (2008) identified *WRKY23* as the negative regulator of plant defense response against the cyst nematode *Heterodera schachtii.* Therefore, the previous research shows that the WRKY TFs can play both a positive and negative role in the regulation of defense responses, thus are part of a complex system of intertwined regulatory elements that orchestrate a finely balanced immunity mechanism in plants.

Here we report the identification and characterization of the *HvWRKY6* gene in barley line CI5791 which is required for the high level of resistance against the necrotrophic pathogen *P. teres* f. *teres* utilizing forward genetics followed by exome capture for mapping-by-sequencing. We hypothesize that the *HvWRKY6* gene functions as a component of the resistant pathway which is required for *Ptt* resistance by contributing to the restriction of lesion growth in the highly resistant barley line CI5791.

Materials and Methods

Mutant Development

The Ethiopian barley line CI5791 is highly resistant to most NFNB isolates collected worldwide. CI5791seed was γ-irradiated to develop a mutant population. Briefly, ~500 gram of seed was hydrated in an air tight container with 60% glycerol for about 7-10 days. The hydrated seed were irradiated with 35 kilorad (350 Gy) gamma rays in a Gammator (M38-4, Radiation

Machinery Corporation, New Jersey, USA) prior to planting. Approximately, 1,400 M_1 seed were planted in trays and allowed to self to generate the M_2 generation. Approximately, 10,000 M_2 seedlings, derived from the original 1,400 M_1 individuals were screened by inoculating with the *Ptt* isolate LDNPtt-19 collected from Langdon, North Dakota. Planting, inoculum preparation, inoculation and disease reading were performed as described in Friesen et al., (2006). After identifying putative mutants these seedlings were transplanted to 15.24 cm (6 inch) pots and allowed to self to generate the M_3 generation seed. The M_3 generation seed was planted in conetainers and screened with *Ptt* isolate 0-1 using three replicates with wt CI5791 and the susceptible barley cultivar Robust as resistant and susceptible checks, respectively. The planting, inoculum preparation, inoculation and disease reading was again performed as described in Friesen et al., (2006).

Mapping Populations and Phenotyping

Two F_2 mapping populations were developed by crossing CI5791-γ3 and CI5791-γ8 homozygous mutant M_3 individuals with the NFNB resistance barley line Heartland. Heartland is a spring six-rowed feed barley that was developed at the Agriculture Canada Research Station, Brandon, Manitoba and registered and released in 1984 (Therrien et al., 1985). Heartland was shown to be resistant to three major Canadian races of *P. teres* f. *teres* before its release and was hypothesized to contain a similar dominant resistance gene as CI5791 at the ch. 6H locus. The planting, inoculum preparation, inoculation and disease reading for the 120 F_2 individuals from each of the CI5791-γ3 and CI5791-γ8 x Heartland populations were performed as described in Friesen et al., (2006). The progenies were phenotyped in the greenhouse at the seedling stage using the *Ptt* isolate 0-1 that has similar virulence as the isolate LDNPtt-19, which was used to identify the original CI5791-γ3 and CI5791-γ8 mutants from the M_2 generation. In short, the

individual F_2 seeds were planted in single cones and placed in a rack bordered with the susceptible barley cv Robust. The barley seedlings were grown in the greenhouse until the secondary leaf was fully expanded (~2 weeks) and then placed in environmentally controlled growth chamber after inoculations were performed as described in Friesen et al., (2006). Disease reading was performed 7 days after inoculation (DAI) using 1-5 rating scale (Neupane et al., 2015) for CI5791-γ3 x Heartland and 1-10 rating scale developed by Tekauz (1985) for CI5791-γ8 x Heartland. The CI5791-γ3 x Heartland F_2 susceptible individuals using a rating cutoff of >2 representing homozygous CI5791 genotype at the mutant region were used for mapping the gene. We utilized PCR-Genotyping by sequencing (PCR-GBS) to genotype CI5791-γ3 x Heartland F_2 homozygous susceptible lines (a total of 34 lines representing 68 recombinant gametes) on the Ion Torrent PGM as described before in chapter 3. A PCR-GBS marker panel designed for polymorphism between Tradition and PI67381 consisiting of 365 markers (Appendix C1) was used to genotype all 34 susceptible F_2 lines. The disease severity of 34 CI5791-γ3 x Heartland F_2 homozygous susceptible lines along with the genotypic data were used for QTL mapping using MapDisto 2.0 (Lorieux, 2012) and Qgene 3.0 (Johanes and Nelson 2008) as described in chapter 3. Single marker regression was used to identify the susceptible QTL in the γ3 x Heartland F_2 population. CI5791 and the two mutants were also phenotyped with the two Moroccan *Ptt* isolates SM36-2 and SM36-3 that were shown to be moderately virulent on CI5791.

Allelism Test

Reciprocal crosses were made between CI5791-γ3 and CI5791-γ8 to determine if the two putative independent mutants were allelic. Six F_1s of CI5791-γ3 x CI5791-γ8 and ten F_1s of

CI5791-γ8 x CI5791-γ3 were phenotyped with the *Ptt* isolate 0-1 and genotyped utilizing primers specific to the putative mutant gene identified in the region delimiting the mutation.

DNA Extraction, Exome Capture and Sequencing

Genomic DNA (gDNA) of the CI5791-γ3 and CI5791-γ8 mutants and wild type (wt) CI5791 were isolated from at least 5 embryos extracted from seeds soaked overnight on petri plates with Whatman filter paper and ~20 ml of H_20. The gDNA extractions were performed using the PowerPlant Pro DNA Isolation Kit (MoBio Laboratories, Inc.) following the manufacturer's protocol. The extracted embryo gDNA was quantified using the Qubit Fluorometer (Life Technologies) with a Qubit dsDNA High Sensitivity Kit. To test if the DNA was degraded it was visualized on a 1% agarose gel containing GelRed (Biotum). DNA that showed an intact band with an ~15-20 kb molecular weight band with minimal lower molecular weight smearing was considered to have adequate integrity for downstream exome capture library preparations.

The NimbleGen SeqCap EZ Library preparation kit and manufacturer's protocol was followed to prepare exome capture libraries of wt CI5791**,** CI5791-γ3, and CI5791-γ8. Approximately 1.2 µg of gDNA of each sample were randomly fragmented to an average size of ~180-220 base pairs (bp) in a 20 µl reaction consisting of 2 µl Fragmentase Reaction Buffer v2 (10X), 1 µl $MgCl_2$ (10 mM), and 2 µl dsDNA Fragmentase enzyme (New England Biolabs). The fragmentation reactions were allowed to proceed at 37^o C for 30 min and inactivated by adding 5 µl of 0.50 M ethylenediaminetetraacetic acid (EDTA). Fragmented DNA samples were then purified with an AMPure XP bead purification kit (Agencourt) following the manufacturer's protocol. The Agilent Bioanalyzer (Agilent Technologies 2100) was used to confirm the target fragmented size of 180-220 bp using the Agilent DNA 1000 kit.

The KAPA DNA Library Preparation Kit for Illumina was used to perform the end repair, A-tailing and adapter ligation to the fragmented DNA for sequencing. A total of 70 µl of end repair reactions (8 µl H_2O, 7 µl end repair buffer (10x), 5 µl end repair enzyme, and 50 µl of fragmented DNA samples) were incubated for 30 min at 20°C to produce blunt-ended 5' phosphorylated fragments. The end repair reaction was purified and eluted using AMpure XP beads as described above. To each tube containing beads with end-repaired DNA, A-tailing reactions (42 µl H_2O, 5 µl A-tailing buffer (10x), and 3 µl A-tailing enzyme) were incubated at 30°C for 30 minutes. The A-tailed DNA was again purified with an AMPure XP bead purification kit (Agencourt) following the manufacturer's protocol. Adapter ligation reactions (50 µl) were prepared (10 µl ligation buffer (5x), 5 µl T4 DNA Ligase, 30 µl H_2O, and 5 µl unique adaptor (10 µM)) and mixed with the beads containing A-tailed DNA and incubated at 20°C for 15 min. The bead bound adapter ligated DNA was immediately purified using 50 µl of PEG/NaCl solution. The samples were mixed thoroughly and incubate for 15 minutes to allow the DNA to bind the beads and placed back on the magnetic block. The cleanup was performed using the Ampure XP bead purification kit (Agencourt) following the manufacturer protocol and 50 µl of DNA was eluted using elution buffer (10 mM Tris-HCL, pH 8.0).

The Pippin Prep instrument (Sage Science) was used to select DNA fragments between 250-450 bp from the exome capture libraries. The library amplification reactions (25 µl of KAPA HiFi HotStart ReadyMix (2X), 5 µl PCR Primer Premix (5 µM), and 20 µl DNA sample library was amplified via PCR. The amplification parameters were 98°C initial denaturation, seven cycles of 98°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec with a final extension of 72°C for 60 sec. The resulting amplicons were purified by mixing with 90 µl of DNA purification beads (SeqCap EZ Pure Capture Kit, Roche Nimblegen) following the same

cleaning process as described above. After 10 minutes of drying, the beads were resuspended in 52 µl of nuclease free water and 50 µl of the DNA was eluted representing the exome capture library ready for hybridization. The Agilent DNA 1000 kit and Agilent Bioanalyzer were used to confirm that the library was free of contaminants and to determine the target fragment size distribution.

All the DNA libraries were pooled together in equal volume by mass. To prepare the hybridization sample, 1 µg of pooled sample was mixed with 2 nmol Multiplex Hybridization Enahancing Oligo pool and 5 µl COT Human DNA. The multiplexed exome capture library pool was dried in a DNA vacuum concentrator for ~45 minutes at high temperature (+60ºC). The dried sample was then mixed with 7.5 µl hybridization buffer (2X) and 3 µl hybridization component A, vortexed for 10 sec and centrifuged at maximum speed for 10 sec followed by incubation at +95ºC for 10 minutes to denature the DNA. After a quick 10 sec centrifugation, the multiplexed exome capture library was mixed with a 4.5 µl aliquot of EZ Library (120426_Barley_BEC_DO4.EZ library) in a 0.2 ml tube, briefly vortexed, centrifuged and incubated at 47ºC for 72 hours (lead +57 ºC).

The hybridized DNA library was washed twice using the Roche Nimblegen wash kit. The bead bound DNA sample was amplified in 50 µl KAPA HiFi HotStart ReadyMix, 10 µl Post-LM-PCR Oligos 1 & 2 (5 µM) and 20 µl of DNA sample with initial denaturing at 98ºC for 45 sec, 14 cycles of 98ºC for 15 sec, 60ºC for 30 sec, and 72ºC for 30 sec, followed by final extension at 72ºC for 1 min. The amplified sample was purified using the SeqCap EZ Pure Capture Bead kit as previously described. The concentration, size distribution, and quality of the amplified captured multiplex DNA sample was performed using the Agilent bioanalyzer 2100 as described before. The amplified library was sequenced on a single Illumina NextSeq flowcell

producing 150 bp single end reads at the USDA-ARS Cereal Genotyping Laboratory (Fargo, North Dakota).

Bioinformatics Analysis

The resulting exome capture sequencing reads from CI5791 wt, CI5791-γ3, and CI5791-γ8 were imported into CLC Genomics Workbench v8.0.1 and quality trimmed using default settings. Trimmed reads were aligned to the barley cultivar Morex draft reference genome (IBGSC 2012) using the Burrows-Wheeler aligner 'mem' algorithm with default settings (Li and Durbin 2010). SAM tools "bedcov' was used to calculate the read depth within each exome capture target region. Coverage was calculated by dividing the depth count by the length of the capture target. Targets were considered putatively absent if coverage was less than 1. Variants were detected in SAMtools (Li et al., 2009) using the 'mpileup' command and the output was filtered with VCFtools (Danecek et al., 2011). Parameters used to filter the data included a genotype quality of >10, read depth of >2, and to only include insertions or deletions. Putative insertions in the mutant lines were deleted from the dataset in MS Excel. Variant calls containing missing data were excluded from further analysis. Additionally, only variants in which the wt CI5791 sample had a homozygous genotype and had an overall variant quality > 100 were retained in the dataset. POPSEQ positions of Morex WGS contigs were obtained from the barley genome database (ftp://ftpmips.helmholtz-muenchen.de/plants/barley/public_data/popseq_IPK/) and used to annotate the variant call data (Mascher et al., 2013). The POPSEQ positions of the markers flanking the QTL identified in the segregating F_2 population (Fig 4.4), described above, were obtained and used to identify exome capture targets within the mapped region. BAM files from the analysis were imported into CLC Genomics Workbench version 8.0.3 (Qiagen) for the visualization of sequence alignments (Fig 4.5).

STS Marker Development and Mutation Validation

Based on the identified nucleotide deletions detected in the mutants, mutant specific sequence tagged site (STS) markers was developed. Two primer sets were developed specific to wt CI5791 and the CI5791- γ8 mutant. Primers WRKY6-F1 (5' GCCGCTGGTTCTCGTCG TTCATGCG 3') and WRKY6-Wt-R1 (5'- TAGTCGACGACGACGGGGCGTCCC -3') only produce an amplicon from wt CI5791 (Fig. 4.8) whereas the primer combination of WRKY6-F1 and WRKY6-Mt-R2 (5'- TAGTCGACGACGACGGGGCGT CCG -3') will only produce an amplicon from the CI5791-γ8 mutant due to designing specificity in the 3 bases at the 3' terminus of the primer that are specific to the 1 bp deletion discovered in the CI5791-γ8 mutant from the exome capture data. The polymerase chain reactions (PCR) were optimized so the discriminant amplicons were specific to the wt or mutant genotypes. The PCR amplification program was set as: denaturation at 95^{o} for 5 min, 25 cycles of 95^{o} for 30 secs, 76^{o} for 1 min, and 76^{o} for 30 secs, and final extension of 72^{o} for 5 min. Wildtype CI5791, Heartland, CI5791-γ3, CI5791-γ8, homozygous susceptible F_2 individuals from two populations, and 15 randomly selected resistance F_2 lines (CI5791-γ8 x Heartland) were genotyped with the wt and mutant specific primers. The F_1 reciprocal cross between CI5791-γ3 and CI5791-γ8 were also genotyped with these primer sets. All PCR amplicons were visualized on 1% agarose gels with GelRed (Biotum).

HvWRKY6 Allele Sequencing and Analysis

To determine if there is allelic variation between resistant and susceptible barley cultivars, we sequenced *HvWRKY6* from CI5791 (resistant), cv Tifang (susceptible) and cv Morex (susceptible). We designed four primer pairs at 1 kb interval to sequence the entire gene including the promoter region (~3,544 bp) (Table B6). The gDNA extractions were performed as

described before and were quantified using the Qubit Fluorometer (Life Technologies) with a Qubit dsDNA Broad Sensitivity Kit. PCR parameters were initial denaturation at 95 °C for 5 minutes, 35 cycles of 94 °C for 30 seconds, 60 °C for 60 seconds, and 72 °C for 60 seconds, followed by a final extension at 72 °C for 5 minutes. PCR amplicons were visualized on a 1% agarose gel containing GelRed (Biotum) and purified using an E.Z.N.A Cycle Pure centrifugation column (Omega Bio-tek) following the manufacturer's protocol. Purified PCR products, ~40 ng, were sent to GenScript for sequencing following their guidelines.

RNA Extraction, cDNA Synthesis, and qPCR

Quantitative PCR (qPCR) was conducted to measure differential regulation of the *HvWRKY6* gene upon interaction with *Ptt* isolates in compatible (susceptible) or incompatible c barley lines. The isolates *Ptt* 0-1, SM36-2, and SM36-3 were used to inoculate CI5791 (resistant) and the barley line Tifang (susceptible) was inoculated with *Ptt* isolate 0-1 only. Primers were designed across exons 1 and exon 2 (Fig 4.13A): wrky6-qpcr-F2 (5'-GTTCCTGCCGTTACTGTCCTCATC-3') and wrky6-qpcr-R2 (5'-TCGCCATCAAGAAGGAGGACCTCAC-3'), that specifically amplify ~120 bp from cDNA and ~270 bp from gDNA. At least three biological replications were collected from each mock (water + tween 20) and *Ptt* inoculated plants. Tissues from the first leaves were collected at time point 0 (non-inoculated control), 5 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 12 hr, 24 hr, 48 hr, 72 hr, 96 hr, 120 hr, 144 hr, 168 hr, post inoculation. Tissue samples were immediately flash frozen in liquid nitrogen and stored at -80ºC for further processing. Total RNA was extracted from the collected tissue using a RNeasy Plant Mini Kit (Qiagen) following the manufacturer's instruction. The total RNA was quantified using the QUBIT fluorometer and the Qubit RNA BR assay kit (Life Technologies) per the manufacturer's instructions. To ensure RNA integrity and

that the RNA was free of gDNA contamination, 1 µl of total RNA was denatured in 4 volumes of denaturing buffer (Formaldehyde Load Dye, Ambion) at 80ºC for 5 min and visualize on a 1% agarose gel with GelRed (Biotum). RNA samples with the four-intact ribosomal RNA (rRNA) bands at the expected molecular weights of ~ 3.4, 1.8, 1.5, and 1.1 kb corresponding to the nuclear 28S and 18S rRNAs and the 23S and 16S plastid rRNAs, respectively, without high molecular weight gDNA contamination were considered as quality RNA and used for cDNA synthesis. The GoScript™ Reverse Transcription System (Promega) was used to synthesize cDNA following the manufacturer's protocol. Briefly, ~1 µg of total RNA was mixed with oligo$(dT)_{15}$ primer (0.5 µg) and incubate at 70ºC for 5 minutes. The RNA sample was then mixed with 15 µl of reverse transcription reaction mix (GoScript™ Reaction Buffer (5X), $MgCl_2$ (1.5 mM), PCR Nucleotide Mix (0.5 mM each dNTP), Recombinant RNasin Ribonuclease Inhibitor (20 units), and Reverse Transcriptase) and incubated at 25ºC for 5 min followed by 42ºC for 60 min and inactivated at 70ºC for 15 minutes. The 20 µl cDNA synthesis reactions were mixed with 80 µl H_2O (1:5). A 10 µl qPCR reaction was prepared by mixing 4 µl of diluted cDNA, 5 µl SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), and 0.5 µl of each forward and reverse primer (10 µM). qPCR was conducted in a CFX96 Real-time system thermalcycler (Bio-Rad) with cycling parameters of 95 °C for 30 sec followed by 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds; 65°C for 30 sec; and 60 cycles of temperature increasing from 60°C to 95°C with fluorescence readings acquired at 0.5°C increments per cycle. Three technical replications were used for each biological rep. The barley *HvSnor14* gene was used as the reference to normalize *HvWRKY6* gene expression. Efficiency of qPCR for *HvWRKY6* and S*nor14* primers were calculated by generating a standard curve with a 10-fold serial dilution starting from 200 pg of PCR amplified template of *HvWRKY6* and S*nor14*.

Differential expression was calculated by using ΔΔCT method on Biorad CFX Manager 3.1 software. A t-test was performed to check the significance of difference at $p<0.05$ using a standard error of mean of 1.

BSMV-VIGS

The barley stripe mosaic virus-virus induced gene silencing (BSMV-VIGS) system was exploited to functionally validate the *HvWRKY6* as required for resistance in the barley line CI5791. A unique 65 bp sequence was selected from the *HvWRKY6* gene by performing a BLASTn search against the low and high confidence gene list in the IPK barley database (http://webblast.ipk-gatersleben.de/barley_ibsc/) to reduce the cross amplification and off target silencing of other WRKY TF homologs in the barley genome. Two primer pairs based on the 5' and 3' termini of this unique sequence were designed with *NotI* and *PacI* adaptor sequences attached to the 5' ends of the respective primers. These adaptors were reciprocally utilized in order to develop sense and antisense constructs. The first primer set was designed with a *NotI* adaptor on the forward primer and *PacI* adaptor on the reverse primer and the second set with the *PacI* adaptor on the forward primer and *NotI* adaptor on the reverse primer.

First Primer set

WRKY6_KD_NtFP1- GGAGCGGCCGCACGCCATGCCGCTAAACGTCG

WRKY6_KD_PcRP1- GGATTAATTAAGCCGGGCATCGGAACATGGAAC

Second Primer set

WRKY6_KD_PcFP1 - GGATTAATTAAACGCCATGCCGCTAAACGTCG

WRKY6_KD_NtRP1- GGAGCGGCCGCGCCGGGCATCGGAACATGGAAC

These two primer sets were used to clone the unique 65 bp *HvWRKY6* fragment into the γRNA strand of the BSMV-VIGS infectious cDNA clone PSL38.1 in both sense and anti-sense

orientations. First, the two primer sets were used to produce the gene specific amplicon from CI5791 cDNA in 20 µl PCR reactions consisting of 2 µl cDNA template, 0.5 µl of each forward and reverse primers (10 µM), 0.3 µl dNTPs (500 µM), 0.2 µl GoTaq (1.25 units), 4 µl GoTaq buffer (10x) and 12.5 µl H_2O. The PCR cycle parameters had an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec followed by a final extension of 72°C for 5 min. The amplicon was purified using an E.Z.N.A Cycle Pure centrifugation column (Omega Bio-tek). The purified PCR product was digested in a 30 µl reaction consisting of 0.5 µl NotI HF (NEB), 0.5 µl PacI (NEB), 3 µl Cut Smart Buffer (NEB), 11 µl H_2O, and 15 µl PCR product. The digestion reaction was allowed to incubate at 37°C for 2 hours followed by inactivation at 65°C for 20 min. The BSMV vector PSL38.1-MCS for cloning the target amplicon was also digested with 3 units of NotI and PacI double digestion reactions using 5 µg of plasmid in a 30 ul reaction. Digested PCR product (2 µl) was mixed thoroughly in an 8 µl ligation reaction mix comprised of 1 µl of predigested vector (~80ng), 1 µl ligation buffer (10X), 1 µl T4 DNA ligase, and 5 µl H_2O and incubate at 4°C for 24 hours. Chemically competent Top 10 *E. coli* cells (ThermoFisher scientific) were then transformed with the ligation mix according to manufacturer protocol and inoculated into 250 µl of Luria Broth (LB) liquid media and incubate at 37°C with 230 rpm shaking for 1 hour. A total of 100 µl of each transformation was plated onto LB agar plates with 100 µg/ml ampicillin and incubated overnight (~12 hrs) at 35°C. Ten random colonies were picked from each transformation and inoculated into 2 ml of LB broth (5g NaCl, 5g tryptone, 2.5g yeast extract, and 500 ml H_2O and ampicillin (100 µg/ml)) in 12 ml borosilicate culture tubes and incubated overnight with shaking at 230 rpm at 37°C. The cell cultures were transferred to a 2-ml microcentrifuge tube and centrifuged at 12000 rcfs for 5 min to pellet the cells and the waste supernatant was discarded.

The plasmid DNA was extracted from the pelleted cells using the PureYield Plasmid miniprep System (Promega) following the manufacturer's protocol.

The BSMV tripartite viral genomic RNAs (α and ß, and γ genomes) with the γ fragment containing the unique 65 bp fragments of the CI5791 *HvWRKY6* allele cloned in both the sense and anti-sense orientations, were synthesized via *in vitro* transcription using the mMESSAGE mMACHINE T7 Transcription Kit (ThermoFisher Scientific) according to manufacturer protocol. 20 µl reactions of each the α genome, ß genome, γ-*HvWRKY6* sense, and γ-*HvWRKY6* antisense genomes were combined with 370 µl FES buffer (100 ml GP buffer, 5 g sodium pyrophosphate decahydrate, 5 g bentonite, 5 g celite, up to 500 ml H_2O) as BSMV-VIGS inoculum. A total of 20 µl of each the α genome, ß genome, and γ genome were combined with 390 µl FES buffer for the BSMV-VIGS control inoculum.

Single seeds of the barley line CI5791 were planted per conetainer and placed on racks. Newly emerged secondary leaves still whorled at ~10-11 days old seedling stage were inoculated with either 5 µl of each tripartite RNAs or BSMV-VIGS control virus (both in FES buffer). Approximately 40 individual plants were inoculated with each BMSV-VIGs experimental RNA and control RNA. Plants were first misted heavily and then inoculated by gently rubbing the leaves with 5 µl of each BSMV-VIGs construct. After incubation in mist chamber for 24 hrs at 100% humidity, inoculated plants were moved back to the growth chamber set at 21^{o}C with a 12 hr photoperiod. Once typical BSMV symptoms, mottling and striping, appeared on the expanded or expanding tertiary leaves, plants were inoculated with *Ptt* isolate 0-1 as previously described in Friesen et al., (2006). Inoculum preparation, inoculation, and disease reading were performed as described before. The barley line CI5791 and cv Robust were used as a resistance and a

susceptible check, respectively. Disease reading was performed 7 and 12 days after *Ptt* inoculation (Fig 4.8, Table B5 and B6) using 1-10 scale developed by Tekauz (1985).

Tissue samples from each of the BSMV-WRKY6 and BSMV-pBS control constructs were collected before and after inoculation with *Ptt* isolate 0-1. At least four biological replications were collected for both BSMV knockdown and BSMV control samples. RNA was extracted as described previously using RNeasy Plant Mini Kit (Qiagen). RNA was visualized on 1% gel with GelRed (Biotum) to ensure RNA integrity. The GoScript^{TM} Reverse Transcription System (Promega) was used to synthesize cDNA following the manufacturer's protocol. qPCR was conducted as described previously to quantify the amount of *HvWrky6* gene silencing in VIGS-WRKY6 plants compared to control construct.

Results

Mutant Identification and Validation

To identify genes involved in the effective and broad NFNB resistance present in barley line CI5791 seed was γ-irradiated and ~10,000 M_2 seedlings originating from ~1,400 M_1 individuals were screened. After inoculation and scoring, 8 putative mutant individuals were identified by phenotyping with *Ptt* isolate LDNPtt-19 and allowed to self to the M_3 generation. After phenotyping M_3 individuals in replicated trials, 2 individual mutant lines were identified that visually had similar susceptible phenotypes that resembled susceptible SFNB reactions. The original barley line CI5791 and the resistant line used in the mutant mapping population development, cv Heartland, exhibited highly resistance reactions (pin point necrotic lesions) to *Ptt* isolate 0-1 with average disease reaction of 1.0 (Fig 4.1, Table B1), respectively, based on the 1-10 rating scale developed by Tekauz (1985). The phenotypes of the two putative independent mutants, CI5791-γ3 and CI5791-γ8, whose resistance had been compromised after γ-irradiation

showed average disease scores of 3.5 and 6.0, respectively (Fig 4.1, Table B1 and B2). The disease reactions on the two putative mutants were consistently atypical NFNB reactions, but rather resembled more typical SFNB symptoms with elliptical lesions often surrounded by a yellow halo. The lesions also appeared distinct with less coalescence over time that is typically observed with NFNB susceptible interactions (Fig. 4.1). However, inoculation of CI5791-γ3 and CI5791-γ8 with the Moroccan *Ptt* isolates SM36-2 and SM36-3 that show virulence on CI5791 resistance had average disease scores of 6.5 and 7, respectively (Fig 4.2 and 4.3, Table B3), and more closely resembled net type symptoms. Wildtype CI5791 exhibited moderately susceptible reaction of 3.5-4 infection types and cv Hockett exhibited resistance reaction of 1 infection type with *Ptt* isolates SM36-2 and SM36-3 (Fig 4.2 and 4.3, Table B3), respectively based on 1-10 rating scale (Tekauz ,1985). The barley cultivar Robust, Hector, and Tifang exhibited susceptible reactions with average disease scores of 8.5, 9.0 and 8.0, respectively, to all isolates used in this study (Fig 4.1, 4.2, and 4.3, Table B1, B2, and B3).

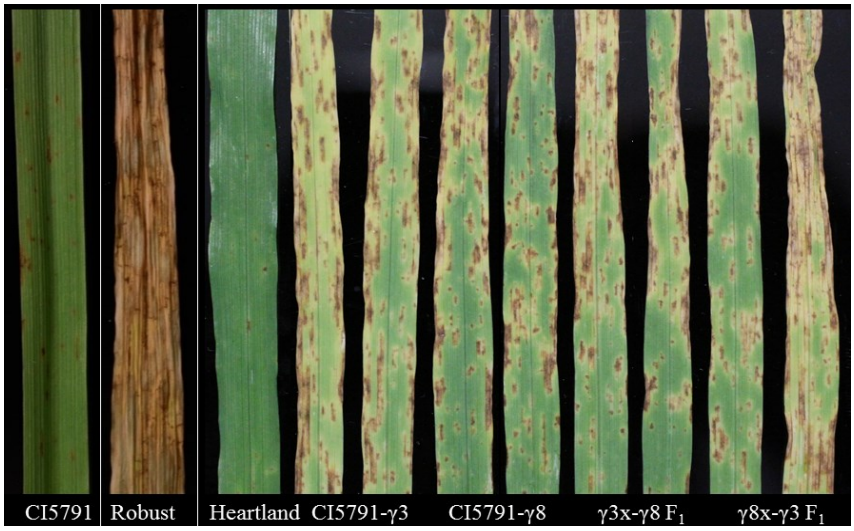

Fig 4.1. Phenotypic reaction of CI5791, Heartland, CI5791-γ3, CI5791-γ8, CI5791-γ3 x -γ8 F_1 and CI5791-γ8 x -γ3 F_1 to *Ptt* isolate 0-1. CI5791 and Heartland showed resistant reactions. Robust, CI5791-γ3, CI5791-γ8, CI5791-γ3 x -γ8 F_1 and CI5791-γ8 x -γ3 F_1 showed susceptible reactions that resembled spot type lesions. The disease was scored based on 1-10 rating scale where 1 is highly resistant and 10 is highly susceptible.

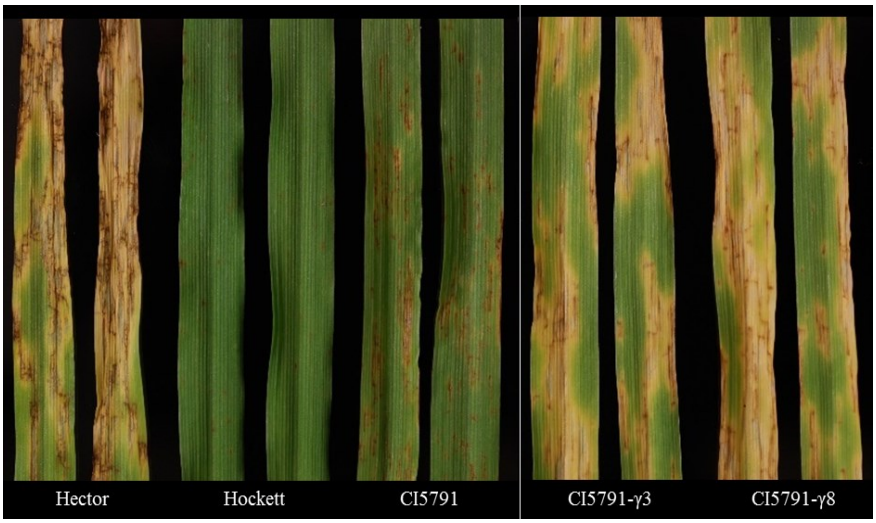

Fig 4.2. Phenotype reactions of Hector, Hockett, CI5791, CI5791-γ3, and CI5791-γ8 with *Ptt* isolate SM36-2. Hockett showed resistant reactions, and CI5791 showed moderately susceptible reactions. Hector, CI5791-γ3, and CI5791-γ8 showed susceptible reactions. The disease was scored based on 1-10 rating scale where 1 is highly resistant and 10 is highly susceptible.

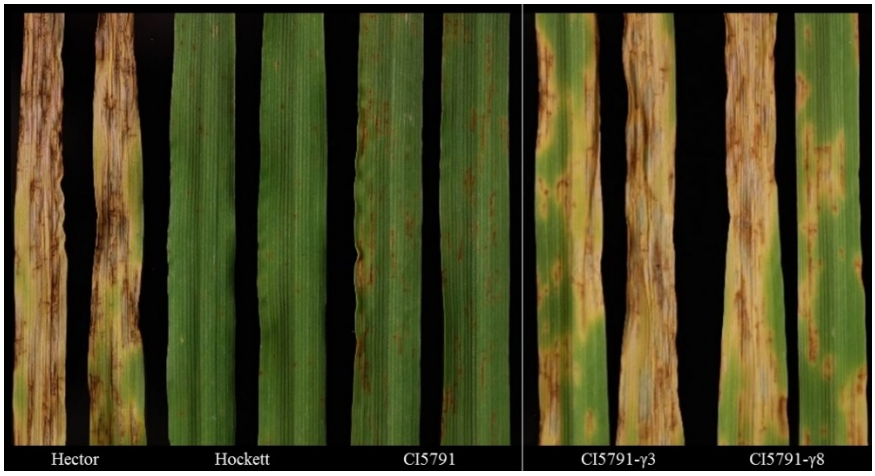

Fig 4.3. Phenotyping reaction of Hector, Hockett, CI5791, CI5791-γ3, and CI5791-γ8 with *Ptt* isolate SM36-3. Hockett showed resistant reactions, and CI5791 showed moderately susceptible reactions. Hector, CI5791-γ3, and CI5791-γ8 showed susceptible reactions. The disease was scored based on 1-10 rating scale where 1 is highly resistant and 10 is highly susceptible.

The validated M_2 mutants, CI5791-γ3 and CI5791-γ8, were crossed with cv Heartland and the F_1 plants were allowed to self to produce a F_2 populations. The F_2 populations, CI5791-γ3 x Heartland and CI5791-γ8 x Heartland, containing 111 and 116 F_2 individuals, respectively, were challenged with *Ptt* isolate 0-1. The phenotype analyses using a 2 reactions type as a cutoff for resistance/susceptibility for CI5791-γ3 x Heartland F_2 with the SFNB 1-5 rating scale and

type 4 reaction type as a cutoff for resistance or susceptibility CI5791-γ8 x Heartland F_2 on the NFNB 1-10 scalec, showed segregation ratios not significantly different from 3 resistant: 1 susceptible as would be expected for a single recessive mutant gene (Table 4.1 and Table B1). This suggested that the mutation that compromised the CI5791 resistance was a single gene in both mutants showing that a single functional gene required for resistance was mutated in each mutant line.

Table 4.1. Segregation of CI5791-γ3 x Heartland and CI5791-γ8 x Heartland F_2 individuals inoculated with *Ptt* isolate 0-1.

Populations	Resistant F_2	Susceptible F_2	χ^2 (3:1)*
CI5791-γ3 F_2s	77	34	1.87
CI5791-γ8 F_2s	89	27	0.26

*Non-significant at p=0.05 level.

Since the F_2 phenotyping data determined that a single recessive mutation was responsible for the susceptible phenotype in both putative independent mutants, reciprocal crosses between CI5791-γ3 and -γ8 were made to determine if these mutated genes were distinct. Six CI5791-γ3 x -γ8 and ten CI5791-γ8 x -γ3 F_1 individuals were challenged with *Ptt* isolate 0-1. It was observed that all the CI5791-γ3 x -γ8 and CI5791-γ8 x -γ3 F_1 individuals showed the susceptible reactions similar to each of the mutant parental lines with an average score of 6.45 and 6.25, respectively using the NFNB 1-10 rating scale (Fig 4.12, Table B3).

Mutant Mapping

The putative homozygous susceptible or homozygous mutant F_2 individuals from the CI5791-γ3 x Heartland population, as determined by their susceptible phenotype showing the characteristic SFNB-like lesions, were genotyped using a PCR-GBS SNP marker panel containing 365 highly polymorphic SNP markers evenly spread throughout the barley genome (Table C1). After utilizing our SNP calling pipeline as described in chapter 3, we identified 123

polymorphic SNP markers spread across the 7 barley chromosomes (Fig 4.4). The QTL mapping utilizing the genotyping of the 34 CI5791-γ3 x Heartland F_2 homozygous susceptible lines, representing 68 recombinant gametes localized the mutation to chromosome 3H within an ~75 cM interval flanked by the SNP markers 11_20742 (POPSEQ position; chr=3H cM=15.15) and 11_21493 (POPSEQ position; chr=3H cM=90.33) (Fig 4.5). The most significant marker 11_10444 (POPSEQ position; chr=3H cM=74.99) had a LOD score of 71.

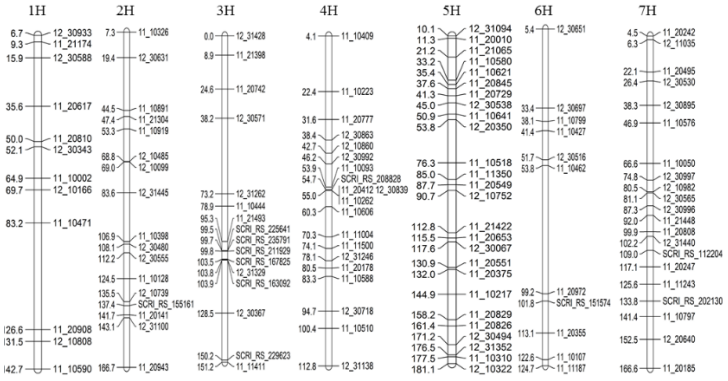

Fig 4.4. Linkage map of 34 CI5791-γ3 x Heartland F_2 susceptible individuals developed with 123 polymorphic SNP markers showing seven barley chromosomes using Qgene software.

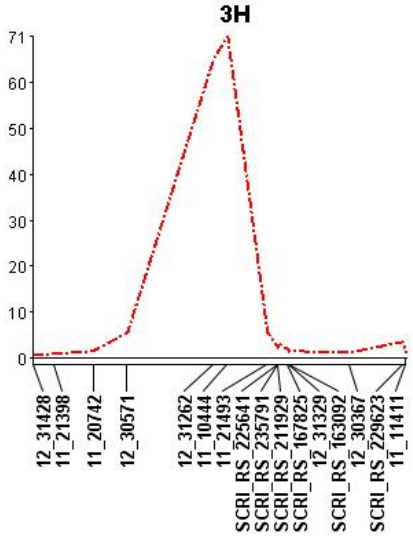

Fig 4.5. The CI5791-γ3 x Heartland F_2 homozygous susceptible lines were used to generate a QTL map of resistance/susceptibility to *Ptt* isolate 0-1 using single marker regression analysis. The Y-axis represents LOD values and X-axis represents the PCR-GBS SNP markers. The most significant marker was 11_10444 (74.99 cM) with a LOD value of 71.

Exome Capture Sequencing and Analysis

Sequencing of wt CI5791, CI5791-γ3 and CI5791-γ8 gDNA enriched via exome capture on a single Illumina NextSeq flowcell resulted in a total of 111,251,482; 103,796,564; and 120,530,567 reads, respectively. This parallel sequencing of the three exome captured genotypes represented a very balanced library. The deletion variant analysis resulted in the identification of a 1 bp deletion in the predicted coding region of the MLOC_68299.2 gene model in CI5791-γ8 that is within the mapped mutant region identified on chr 3H (Fig 4.5). The single base deletion in the MLOC_68299.2 gene model is within the second predicted exon, of the barley paralog of the *Arabidopsis* WRKY transcription factor 6 gene, *HvWrky6*. Coverage analysis and visualization of read alignments showed that MLOC_68299.2 (*HvWrky6*) is completely deleted from the CI5791-γ3 mutant (Fig 4.7).

Characterization of the Candidate Gene

The variant analysis of the exome capture data pinpointed a single base pair deletion in the barley gene model MLOC_68299.2 from the CI5791-γ8 mutant in the second exon, which results in a frame shift and predicted translation of a non-functional 148 amino acid (aa) truncated protein. The analysis also showed that MLOC_68299.2 was completely deleted from the CI5791-γ3 mutant, as no sequence reads mapped to the gene model from the reference sequences. The MLOC_68299.2 gene spans 8,026 bp of genomic DNA localized to barley chr 3H at ~50.7 cM based on POPSEQ positions (Mascher et al., 2013). MLOC_68299.2 is predicted to transcribe a 1,707 nucleotides mRNA consisting of 6 exons (Fig 4.13A) that is predicted to encode a 569 aa functional protein (~59.67 kDa). The MLOC_68299.2 gene model is predicted to contain WRKY transcription factor domains including the highly conserved WRKYGQK DNA binding aa motif (Fig 4.13A). Homology searches utilizing NCBI BLASTP

identified the candidate MLOC_68299.2 predicted protein as an ortholog of the *Arabidopsis* WRKY transcription factor 6, thus, MLOC_68299.2 was designated as *HvWRKY6*. The predicted *HvWRKY6* protein has 50% aa identity and 59% aa similarity with the *Arabidopsis* WRKY6 protein (query cover 89% and e-value $4e^{-130}$) (Fig 4.6). A reciprocal result was obtained when the *AtWRKY6* protein was used as the query on the IPK barley blast server, identifying only one matching WRKY protein corresponding to the candidate MLOC_68299.2 gene. Thus, MLOC_68299.2 appears to represent the only *AtWRKY6* orthalog in the barley genome. Interproscan SMART domain identified a conserved WRKY domain (300-360 aa) in *HvWRKY6* with high confidence prediction (http://smart.embl.de/) (Letunic et al., 2015). Analysis of the full length *HvWRKY6* gene sequence from CI5791, Morex, and Tifang were identical suggesting that the gene is conserved across both resistant and susceptible barley genotypes.

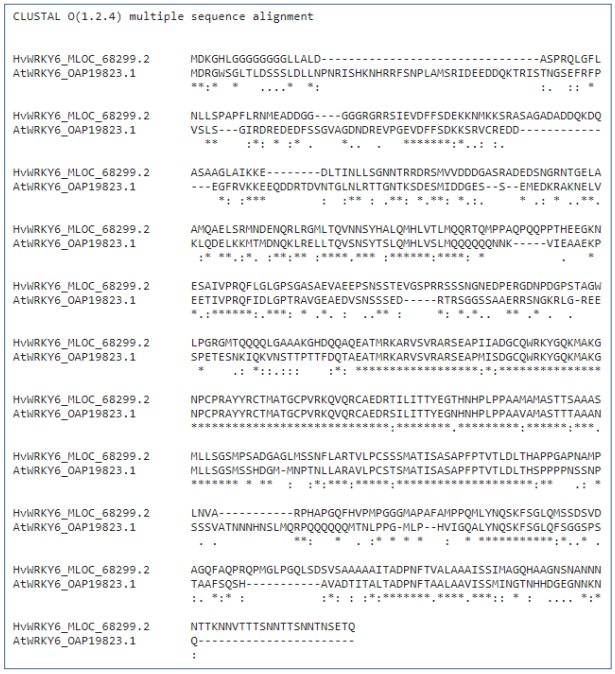

Fig 4.6. Amino acid alignment of *HvWRKY6* and *AtWRKY6.* An * (asterisk) indicates fully conserved residue, A : (colon) indicates conservation between groups of strongly similar properties roughly equivalent to scoring > 0.5 in the Gonnet PAM 250 matrix, and a . (period) indicates conservation between groups of weakly similar properties roughly equivalent to scoring ≤0.5 and > 0 in the Gonnet PAM 250 matrix.

Validation of HvWRKY6 Function in CI5791 NFNB Resistance

The primers (WRKY6-F1 and WRKY6-Mt-R2) designed to specifically produce a *HvWRKY6* amplicon from the CI5791-γ8 mutant utilizing 3' terminus specificity of the WRKY6-Mt-R2 primer to the single nucleotide deletion specifically produced an amplicon from CI5791-γ8 gDNA and did not produce any amplicons from wt CI5791, Heartland or CI5791-γ3 (complete gene deletion) gDNA (Fig 4.7 and 4.8). Whereas, the wild type specific primer pair (WRKY6-F1+WRKY6-Wt-R1) amplified only from wt CI5791 and Heartland (Fig 4.8) with no amplicons produced in either mutant.

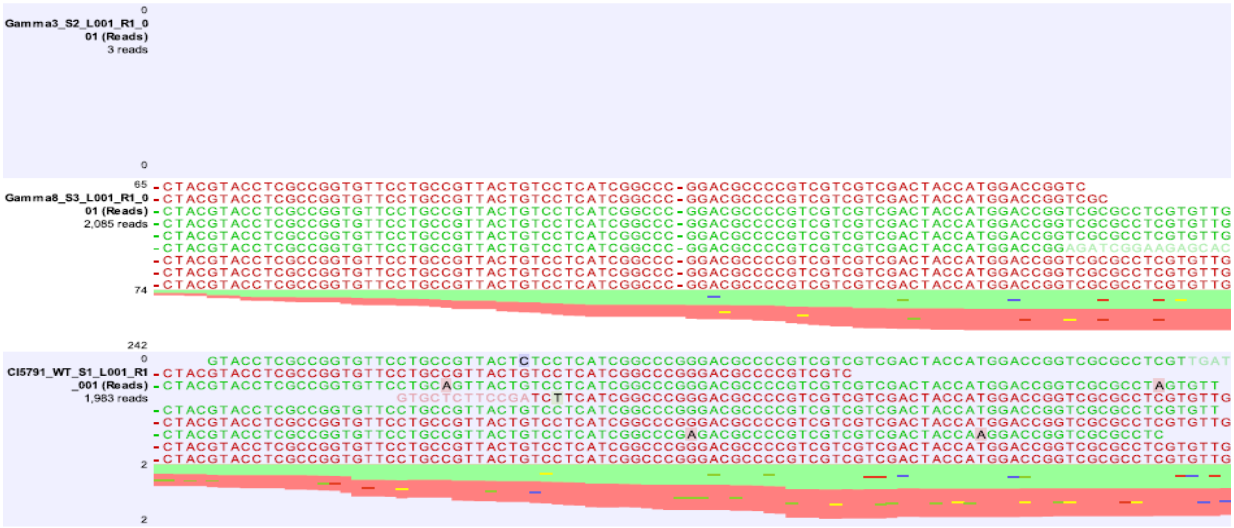

Fig 4.7. Sequence alignment of MLOC_68299.2 sequence reads from CI5791-γ3, CI5791-γ8 and wt CI5791 with the reference genome sequence of cv Morex. There are only 3 reads in CI5791-γ3, and ~2,000 reads in CI5791-γ8 and wt CI5791 confirming a complete gene deletion in CI5791-γ3 and a single bp deletion in CI5791-γ8.

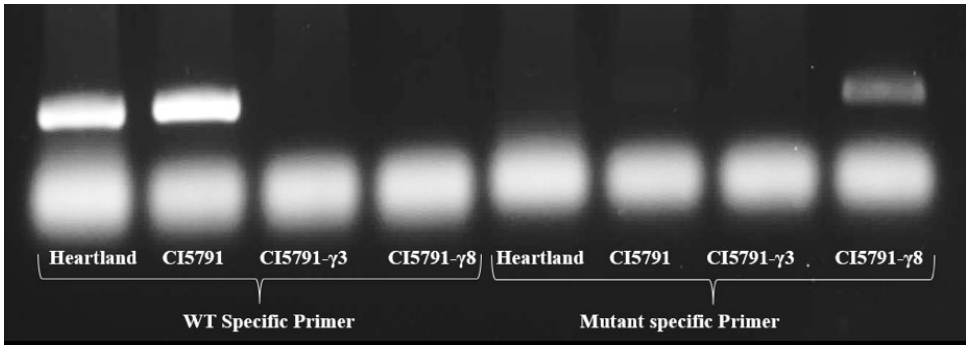

Fig 4.8. Genotyping of Heartland, CI5791, CI5791-γ3, and CI5791-γ8 with wt and CI5791-γ8 mutant specific primers. The wt specific primers amplified only from Heartland and wt CI5791. The mutant specific primers amplified only from CI5791-γ8 and no amplification in CI5791-γ3 as it is missing the entire gene sequence.

All homozygous susceptible F_2 individuals from both the CI5791-γ3 x Heartland and CI5791-γ8 x Heartland populations showed mutant *HvWRKY6* genotype when genotyped with CI5791-γ8 mutant specific primers (WRKY6-F1+WRKY6-Mt-R2) or wt specific primers (Fig 4.9 and 4.10). This was determined by no observed amplification with either primer pair on the CI5791-γ3 x Heartland F_2 individuals which is consistent with the entire gene deletion detected with the exome capture experiment (Fig 4.9). With the CI5791-γ8 x Heartland F_2 individuals there was amplicons produced with the mutant specific primer pair (WRKY6-F1+WRKY6-Mt-R2) but no observed amplification with the wt specific primer pair, which is consistent with 1 bp deletion detected with the exome capture experiment (Fig 4.10). Fifteen randomly selected resistant F_2 individuals from the CI5791-γ8 x Heartland showed a 1 homozygous: 2 heterozygous genotype segregations (Fig 4.11). This genotyping perfectly linked the genetic mutation with the mutant phenotype in this small F_2 population representing 68 recombinant gametes. Also, the genotypes of all the reciprocal F_1 (CI5791-γ3 x CI5791-γ8 or CI5791-γ8 x CI5791-γ3) individuals had CI5791-γ8 mutant like genotype, lacking a wt allele, further providing the evidence that these two mutants CI5791-γ3 and CI5791-γ8 are allelic to each other (Fig 4.1 and 4.12).

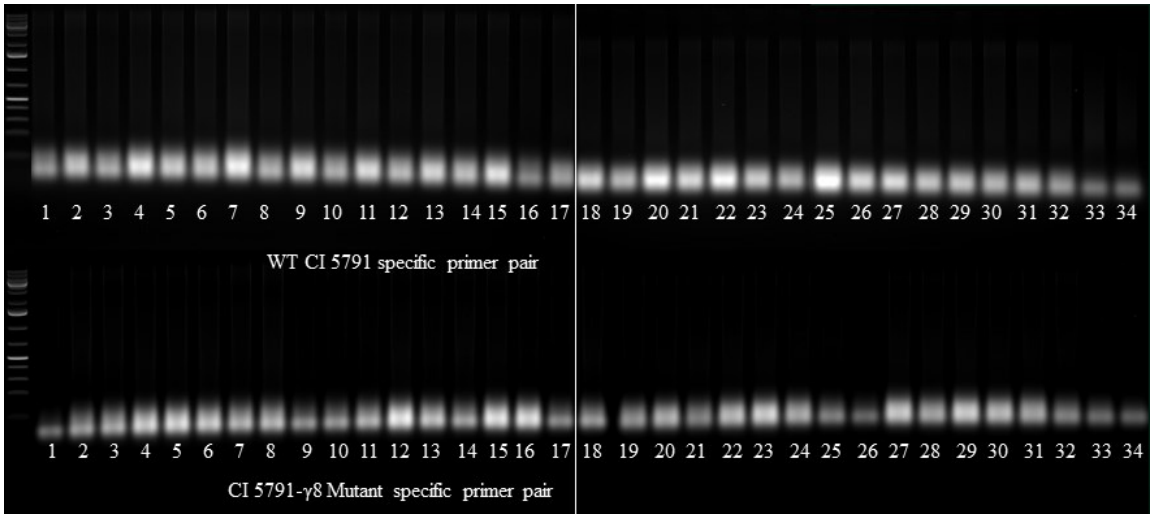

Fig 4.9. Genotyping of 34 susceptible F_2 individuals of the CI5791-γ3 x Heartland with wild type specific (top part) and mutant specific primers (bottom part). No amplification was observed with either primer pairs on all susceptible F_2 individuals, which is consistent with the entire gene deletion detected in CI5791-γ3.

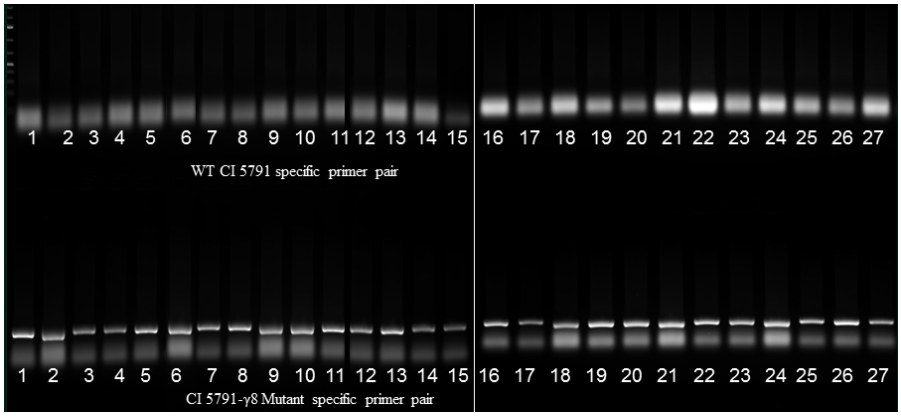

Fig 4.10. Genotyping of 27 susceptible F_2 individuals of the CI5791-γ8 x Heartland with wild type specific (top part) and mutant specific primers (bottom part). WT primer pairs did not amplify from any of the 27 individuals whereas the CI5791-γ8 mutant primer pair amplified from all 27 indviduals suggesting that the CI5791-γ8 mutation corresponds with the mutant phenotype.

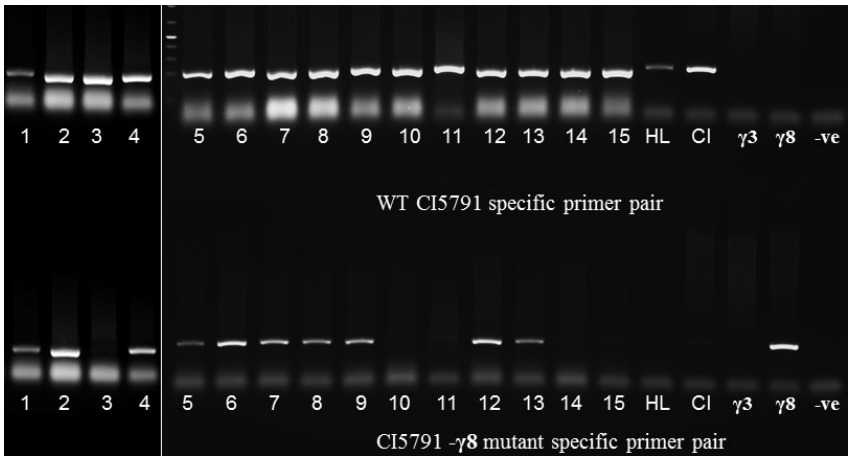

Fig 4.11. Genotyping of 15 CI5791-γ8 x Heartland F_2 resistant individuals with the primer pair specific to 1 bp deletion within MLOC_68299.2 in the CI5791-γ8 mutant which segregated in 1 homozgyous to 2 heterozygous resistant individuals. Sample 3, 10, 11, 14, and 15 are homozygous resistant and the remaining 10 are heterozygous resistant F_2 individuals. CI5791, Heartland, CI5791-γ3, and CI5791-γ8 were also genotyped as a positive control.

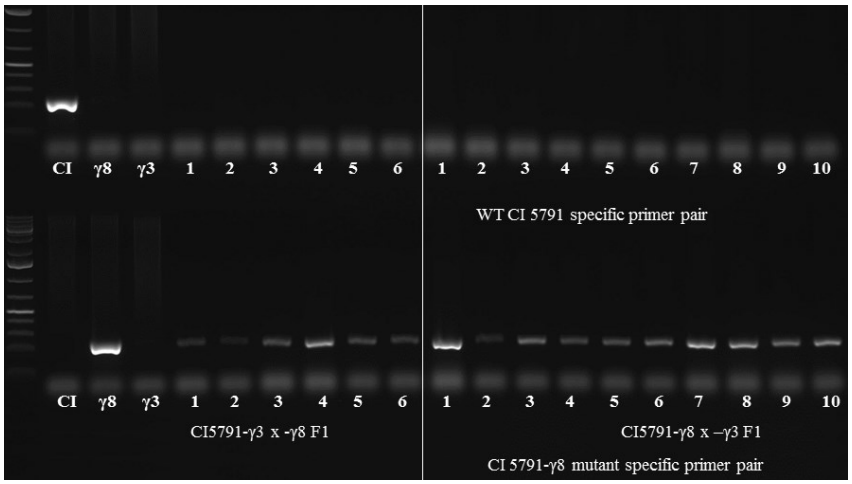

Fig 4.12. Genotyping of 6 CI5791-γ3 x-γ8 F_1 (left) and 10 CI5791-γ8 x-γ3 F_1 (right) individuals with wild type (wt) specific (top part) and mutant specific primers (bottom part). The wt primer pair did not produce amplicons from any of the F_1 individuals (top) whereas the CI5791-γ8 mutant specific primer pair produced amplicons from all F_1 indviduals (bottom) supporting the the conclusion that the two independent mutants are allelic.

BSMV-VIGS

The barley stripe mosaic virus (BSMV) tripartite genome was utilized to develop *HvWRKY6* post transcriptional gene silencing constructs (Fig 4.13B). The disease reaction in the BSMV-*WRKY6* inoculated plants targeted for post transcriptional gene silencing of *HvWRKY6*

were significantly more susceptible than the BSMV-*pBS* virus inoculated controls at both 7 and 12 days post inoculation (dpi) when inoculated with *Ptt* isolate 0-1 (Fig 4.13C, Table 4.2). The BSMV-*pBs* virus control inoculations did not show the shift from resistance towards susceptibility. However, qPCR examining *HvWRKY6* transcript levels did not show a significant difference in *HvWRKY6* gene expression between the BSMV- *WRKY6* and BSMV-*pBS* inoculated plants post NFNB reading.

Table 4.2. Phenotypic analysis of BSMBV-VIGS plants inoculated with *Ptt* isolate 0-1.

Days post inoculation (dpi)	VIGS Knockdown	VIGS Control	Pr > |t|
7 dpi	4.67 ± 1.17	3.06 ± 1.09	<.0001
12 dpi	6.00 ± 1.79	2.99 ± 1.23	<.0001

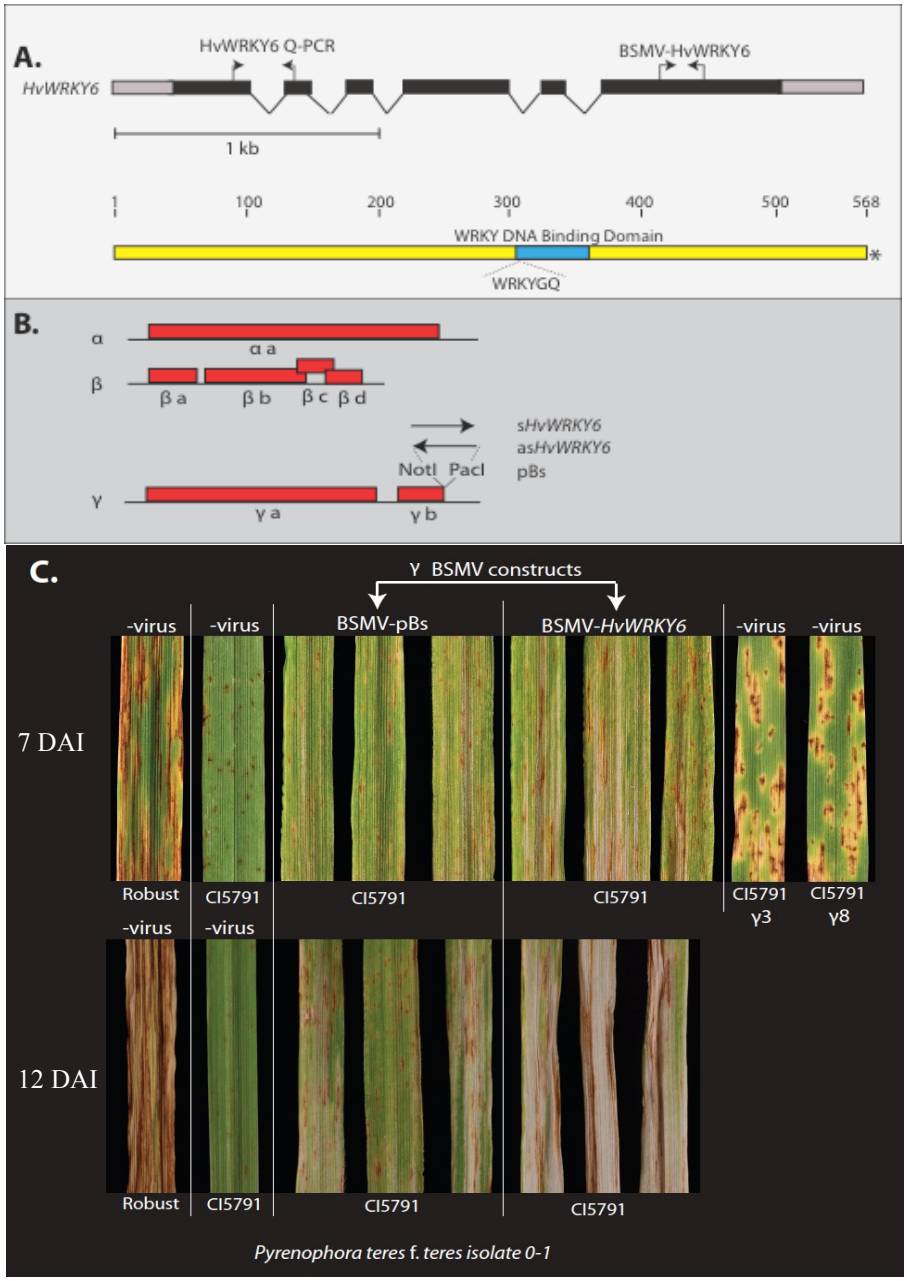

Fig 4.13. A. *HvWRKY6* transcription factor gene and protein structure showing location of primers used for qPCR and BSMV-VIGS constructs. **B.** Barley stripe mosaic virus tripartite genome utilized to develop *HvWRKY6* post transcriptional gene silencing constructs. **C.** Results of BSMV-VIGS experiments showing that the specific silencing of the candidate *HvWRKY6* gene results in susceptible reaction when inoculated with *P. teres* f. *teres* isolate 0-1. The BSMV-VIGS pBs vector control does not show the shift from resistance towards susceptibility.

qPCR

The qPCR experiment conducted on wt CI5791 inoculated with the *Ptt* isolate 0-1 showed that *HvWRKY6* is upregulated at 4 hours post inoculation (hpi) at least 5 fold until 6 hpi,

then it gradually decreases and maintains a level of ~1-fold upregulation until 168 hpi (Fig 4.14). With the Morrocan *Ptt* isolate SM36-3, which is moderately virulent on CI5791, *HvWRKY6* was upregulated 1 hpi by 1.6 fold and increases to 12.6 fold upregulation at 4 hpi, 4 fold upregulation at 6 hpi, 16 fold at 12 hpi, and maintained at least 5 fold upregulation after 96 through 168 hpi (Fig 4.14). When susceptible cultivar Tifang was challenged with *Ptt* isolate 0-1, *HvWRKY6* was upregulated 2.8 folds at 30 minutes post inoculation to 22 fold at 6 hpi, 4.8 fold at 12 hpi, 9 folds at 24 hpi, and maintained at least 8 folds after 96 through 168 hpi (Fig 4.14). The qPCR analyses confirmed that the expression of *HvWRKY6* in line CI5791 was significantly higher with the moderately virulent *Ptt* isolate 36-3 than the avirulent isolate 0-1 between 1-4hrs, and 96 -168 hrs. Similarly, the expression of *HvWRKY6* in the susceptible line Tifang challenged with the virulent *Ptt* isolate 0-1 was much higher and significantly different than 36-3 and 0-1 on CI5791 at the times between 30 min through 2 hrs and 96 hrs through 168 hrs. This suggested that the *HvWRKY6* gene may function as a component of signaling pathway which is required for resistance by restricting the lesion growth in CI5791 and mediates defense response when not or slightly expressed whereas acts as a negative regulator of the plant basal defense response when highly expressed. This result of negative regulator of plant defense response when highly expressed is consistence with previous studies where overexpression of WRKY TF function as negative regulator of the plant defense response (Journot-Catalino et al., 2006, Li et al., 2004, Mao et al., 2007, Kim et al., 2008, Xing et al., 2008).

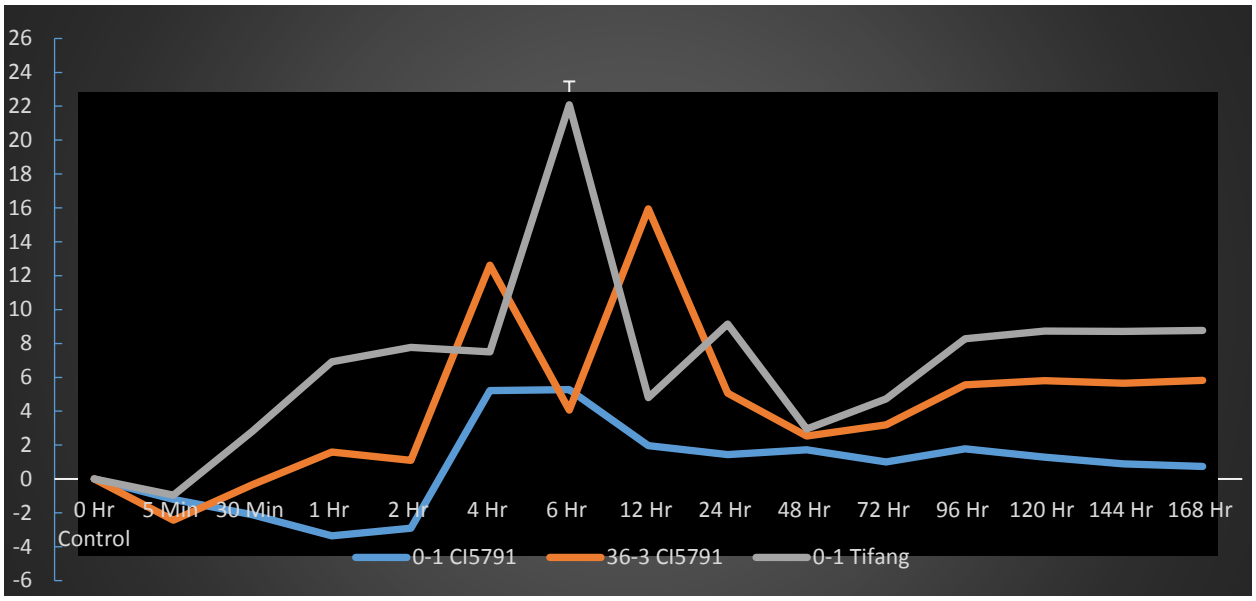

Fig 4.14. Relative expression of *HvWRKY6* in resistant cv CI5791 inoculated with *Ptt* isolate 0-1 (blue) and moderatly virulent isolate SM36-3 (orange); and the susceptible cv Tifang inoculated with *Ptt* isolate 0-1 (Gray). Y-axis represents the fold change and x-axis represents the time point at which the leaf samples were collected. Overall, the expression of *HvWRKY6* is higher during the Tifang compatible reaction challenged with isolate 0-1 compared to CI5791 with isolate 0-1 and SM36-3.

Discussion

Utilizing forward genetics, genetic mapping, and mapping-by-sequencing, the CI5791-γ3 and CI5791-γ8 mutation was localized to a region of barley chromosome 3H and a candidate gene identified based on two independent mutations, a single bp and whole gene deletion, in the CI5791-γ8 and CI5791-γ3 mutants, respectively, using exome capture. The 1 bp and whole gene deletion are in line with the reports that γ -irradiation cause 1 to 10 kb deletions (Morita et al., 2009). The independent mutations underlying the mutant phenotype were within the *HvWRKY6* gene, an ortholog of the *Arabidopsis* WRKY TF 6. We further validated the gene via post transcriptional gene silencing as being required for the high level of CI5791 resistance, thus, we hypothesize that *HvWRKY6* is required for NFNB resistance responses.

The two independent mutants CI5791-γ3 and CI5791-γ8 exhibited susceptible symptoms to *Ptt* isolates that are not typical NFNB symptoms, but rather resemble SFNB lesions when inoculated with the *Ptt* isolates LDNPtt-19 and 0-1. The symptoms exhibited on the mutants are dark brown elliptical necrotic lesions that are surrounded by an expanding yellow chlorotic margin. The chlorosis expands and eventually coalesces with other lesions suggesting underlying pathogen growth, yet the necrotic regions remain relatively confined and elliptical resembling a SFNB type of susceptible reaction. Therefore, we initially phenotyped CI5791-γ3 x Heartland F_2 population using a 1-5 SFNB rating scale as described in Neupane et al., (2015). However, the CI5791-γ8 x Heartland F_2 population, BSMV-VIGS experiment, and CI5791-γ3 x CI5791-γ8 and reciprocal cross F_1s were phenotyped using the NFNB 1-10 rating scale as described by Tekauz et al., (1985) as this mutant is related with NFNB disease resistance/ susceptibility. Interestingly, the mutant symptoms when inoculated with the two Moroccan isolates resemble typical net type lesions with enlarged chlorosis that coalesced and longitudinal and vertical striation visible but not as prominent as seen in typical NFNB susceptible interactions. Thus, we speculate that the *HvWRKY6* transcription factor may function in restricting the growth of the pathogen. In a typical CI5791 resistance reaction the pathogen apparently penetrates the host as indicated by the formation of the pin point lesions, yet the pathogen growth is arrested early in the infection process and the lesion growth is stopped. This CI5791 major resistance gene maps to the centromeric region of chromosome 6H, which was the initial gene we were targeting in the mutant screening and we suspected would represent some form of immunity receptor. However, the first two mutants we identified were allelic and mapped to the 3H QTL locus and appear to be transcription factors that could play a role in arresting pathogen colonization and spread.

Necrotrophic pathogens often produce several host specific necrotrophic effectors (NE) including low molecular weight metabolites and small secreted proteins that interact with dominant host susceptibility genes (Wolpert et al., 2002, Liu et al 2011, Stergiopoulos et al., 2013, Liu et al., 2012 and 2015, Shjerve et al., 2014). These interactions often follow the inverse-gene-for-gene model triggering programmed cell death (PCD) to facilitate necrotrophic fungal growth resulting in compatible interactions or a susceptible reaction called necrotrophic-effector triggered susceptibility (NETS) (Friesen and Faris 2010, Faris et al., 2010, Liu et al., 2015). *Ptt* is a necrotrophic pathogen that has been shown to produce proteinaceous effector designated as PttNE1 that targets dominant susceptibility gene/s on chr 6H in barley (Liu et al., 2015) in an inverse gene-for-gene manner resulting in NETS. However, the CI5791 dominant resistance mechanism appears to follow the gene-for-gene model and possibly represents an R-gene-Avr gene interaction that results in an early dominant resistance response. The *HvWRKY6* gene appears to be a highly conserved transcription factor that is required for arresting pathogen spread after penetration. Thus, we hypothesize that it may be activated early in the response providing early resistance, which translates into preventing further proliferation of the fungus after penetration and thereby limiting the growth of the lesions. *Ptt* 36-2 and 36-3 isolates may have produced some other virulence effectors or have a variable avr gene that evades early recognition and activation of the resistance signaling pathway resulting in a moderately susceptible reaction in CI5791. Yet, this more prolific early pathogen growth and the chlorosis results in a high level of susceptibility in the *HvWRKY6* mutants suggesting that *HvWRKY6* plays a role in sequestering pathogen spread after penetration and pathogen establishment in the host.

The qPCR analysis was performed because there appeared to be no polymorphism in the primary amino acid sequence of the *HvWRKY6* protein from a small number of resistant and

susceptible genotypes, suggesting that functional polymorphism between resistant and susceptible interactions may be occurring at a differential transcription level. The qPCR data showed that differential expression of *HvWRKY6* occurrs in the barley line CI5791 in response to pathogen challenge as early as 1 hpi with the moderately virulent *Ptt* isolates 36-3 and 4 hpi with the avirulent *Ptt* isolate 0-1. Similary, in the susceptible cultivar Tifang, the differential expression occurred as early as 30 minutes after inoculation and reached a maximum of 22 fold upregulation at 6 hpi. Overall, the expression level in the susceptible cultivar Tifang with *Ptt* isolate 0-1 was significantly higher than CI5791 with 0-1 and 36-3. BSMV-VIGS experiment showed the specific silencing of the candidate *HvWRKY6* gene results in a susceptible phenotype when inoculated with *Ptt* isolate 0-1, however, VIGS qPCR result showed no significant differences in *HvWRKY6* gene suppression/ expression in both the BSMV- *HvWRKY6* or BSMV-*pBs* control virus post transcriptional gene silencing plants. However, BSMV-VIGS experiments are notorious for inconsistent silencing levels, thus these expression analyses need to be repeated with more biological replications.

The data generated in this study shows that the *HvWRKY6* gene functions in NFNB resistance and probably plays a role in the activation of defense genes that are required to restrict lesion growth. However, the data showing that the gene is expressed at higher levels at the later time points in compatible interactions shows that the differential upregulation of this gene does not correlate with resistance. The loss of function in mutants suggested a positive role of *HvWRKY6* but the time course qRCR analysis doesn't suggest a role of differential regulation describing its positive role. Since, the disruption of the *HvWRKY6* gene produces a predicted non-functional protein in the two independent mutants, which were susceptible to *Ptt* isolate 0-1 we must posite a predominantely positive role of *HvWRKY6* in the NFNB resistance responses.

Thus, it is likely that *HvWRKY6* is involved in a basal resistance and doesn't represent the gene that underlies the 3H dominant resistance QTL reported by Koladia et al., (2017) as there appears to be no primary gene polymorphism between CI5791 and Tifang or expression differences that could explain its functional polymorphism in a CI5791 x Tifang population, but the possibility can't be ruled out.

Several studies have provided evidence that WRKY TFs are an integral part of the plant immune system including roles in PTI, ETI, and systemic acquired resistance (SAR) (Eulgem and Somssich, 2007, Li et al., 2004, Rushton et al., 2010). Certain WRKY DNA-binding factor may serve as a component in a signal transduction pathway in plant cells in response to pathogens and regulate the expression of certain plant defense genes (Reichmann et al., 2000). *AtWRKY6* regulates both plant defense responses against *Pseudomonas syringae* pv. *tomato* as well as senescence in *Arabidopsis,* which regulates the *SIRK* gene (Senescence-Induced Receptor like serine/threonine protein Kinase) that encodes a receptor-like kinase that is exclusively localized to the plant cell nucleus (Robatzek et al., 2002). In wheat, *TaWRKY70* TF was identified as a strong candidate gene within Fusarium head blight *QTL-2DL* that governs resistance against *Fusarium graminarium* by regulating the three downstream resistance genes *TaACT*, *TaDGK*, and *TaGLI* (Kage et al., 2017). Thus, we hypothesize that *HvWRKY6* may regulate other defense related genes that are required to restrict pathogen/ lesion growth.

Thus, utilizing forward genetics, mapping-by-sequencing, exome capture and next generation sequencing data, we identified the *HvWRKY6* gene that is required for NFNB resistance in barley line CI5791. We propose that the *HvWRKY6* transcription factor positively functions to regulate defense response genes, which are required for resistance in CI5791 to delimit the growth of the pathogen in the host.

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CHAPTER 5. CONCLUSION

Net blotch is an economically important foliar disease of barley that occurs in two distinct forms: Spot Form Net blotch (SFNB) and Net Form Net Blotch (NFNB) caused by *Pyrenophora teres* f. *maculata* (*Ptm*) and *Pyrenophora teres* f. *teres* (*Ptt*), respectively. This disease has a potential to cause an average yield loss of 10-40%. Chemical fungicides and cultural practices can help manage the disease; yet host resistance is the most economic method of managing this disease to reduce yield and quality losses. However, historically Upper Midwestern barley breeding programs have devoted less time and limited resources into deploying resistances to both forms of net blotch because it was not considered economically important to barley production as compared to other diseases like fusarium head blight, spot blotch and stem rust. Thus, the commercial cultivars grown in the region are moderately susceptible to susceptible to both of these diseases, which are current threats to barley production in the Northern Great Plains (ND, Montana, and Eastern Idaho) and Canada. The presence of highly virulent isolates is being detected in this region compared to isolates collected from other regions around the world. Therefore, it is critical to identify and deploy resistance against net blotch in this region to minimize the threat posed by these two diseases.

Genome-wide association mapping was conducted on 2,062 world barley core collection accessions which were phenotyped at the seedling stage with 4 geographically distinct *Ptm* isolates collected from the United States (FGO), New Zealand (NZKF2), Australia (SG1), and Denmark (DEN 2.6 and genotyped with the 9k Illumina barley iSelect chip. The association mapping study identified 27 distinct loci associated with SFNB resistance/ susceptibility of which 6 loci were previously reported and the remaining 21 were novel loci representing a broad spectrum of resistance/ susceptibility loci. Further, the two higly resistant lines PI67381 (2-

rowed) and PI84314 (6-rowed) that were identified from the world barley core collections were crossed with two popular malting cultivars Tradition (6-rowed) and Pinnacle (2-rowed) grown in the Upper Midwestern US. Three recombinant inbred line (RIL) mapping populations of Tradition x PI67381, Pinnacle x PI67381 and Pinnacle x PI84314 were developed. The RIL populations were genotyped using PCR-GBS and phenotyped at the seedling stage with six diverse SFNB isolates from the United States (FGO, PA14 and CA17), New Zealand (NZKF2), Australia (SG1), and Denmark (DEN 2.6). QTL analysis identified a total of twelve quantitative trait loci (QTL) on chromosome 2H, 3H, 4H, 6H and 7H, of which nine were previously reported and the remaining three QTL: *QRptm-2H-77-83, QRptm-2H-141-152*, and *QRptm-7H-92-95* are novel. These resistances and the markers delimiting the QTL are being utilized to develop prebreeding lines by introgressing SFNB resistance into the cultivars Pinnacle and Tradition utilizing marker assisted selection.

The barley line CI5791 exhibits a high level of resistance to diverse *Ptt* isolates collected from around the world. To identify genes involved in this CI5791 resistance, a forward genetic approach was used to identify two mutants designated as CI5791-γ3 and CI5791-γ8 by phentotyping with *Ptt* isolates. The mutation was mapped to chr 3H in CI5791-γ3 x Heartland F_2 population. Exome capture mediated mapping-by-sequencing identified a candidate *HvWRKY6* transcription factor gene required for NFNB resistance. We hypothesize that the *HvWRKY6* gene function as a component of a resistance pathway, which is required for resistance by restricting lesion growth in CI5791. The resistance/susceptibility loci identified in this study will facilitate the development of net blotch resistant cultivars.

APPENDIX A. DISEASE PHENOTYPE OF THREE RIL POPULATIONS TO SIX GEOGRAPHICALLY DIVERSE *P. TERES* F. *MACULATA* ISOLATES

Table A1. The average seedling infection types for the Tradition X PI67381 RIL population with the global collection of *P. teres f. maculata* isolates using a 1-5 rating scale (Neupane et al., 2015).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
1	T67381-1	1.8	2.0	1.7	1.8	1.7	1.7
2	T67381-2	3.2	3.3	3.3	2.8	2.3	2.0
3	T67381-3	2.2	1.8	1.7	2.0	1.8	1.8
4	T67381-4	2.0	2.3	2.3	2.2	1.7	1.5
5	T67381-5	1.8	2.0	1.7	1.7	1.7	1.0
6	T67381-6	1.3	1.2	1.0	1.2	1.5	1.0
7	T67381-7	3.7	3.2	3.3	2.5	2.0	1.5
8	T67381-9	3.0	3.3	3.3	2.7	1.7	2.0
9	T67381-10	1.8	2.3	2.3	2.0	1.2	1.2
10	T67381-11	2.5	3.0	3.0	2.3	1.8	2.0
11	T67381-12	3.0	3.0	3.0	3.2	2.2	1.8
12	T67381-13	2.2	2.5	2.7	2.3	2.0	2.0
13	T67381-14	1.7	1.5	1.7	1.2	1.7	1.5
14	T67381-16	2.8	2.8	2.8	2.5	1.0	1.5
15	T67381-17	1.3	2.0	2.0	1.5	1.7	2.0
16	T67381-18	2.8	3.2	3.0	2.5	1.3	2.0
17	T67381-19	2.8	3.5	3.5	2.8	2.0	1.7
18	T67381-21	2.7	2.7	2.3	2.0	2.0	1.7
19	T67381-22	2.2	2.8	2.7	2.2	1.8	1.3
20	T67381-23	2.5	3.0	2.3	2.3	2.2	1.8
21	T67381-24	1.3	1.3	1.2	1.2	1.7	1.0
22	T67381-25	2.3	3.0	2.5	2.5	1.3	1.3
23	T67381-26	1.7	1.8	1.8	1.8	1.8	1.7
24	T67381-27	3.2	3.2	3.0	2.5	1.7	1.3
25	T67381-28	1.8	2.0	2.0	1.3	2.2	2.2
26	T67381-29	3.0	2.8	2.8	2.5	1.3	1.0
27	T67381-30	2.8	3.3	3.2	2.8	1.8	1.7
28	T67381-31	2.8	3.7	3.2	2.5	1.8	1.5
29	T67381-32	2.3	3.0	2.7	2.5	1.7	1.3
30	T67381-33	2.2	2.3	2.3	2.3	1.2	1.3
31	T67381-34	1.7	1.7	1.7	1.5	2.0	2.0
32	T67381-37	2.3	2.8	3.0	2.2	1.5	1.7
33	T67381-38	3.0	3.2	3.0	2.5	2.2	2.3
34	T67381-39	1.7	2.0	1.8	1.3	1.7	1.7
35	T67381-40	3.3	3.8	3.0	2.5	2.2	1.8
36	T67381-41	2.8	3.0	3.0	2.5	1.7	2.0
37	T67381-42	3.0	3.0	3.0	2.3	1.8	1.8

Table A1. The average seedling infection types for the Tradition X PI67381 RIL population with the global collection of *P. teres f. maculata* isolates using a 1-5 rating scale (Neupane et al., 2015) (continued).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
38	T67381-43	2.7	2.3	2.5	2.0	1.8	1.5
39	T67381-44	2.0	1.8	2.2	1.7	1.5	2.0
40	T67381-45	2.8	3.3	3.0	2.7	1.5	1.8
41	T67381-46	2.3	3.0	2.8	2.2	1.7	1.5
42	T67381-47	2.0	2.2	2.0	1.7	1.5	1.3
43	T67381-48	2.5	2.8	2.7	2.5	1.5	1.8
44	T67381-49	3.0	3.2	3.3	2.7	1.7	2.0
45	T67381-50	1.7	1.7	1.5	1.2	1.3	1.5
46	T67381-51	2.2	2.0	1.8	1.5	1.8	1.5
47	T67381-52	3.2	3.5	3.2	3.0	1.3	2.0
48	T67381-53	1.5	2.0	2.0	1.2	1.5	1.7
49	T67381-54	2.7	3.5	3.0	2.7	1.5	1.7
50	T67381-55	1.8	1.7	1.5	1.5	1.2	1.5
51	T67381-56	2.2	2.0	1.8	1.7	1.7	2.0
52	T67381-57	3.0	3.0	3.0	2.5	1.7	1.5
53	T67381-58	1.8	2.2	2.0	1.5	1.5	1.7
54	T67381-59	1.5	1.5	1.5	1.8	2.0	1.5
55	T67381-60	2.0	2.3	2.3	1.5	1.3	1.7
56	T67381-61	2.0	2.3	2.7	2.3	1.8	1.8
57	T67381-62	3.0	3.3	3.3	2.5	1.8	2.0
58	T67381-63	1.8	2.0	1.8	2.0	1.8	2.2
59	T67381-64	1.8	1.8	2.0	1.7	2.0	1.8
60	T67381-65	1.8	1.8	1.8	1.8	1.8	2.0
61	T67381-66	2.7	2.7	3.2	2.5	2.0	1.7
62	T67381-67	1.8	1.8	1.7	1.7	2.3	2.0
63	T67381-68	2.0	1.8	1.7	1.5	1.8	1.7
64	T67381-69	1.8	1.8	1.8	1.8	1.7	1.5
65	T67381-72	2.7	2.8	2.7	2.5	1.8	1.5
66	T67381-73	1.8	2.3	2.3	2.2	1.2	1.2
67	T67381-74	1.5	1.2	1.3	1.2	1.0	1.3
68	T67381-75	2.3	3.0	2.8	2.5	1.5	1.0
69	T67381-76	2.8	3.0	3.0	2.5	1.7	1.3
70	T67381-77	1.8	1.8	1.7	1.3	2.0	1.7
71	T67381-78	2.0	1.7	1.8	1.8	1.7	2.2
72	T67381-79	1.7	1.7	2.0	1.3	1.3	1.2
73	T67381-80	1.5	1.2	1.3	1.3	1.8	1.7
74	T67381-81	2.7	2.8	2.3	2.7	1.7	1.5
75	T67381-82	2.3	2.8	2.8	1.8	2.2	1.8
76	T67381-83	3.0	3.3	3.0	3.0	1.7	1.7
77	T67381-85	3.2	3.3	3.0	2.7	1.8	1.7
78	T67381-86	1.5	1.7	1.5	1.2	1.2	1.5
79	T67381-87	3.0	3.0	3.0	2.5	1.7	1.5

Table A1. The average seedling infection types for the Tradition X PI67381 RIL population with the global collection of *P. teres f. maculata* isolates using a 1-5 rating scale (Neupane et al., 2015) (continued).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
80	T67381-88	2.0	2.2	2.0	1.5	1.7	1.5
81	T67381-89	1.7	1.8	1.5	1.8	2.0	1.5
82	T67381-90	1.7	2.0	1.8	1.7	1.5	1.2
83	T67381-91	1.5	1.8	2.0	1.7	1.3	1.0
84	T67381-92	2.5	2.5	2.8	2.5	1.2	1.2
85	T67381-93	3.0	3.2	3.0	3.0	2.0	1.5
86	T67381-94	3.0	3.3	3.0	2.5	1.3	1.5
87	T67381-97	3.0	3.3	2.8	2.8	2.2	1.7
88	T67381-98	2.2	2.0	2.0	1.7	1.3	1.5
89	T67381-100	2.5	3.0	3.0	2.2	2.0	1.5
90	T67381-101	2.8	3.3	3.0	2.8	1.5	1.2
91	T67381-102	2.8	3.0	3.0	2.5	1.5	1.3
92	T67381-103	1.7	1.3	1.5	1.3	1.8	1.7
93	T67381-104	2.7	3.0	3.0	2.5	1.8	1.3
94	T67381-105	3.0	2.7	2.8	2.7	2.0	1.7
95	T67381-106	2.0	2.0	2.0	1.5	1.7	1.3
96	T67381-107	2.1	2.5	2.5	2.0	1.7	1.3
97	T67381-109	2.8	3.3	3.0	3.0	1.8	1.8
98	T67381-110	2.3	2.5	2.5	2.2	1.8	1.8
99	T67381-111	1.7	1.5	1.7	1.5	1.8	1.5
100	T67381-112	2.5	2.5	2.5	2.3	1.5	1.3
101	T67381-113	2.0	1.5	1.8	1.7	2.0	1.5
102	T67381-114	1.5	1.5	1.3	1.8	1.5	1.5
103	T67381-115	1.8	2.5	2.2	2.2	1.7	1.3
104	T67381-117	2.0	2.0	1.8	2.0	2.0	1.8
105	T67381-118	3.3	3.0	3.0	2.3	1.3	1.3
106	T67381-119	1.3	1.2	1.0	1.3	1.2	1.2
107	T67381-120	2.2	2.2	2.0	1.8	1.8	1.7
108	T67381-121	2.7	3.0	2.8	2.7	1.5	1.7
109	T67381-122	2.3	2.7	2.3	2.2	2.2	2.0
110	T67381-123	3.0	3.0	3.0	2.5	1.3	1.3
111	T67381-124	2.0	1.8	1.7	1.5	1.8	2.0
112	T67381-125	2.7	2.5	2.3	2.7	2.0	1.5
113	T67381-126	2.2	2.5	2.3	2.3	1.2	1.2
114	T67381-127	1.8	2.0	1.8	1.3	1.3	1.3
115	T67381-128	1.7	1.8	2.0	1.5	1.5	1.7
116	T67381-129	2.0	2.0	2.0	1.7	2.0	1.8
117	T67381-132	1.7	1.8	1.8	2.0	2.0	1.3
118	T67381-133	2.0	2.0	2.0	2.0	1.7	1.5
119	T67381-134	2.0	2.3	2.2	1.8	1.5	1.2
120	T67381-135	2.5	3.0	2.5	2.3	1.5	1.5
121	Tradition	3.0	3.0	3.0	2.7	2.3	2.3
122	PI67381	1.5	1.5	1.3	1.5	1.3	1.2

Table A2. The average seedling infection types for the Pinnacle X PI67381 RIL population with the global collection of *P. teres* f. *maculata* isolates using a 1-5 rating scale (Neupane et al., 2015).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
1	P67381-1	2.8	2.8	2.8	2.0	1.7	1.5
2	P67381-2	2.0	1.7	2.0	1.8	1.5	1.2
3	P67381-3	3.0	3.0	3.0	2.7	2.0	1.8
4	P67381-4	2.5	2.0	2.0	2.2	2.2	1.5
5	P67381-5	3.7	3.3	3.0	3.2	2.3	2.0
6	P67381-6	3.0	2.5	2.8	3.0	1.8	1.5
7	P67381-7	2.2	-	-	-	1.5	1.8
8	P67381-8	2.8	3.0	3.0	2.7	1.7	1.7
9	P67381-9	2.7	2.5	2.7	2.8	1.3	1.0
10	P67381-10	2.5	2.0	2.0	2.0	1.8	1.7
11	P67381-11	2.0	1.5	1.7	1.7	1.3	1.2
12	P67381-12	2.7	1.8	2.2	2.2	1.7	2.0
13	P67381-13	2.8	2.5	3.0	3.0	2.0	1.5
14	P67381-14	3.2	2.3	2.8	2.5	1.5	1.5
15	P67381-15	2.2	2.0	1.5	2.0	1.3	1.3
16	P67381-16	1.5	-	-	-	2.0	1.8
17	P67381-17	4.0	3.3	3.5	3.0	2.3	1.8
18	P67381-18	3.5	-	2.0	-	-	2.0
19	P67381-19	2.2	2.5	2.0	2.7	1.2	1.5
20	P67381-20	2.7	2.2	2.5	2.2	2.3	1.8
21	P67381-21	2.7	2.3	2.3	2.7	1.8	1.7
22	P67381-22	3.3	3.3	3.0	3.2	2.3	1.5
23	P67381-23	3.2	2.7	2.5	3.3	1.5	1.2
24	P67381-24	2.7	2.8	3.0	2.8	1.8	1.7
25	P67381-25	2.3	2.0	2.0	2.0	1.3	1.2
26	P67381-26	3.3	2.7	2.7	3.2	1.5	1.2
27	P67381-27	2.3	2.3	2.2	2.8	1.3	1.5
28	P67381-28	2.8	2.5	2.2	2.7	1.8	1.8
29	P67381-29	2.5	2.8	2.7	2.7	1.8	1.3
30	P67381-30	3.3	3.2	3.0	3.0	1.5	1.7
31	P67381-31	2.8	2.0	2.7	2.2	1.5	2.0
32	P67381-32	2.3	1.5	2.0	2.0	1.3	1.3
33	P67381-33	3.2	3.0	2.8	3.2	1.7	1.8
34	P67381-34	3.2	2.7	3.0	2.7	1.5	1.8
35	P67381-35	1.5	1.5	1.5	1.8	1.3	1.5
36	P67381-36	2.8	1.7	1.8	2.0	1.7	1.2
37	P67381-37	2.8	2.5	2.7	2.3	1.8	2.0
38	P67381-38	3.8	3.3	3.0	3.3	1.7	1.8
39	P67381-39	3.0	2.8	3.0	3.0	1.3	1.8
40	P67381-40	2.7	2.5	2.7	2.3	1.5	1.5
41	P67381-41	3.3	-	-	-	1.5	1.5
42	P67381-42	2.7	2.3	3.0	3.0	2.0	1.8

Table A2. The average seedling infection types for the Pinnacle X PI67381 RIL population with the global collection of *P. teres* f. *maculata* isolates using a 1-5 rating scale (Neupane et al., 2015) (continued).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
43	P67381-43	2.8	2.3	2.0	2.7	2.3	2.0
44	P67381-44	2.5	2.3	2.0	2.5	1.8	1.5
45	P67381-45	1.7	1.5	1.5	2.0	1.5	1.3
46	P67381-46	3.5	3.2	3.0	3.2	1.8	1.8
47	P67381-47	1.8	1.3	2.0	1.8	1.0	1.0
48	P67381-48	2.8	2.5	2.7	2.8	2.0	1.7
49	P67381-49	2.5	2.2	2.2	2.7	1.5	1.2
50	P67381-50	-	-	3.0	-	1.5	1.5
51	P67381-51	3.0	2.7	2.8	2.8	1.5	1.7
52	P67381-52	2.0	2.3	2.3	1.7	1.5	1.5
53	P67381-53	3.0	2.5	2.3	2.5	1.8	1.7
54	P67381-54	3.2	3.0	3.0	2.5	2.0	1.8
55	P67381-55	1.8	2.0	1.8	2.0	1.5	1.3
56	P67381-56	2.3	1.8	2.0	1.8	1.2	1.2
57	P67381-57	3.0	3.0	2.8	3.0	1.7	1.8
58	P67381-58	2.5	2.3	2.8	2.7	1.8	1.8
59	P67381-59	3.7	3.5	3.2	3.3	2.3	2.0
60	P67381-60	2.3	2.2	2.0	2.2	2.2	2.0
61	P67381-61	3.0	2.7	2.8	2.8	1.7	1.7
62	P67381-62	3.0	2.8	3.0	3.0	1.7	1.7
63	P67381-63	3.0	2.5	2.5	2.3	2.0	1.7
64	P67381-64	2.8	2.2	2.5	2.0	2.0	1.8
65	P67381-65	2.8	2.2	2.5	2.2	1.7	2.0
66	P67381-66	2.5	3.0	3.0	3.3	1.5	1.5
67	P67381-67	1.8	1.7	2.2	2.0	1.5	1.2
68	P67381-68	3.2	2.5	2.5	3.2	1.7	1.7
69	P67381-69	2.0	1.5	1.5	1.5	1.8	1.7
70	P67381-70	2.2	2.2	2.3	2.0	1.7	1.3
71	P67381-71	3.0	2.3	2.2	2.3	1.8	2.0
72	P67381-72	1.8	-	2.8	-	1.5	1.5
73	P67381-73	1.8	1.7	1.8	1.8	1.5	1.0
74	P67381-74	3.5	2.7	2.8	2.8	1.8	1.8
75	P67381-75	3.8	3.2	3.0	3.0	1.8	1.8
76	P67381-76	3.8	3.3	2.7	3.5	2.0	2.0
77	P67381-77	2.8	2.0	2.7	2.5	1.8	2.0
78	P67381-78	3.2	2.8	2.8	3.0	1.8	2.2
79	P67381-79	2.5	2.5	2.3	2.8	2.0	-
80	P67381-80	2.5	2.2	2.2	2.2	2.0	2.0
81	P67381-81	2.0	2.7	2.7	3.2	1.2	1.3
82	P67381-82	2.2	2.0	2.0	1.8	1.8	1.7
83	P67381-83	3.2	3.0	2.8	3.3	1.8	2.0
84	P67381-84	2.0	2.0	2.2	2.0	1.7	1.7

Table A2. The average seedling infection types for the Pinnacle X PI67381 RIL population with the global collection of *P. teres* f. *maculata* isolates using a 1-5 rating scale (Neupane et al., 2015) (continued).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
85	P67381-85	2.0	1.8	2.2	1.7	1.8	1.5
86	P67381-86	3.0	2.8	2.8	3.0	1.5	1.0
87	P67381-87	2.0	1.8	2.0	1.8	1.3	1.5
88	P67381-88	2.7	2.3	2.0	2.0	1.8	1.8
89	P67381-89	2.0	1.8	1.8	2.0	1.0	1.2
90	P67381-90	2.2	2.2	2.3	2.2	1.7	1.5
91	P67381-91	2.2	2.3	2.0	2.0	1.8	1.5
92	P67381-92	2.7	2.5	2.2	2.5	2.0	1.8
93	P67381-93	-	-	-	3.5	2.0	2.0
94	P67381-94	2.5	2.7	3.2	2.3	1.8	1.5
95	P67381-95	2.7	2.2	2.0	2.3	1.5	1.5
96	P67381-96	-	2.5	-	-	2.0	1.5
97	P67381-97	3.0	2.7	3.0	3.0	1.5	1.3
98	P67381-98	2.7	2.3	2.3	2.3	1.7	1.5
99	P67381-99	2.2	2.2	2.3	2.0	1.3	1.2
100	P67381-100	-	2.5	-	-	1.5	1.0
101	P67381-101	2.7	2.3	2.3	2.7	1.7	1.7
102	P67381-102	2.0	2.0	1.8	1.8	1.7	1.3
103	P67381-103	3.2	3.0	2.8	3.0	1.5	1.5
104	P67381-104	2.5	2.3	1.8	2.0	1.7	1.8
105	P67381-105	2.5	2.0	1.8	1.8	1.8	2.0
106	P67381-106	3.0	2.7	2.8	3.0	1.5	1.7
107	P67381-107	2.0	2.0	1.5	2.0	1.7	1.8
108	P67381-108	2.8	1.8	2.2	3.0	1.7	1.2
109	P67381-109	3.0	2.8	2.8	3.2	1.8	1.7
110	P67381-110	3.0	2.8	2.7	3.0	1.8	2.0
111	P67381-111	2.5	3.0	2.8	3.2	1.7	1.5
112	P67381-112	2.5	2.3	2.0	2.3	1.7	1.8
113	P67381-113	2.5	-	-	-	1.5	1.5
114	P67381-114	2.5	2.2	2.3	2.7	1.5	1.3
115	P67381-115	3.0	2.8	3.0	3.3	1.7	1.5
116	P67381-116	3.2	3.0	2.5	3.0	1.7	1.5
117	P67381-117	3.3	3.0	3.2	3.0	1.7	2.0
118	Pinnacle	3.3	3.2	3.0	2.8	2.3	2.3
119	PI67381	1.5	1.2	1.5	1.5	1.3	1.0

Table A3. The average seedling infection types for the Pinnacle X PI84314 RIL population with the global collection of *P. teres f. maculata* isolates using a 1-5 rating scale (Neupane et al., 2015).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
1	P84314-1	1.8	2.2	2.0	2.2	1.5	1.5
2	P84314-2	2.7	2.8	2.8	2.5	2.0	2.2
3	P84314-3	2.0	2.0	2.0	-	1.5	1.3
4	P84314-4	2.2	1.8	1.7	1.8	1.5	1.3
5	P84314-5	2.7	2.8	2.5	2.5	1.2	1.7
6	P84314-6	-	-	-	-	-	-
7	P84314-7	2.8	3.0	2.7	2.5	1.8	1.8
8	P84314-8	2.2	2.5	2.3	2.0	1.2	1.2
9	P84314-9	2.5	-	-	-	1.5	1.5
10	P84314-10	3.0	-	-	-	2.0	2.3
11	P84314-11	3.0	-	-	-	1.5	-
12	P84314-12	2.3	2.7	2.7	2.2	1.7	1.5
13	P84314-13	2.2	2.5	3.0	2.3	1.3	1.2
14	P84314-14	2.8	3.2	3.0	2.7	1.7	1.7
15	P84314-15	2.7	2.5	2.5	2.3	1.8	2.0
16	P84314-16	2.7	3.0	2.5	2.3	1.8	1.8
17	P84314-17	1.8	2.2	2.0	2.7	1.3	1.3
18	P84314-18	3.2	3.0	2.8	3.0	1.8	1.7
19	P84314-19	2.3	2.7	2.2	2.0	1.3	1.3
20	P84314-20	2.5	2.8	2.8	2.8	1.7	1.7
21	P84314-21	2.8	2.7	2.5	2.3	2.0	1.8
22	P84314-22	3.7	3.3	2.8	3.0	1.8	2.0
23	P84314-23	2.3	2.5	2.5	2.3	1.7	1.5
24	P84314-24	2.8	2.7	2.5	2.7	1.8	2.0
25	P84314-25	3.2	3.2	2.5	2.8	1.7	1.8
26	P84314-26	2.5	3.0	2.0	-	1.5	1.8
27	P84314-27	2.5	2.0	1.5	-	1.5	1.8
28	P84314-28	2.5	-	-	-	1.5	1.5
29	P84314-29	3.3	2.8	3.0	2.0	1.8	2.0
30	P84314-30	3.0	2.5	2.3	-	1.5	1.3
31	P84314-31	3.2	3.2	2.8	2.8	1.8	1.8
32	P84314-32	2.5	3.2	2.5	2.3	1.8	1.8
33	P84314-33	2.3	2.5	2.5	2.0	1.8	2.0
34	P84314-34	2.5	2.8	2.5	3.0	1.5	1.7
35	P84314-35	2.8	2.5	1.8	2.3	2.3	2.0
36	P84314-36	2.5	2.8	2.3	2.8	2.2	1.5
37	P84314-37	2.0	2.2	2.0	2.0	1.5	1.3
38	P84314-38	2.3	2.2	2.0	2.5	1.7	1.2
39	P84314-39	3.0	3.2	2.5	2.7	2.3	2.3
40	P84314-40	2.0	-	-	-	1.5	2.0
41	P84314-41	2.7	2.3	2.2	2.8	1.5	1.5
42	P84314-42	2.3	2.7	2.8	2.5	1.8	1.8

Table A3. The average seedling infection types for the Pinnacle X PI84314 population with the global collection of *P. teres f. maculata* isolates using a 1-5 rating scale (Neupane et al., 2015) (continued).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
43	P84314-43	3.2	2.0	1.8	2.2	1.8	1.3
44	P84314-44	2.5	2.2	1.8	2.3	1.5	1.7
45	P84314-45	2.7	2.8	3.0	2.7	2.3	2.0
46	P84314-46	2.2	2.3	2.7	2.0	1.5	1.3
47	P84314-47	3.2	3.2	2.3	2.3	1.8	1.8
48	P84314-48	3.0	3.3	3.0	3.0	1.7	1.3
49	P84314-49	2.2	2.0	2.0	2.0	1.5	1.2
50	P84314-50	3.2	2.5	2.7	2.3	1.3	1.3
51	P84314-51	2.7	2.8	2.5	2.8	2.0	1.7
52	P84314-52	3.7	2.8	2.5	2.5	1.7	1.5
53	P84314-53	3.5	3.2	2.8	2.8	1.8	2.0
54	P84314-54	3.0	-	-	-	2.0	2.0
55	P84314-55	2.8	3.0	2.3	2.5	1.7	1.8
56	P84314-56	2.3	3.2	2.3	3.0	2.2	1.8
57	P84314-57	3.2	2.8	2.3	2.5	1.7	1.5
58	P84314-58	-	-	-	-	-	-
59	P84314-59	2.5	2.2	2.2	2.3	1.7	1.3
60	P84314-60	2.0	2.2	2.2	2.3	1.8	1.7
61	P84314-61	1.5	-	-	-	1.8	1.8
62	P84314-62	2.0	2.5	1.8	2.0	1.2	1.7
63	P84314-63	3.2	3.2	3.0	2.7	2.2	2.2
64	P84314-64	2.5	3.0	2.3	2.8	1.8	1.7
65	P84314-65	1.5	-	-	-	1.0	1.0
66	P84314-66	2.5	2.5	2.2	2.3	1.2	1.3
67	P84314-67	2.8	3.0	2.8	2.7	1.3	1.5
68	P84314-68	2.5	-	-	-	1.5	1.8
69	P84314-69	3.0	3.0	2.8	2.5	1.5	1.3
70	P84314-70	3.0	3.0	3.0	-	2.0	1.8
71	P84314-71	2.7	2.8	2.7	2.8	1.8	2.0
72	P84314-72	2.7	2.7	1.8	2.3	1.2	1.2
73	P84314-73	2.5	3.0	3.0	-	2.0	2.0
74	P84314-74	2.7	2.7	2.3	2.8	1.3	1.5
75	P84314-75	2.8	2.5	3.0	2.3	1.7	1.7
76	P84314-76	3.3	2.8	2.7	2.3	2.0	1.8
77	P84314-77	2.3	2.5	2.5	2.5	1.5	1.3
78	P84314-78	1.8	2.0	2.0	2.0	1.3	1.3
79	P84314-79	3.3	3.3	3.0	2.8	2.3	2.2
80	P84314-80	3.0	2.8	2.2	2.8	1.3	1.5
81	P84314-81	2.8	2.8	2.5	2.5	1.3	1.5
82	P84314-82	2.5	2.8	2.7	2.7	2.0	1.5
83	P84314-83	2.3	2.5	2.7	2.8	1.5	1.8
84	P84314-84	2.3	2.0	2.2	2.0	1.2	1.3
85	P84314-85	3.3	3.3	3.0	2.8	1.7	1.5
86	P84314-86	3.3	3.5	2.8	3.0	1.8	2.0

Table A3. The average seedling infection types for the Pinnacle X PI84314 population with the global collection of *P. teres f. maculata* isolates using a 1-5 rating scale (Neupane et al., 2015) (continued).

SN	Lines	FGO	PA14	CA17	SG1	DEN2.6	NZKF2
87	P84314-87	2.3	2.8	2.5	2.5	1.5	1.5
88	P84314-88	1.8	1.5	1.7	2.0	1.0	1.0
89	P84314-89	2.2	2.7	2.0	2.5	1.3	1.2
90	P84314-90	2.8	3.0	2.5	-	1.8	2.0
91	P84314-91	3.0	3.0	3.0	-	1.5	1.5
92	P84314-92	2.0	2.2	1.8	2.0	1.3	1.3
93	P84314-93	2.7	2.3	2.0	2.0	1.8	1.7
94	P84314-94	2.8	3.0	2.7	2.7	1.5	1.5
95	P84314-95	2.5	2.0	2.0	2.0	1.2	1.3
96	P84314-96	3.3	3.5	3.2	3.3	1.8	1.8
97	P84314-97	3.0	2.8	2.5	2.5	1.7	1.8
98	P84314-98	3.2	3.2	2.8	3.0	2.5	2.2
99	P84314-99	2.8	3.2	2.7	2.3	1.5	1.8
100	P84314-100	3.0	3.2	3.0	3.0	1.7	1.8
101	P84314-101	2.2	2.3	2.0	2.5	1.2	1.0
102	P84314-102	2.5	2.5	2.3	2.5	1.7	1.5
103	P84314-103	3.3	2.8	2.2	2.5	1.7	1.7
104	P84314-104	3.2	2.8	2.5	2.5	1.8	1.7
105	P84314-105	2.2	2.2	2.2	2.2	1.3	1.5
106	P84314-106	2.3	2.2	2.2	2.5	1.8	1.8
107	P84314-107	2.3	2.0	2.0	-	1.0	1.3
108	P84314-108	2.5	2.8	2.3	2.5	1.5	1.8
109	P84314-109	3.3	3.0	2.7	2.8	2.0	1.7
110	P84314-110	2.2	2.5	2.0	-	1.5	1.5
111	P84314-111	2.0	-	-	-	1.8	1.5
112	P84314-112	3.0	2.8	2.0	2.8	1.7	1.7
113	P84314-113	2.5	2.2	2.0	2.3	1.7	1.7
114	P84314-114	3.0	3.2	3.2	2.8	1.8	1.8
115	P84314-115	3.0	3.0	3.0	2.7	1.7	1.7
116	Pinnacle	2.8	3.3	3.0	2.8	2.5	2.3
117	PI84314	1.3	1.8	1.5	1.5	1.0	1.0

Table A4. Average seedling reaction type of Tradition X PI67381 F_2 population to *Ptm* isolate FG0 using 1-5 rating scale (Neupane et al., 2015).

SN	Entry	Dis. Score
1	PI67381xTradition F_2-1	1.5
2	PI67381xTradition F_2-2	1.5
3	PI67381xTradition F_2-3	1.5
4	PI67381xTradition F_2-4	2.0
5	PI67381xTradition F_2-5	1.5
6	PI67381xTradition F_2-6	1.5
7	PI67381xTradition F_2-7	1.5
8	PI67381xTradition F_2-8	1.5
9	PI67381xTradition F_2-9	2.0
10	PI67381xTradition F_2-10	1.5
11	PI67381xTradition F_2-11	1.5
12	PI67381xTradition F_2-12	1.5
13	PI67381xTradition F_2-13	1.5
14	PI67381xTradition F_2-14	1.5
15	PI67381xTradition F_2-15	1.5
16	PI67381xTradition F_2-16	2.0
17	PI67381xTradition F_2-17	1.5
18	PI67381xTradition F_2-18	1.5
19	PI67381xTradition F_2-19	2.0
20	PI67381xTradition F_2-20	1.5
21	PI67381xTradition F_2-21	1.5
22	PI67381xTradition F_2-22	1.5
23	PI67381xTradition F_2-23	1.5
24	PI67381xTradition F_2-24	1.5
25	PI67381xTradition F_2-25	1.5
26	PI67381xTradition F_2-26	1.5
27	PI67381xTradition F_2-27	2.0
28	PI67381xTradition F_2-28	1.5
29	PI67381xTradition F_2-29	4.0
30	PI67381xTradition F_2-30	3.0
31	PI67381xTradition F_2-31	3.0
32	PI67381xTradition F_2-32	3.0
33	PI67381xTradition F_2-33	3.5
34	PI67381xTradition F_2-34	3.0
35	PI67381xTradition F_2-35	3.0
36	PI67381xTradition F_2-36	3.0
37	PI67381xTradition F_2-37	3.5
38	PI67381xTradition F_2-38	3.0
39	PI67381xTradition F_2-39	3.5
40	PI67381xTradition F_2-40	3.0
41	PI67381xTradition F_2-41	3.5

Table A4. Average seedling reaction type of Tradition X PI67381 F2 population to *Ptm* isolate FG0 using 1-5 rating scale (Neupane et al., 2015) (continued).

SN	Entry	Dis. Score
42	PI67381xTradition F_2-42	3.5
43	PI67381xTradition F_2-43	3.5
44	PI67381xTradition F_2-44	3.0
45	PI67381xTradition F_2-45	3.0
46	PI67381xTradition F_2-46	3.0
47	PI67381xTradition F_2-47	3.5
48	PI67381xTradition F_2-48	3.5
49	PI67381xTradition F_2-49	3.5
50	PI67381xTradition F_2-50	3.0
51	PI67381xTradition F_2-51	3.5
52	PI67381xTradition F_2-52	3.0
53	PI67381xTradition F_2-53	3.0
54	PI67381xTradition F_2-54	3.0
55	PI67381xTradition F_2-55	3.0
56	PI67381xTradition F_2-56	3.5
57	PI67381xTradition F_2-57	3.5
58	PI67381xTradition F2-58	3.5
59	PI67381xTradition F2-59	3.5
60	PI67381xTradition F2-60	3.0
61	PI67381xTradition F2-61	3.0
62	PI67381xTradition F2-62	3.0
63	PI67381xTradition F2-63	3.5
64	PI67381xTradition F2-64	3.5
65	PI67381xTradition F2-65	3.5
66	PI67381xTradition F2-66	3.0
67	PI67381xTradition F2-67	3.0
68	PI67381xTradition F2-68	3.5
69	PI67381xTradition F2-69	3.0
70	PI67381xTradition F2-70	3.5
71	PI67381xTradition F2-71	3.5
72	PI67381xTradition F2-72	3.0
73	PI67381xTradition F2-73	3.0
74	PI67381xTradition F2-74	3.0
75	PI67381xTradition F2-75	3.0
76	PI67381xTradition F2-76	3.0
77	PI67381xTradition F2-77	3.0
78	PI67381xTradition F2-78	3.0
79	PI67381xTradition F2-79	3.0
80	PI67381xTradition F2-80	3.5
81	PI67381xTradition F2-81	3.0
82	PI67381xTradition F2-82	3.0
83	PI67381xTradition F2-83	3.5

Table A4. Average seedling reaction type of Tradition X PI67381 F2 population to *Ptm* isolate FG0 using 1-5 rating scale (Neupane et al., 2015) (continued).

SN	Entry	Dis. Score
84	PI67381xTradition F2-84	3.0
85	PI67381xTradition F2-85	3.5
86	PI67381xTradition F2-86	3.0
87	PI67381xTradition F2-87	3.0
88	PI67381xTradition F2-88	3.0
89	PI67381xTradition F2-89	3.5
90	PI67381xTradition F2-90	3.0
91	PI67381xTradition F2-91	3.0
92	PI67381xTradition F2-92	3.5
93	PI67381xTradition F2-93	3.0
94	PI67381xTradition F2-94	3.0
95	PI67381xTradition F2-95	3.0
96	PI67381xTradition F2-96	3.0
97	PI67381xTradition F2-97	3.5
98	PI67381xTradition F2-98	3.5
99	PI67381xTradition F2-99	3.0
100	PI67381xTradition F2-100	3.0
101	PI67381xTradition F2-101	3.0
102	PI67381xTradition F2-102	3.0
103	PI67381xTradition F2-103	3.5
104	PI67381xTradition F2-104	3.0
105	PI67381xTradition F2-105	3.0
106	PI67381xTradition F2-106	3.0
107	PI67381xTradition F2-107	3.0
108	PI67381xTradition F2-108	3.0
109	PI67381xTradition F2-109	3.0
110	PI67381xTradition F2-110	3.5
111	PI67381xTradition F2-111	3.5
112	PI67381xTradition F2-112	3.5
113	PI67381xTradition F2-113	3.0

APPENDIX B. DISEASE PHENOTYPE OF CI5791, HEARTLAND, CI5791-γ3, CI5791-γ8, CI5791-γ3 X -γ8 F_1, CI5791-γ8 X -γ3 F_1, CI5791-γ3 X HEARTLAND F_2 AND CI5791-γ8 X HEARTLAND F_2 POPULATIONS PHENOTYPED WITH *PTT* ISOLATES

Table B1. Seedling disease reactions of 111 CI5791-γ3 X Heartland F_2 individuals to *Ptt* isolate 0-1 based on the 1-5 SFNB rating scale. Disease reactions above 2 were considered susceptible.

SN	CI5791-γ3 X Heartland F2	Score
1	γ3/ HL-1	2.0
2	γ3/ HL-2	1.0
3	γ3/ HL-3	1.5
4	γ3/ HL-4	1.0
5	γ3/ HL-5	1.0
6	γ3/ HL-6	1.0
7	γ3/ HL-7	1.0
8	γ3/ HL-8	2.0
9	γ3/ HL-9	1.0
10	γ3/ HL-10	1.0
11	γ3/ HL-11	1.0
12	γ3/ HL-12	1.5
13	γ3/ HL-13	1.0
14	γ3/ HL-14	1.5
15	γ3/ HL-15	1.0
16	γ3/ HL-16	1.5
17	γ3/ HL-17	1.0
18	γ3/ HL-18	1.0
19	γ3/ HL-19	1.0
20	γ3/ HL-20	1.0
21	γ3/ HL-21	2.0
22	γ3/ HL-22	1.0
23	γ3/ HL-23	1.0
24	γ3/ HL-24	1.0
25	γ3/ HL-25	3.0
26	γ3/ HL-26	3.5
27	γ3/ HL-27	4.0
28	γ3/ HL-28	4.0
29	γ3/ HL-29	4.5
30	γ3/ HL-30	4.0
31	γ3/ HL-31	4.5
32	γ3/ HL-32	4.5
33	γ3/ HL-33	4.5
34	γ3/ HL-34	3.5

Table B1. Seedling disease reactions of 111 CI5791-γ3 X Heartland F2 individuals to *Ptt* isolate 0-1 based on the 1-5 SFNB rating scale. Disease reactions above 2 were considered susceptible (continued).

SN	CI5791-γ3 X Heartland F2	Score
35	γ3/ HL-35	3.5
36	γ3/ HL-36	4.5
37	γ3/ HL-38	3.5
38	γ3/ HL-39	4.0
39	γ3/ HL-40	4.0
40	γ3/ HL-41	4.5
41	γ3/ HL-42	4.0
42	γ3/ HL-43	4.0
43	γ3/ HL-44	4.5
44	γ3/ HL-45	1.0
45	γ3/ HL-46	1.0
46	γ3/ HL-47	1.5
47	γ3/ HL-48	1.0
48	γ3/ HL-49	1.0
49	γ3/ HL-50	1.0
50	γ3/ HL-51	2.5
51	γ3/ HL-1	1.0
52	γ3/ HL-2	1.0
53	γ3/ HL-3	2.0
54	γ3/ HL-4	1.0
55	γ3/ HL-5	1.0
56	γ3/ HL-6	1.5
57	γ3/ HL-7	1.0
58	γ3/ HL-8	1.0
59	γ3/ HL-9	1.0
60	γ3/ HL-10	1.0
61	γ3/ HL-11	1.5
62	γ3/ HL-12	1.0
63	γ3/ HL-13	1.0
64	γ3/ HL-14	1.0
65	γ3/ HL-15	1.0
66	γ3/ HL-16	1.0
67	γ3/ HL-17	1.0
68	γ3/ HL-18	1.0
69	γ3/ HL-19	1.0
70	γ3/ HL-20	1.0
71	γ3/ HL-21	1.0
72	γ3/ HL-22	1.0
73	γ3/ HL-23	1.0
74	γ3/ HL-24	1.0
75	γ3/ HL-25	1.0

Table B1. Seedling disease reactions of 111 CI5791-γ3 X Heartland F2 individuals to *Ptt* isolate 0-1 based on the 1-5 SFNB rating scale. Disease reactions above 2 were considered susceptible (continued).

SN	CI5791-γ3 X Heartland F2	Score
76	γ3/ HL-26	1.0
77	γ3/ HL-27	1.0
78	γ3/ HL-28	1.5
79	γ3/ HL-29	1.0
80	γ3/ HL-30	1.5
81	γ3/ HL-31	1.0
82	γ3/ HL-32	1.5
83	γ3/ HL-33	1.5
84	γ3/ HL-34	1.0
85	γ3/ HL-35	1.0
86	γ3/ HL-36	1.5
87	γ3/ HL-37	1.0
88	γ3/ HL-38	2.0
89	γ3/ HL-40	3.5
90	γ3/ HL-41	4.0
91	γ3/ HL-42	4.0
92	γ3/ HL-43	4.0
93	γ3/ HL-44	3.5
94	γ3/ HL-45	3.0
95	γ3/ HL-46	3.5
96	γ3/ HL-47	4.0
97	γ3/ HL-48	5.0
98	γ3/ HL-49	4.5
99	γ3/ HL-50	4.0
100	γ3/ HL-51	4.0
101	γ3/ HL-52	1.0
102	γ3/ HL-53	1.0
103	γ3/ HL-54	1.0
104	γ3/ HL-55	1.0
105	γ3/ HL-56	1.0
106	γ3/ HL-57	1.0
107	γ3/ HL-58	1.0
108	γ3/ HL-59	1.0
109	γ3/ HL-60	1.0
110	γ3/ HL-61	3.0
111	γ3/ HL-62	3.5
112	CI5791-γ3	3.5
113	CI 5791	1.0
114	Heartland	1.0

Table B2. Seedling disease reactions of 116 CI5791-γ8 X Heartland F_2 individuals to *Ptt* isolate 0-1 based on the 1-10 NFNB rating scale. Disease reactions above 3 were considered susceptible.

SN	CI5791-γ8 X Heartland	Score
1	γ8/ HL - 1	6.0
2	γ8/ HL - 2	7.0
3	γ8/ HL - 3	6.5
4	γ8/ HL - 4	6.0
5	γ8/ HL - 5	5.0
6	γ8/ HL - 6	5.5
7	γ8/ HL - 7	8.0
8	γ8/ HL - 8	6.0
9	γ8/ HL - 9	6.5
10	γ8/ HL - 10	7.5
11	γ8/ HL - 12	4.5
12	γ8/ HL - 13	6.0
13	γ8/ HL - 14	6.5
14	γ8/ HL - 15	7.0
15	γ8/ HL - 16	8.5
16	γ8/ HL - 17	6.5
17	γ8/ HL - 18	9.0
18	γ8/ HL - 19	7.5
19	γ8/ HL - 20	6.5
20	γ8/ HL - 21	7.0
21	γ8/ HL - 22	5.5
22	γ8/ HL - 23	8.0
23	γ8/ HL - 24	6.5
24	γ8/ HL - 25	6.0
25	γ8/ HL - 26	6.0
26	γ8/ HL - 27	7.5
27	γ8/ HL - 28	7.5
28	γ8/ HL - 29	2.0
29	γ8/ HL - 30	1.0
30	γ8/ HL - 31	1.5
31	γ8/ HL - 32	1.0
32	γ8/ HL - 33	1.5
33	γ8/ HL - 34	1.5
34	γ8/ HL - 35	1.0
35	γ8/ HL - 36	1.5
36	γ8/ HL - 37	1.5
37	γ8/ HL - 38	1.0
38	γ8/ HL - 39	1.5
39	γ8/ HL - 40	1.5
40	γ8/ HL - 41	1.5
41	γ8/ HL - 42	1.5

Table B2. Seedling disease reactions of 116 CI5791-γ8 X Heartland F2 individuals to *Ptt* isolate 0-1 based on the 1-10 NFNB rating scale. Disease reactions above 3 were considered susceptible (continued).

SN	CI5791-γ8 X Heartland	Score
42	γ8/ HL - 43	1.0
43	γ8/ HL - 44	2.5
44	γ8/ HL - 45	2.0
45	γ8/ HL - 46	2.0
46	γ8/ HL - 47	2.2
47	γ8/ HL - 48	1.0
48	γ8/ HL - 49	1.0
49	γ8/ HL - 50	1.0
50	γ8/ HL - 51	1.5
51	γ8/ HL - 52	1.0
52	γ8/ HL - 53	1.5
53	γ8/ HL - 54	1.0
54	γ8/ HL - 55	2.0
55	γ8/ HL - 56	1.5
56	γ8/ HL - 57	2.0
57	γ8/ HL - 58	1.5
58	γ8/ HL - 59	2.0
59	γ8/ HL - 60	1.5
60	γ8/ HL - 61	1.0
61	γ8/ HL - 62	1.0
62	γ8/ HL - 63	1.5
63	γ8/ HL - 64	2.5
64	γ8/ HL - 65	2.0
65	γ8/ HL - 66	1.0
66	γ8/ HL - 67	1.5
67	γ8/ HL - 68	1.0
68	γ8/ HL - 69	1.0
69	γ8/ HL - 70	1.5
70	γ8/ HL - 71	1.0
71	γ8/ HL - 72	1.0
72	γ8/ HL - 73	1.0
73	γ8/ HL - 74	1.0
74	γ8/ HL - 75	2.0
75	γ8/ HL - 76	1.5
76	γ8/ HL - 77	1.5
77	γ8/ HL - 78	1.0
78	γ8/ HL - 79	1.5
79	γ8/ HL - 80	1.5
80	γ8/ HL - 81	1.0
81	γ8/ HL - 82	2.0
82	γ8/ HL - 83	1.5

Table B2. Seedling disease reactions of 116 CI5791-γ8 X Heartland F2 individuals to *Ptt* isolate 0-1 based on the 1-10 NFNB rating scale. Disease reactions above 3 were considered susceptible (continued).

SN	CI5791-γ8 X Heartland	Score
83	γ8/ HL - 84	1.5
85	γ8/ HL - 86	1.0
86	γ8/ HL - 87	1.0
87	γ8/ HL - 88	1.0
88	γ8/ HL - 89	1.5
89	γ8/ HL - 90	1.0
90	γ8/ HL - 91	2.0
91	γ8/ HL - 92	2.5
92	γ8/ HL - 93	2.0
93	γ8/ HL - 94	1.5
94	γ8/ HL - 95	1.5
95	γ8/ HL - 96	2.0
96	γ8/ HL - 97	2.0
97	γ8/ HL - 98	1.5
98	γ8/ HL - 99	1.5
99	γ8/ HL - 100	1.5
100	γ8/ HL - 101	1.0
101	γ8/ HL - 102	1.0
102	γ8/ HL - 103	1.5
103	γ8/ HL - 104	1.0
104	γ8/ HL - 105	1.0
105	γ8/ HL - 106	2.0
106	γ8/ HL - 107	2.0
107	γ8/ HL - 108	2.0
108	γ8/ HL - 109	1.5
109	γ8/ HL - 110	1.5
110	γ8/ HL - 111	2.0
111	γ8/ HL - 112	1.0
112	γ8/ HL - 113	1.5
113	γ8/ HL - 114	1.5
114	γ8/ HL - 115	1.0
115	γ8/ HL - 116	1.5
116	γ8/ HL - 117	1.0
117	CI5791-γ3	6.5
118	CI5791-γ8	6.0
119	Heartland	1.5
120	CI5791	1.5
121	Robust	8.5
122	Tifang	8.0

Table B3. Seedling disease phenotype of CI5791-γ3, CI5791-γ8, CI5791, Hockett, and Hector to the *Ptt* isolates SM36-2 and SM36-3 collected in Morocco.

Lines	*Ptt* SM-36-2	*Ptt* SM36-3
CI5791-γ3	6.5	6.0
CI5791-γ8	7.0	6.5
CI5791	4.5	3.5
Hockett	1.0	1.0
Hector	9.5	8.0

Table B4. Seedling disease phenotype of reciprocal cross between CI5791-γ3 and CI5791-γ8 F_1s to *Ptt* isolate 0-1.

SN	Plants	Disease score
1	CI5791-γ8/-γ3 - 1	6.5
2	CI5791-γ8/-γ3 - 2	7.5
3	CI5791-γ8/-γ3 - 3	7.0
4	CI5791-γ8/-γ3 - 4	6.0
5	CI5791-γ8/-γ3 - 5	5.5
6	CI5791-γ8/-γ3 - 6	6.5
7	CI5791-γ8/-γ3 - 7	5.5
8	CI5791-γ8/-γ3 - 8	7.0
9	CI5791-γ8/-γ3 - 9	7.0
10	CI5791-γ8/-γ3 - 10	6.0
11	CI5791-γ3/-γ8 - 1	5.5
12	CI5791-γ3/-γ8 - 2	9.0
13	CI5791-γ3/-γ8 - 3	5.5
14	CI5791-γ3/-γ8 - 4	6.0
15	CI5791-γ3/-γ8 - 5	6.0
16	CI5791-γ3/-γ8 - 6	5.5
17	CI5791-γ3	6.0
18	CI5791-γ8	5.5
19	CI5791	1.5
20	Heartland	1.5
21	Robust	8.5

Table B5. Phenotypic analysis of BSMV-VIGS plants inoculated with *Ptt* isolate 0-1 at 7 DAI (days after inoculation).

Plants	VIGS Knockdown	Plants	MCS
1	5.3	1	2.3
2	5.3	2	2.0
3	5.3	3	5.3
4	2.0	4	3.3
5	4.3	5	2.3
6	4.3	6	2.0
7	5.0	7	2.0
8	6.0	8	2.3
9	6.8	9	4.8
10	5.8	10	2.3
11	3.3	11	2.0
12	5.0	12	4.3
13	4.3	13	3.0
14	2.3	14	2.8
15	5.3	15	3.3
16	4.3	16	2.3
17	4.8	17	4.3
18	3.0	18	3.0
19	5.3	19	4.3
20	2.3	20	5.0
21	4.8	21	2.0
22	6.0	22	4.0
23	5.0	23	3.0
24	3.3	24	5.0
25	6.0	25	3.8
26	5.8	26	3.0
27	6.3	27	2.3
28	5.0	28	2.3
29	4.3	29	3.3
30	3.0	30	2.0
31	6.0	31	2.8
32	3.8	32	2.8
33	5.0	33	2.8
34	6.0	CI 5791	1.3
35	4.8	Robust	8.0
36	5.0		
37	5.0		
38	3.3		
39	4.8		

Table B6. Phenotypic analysis of BSMV-VIGS plants inoculated with *Ptt* isolate 0-1 at 12 DAI (days after inoculation).

Plants	VIGS Knockdown	Plants	MCS
1	5.0	1	1.0
2	5.0	2	2.0
3	7.3	3	2.0
4	6.3	4	2.0
5	4.3	5	1.3
6	3.8	6	4.3
7	6.3	7	2.8
8	4.8	8	2.3
9	3.3	9	2.3
10	5.3	10	2.3
11	8.3	11	2.3
12	5.3	12	2.3
13	7.3	13	2.3
14	10.0	14	5.3
15	9.0	15	3.3
16	8.3	16	4.3
17	9.0	17	2.3
18	7.3	18	4.3
19	3.3	19	4.3
20	4.3	20	3.0
21	5.0	21	-
22	3.3	22	4.3
23	6.8	23	3.3
24	3.3	24	3.3
25	7.3	25	5.0
26	5.0	26	2.0
27	7.0	27	2.0
28	4.3	28	2.3
29	5.3	29	6.3
30	6.3	30	2.8
31	7.3	31	3.3
32	5.3	32	4.3
33	7.3	33	2.0
34	7.3	CI 5791	1.3
35	7.0	Robust	9.5

Table B7. Primer sequences used to amplify the whole *HvWRKY6* gene for sequencing.

Primers	Sequence (5'-3')
Wrky6-gen-F1	GATGAGCATCCAGTAAGCTGTCACTG
Wrky6-gen-R1	GACTAACAGGTGCAACGATGCG
Wrky6-gen-F2	CGAGGAAGTTGGAGCTCATGAGC
Wrky6-gen-R2	CTCCGACTCACGAGGTACTATTC
Wrky6-gen-F3	CACGATGGCACTCTCGTTCTTG
Wrky6-gen-R3	GGCAGCTTGGCTTCTTGAACTTG
Wrky6-gen-F4	CTTCTTCTCGTCGGAGAAGAAGTC
Wrky6-gen-R4	CTACTATTCCTCGTGCAGTACGTG

APPENDIX C. RIL POPULATION SPECIFIC PCR GBS SNP MARKERS PANELS

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information.

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
1	11_21067	1H	2.44	ACACTGACGACATGGTTCTACATTGTTCCCAACAGGGAAAGG	TACGGTAGCAGAGACTTGGTCTCTAGCCTGATGAATGAAGAC
2	12_31144	1H	4.98	ACACTGACGACATGGTTCTACAGAAAAGAAGTGATTCGCCCG	TACGGTAGCAGAGACTTGGTCTTGCAGAAGTTCAAGACCCTG
3	12_30933	1H	6.68	ACACTGACGACATGGTTCTACATCAGAAGAGTGCAGCATAAC	TACGGTAGCAGAGACTTGGTCTTCAGTTTGATACTTTGCCTG
4	11_21174	1H	9.34	ACACTGACGACATGGTTCTACAAGAAAGGACGTCGAAACGAG	TACGGTAGCAGAGACTTGGTCTATCGCCGGGTGATACAGATG
5	12_30919	1H	12.50	ACACTGACGACATGGTTCTACAAAGATCAGTGCGCAATAACG	TACGGTAGCAGAGACTTGGTCTGCTAGGTGCTGCTGATGTTT
6	12_30588	1H	15.90	ACACTGACGACATGGTTCTACAAGTTTCGACACAGACACAGG	TACGGTAGCAGAGACTTGGTCTCTGACATGTACGATGATGTG
7	12_30948	1H	20.31	ACACTGACGACATGGTTCTACAATGGCGAGCTCGACTTGTTC	TACGGTAGCAGAGACTTGGTCTCGGGTGCGTGTTTCTGAAC
8	11_20371	1H	21.97	ACACTGACGACATGGTTCTACATGGTTGTAACAAGTCGCGAG	TACGGTAGCAGAGACTTGGTCTGAGACCGATAATCGACAAGC
9	11_20712	1H	26.32	ACACTGACGACATGGTTCTACATCATTTGTTGCCCTCTGGTG	TACGGTAGCAGAGACTTGGTCTAAACCCTAGAATGTACACGG
10	11_21048	1H	31.24	ACACTGACGACATGGTTCTACAAGGAGAAGAAGGCGGAGGTG	TACGGTAGCAGAGACTTGGTCTTTCCAGAGCTGATCCATGTC
11	11_20617	1H	35.60	ACACTGACGACATGGTTCTACATCTCCCTGAAACATGGAACC	TACGGTAGCAGAGACTTGGTCTACAGATAGGAAATTCACTGC
12	11_21072	1H	36.71	ACACTGACGACATGGTTCTACATTGCCGAAGATGGTGGTGAG	TACGGTAGCAGAGACTTGGTCTATCCCGGCATAGACGACAG
13	12_30336	1H	42.17	ACACTGACGACATGGTTCTACAGCAGTTTATATTCCGGTGTG	TACGGTAGCAGAGACTTGGTCTACACTGAGTCAACGTAGTGC
14	12_10314	1H	46.53	ACACTGACGACATGGTTCTACATGGCTTGTGGAATTGAGGAC	TACGGTAGCAGAGACTTGGTCTTATAACTTCGGTGGACGCTG
15	12_30683	1H	49.10	ACACTGACGACATGGTTCTACAAGAGCCCACTGTACACTATC	TACGGTAGCAGAGACTTGGTCTCTGGATGACAGACTACAAGC
16	11_20810	1H	50.00	ACACTGACGACATGGTTCTACAAAGGCGGCTAGTGCTAATTC	TACGGTAGCAGAGACTTGGTCTATTCTTGCTACGCCATCGAG
17	12_30110	1H	50.30	ACACTGACGACATGGTTCTACAGTTCTCCTCTACCTCTAGTG	TACGGTAGCAGAGACTTGGTCTTATCATCCATGCTCGCTCTG
18	12_30343	1H	52.08	ACACTGACGACATGGTTCTACATCTGCTGGAGAACAAGGTAG	TACGGTAGCAGAGACTTGGTCTTTTTTGCACATTCCAGCGCC
19	11_20997	1H	54.14	ACACTGACGACATGGTTCTACACTTATCGTTGGTGGGATTGC	TACGGTAGCAGAGACTTGGTCTCGCACATCCTACTTCATCAG
20	12_30753	1H	56.50	ACACTGACGACATGGTTCTACAGCAGATTATCATGCTATGCC	TACGGTAGCAGAGACTTGGTCTGATCGCTAGCCATCATTCAG
21	12_30304	1H	59.07	ACACTGACGACATGGTTCTACACTCCTTGCAAGATGCAGATG	TACGGTAGCAGAGACTTGGTCTGATTCTAGCGGCGAACCATC
22	11_10617	1H	59.07	ACACTGACGACATGGTTCTACACATTCCATGTGTCCGGCAAG	TACGGTAGCAGAGACTTGGTCTAGGGTAAGCTCCTGACGAAG
23	11_10002	1H	64.93	ACACTGACGACATGGTTCTACACGACGACAATACACAACACC	TACGGTAGCAGAGACTTGGTCTGTTCGGTGCGTCTGTTTTTG
24	12_10166	1H	69.73	ACACTGACGACATGGTTCTACAAGTGGGCGTTGATGTGATTC	TACGGTAGCAGAGACTTGGTCTAACGGTCGACAAACCGTAAG

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
25	11_10279	1H	72.86	ACACTGACGACATGGTTCTACATGTACTCTTTTGCGACGCTG	TACGGTAGCAGAGACTTGGTCTACCACATGAATGTCCAGCAG
26	11_20990	1H	74.12	ACACTGACGACATGGTTCTACAAGTAACACTGGACACACACC	TACGGTAGCAGAGACTTGGTCTGCCATAATTGAGAGGCTGTG
27	11_10471	1H	83.15	ACACTGACGACATGGTTCTACAGACAGGAGGTTCAACATAGC	TACGGTAGCAGAGACTTGGTCTTGGTGGCCAGTTTTTACTAC
28	11_20149	1H	94.48	ACACTGACGACATGGTTCTACATAATGTGAGAAATTTTGATAT	TACGGTAGCAGAGACTTGGTCTTCAAGACCTTGTGGTTTGGC
29	SCRI_RS_188909	1H	98.45	ACACTGACGACATGGTTCTACACAGCACGTAACATCTGCATC	TACGGTAGCAGAGACTTGGTCTATCCAGCTACTAGAGTGGAC
30	12_20187	1H	101.05	ACACTGACGACATGGTTCTACAAAGGACCTCGACAAGGAGGA	TACGGTAGCAGAGACTTGGTCTGAGCTCCAGCACAGTCTTG
31	11_20909	1H	104.10	ACACTGACGACATGGTTCTACATCGGCGGCGGCGATGTCCT	TACGGTAGCAGAGACTTGGTCTGCCACGAGAACGGCTTCCT
32	11_20844	1H	109.53	ACACTGACGACATGGTTCTACACATTCAGCACAAGACACTAC	TACGGTAGCAGAGACTTGGTCTGATGTCTCTTCGTGGTTCTC
33	12_30014	1H	114.98	ACACTGACGACATGGTTCTACAATATATCCAAAGTGCTGTCG	TACGGTAGCAGAGACTTGGTCTATCAGCTCTGGGACGGCTTG
34	11_10854	1H	122.29	ACACTGACGACATGGTTCTACACAGGGATTCCAATACCACAC	TACGGTAGCAGAGACTTGGTCTTTGTTCAACCAAACGAGTGC
35	11_20908	1H	126.60	ACACTGACGACATGGTTCTACAGATTGACGAGGCGGTGATAC	TACGGTAGCAGAGACTTGGTCTCAAAGGAAGGAACCGAATGC
36	12_11443	1H	128.27	ACACTGACGACATGGTTCTACACACTGTACTCAGAAGAATAGG	TACGGTAGCAGAGACTTGGTCTATGATGGTGGTGGTAGTCAG
37	12_10808	1H	131.46	ACACTGACGACATGGTTCTACATTATACCCTTCAAGCAGCGG	TACGGTAGCAGAGACTTGGTCTGCAGAGGCTAATAAAGCAAC
38	12_10693	1H	132.54	ACACTGACGACATGGTTCTACAAAGTCCCATGGGAGAATCAG	TACGGTAGCAGAGACTTGGTCTTCTTGCCACCAATGGTGAAC
39	11_11105	1H	142.16	ACACTGACGACATGGTTCTACAGAGATCTGGGAAGCTTAGAC	TACGGTAGCAGAGACTTGGTCTTTTGGTGCTCTGTACAAGGG
40	11_10590	1H	142.74	ACACTGACGACATGGTTCTACAACAACACAGCGAAAACGAAC	TACGGTAGCAGAGACTTGGTCTTGACGAGACTGCACTAGGTA
41	12_31081	1H	145.82	ACACTGACGACATGGTTCTACAAACTGCTTGGATGGAACCAC	TACGGTAGCAGAGACTTGGTCTGCCTCATTTCTGAGATAGTG
42	11_10326	2H	7.29	ACACTGACGACATGGTTCTACACTCCATGGGATACCCATGTC	TACGGTAGCAGAGACTTGGTCTTCAAGAAACGGTGATGGTGC
43	11_21377	2H	13.19	ACACTGACGACATGGTTCTACAAGCAGCAGCTACTTGCAAAC	TACGGTAGCAGAGACTTGGTCTACTGCCAAAGAGACGATTGC
44	12_30631	2H	19.42	ACACTGACGACATGGTTCTACAATTTATGGACGAGGCAACTG	TACGGTAGCAGAGACTTGGTCTATGAGAACTGCTCTCGCGTG
45	SCRI_RS_152744	2H	23.76	ACACTGACGACATGGTTCTACACATCAAGAAAGAAGCCGGAG	TACGGTAGCAGAGACTTGGTCTACGTACTCGGCGTCCACCA
46	11_10943	2H	25.53	ACACTGACGACATGGTTCTACACCAACACTAACGGTAACAGC	TACGGTAGCAGAGACTTGGTCTTCGCCGTTTTCACTCTTCAG
47	12_10777	2H	30.36	ACACTGACGACATGGTTCTACAGAGAGGCCACGTCAATCAAT	TACGGTAGCAGAGACTTGGTCTTCTGAGGTTGGTAGATAGGG
48	11_10216	2H	40.73	ACACTGACGACATGGTTCTACAAAGTCTTGATCCAGCCTTGC	TACGGTAGCAGAGACTTGGTCTCGCCTAGTCTTAATGTCTGG

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
49	11_10891	2H	44.54	ACACTGACGACATGGTTCTACAGGAGATGACAAGAAACCCAC	TACGGTAGCAGAGACTTGGTCTATTGCTGGATCATCTGGCAC
50	11_21304	2H	47.35	ACACTGACGACATGGTTCTACATCAAGGACATGCGCTTCAG	TACGGTAGCAGAGACTTGGTCTCTTGTTGACGAGCGCGAAG
51	11_10919	2H	53.26	ACACTGACGACATGGTTCTACATAAAGGGCAAGGAAAAGCGG	TACGGTAGCAGAGACTTGGTCTCCTGATAAGCTACAGCATGA
52	12_30432	2H	54.31	ACACTGACGACATGGTTCTACAAGAACAACCGAGTCATGTGC	TACGGTAGCAGAGACTTGGTCTGTTCCATGCACCCATGATGA
53	12_30703	2H	63.08	ACACTGACGACATGGTTCTACAGATCTGTAGCGTTGTACTCC	TACGGTAGCAGAGACTTGGTCTCTCACATTGTCATGCTTCCC
54	11_21005	2H	64.70	ACACTGACGACATGGTTCTACATAAACACCACACGACCGAAG	TACGGTAGCAGAGACTTGGTCTGTTCCATTGGTGCTTGCTTG
55	SCRI_RS_151535	2H	65.71	ACACTGACGACATGGTTCTACAGACCTTGACACAGTAAAAGC	TACGGTAGCAGAGACTTGGTCTAGGTCATCTGCTGTTGCATC
56	SCRI_RS_175065	2H	65.81	ACACTGACGACATGGTTCTACACACATCATCACAAGCTAACG	TACGGTAGCAGAGACTTGGTCTTTCCCTTAGCTATGGGAGTG
57	SCRI_RS_154617	2H	66.11	ACACTGACGACATGGTTCTACAAGTTCGTGACGCACGAGATG	TACGGTAGCAGAGACTTGGTCTATCCAGATGATGCACCTGAG
58	11_10733	2H	66.11	ACACTGACGACATGGTTCTACACACAGAGACGCACACAAATC	TACGGTAGCAGAGACTTGGTCTGATGCCACAGATGAGCTATG
59	11_10325	2H	66.11	ACACTGACGACATGGTTCTACAAATGGAGTCGAATGGACGAG	TACGGTAGCAGAGACTTGGTCTGTCACATGGATTACTGACCC
60	12_10927	2H	67.89	ACACTGACGACATGGTTCTACATCATGAAGGTATGGCTTCCG	TACGGTAGCAGAGACTTGGTCTCAATTCTTAGGAGGAGACAG
61	12_10485	2H	68.80	ACACTGACGACATGGTTCTACAAAAGAAACCCACTGCTCCAG	TACGGTAGCAGAGACTTGGTCTTGTGGTTGTCTCTCAAACCG
62	12_10099	2H	69.00	ACACTGACGACATGGTTCTACAATGTAGTCGCGGCGCTGGAA	TACGGTAGCAGAGACTTGGTCTTGGGCGTCGAGTACAAAGTC
63	11_20532	2H	69.55	ACACTGACGACATGGTTCTACAGCAGATCCCAACAAAAGACG	TACGGTAGCAGAGACTTGGTCTATCCCTCGATGTAGTGTCTG
64	11_10265	2H	75.18	ACACTGACGACATGGTTCTACACCACGGTACAGCGAAAATTC	TACGGTAGCAGAGACTTGGTCTCTCTCGATCCTTCTAGGTTG
65	11_20947	2H	77.34	ACACTGACGACATGGTTCTACATCGATGTGTAGATAGACCGC	TACGGTAGCAGAGACTTGGTCTTACCCCCCAGAAAAACGTTC
66	11_10196	2H	81.26	ACACTGACGACATGGTTCTACACAACTGCATTCCCTGGTTAC	TACGGTAGCAGAGACTTGGTCTCGTTCTCGTATCTCTGGTTG
67	12_31445	2H	83.59	ACACTGACGACATGGTTCTACAAAAGCACATATGTTGATGGC	TACGGTAGCAGAGACTTGGTCTCCGTTCCTTTCTAGTTTCTC
68	11_21242	2H	86.84	ACACTGACGACATGGTTCTACATCGGGAAAAAGGTCCAATAG	TACGGTAGCAGAGACTTGGTCTTGTTTGCACAGCAAGCTTCC
69	12_30900	2H	90.99	ACACTGACGACATGGTTCTACAAGCAGGCCGAGATTCTGGAG	TACGGTAGCAGAGACTTGGTCTAGATACACCTTGCGGGCTG
70	12_10936	2H	93.14	ACACTGACGACATGGTTCTACAAGTAATCCTGGAGCCACCTG	TACGGTAGCAGAGACTTGGTCTCTGGACAGGAGGCTGTACAT
71	11_10214	2H	99.04	ACACTGACGACATGGTTCTACACATGGCAGCTAAGCCCTAAG	TACGGTAGCAGAGACTTGGTCTGGACGACGAGGAGTAAATAG
72	11_21175	2H	101.98	ACACTGACGACATGGTTCTACACCACCGCGTACTTAATTGTG	TACGGTAGCAGAGACTTGGTCTCGCAATCCAAATCCAGTCAC
73	11_10398	2H	106.90	ACACTGACGACATGGTTCTACATAGACGGCTTTATTTGGCAG	TACGGTAGCAGAGACTTGGTCTAAGCCACGGTGATTGATGAC

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
74	12_30480	2H	108.14	ACACTGACGACATGGTTCTACAATGCGGCTCGGATGTGTATC	TACGGTAGCAGAGACTTGGTCTACGAACTTACCTTGATGCGG
75	12_30555	2H	112.22	ACACTGACGACATGGTTCTACATTCCGACCATCACTTGAGAG	TACGGTAGCAGAGACTTGGTCTTCAATCCTGAGAACAAGGAC
76	11_20099	2H	121.76	ACACTGACGACATGGTTCTACACGTTTTCGACGCAGAGTTGT	TACGGTAGCAGAGACTTGGTCTCGTGCAAGTCGCAGAAGAG
77	11_10128	2H	124.50	ACACTGACGACATGGTTCTACATTCTGGACGGTGAGCTTGAC	TACGGTAGCAGAGACTTGGTCTCCAAGAAGATCGGTGAGGAC
78	11_10429	2H	126.63	ACACTGACGACATGGTTCTACATGATGGGCGCATTGACTATG	TACGGTAGCAGAGACTTGGTCTTTCTCATTGTTCTTCGCCCG
79	12_31095	2H	131.66	ACACTGACGACATGGTTCTACAAGTGGGCGTTCTTCTTGATG	TACGGTAGCAGAGACTTGGTCTAGAAGGACTGCTACCACGAG
80	12_10739	2H	135.51	ACACTGACGACATGGTTCTACACTGAAACACCCAACACTTGC	TACGGTAGCAGAGACTTGGTCTTTTGACTAGCAACCCATGCC
81	SCRI_RS_155161	2H	137.44	ACACTGACGACATGGTTCTACATGCTGTCCAATCTGAAGCTG	TACGGTAGCAGAGACTTGGTCTTCTTGATGCTCAATGTGCTG
82	11_10092	2H	137.44	ACACTGACGACATGGTTCTACATCGACTGCAACAAGCTTCAC	TACGGTAGCAGAGACTTGGTCTCGAACACATGCAGAAAGCAG
83	11_20141	2H	141.70	ACACTGACGACATGGTTCTACAAGCAGCAGTGATGAAGTTGG	TACGGTAGCAGAGACTTGGTCTAGAGACGATGCAGTCGTTGG
84	12_31100	2H	143.07	ACACTGACGACATGGTTCTACAATGGCCTTAGTGTCTCTTCC	TACGGTAGCAGAGACTTGGTCTAGAAAAGGCTGTCCCTTTGG
85	11_21125	2H	145.20	ACACTGACGACATGGTTCTACAGCCTCAGCCAGAATAGTAAG	TACGGTAGCAGAGACTTGGTCTAAAGTCTTCCATGGAAGCCC
86	12_30106	2H	150.78	ACACTGACGACATGGTTCTACAGATGCCGACCTCCATGAATC	TACGGTAGCAGAGACTTGGTCTGTGGCATACGGGTTCAACTG
87	12_30341	2H	155.26	ACACTGACGACATGGTTCTACATGTCCATGTGATAGGTGACG	TACGGTAGCAGAGACTTGGTCTATAGGAAATGGCTTGGGCTG
88	12_30352	2H	157.42	ACACTGACGACATGGTTCTACATGCTGAAGAGCTCCTGGTAG	TACGGTAGCAGAGACTTGGTCTTACTGCGGGTTCTGCCGGT
89	12_10181	2H	162.06	ACACTGACGACATGGTTCTACACACCGCAAATCCCATGGATG	TACGGTAGCAGAGACTTGGTCTGGCGTAAACATGATAATTACC
90	11_20943	2H	166.65	ACACTGACGACATGGTTCTACAGGGTTATCGATCTGTTCTGC	TACGGTAGCAGAGACTTGGTCTCGAAGAAGGCATGCAAGAAC
91	12_30823	2H	168.93	ACACTGACGACATGGTTCTACAACCATCACAACCAAGGTGAG	TACGGTAGCAGAGACTTGGTCTTGATGACCACCTTGTCGATG
92	11_10072	2H	171.55	ACACTGACGACATGGTTCTACAGAGTGTCTTGTCCTTGTTGC	TACGGTAGCAGAGACTTGGTCTGTATCCAAACCTCTGAGGAC
93	12_31180	2H	175.48	ACACTGACGACATGGTTCTACAACGGATGAGATTCAGGAAGC	TACGGTAGCAGAGACTTGGTCTAACCATGTGCATGCATGTCC
94	11_10085	2H	177.38	ACACTGACGACATGGTTCTACACACATAGTCTCGGCTATTCC	TACGGTAGCAGAGACTTGGTCTCAGTAGCATGTTGGTGGTTG
95	12_31428	3H	0.00	ACACTGACGACATGGTTCTACAGGCGGTGAGGAGGTAGGTG	TACGGTAGCAGAGACTTGGTCTGCATCCTCTCCGGATTCTTC
96	11_20252	3H	6.46	ACACTGACGACATGGTTCTACATTCCTGAATGTCTCCTATGC	TACGGTAGCAGAGACTTGGTCTAAACGCCCTGCATTAGCGAC
97	11_21398	3H	8.86	ACACTGACGACATGGTTCTACAGCTCACTTGTTCAATGCACC	TACGGTAGCAGAGACTTGGTCTATGATTGCTTCCGAAGACCC
98	12_30818	3H	12.23	ACACTGACGACATGGTTCTACACTACTTCTTGGCCGCGGAC	TACGGTAGCAGAGACTTGGTCTATCGTCACGCCCCTCTTCTC

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
99	11_20742	3H	24.63	ACACTGACGACATGGTTCTACAATCAGCACCGTGTACTTGGG	TACGGTAGCAGAGACTTGGTCTCTTGGTCTCGACCACATTGC
100	11_20968	3H	35.40	ACACTGACGACATGGTTCTACATCGTCAATGGTGATCTCCTC	TACGGTAGCAGAGACTTGGTCTGAAGGTGATAGTCTCCAAGG
101	12_30571	3H	38.19	ACACTGACGACATGGTTCTACACAAGGCGTCGATTTCTTCTC	TACGGTAGCAGAGACTTGGTCTGAAGTCGCCAGCTGTTGAAG
102	11_10026	3H	40.34	ACACTGACGACATGGTTCTACAAGGAGCCACGTCGAGATTTC	TACGGTAGCAGAGACTTGGTCTCTTTGAAGCCGGAGTTCATC
103	12_10968	3H	44.82	ACACTGACGACATGGTTCTACACCGAGATGCTTCTACTGTTC	TACGGTAGCAGAGACTTGGTCTGAATCCCGCCCTGCTGAAC
104	SCRI_RS_127994	3H	53.42	ACACTGACGACATGGTTCTACACAACATCACCACCGGGGTC	TACGGTAGCAGAGACTTGGTCTTTGTTCACCTGGTACACCAC
105	12_30467	3H	56.79	ACACTGACGACATGGTTCTACAATTCTTCGATGATGGCGGAC	TACGGTAGCAGAGACTTGGTCTGCAAAATGGCCTCAACTTCG
106	11_20583	3H	58.31	ACACTGACGACATGGTTCTACATTCAATCGCTGACCCGCAC	TACGGTAGCAGAGACTTGGTCTCGGATGGGGATCTCATATAC
107	12_30009	3H	59.39	ACACTGACGACATGGTTCTACAACTCCCTCCTTACAAGCAAC	TACGGTAGCAGAGACTTGGTCTCTCTACGTACATATCCTGGC
108	11_10373	3H	61.94	ACACTGACGACATGGTTCTACACCGAATCACCGAGAAATAGG	TACGGTAGCAGAGACTTGGTCTCTGCAAGCAAATAAAGTGGC
109	12_30616	3H	65.44	ACACTGACGACATGGTTCTACAGGTGAAGCAATCACGAGTTC	TACGGTAGCAGAGACTTGGTCTCTAGGGTTGAGCTTACTAGG
110	12_30399	3H	67.53	ACACTGACGACATGGTTCTACAACTCTTGTCGTGTGGTACTC	TACGGTAGCAGAGACTTGGTCTGCTTTAGAGATATTGTACAG
111	11_20115	3H	71.74	ACACTGACGACATGGTTCTACAGTGAACCTTGTATATTCTG	TACGGTAGCAGAGACTTGGTCTCAGAACCACAAGCTGTAGAC
112	12_31262	3H	73.21	ACACTGACGACATGGTTCTACACATCCTAGCATATCCCATCC	TACGGTAGCAGAGACTTGGTCTGCCGCTTCAATCACATCTGC
113	11_10444	3H	78.93	ACACTGACGACATGGTTCTACATTCTCGTAGTTCCTGAGCTG	TACGGTAGCAGAGACTTGGTCTTAGGGTGGATCTCCTCCTAC
114	12_31018	3H	81.11	ACACTGACGACATGGTTCTACATGGCTATGGCGTTGTTGAAG	TACGGTAGCAGAGACTTGGTCTTCCTGAAAACTTGAGGCCAG
115	12_31367	3H	85.85	ACACTGACGACATGGTTCTACAGCTTTATCGACCATTCCAGC	TACGGTAGCAGAGACTTGGTCTCAAGGGTCGAATTGTGCAAG
116	SCRI_RS_159340	3H	88.17	ACACTGACGACATGGTTCTACAGACTGCCTACGTTTCTTTGG	TACGGTAGCAGAGACTTGGTCTCATTGGTGGCTTAGTAGCAG
117	12_30250	3H	88.17	ACACTGACGACATGGTTCTACAACAGATCCAAGGTGAAGAGC	TACGGTAGCAGAGACTTGGTCTTTCTCTTCTATCGGGGACTC
118	11_20626	3H	91.33	ACACTGACGACATGGTTCTACAAACAGGCTTCTAGCTTTGGG	TACGGTAGCAGAGACTTGGTCTGAGTTTGCGAAAGGTCAAGC
119	11_21493	3H	95.25	ACACTGACGACATGGTTCTACACGGGCCTTGATTGATTTTGC	TACGGTAGCAGAGACTTGGTCTCCAGGTGGCAGTGAAAAAAC
120	SCRI_RS_221787	3H	99.26	ACACTGACGACATGGTTCTACAGAGTGGAGTGGTTGCTGCTT	TACGGTAGCAGAGACTTGGTCTACCCGCGCGTGGGTGAGTC
121	SCRI_RS_164704	3H	99.26	ACACTGACGACATGGTTCTACATTTCGTAGAGGCCATCGTAG	TACGGTAGCAGAGACTTGGTCTCACATGGTATTATTATTTCCC
122	SCRI_RS_225641	3H	99.46	ACACTGACGACATGGTTCTACAAAGTGATGATGATGCTGCCG	TACGGTAGCAGAGACTTGGTCTCCCCTTCACCAGATCTGCAT
123	SCRI_RS_133339	3H	99.56	ACACTGACGACATGGTTCTACATGACAGAGAGAACGAACTCC	TACGGTAGCAGAGACTTGGTCTTTTGGCCCATGGATCAAGTG

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
124	12_30423	3H	99.66	ACACTGACGACATGGTTCTACAGAAGTCAATACCGATTGTAG	TACGGTAGCAGAGACTTGGTCTTGTTGGCAAGAGTTGCCTCC
125	SCRI_RS_211929	3H	99.66	ACACTGACGACATGGTTCTACAAAGTACTACGCCGACGACTG	TACGGTAGCAGAGACTTGGTCTTGGTTTTCTGGGTCTTCACG
126	SCRI_RS_235791	3H	99.66	ACACTGACGACATGGTTCTACATGGCGACCATCAAAGCAAAG	TACGGTAGCAGAGACTTGGTCTGCACATGAGGATGTGTGTTG
127	SCRI_RS_167825	3H	103.46	ACACTGACGACATGGTTCTACACTCGGGTACAGACACCATTC	TACGGTAGCAGAGACTTGGTCTTAGCAGCGGCCATCTATCTC
128	12_31329	3H	103.83	ACACTGACGACATGGTTCTACAAATGAAGTGTTCGGCGACAG	TACGGTAGCAGAGACTTGGTCTTCAGAGTTCATGTTCTGGG
129	SCRI_RS_163092	3H	103.86	ACACTGACGACATGGTTCTACAACAGGTTCTTAGTTGCGGTG	TACGGTAGCAGAGACTTGGTCTAAGAACCAGTGCTGTCAGAG
130	11_10584	3H	105.98	ACACTGACGACATGGTTCTACATGCTCGGACAGAGACGTGA	TACGGTAGCAGAGACTTGGTCTTGCTGGGCTTTCTCGACGAT
131	11_20944	3H	109.12	ACACTGACGACATGGTTCTACAGGAGAACATTCCACATTAG	TACGGTAGCAGAGACTTGGTCTATACAACGAGGCATCTGAGC
132	11_21405	3H	111.70	ACACTGACGACATGGTTCTACAAATTGATCCCCATGACTCCC	TACGGTAGCAGAGACTTGGTCTTGCAGTCATCAAATTCAGCG
133	12_30081	3H	115.86	ACACTGACGACATGGTTCTACATGAATGGCCATTGCCATGAG	TACGGTAGCAGAGACTTGGTCTCACACTTGCAAGTACCACAC
134	12_10188	3H	118.27	ACACTGACGACATGGTTCTACAGAAGATCCATTCTCATGCTG	TACGGTAGCAGAGACTTGGTCTAATGCCTAAATGTATGCAG
135	12_30973	3H	121.02	ACACTGACGACATGGTTCTACACTGAAAACAGATGGTGTAA	TACGGTAGCAGAGACTTGGTCTCCACATATCATTGTTACCTTG
136	11_20085	3H	126.41	ACACTGACGACATGGTTCTACAATGGAGGACGAATAGGGAGG	TACGGTAGCAGAGACTTGGTCTCTCCCGTTTGTCCAAAACTG
137	12_30367	3H	128.53	ACACTGACGACATGGTTCTACACAACGGTCCGATTTGCACTC	TACGGTAGCAGAGACTTGGTCTTTGTGTTTGTGGACACTAGC
138	12_21386	3H	133.66	ACACTGACGACATGGTTCTACAACATAGTACAGTAGCAAGGG	TACGGTAGCAGAGACTTGGTCTTTCGCTGAAGAACCCACTTG
139	11_11436	3H	145.65	ACACTGACGACATGGTTCTACATGCATCGGACGGTATACTTC	TACGGTAGCAGAGACTTGGTCTGGATTGCTTGTTCCTAATGG
140	12_20198	3H	147.80	ACACTGACGACATGGTTCTACAAGCAGCAGCTTGCCATCGAG	TACGGTAGCAGAGACTTGGTCTTTGGCAAGCTAGGTAGCAAG
141	SCRI_RS_229623	3H	150.19	ACACTGACGACATGGTTCTACAAGAGAAGAAGAGGAGCAACC	TACGGTAGCAGAGACTTGGTCTATGAGACCGGGTCGAGAATG
142	11_11411	3H	151.23	ACACTGACGACATGGTTCTACACACTCCCATATGGTGGATCA	TACGGTAGCAGAGACTTGGTCTGCTTGGGGTGAACGTCATTG
143	SCRI_RS_156315	3H	153.39	ACACTGACGACATGGTTCTACACATCATAGATACAGCCTGCC	TACGGTAGCAGAGACTTGGTCTTATCAGCAGTGGATGAAGCC
144	12_30055	3H	162.85	ACACTGACGACATGGTTCTACACCACCCACAGTTCAGGAAAC	TACGGTAGCAGAGACTTGGTCTAAGGATTAAAGGCGCTGACC
145	12_30135	3H	164.42	ACACTGACGACATGGTTCTACACAGGGTCCCAGAATTTATAG	TACGGTAGCAGAGACTTGGTCTGTACTGGTCATCAAAGTGAA
146	12_30764	4H	0.71	ACACTGACGACATGGTTCTACATCCAGGACGCCGTGCTCTA	TACGGTAGCAGAGACTTGGTCTCGGAGCCGACGAGGGGCTT
147	11_10409	4H	4.11	ACACTGACGACATGGTTCTACAAGCACTTTGGAAACAGTGGG	TACGGTAGCAGAGACTTGGTCTGAATTTCCCATGAAGAGGTG
148	12_31458	4H	14.13	ACACTGACGACATGGTTCTACAGCAGTTTCAAAAGCAATCTC	TACGGTAGCAGAGACTTGGTCTCAGCTTTCTGCTGTTATGTTC

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
149	12_30540	4H	16.13	ACACTGACGACATGGTTCTACATGTATGGAGTGTCCATGGAG	TACGGTAGCAGAGACTTGGTCTCACACCGTTTTATCAAATC
150	12_30150	4H	18.47	ACACTGACGACATGGTTCTACAGGTAAACCACCACTTCTTGC	TACGGTAGCAGAGACTTGGTCTCATCCCATCTATCCTGAAAC
151	11_10223	4H	22.43	ACACTGACGACATGGTTCTACACGTCGGTAGACGAGAGAAG	TACGGTAGCAGAGACTTGGTCTTTTCGCTCGGTGTCCAAAAC
152	11_20777	4H	31.64	ACACTGACGACATGGTTCTACAGATGAGGATGTCCTCCATTG	TACGGTAGCAGAGACTTGGTCTTCCAAGAAGATTCCCGATGC
153	11_21374	4H	32.68	ACACTGACGACATGGTTCTACAGGAGCAAACGTGTTAGTTGG	TACGGTAGCAGAGACTTGGTCTATACCCAAGATTGTCGGCAC
154	12_30863	4H	38.44	ACACTGACGACATGGTTCTACACTACCAGCTCTTCCAGCAG	TACGGTAGCAGAGACTTGGTCTATTGAGTTCAGCATCAAGGG
155	12_10860	4H	42.69	ACACTGACGACATGGTTCTACACGCATGAATGATGCTACCAC	TACGGTAGCAGAGACTTGGTCTTCGCTAACCTGCAAGCACAC
156	12_30992	4H	46.19	ACACTGACGACATGGTTCTACATTGAACTGAACTGCAGCAGG	TACGGTAGCAGAGACTTGGTCTGAGATGGACGTGCTCTTTTC
157	SCRI_RS_9296	4H	47.17	ACACTGACGACATGGTTCTACATCTCCCATTTCCTGCTCTTC	TACGGTAGCAGAGACTTGGTCTTCCATTTCGAGAAGCTCACC
158	12_30328	4H	47.94	ACACTGACGACATGGTTCTACAAACAGCTACCTCCGGCAGA	TACGGTAGCAGAGACTTGGTCTACGTAGAGCGCGTCGGCGT
159	11_11180	4H	50.70	ACACTGACGACATGGTTCTACATGTTAGGAGGTGAGTTGTCG	TACGGTAGCAGAGACTTGGTCTGTCTTATCAGCACACCTATC
160	11_20939	4H	52.67	ACACTGACGACATGGTTCTACATGTTCCTGCCATACGTCGAG	TACGGTAGCAGAGACTTGGTCTTCACTGCCTCGATGTCAATG
161	SCRI_RS_157310	4H	53.67	ACACTGACGACATGGTTCTACAATGTCCTCCCCGTCCTTCAC	TACGGTAGCAGAGACTTGGTCTCTCCTCGCCGCCGAAGAAG
162	SCRI_RS_184107	4H	53.77	ACACTGACGACATGGTTCTACAATGTACTCAAGCTACACGCC	TACGGTAGCAGAGACTTGGTCTTAGGGCCTGGTACAGGTTG
163	11_10093	4H	53.87	ACACTGACGACATGGTTCTACAAGTTTCATGAGGACTGCATC	TACGGTAGCAGAGACTTGGTCTTTTCAATTCTACCGGCGCCC
164	SCRI_RS_128723	4H	54.66	ACACTGACGACATGGTTCTACACATTGTCAACGGGAAAGAGG	TACGGTAGCAGAGACTTGGTCTCTCTGCAACATCCTTTTCCC
165	SCRI_RS_155554	4H	54.66	ACACTGACGACATGGTTCTACACGCAAATCGGTTTCAAGCAC	TACGGTAGCAGAGACTTGGTCTTCCAGAGAGACTGATACAAC
166	SCRI_RS_221172	4H	54.66	ACACTGACGACATGGTTCTACAACCAGCTGCTAAGATTGCTC	TACGGTAGCAGAGACTTGGTCTAGTGGAAGGCCATAACGAAC
167	SCRI_RS_208828	4H	54.66	ACACTGACGACATGGTTCTACAGGGAACTGTTGTAGTACTGG	TACGGTAGCAGAGACTTGGTCTAACAATGTAGCACCAGCAAC
168	11_20472	4H	54.95	ACACTGACGACATGGTTCTACAACGAAATGGACGCGTCAAAG	TACGGTAGCAGAGACTTGGTCTCCTTCCTGTTTGGCTCAAAG
169	11_10262	4H	54.95	ACACTGACGACATGGTTCTACATAGAGACCCAGAGACTAACC	TACGGTAGCAGAGACTTGGTCTGCCAGTGCTTATGAAGGAAC
170	11_20412	4H	54.95	ACACTGACGACATGGTTCTACATCTGCTTCGAGACCCTGAAC	TACGGTAGCAGAGACTTGGTCTTGCCTAACCCGGCGAAGTG
171	SCRI_RS_228477	4H	54.95	ACACTGACGACATGGTTCTACAGGGAGGAAGAGATGTGTTTG	TACGGTAGCAGAGACTTGGTCTACTGCTACGTAATGAACTCC
172	SCRI_RS_168496	4H	54.95	ACACTGACGACATGGTTCTACACCACGACTCCATTAGGGATG	TACGGTAGCAGAGACTTGGTCTTATGCGTACAAGTCACCACC
173	12_30839	4H	54.95	ACACTGACGACATGGTTCTACAGTCAGTGAAGCCTTTGAGTC	TACGGTAGCAGAGACTTGGTCTGAAAGTCACATAAGCATGCC

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
174	11_10509	4H	55.64	ACACTGACGACATGGTTCTACATGGCCCTCTTATATATCCCC	TACGGTAGCAGAGACTTGGTCTGCAAACTGCTTGGCAAAACC
175	SCRI_RS_189180	4H	57.32	ACACTGACGACATGGTTCTACAGTCGAATTTCTTGTGTGGGC	TACGGTAGCAGAGACTTGGTCTTGCTAGCTATCCAGCTCAAG
176	11_20924	4H	58.82	ACACTGACGACATGGTTCTACATCAGTGGAGTGTGATACCAG	TACGGTAGCAGAGACTTGGTCTTCAGTGGACCTTTCAGAAGC
177	SCRI_RS_163033	4H	59.22	ACACTGACGACATGGTTCTACAATACCAAAGGTGCTTGAGCG	TACGGTAGCAGAGACTTGGTCTATGTCTACCGCCGATGTATC
178	SCRI_RS_147712	4H	59.22	ACACTGACGACATGGTTCTACATACCACACCTCAGCAAACTC	TACGGTAGCAGAGACTTGGTCTGTCAAATGTCCAGGTAAAGG
179	11_10606	4H	60.28	ACACTGACGACATGGTTCTACAGGGCACAACTTACTTGCTTA	TACGGTAGCAGAGACTTGGTCTATGTTCATTGCTCCCATCTC
180	11_20072	4H	62.96	ACACTGACGACATGGTTCTACACAGCAGTCTCTTACAATCAC	TACGGTAGCAGAGACTTGGTCTACGGTGGTACACTTTCAGAG
181	11_11513	4H	64.45	ACACTGACGACATGGTTCTACAGTTCAACCACCATCATCCAC	TACGGTAGCAGAGACTTGGTCTGGACAGAGCACCCAAGTTTG
182	11_10309	4H	67.91	ACACTGACGACATGGTTCTACAACACACACAACACACAAGGG	TACGGTAGCAGAGACTTGGTCTAGATCGGCCGTGGAACCATT
183	11_11004	4H	70.33	ACACTGACGACATGGTTCTACATGTCGAGCTAGACATGTCTG	TACGGTAGCAGAGACTTGGTCTGCAAATGAAAGAGACGCTCC
184	11_11500	4H	74.05	ACACTGACGACATGGTTCTACAGGTGCAGACTACCACACAAG	TACGGTAGCAGAGACTTGGTCTATAAGATGTGTGTGATTGGC
185	12_31246	4H	78.11	ACACTGACGACATGGTTCTACAGGATCGAGATTATGACAGGC	TACGGTAGCAGAGACTTGGTCTGGCTAACAGAAGAGGTTTCC
186	11_20178	4H	80.52	ACACTGACGACATGGTTCTACAGCATGAACGTACAACATCCC	TACGGTAGCAGAGACTTGGTCTTGACGAATGTGTCACCACTG
187	11_10588	4H	83.34	ACACTGACGACATGGTTCTACAGACCTTGGTGTCTTCACAAC	TACGGTAGCAGAGACTTGGTCTGCTAGCCAAGTTGTTTCCAG
188	12_30718	4H	94.74	ACACTGACGACATGGTTCTACAAAGGGCACAATGTCAACCTG	TACGGTAGCAGAGACTTGGTCTTAAGGCCGCATTGATCACCG
189	11_20762	4H	96.60	ACACTGACGACATGGTTCTACAGTTATGGAAAGTAGAGGGAC	TACGGTAGCAGAGACTTGGTCTGGCAAAGTTGACGAAATCTG
190	SCRI_RS_131671	4H	99.68	ACACTGACGACATGGTTCTACACAGTGAAACTCATGATCCCC	TACGGTAGCAGAGACTTGGTCTGCTGCCAACATAAACTCTTC
191	11_10510	4H	100.38	ACACTGACGACATGGTTCTACAAGCCGGAAAATTTCCTCCTC	TACGGTAGCAGAGACTTGGTCTCTTGAAGTAGACGGATAGGC
192	SCRI_RS_148330	4H	103.48	ACACTGACGACATGGTTCTACAAAATCTGCTTGGCCTGTACG	TACGGTAGCAGAGACTTGGTCTTACGATTTGTGCCTGTCCTC
193	SCRI_RS_192689	4H	103.58	ACACTGACGACATGGTTCTACAATTTCGTGTGCTCCAGAACC	TACGGTAGCAGAGACTTGGTCTTACCACGAGCACGACGAAG
194	11_20974	4H	105.14	ACACTGACGACATGGTTCTACAATGGCGACTTGACCACAAAC	TACGGTAGCAGAGACTTGGTCTAAGTACAAGGCAGAGACTCC
195	12_30385	4H	108.85	ACACTGACGACATGGTTCTACATAGCTTGTGTACACTCGGAC	TACGGTAGCAGAGACTTGGTCTACACTATGTCCCCTTGGGAG
196	12_31138	4H	112.79	ACACTGACGACATGGTTCTACAGCTTGGTTGGGACTTTTAGG	TACGGTAGCAGAGACTTGGTCTTACACACCGGTATACATTGC
197	11_10697	4H	117.33	ACACTGACGACATGGTTCTACACTCTAACCACTTCTACGTGC	TACGGTAGCAGAGACTTGGTCTCCCGTTCGATGTCGATTTAC
198	11_11186	4H	123.54	ACACTGACGACATGGTTCTACATACTCCAGTCCTGTGCTTAG	TACGGTAGCAGAGACTTGGTCTAGCGGTGGATGGACAATTGG

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
199	12_30873	4H	125.11	ACACTGACGACATGGTTCTACAACAGCAAATTTGAATGGGTG	TACGGTAGCAGAGACTTGGTCTGAAACTTGAGGTGTATCTTTG
200	12_31422	4H	127.26	ACACTGACGACATGGTTCTACACAGTGAGTACATTAGCTCTAC	TACGGTAGCAGAGACTTGGTCTAAAAGGAAACAAACTGCTC
201	12_30824	4H	129.68	ACACTGACGACATGGTTCTACAGACCGTCGGTGTAGAAAATG	TACGGTAGCAGAGACTTGGTCTTCAACATCCCAATCCCACAG
202	12_30975	5H	3.66	ACACTGACGACATGGTTCTACACATACAATGAGTAATGACGTG	TACGGTAGCAGAGACTTGGTCTGGTACAATACAATACCAAAAG
203	11_20206	5H	6.55	ACACTGACGACATGGTTCTACACCGTCTTGGTTGGTTTCGAC	TACGGTAGCAGAGACTTGGTCTAGGTCCATATCACCTCTTCC
204	12_31094	5H	10.10	ACACTGACGACATGGTTCTACACCTCAAATCCTACGAGCTTC	TACGGTAGCAGAGACTTGGTCTCCAGGTTTTTGCGAAAACCG
205	11_20010	5H	11.32	ACACTGACGACATGGTTCTACAAACACCTTTCGAAGGGCAAC	TACGGTAGCAGAGACTTGGTCTGGAATTCACAGGCAATCTCG
206	11_21065	5H	21.24	ACACTGACGACATGGTTCTACACCATGGTGGTGATCAGCAG	TACGGTAGCAGAGACTTGGTCTTCTGGTTCGTCGGCCTCGGT
207	11_20386	5H	28.56	ACACTGACGACATGGTTCTACAAGAACTCCAGGCTAGGTTAC	TACGGTAGCAGAGACTTGGTCTTCTCCATCGATCTGACCTAC
208	SCRI_RS_108416	5H	31.86	ACACTGACGACATGGTTCTACACTACTGCATTAGCAACAAGG	TACGGTAGCAGAGACTTGGTCTAACTCCCCTCCTTGAGAAAG
209	SCRI_RS_205100	5H	31.86	ACACTGACGACATGGTTCTACACAGCTTCAAGTCGCTTATGG	TACGGTAGCAGAGACTTGGTCTCTATGATCTCAAGCAGCAGG
210	11_10580	5H	33.22	ACACTGACGACATGGTTCTACACAGAGCACATGCTACTAAAC	TACGGTAGCAGAGACTTGGTCTGCCGATGGTCAGATTTGCTC
211	11_10621	5H	35.35	ACACTGACGACATGGTTCTACACCTTTCCAACCTTAAGAAGC	TACGGTAGCAGAGACTTGGTCTTGTCAGGAACTTGATCAGGG
212	11_20845	5H	37.62	ACACTGACGACATGGTTCTACACGATCGGCTTTATGATAGGC	TACGGTAGCAGAGACTTGGTCTTCTGCTCCGAAGCAGGAAAG
213	11_20729	5H	41.33	ACACTGACGACATGGTTCTACACAAGCATTGGATTGTTGCCG	TACGGTAGCAGAGACTTGGTCTCACCAGAAGCTTTTGGTGC
214	11_10252	5H	43.92	ACACTGACGACATGGTTCTACATCCTTGAACTTCTCCGTCAC	TACGGTAGCAGAGACTTGGTCTCGAACATAATGCTGCAGGAG
215	11_20239	5H	44.20	ACACTGACGACATGGTTCTACAACAACAGCTTCATTGCTGCC	TACGGTAGCAGAGACTTGGTCTTGATGATATCCACACCGACC
216	12_30538	5H	44.99	ACACTGACGACATGGTTCTACATTCGATCAAACCCCTCATGC	TACGGTAGCAGAGACTTGGTCTTGGAGGGTGATTGATCTTTG
217	12_30745	5H	46.21	ACACTGACGACATGGTTCTACATGTTAAGCAAGCCGGTGAAC	TACGGTAGCAGAGACTTGGTCTTGAAGGCCTAGTACCTTCTG
218	11_10641	5H	50.88	ACACTGACGACATGGTTCTACAACTCCTACTTCAACAAGGTC	TACGGTAGCAGAGACTTGGTCTGAAGAGGCCCAACAATCTTG
219	12_20350	5H	53.77	ACACTGACGACATGGTTCTACAACTAGCTTTCTTGCCGACAC	TACGGTAGCAGAGACTTGGTCTTACATGTCCAGATGTCCTAC
220	11_21309	5H	55.83	ACACTGACGACATGGTTCTACAGAGATAGTGAAGTTTGGGAG	TACGGTAGCAGAGACTTGGTCTTCCTCATTGGAAAGGGCTTC
221	11_20236	5H	59.03	ACACTGACGACATGGTTCTACATGCCGATGAGGCGATTATTC	TACGGTAGCAGAGACTTGGTCTGCAATTGAATCGACCCTGTG
222	11_20645	5H	64.25	ACACTGACGACATGGTTCTACACCACACGCTGCAAATACATC	TACGGTAGCAGAGACTTGGTCTCCTGTACCTTCGCTTTCTTG
223	12_10674	5H	68.83	ACACTGACGACATGGTTCTACATAATAAGGCTTCCGACGGAG	TACGGTAGCAGAGACTTGGTCTGCCACCTGCTTGAATGGATG

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
224	11_20497	5H	73.15	ACACTGACGACATGGTTCTACATCGGATACAACCATGAGAGC	TACGGTAGCAGAGACTTGGTCTCGGTCTGGTTGATCTTCTTG
225	11_10518	5H	76.34	ACACTGACGACATGGTTCTACAAAGACAGCCTCGACATCATC	TACGGTAGCAGAGACTTGGTCTAGTAGTTTCCTCAACACGTC
226	11_21421	5H	83.08	ACACTGACGACATGGTTCTACATGCTGCTGCTGTTTATGGTG	TACGGTAGCAGAGACTTGGTCTGATTGACTAGATACTGTGGC
227	11_11350	5H	84.96	ACACTGACGACATGGTTCTACAGAATCTCTTCGTCGTCGATG	TACGGTAGCAGAGACTTGGTCTCGCGAGGGTAGAACATCATT
228	11_20549	5H	87.71	ACACTGACGACATGGTTCTACAAGTTGGAGATGCAGATGCAG	TACGGTAGCAGAGACTTGGTCTCATGAGGAGATGAGAAGAAC
229	12_10752	5H	90.68	ACACTGACGACATGGTTCTACAGGTCAAGTGCTCACAACAAC	TACGGTAGCAGAGACTTGGTCTTCTCGCTCTTTCTTCCTTCC
230	12_30456	5H	95.65	ACACTGACGACATGGTTCTACACTTCTGCAGGAGTGACATTG	TACGGTAGCAGAGACTTGGTCTTCATTGCAGTGTCTGCTCTC
231	11_11200	5H	99.58	ACACTGACGACATGGTTCTACAACCTTTGTTTTGCTTGCAGG	TACGGTAGCAGAGACTTGGTCTGAAGCGCTCATCAACCATAC
232	11_11507	5H	111.56	ACACTGACGACATGGTTCTACAGGGCACAATTTGTTACATAG	TACGGTAGCAGAGACTTGGTCTTAGCCTTCCTTCATTGTGCC
233	11_21422	5H	112.78	ACACTGACGACATGGTTCTACACTGAAAATGACCTCCAAGGG	TACGGTAGCAGAGACTTGGTCTAGCAGGGCTATGATTCCTCT
234	11_20653	5H	115.53	ACACTGACGACATGGTTCTACAATACCACTTGTGATCCGAGG	TACGGTAGCAGAGACTTGGTCTGACCAGGAATTGACTGGAAG
235	12_30067	5H	117.63	ACACTGACGACATGGTTCTACAATTGATGTCGAGAACCGGAG	TACGGTAGCAGAGACTTGGTCTCGAGAAGAACAAAGCACCTG
236	11_21247	5H	119.72	ACACTGACGACATGGTTCTACATCCGTTCCCGTTTGTTACAC	TACGGTAGCAGAGACTTGGTCTGGCTCCATTTTTATGTAACTG
237	11_10845	5H	128.80	ACACTGACGACATGGTTCTACACAACAGCGATCCAAGCTTCC	TACGGTAGCAGAGACTTGGTCTCATGGACTAGCCTTGACTTC
238	11_20551	5H	130.93	ACACTGACGACATGGTTCTACATCCAGAAAGCTGAGAGCATC	TACGGTAGCAGAGACTTGGTCTCAATGATCATATCTGAGGCG
239	11_20375	5H	132.00	ACACTGACGACATGGTTCTACAAAAGGGCCTCAGACTTCAAG	TACGGTAGCAGAGACTTGGTCTGTACGACAAGGAGAAACTGC
240	11_20100	5H	135.42	ACACTGACGACATGGTTCTACATGGTGAAGAGGGCCGAGAAG	TACGGTAGCAGAGACTTGGTCTTTCTTGATGTCGGCTTCGC
241	12_31050	5H	137.22	ACACTGACGACATGGTTCTACATTGTCGTGCTGCCCTTGGAA	TACGGTAGCAGAGACTTGGTCTTTCAGGGAGAAGAGTCCATC
242	12_31165	5H	138.44	ACACTGACGACATGGTTCTACAGCCCAACGTCATCGTACGAA	TACGGTAGCAGAGACTTGGTCTTCCAGAAGAAGGCCAAGGAC
243	12_31221	5H	142.71	ACACTGACGACATGGTTCTACACTTTGCGAAGCACGTTTCTC	TACGGTAGCAGAGACTTGGTCTAACTTTGCCATGGAAGGAAG
244	11_10217	5H	144.86	ACACTGACGACATGGTTCTACATGGTCTCCACAAACATGACG	TACGGTAGCAGAGACTTGGTCTGATAGGCTCCGTAGATCAAG
245	11_10582	5H	149.41	ACACTGACGACATGGTTCTACAACGTAGCTAAGAGACCAAAC	TACGGTAGCAGAGACTTGGTCTTGGAGTTGTTCTCCATGACC
246	11_21018	5H	153.47	ACACTGACGACATGGTTCTACAGCGAATGTTCTAGACCTTAC	TACGGTAGCAGAGACTTGGTCTCTCTGGGACAATGGAAGTAG
247	12_30162	5H	156.70	ACACTGACGACATGGTTCTACAATGTGAAGACGGAGCTGTAG	TACGGTAGCAGAGACTTGGTCTAAACAACACCCAAGGTCCAC
248	11_20829	5H	158.18	ACACTGACGACATGGTTCTACATTCTCCTCCTTGATGTCACC	TACGGTAGCAGAGACTTGGTCTTGTTCCTTGTGATGAGCACG

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
249	11_20826	5H	161.41	ACACTGACGACATGGTTCTACAACCAAGGCGAGGAGGAGAGA	TACGGTAGCAGAGACTTGGTCTGCTCCTCCCCTTTCTTTTTG
250	11_10869	5H	163.16	ACACTGACGACATGGTTCTACAGCTGCTACACACATGAATTG	TACGGTAGCAGAGACTTGGTCTCTAAGATGAAGCTCTGGCTC
251	11_10600	5H	165.57	ACACTGACGACATGGTTCTACAACGATGCCCTCCTTGACGA	TACGGTAGCAGAGACTTGGTCTAAAAGTGTGCCGGGACAATG
252	11_20536	5H	167.35	ACACTGACGACATGGTTCTACAACACATTGGGCAAGGTTCAC	TACGGTAGCAGAGACTTGGTCTGAGGCTTGCAAAAAGCTTGG
253	11_21138	5H	169.97	ACACTGACGACATGGTTCTACATCCAGCTCAGCAATGTTGTC	TACGGTAGCAGAGACTTGGTCTCAGGAGGTCAGTTTAAGTGC
254	12_30494	5H	171.16	ACACTGACGACATGGTTCTACATCATCCAGTTCAGCGCCTTC	TACGGTAGCAGAGACTTGGTCTTCTTGGTGTGCATGGTGAAG
255	12_30504	5H	172.25	ACACTGACGACATGGTTCTACAGCACCATCACTATCATGCAG	TACGGTAGCAGAGACTTGGTCTAATCTGTTGCTCCATGGCTG
256	12_31352	5H	176.52	ACACTGACGACATGGTTCTACAATCGAGTTCTACCGGCACTG	TACGGTAGCAGAGACTTGGTCTGCTTGATGAGGTTGAACAC
257	11_10310	5H	177.50	ACACTGACGACATGGTTCTACATAGGAGAGGGAGCAAAACAG	TACGGTAGCAGAGACTTGGTCTGAGAATCTTTACTTGACCCG
258	11_11364	5H	179.67	ACACTGACGACATGGTTCTACAGTTCTCCAGGAAACAACCAG	TACGGTAGCAGAGACTTGGTCTCGGACGGAGTAACTTTTTA
259	12_10322	5H	181.11	ACACTGACGACATGGTTCTACAGCGCCACCATGTTACGACC	TACGGTAGCAGAGACTTGGTCTAGTGAGTGGCAGACACAGAG
260	12_31123	5H	184.75	ACACTGACGACATGGTTCTACACACCGTGCCTTTCTTAGAAG	TACGGTAGCAGAGACTTGGTCTTCCATCGACATCCTTAAGGG
261	11_20232	6H	0.00	ACACTGACGACATGGTTCTACATGTGACGAATTTCTCGAGCC	TACGGTAGCAGAGACTTGGTCTCAAGGATGATGATTGAGGGC
262	11_20212	6H	2.86	ACACTGACGACATGGTTCTACACTTGTCGTCAATGGCGTAAG	TACGGTAGCAGAGACTTGGTCTTCATCGACCGAGGCCGAGAA
263	12_30651	6H	5.44	ACACTGACGACATGGTTCTACAAGCTCCATGCTACCTATGAG	TACGGTAGCAGAGACTTGGTCTTGGCCAATTCCTTCATCTCC
264	11_21204	6H	7.56	ACACTGACGACATGGTTCTACACTCTATCTTCTATTCTCATC	TACGGTAGCAGAGACTTGGTCTACAGATAATCCGCCTCTACC
265	11_21032	6H	11.35	ACACTGACGACATGGTTCTACAAATCTCTGCATAAGAGCAGG	TACGGTAGCAGAGACTTGGTCTCATCAAGGAAGCTGGAGGTG
266	11_20415	6H	15.16	ACACTGACGACATGGTTCTACAGCTGTCATCTTTCTCGAGTC	TACGGTAGCAGAGACTTGGTCTGTAAGAAACTTCTCCACCAG
267	11_20315	6H	25.77	ACACTGACGACATGGTTCTACACTAGGAGGAAAGTGCCGTC	TACGGTAGCAGAGACTTGGTCTTCAAGAAGAACGTGGAGCTG
268	11_10136	6H	27.19	ACACTGACGACATGGTTCTACAACAGGTCGCTTGAGGGTATG	TACGGTAGCAGAGACTTGGTCTGCCTTGGGAAGATAACAAGC
269	11_20745	6H	31.59	ACACTGACGACATGGTTCTACACATTCAGATTCATTCCTTGC	TACGGTAGCAGAGACTTGGTCTCGTCGGTCTGTGTAGTTAGC
270	12_30697	6H	33.39	ACACTGACGACATGGTTCTACATAGGACGGTGCATCCATTTG	TACGGTAGCAGAGACTTGGTCTGAAGATAGGGACTGAAGCTG
271	12_31485	6H	35.29	ACACTGACGACATGGTTCTACATCCTGATAAAGGCAGGAGTC	TACGGTAGCAGAGACTTGGTCTAGTTGTGCGGCTGTTTGTCC
272	11_10799	6H	38.12	ACACTGACGACATGGTTCTACACCATTTCCCCTAGGAATCAG	TACGGTAGCAGAGACTTGGTCTCTTCCATCTCACCCAAGAAC
273	11_10427	6H	41.35	ACACTGACGACATGGTTCTACAGAACAGGTACCACAAATGGG	TACGGTAGCAGAGACTTGGTCTAGAACCACTGTCTACGAAAC

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
274	12_30521	6H	44.61	ACACTGACGACATGGTTCTACAGTTCCCATCACAAAACCAGC	TACGGTAGCAGAGACTTGGTCTTTCGCATACCTGCAATGCTG
275	12_30516	6H	51.74	ACACTGACGACATGGTTCTACAACATTCCCGATTCAGACGAC	TACGGTAGCAGAGACTTGGTCTATCCTTTCTTCGGAGTCCTC
276	11_10462	6H	53.84	ACACTGACGACATGGTTCTACATGTCCCAACACCATTCAGAG	TACGGTAGCAGAGACTTGGTCTAGTGGCACAGAAGCAAAGAG
277	11_20675	6H	55.90	ACACTGACGACATGGTTCTACATTGGGCGTACGAGGAGTATG	TACGGTAGCAGAGACTTGGTCTTTCACCAGCACCTGGTCCTC
278	11_10962	6H	58.34	ACACTGACGACATGGTTCTACATCTTGCCATCAGGCCTCAAC	TACGGTAGCAGAGACTTGGTCTTCAGCCATGTTCTTGCTACC
279	SCRI_RS_213566	6H	59.01	ACACTGACGACATGGTTCTACACGGTCATTATCATGGTTAGG	TACGGTAGCAGAGACTTGGTCTGGATCCCCAACAAATCAAAC
280	SCRI_RS_176650	6H	59.21	ACACTGACGACATGGTTCTACAAAATGAGGATGTCGCAGGTG	TACGGTAGCAGAGACTTGGTCTAATCCAGCACAGCTTCTGTC
281	SCRI_RS_186193	6H	59.21	ACACTGACGACATGGTTCTACATACGAGTCGGAGAAGTCAGC	TACGGTAGCAGAGACTTGGTCTAAGTACACCTTCCGCTTCGC
282	SCRI_RS_188243	6H	59.21	ACACTGACGACATGGTTCTACACCAAGGTCAAACTCTTTGCC	TACGGTAGCAGAGACTTGGTCTGCAGCTCTCAGGATTTGAAG
283	11_10377	6H	59.33	ACACTGACGACATGGTTCTACACTCCATGGCCTTGATCAAAG	TACGGTAGCAGAGACTTGGTCTCCAGATGAACTTCAAGCGTC
284	12_11253	6H	62.91	ACACTGACGACATGGTTCTACATAAGCTTTGCTTGCTGTGCC	TACGGTAGCAGAGACTTGGTCTAGTCCACCGTTTAGTGTCTC
285	12_10758	6H	64.29	ACACTGACGACATGGTTCTACATAGGCAACGAACCAAGTCTC	TACGGTAGCAGAGACTTGGTCTTGGAACGGATAGGTTTGGTC
286	11_20058	6H	65.38	ACACTGACGACATGGTTCTACAGAGTTGGGAACTTGGCATAC	TACGGTAGCAGAGACTTGGTCTGATTTGTGCTGCCAAATGCC
287	11_11483	6H	69.79	ACACTGACGACATGGTTCTACATCATCGTGGAGCTCTAGGAC	TACGGTAGCAGAGACTTGGTCTGTATACAGTAGGCTAGAGCG
288	11_10455	6H	72.29	ACACTGACGACATGGTTCTACAGCTGTAAGCAATGTCTTCCG	TACGGTAGCAGAGACTTGGTCTGACGACAAAATGGTACAAGG
289	11_10124	6H	73.83	ACACTGACGACATGGTTCTACAAGGACTAAACCCTCTGTCTG	TACGGTAGCAGAGACTTGGTCTCAATAAGCTAAAGCAAGAC
290	11_20892	6H	74.18	ACACTGACGACATGGTTCTACATGAAAACCATTGCCCCGAAG	TACGGTAGCAGAGACTTGGTCTTATGAACATCTCGGGTCCTG
291	12_31111	6H	76.62	ACACTGACGACATGGTTCTACAGGCGAAACCACAGATGGTTG	TACGGTAGCAGAGACTTGGTCTAACAGCAACTGGAGGATTGG
292	11_20682	6H	77.70	ACACTGACGACATGGTTCTACAAGGAGGACGAGCAGCAGC	TACGGTAGCAGAGACTTGGTCTTGACACTTGTGACAGTTCGG
293	11_10220	6H	80.31	ACACTGACGACATGGTTCTACAGTGTTTGTGTACATGGTGCG	TACGGTAGCAGAGACTTGGTCTTTTCCCTGCACATGCTTCTC
294	11_11246	6H	81.48	ACACTGACGACATGGTTCTACAGGTGCAATCCATTGTTGTTG	TACGGTAGCAGAGACTTGGTCTAGTTCAGCATCCCCAGTGAC
295	12_30698	6H	88.85	ACACTGACGACATGGTTCTACAAAGTACACACGGCAAGGAAG	TACGGTAGCAGAGACTTGGTCTACTGCTAGCTAACCGGAAAC
296	11_10400	6H	92.12	ACACTGACGACATGGTTCTACATTATTCGCGCCACCAGATTG	TACGGTAGCAGAGACTTGGTCTGACCATGCTTCTTCATCTGC
297	12_30151	6H	97.15	ACACTGACGACATGGTTCTACAACGGCGGCTACTACACGCT	TACGGTAGCAGAGACTTGGTCTTTGCACTCCACGAACGGCTC
298	11_20972	6H	99.15	ACACTGACGACATGGTTCTACAGAGTTTGACAACAGCGCTTC	TACGGTAGCAGAGACTTGGTCTAGTCATGAGATCCTGTACAC

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
299	12_31353	6H	100.53	ACACTGACGACATGGTTCTACAGTTTTGCCACATTGAAAGGAG	TACGGTAGCAGAGACTTGGTCTAGCAACGGTAAAATACGGGC
300	SCRI_RS_151574	6H	101.83	ACACTGACGACATGGTTCTACAACGCTCATGACGTGCCACTA	TACGGTAGCAGAGACTTGGTCTATCGGCGGTCGGGATCAGAA
301	12_31115	6H	102.03	ACACTGACGACATGGTTCTACAAACCACACCAACTGACTTGC	TACGGTAGCAGAGACTTGGTCTGCATGAACAAAACCGACGAG
302	11_20379	6H	105.23	ACACTGACGACATGGTTCTACAATAAGCCACTGCTCCCCTTC	TACGGTAGCAGAGACTTGGTCTCGAGTAGGAGTATGTCACTG
303	11_20036	6H	110.59	ACACTGACGACATGGTTCTACATCCTATCGTTTGGCTTTCGG	TACGGTAGCAGAGACTTGGTCTTAGGAGAGTCAGCGCAGAAG
304	11_20355	6H	113.05	ACACTGACGACATGGTTCTACAATCCATGATACAGCCTAGTG	TACGGTAGCAGAGACTTGGTCTCCCCAGTTTCAATTGATTCC
305	12_30734	6H	116.37	ACACTGACGACATGGTTCTACACTTCATCCGTTTCACCGTTC	TACGGTAGCAGAGACTTGGTCTTCAGACAGAGCAGAATGCAG
306	11_10107	6H	122.64	ACACTGACGACATGGTTCTACATTGAGGTGGTCTAGATGCAG	TACGGTAGCAGAGACTTGGTCTACCGCATACCCTCCAAAAAG
307	11_11187	6H	124.72	ACACTGACGACATGGTTCTACAAATTGCTCCCTTGAAACCGC	TACGGTAGCAGAGACTTGGTCTTCAGTCAGGTGATTATGTTG
308	11_20868	6H	129.12	ACACTGACGACATGGTTCTACAGGTAGCTTTATGTATGGCGG	TACGGTAGCAGAGACTTGGTCTTTCGCCGCCGTTCTGCTCTT
309	11_20537	6H	139.39	ACACTGACGACATGGTTCTACAGCAAGGACGATAGGTAGATG	TACGGTAGCAGAGACTTGGTCTCATCCAGCATGCCTACAATC
310	11_21419	7H	0.00	ACACTGACGACATGGTTCTACACATGGTCTTGCAGACATTCC	TACGGTAGCAGAGACTTGGTCTCTTGATCGTCAAGAGAAGTG
311	11_10682	7H	1.08	ACACTGACGACATGGTTCTACACGCCTAGTTTAGTGGCTGGT	TACGGTAGCAGAGACTTGGTCTCTGGAAATCTAAGATGAACC
312	11_20710	7H	2.47	ACACTGACGACATGGTTCTACATGCCATTGCTGCAAGGATAG	TACGGTAGCAGAGACTTGGTCTACAATTCATCCCCACCTCTC
313	11_20242	7H	4.52	ACACTGACGACATGGTTCTACACCTGGTCATCCTTGATGCTG	TACGGTAGCAGAGACTTGGTCTTTTTCGCCGAGTGGAACAAG
314	12_11035	7H	6.29	ACACTGACGACATGGTTCTACACCAGGTGTATCTGAATAAGAC	TACGGTAGCAGAGACTTGGTCTTGCAGCTGAAAATCCAATAG
315	11_20245	7H	9.82	ACACTGACGACATGGTTCTACATGCACTAGTTCTTGCCCATC	TACGGTAGCAGAGACTTGGTCTGGAGAAATTCCAGGGCTATC
316	11_10841	7H	12.97	ACACTGACGACATGGTTCTACAGCTTTGAAGCATGATCGGAC	TACGGTAGCAGAGACTTGGTCTATTCGGTTGACCACAAGCTC
317	11_21437	7H	15.44	ACACTGACGACATGGTTCTACACCAGACAATGTTCAAGGTCC	TACGGTAGCAGAGACTTGGTCTTATCTCATTCTCGCACCCTC
318	11_21050	7H	17.23	ACACTGACGACATGGTTCTACACTTGTCCAATGGACGAAAGG	TACGGTAGCAGAGACTTGGTCTTGTAAACAGAAGGCGTGGTG
319	11_20722	7H	19.22	ACACTGACGACATGGTTCTACAGGAGGCAACATCTCTGATAG	TACGGTAGCAGAGACTTGGTCTAAATGCCTGATGTCGCGATG
320	11_20495	7H	22.12	ACACTGACGACATGGTTCTACACGCATTGACGCTTCCTTTTG	TACGGTAGCAGAGACTTGGTCTAGTCTAAGGACAACGGACAC
321	12_30530	7H	26.35	ACACTGACGACATGGTTCTACATACGAGGTGCACGGCCTCAT	TACGGTAGCAGAGACTTGGTCTGGTCGTGGTAGATGTGGTC
322	12_30780	7H	29.62	ACACTGACGACATGGTTCTACATTGGTGAAGGCCGCAGCAAC	TACGGTAGCAGAGACTTGGTCTGGTGGGGTTCTACCAGAAG
323	12_30219	7H	32.88	ACACTGACGACATGGTTCTACAGAAACTCACCAAATCCATCG	TACGGTAGCAGAGACTTGGTCTAAGTCCGTGACATACCCTAC

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
324	11_20993	7H	34.74	ACACTGACGACATGGTTCTACATACAGCGAATGGATCGATGA	TACGGTAGCAGAGACTTGGTCTTTTGGAAGCGGGACATGCTC
325	12_30895	7H	38.31	ACACTGACGACATGGTTCTACACACCTGCAGGCAGTATAAAG	TACGGTAGCAGAGACTTGGTCTCAGCTTATGTACGTACTCTC
326	11_10838	7H	40.46	ACACTGACGACATGGTTCTACACGTTGAGAATTGCGAAGGAC	TACGGTAGCAGAGACTTGGTCTCAGTCATCAGCATAAGTTCC
327	11_10576	7H	46.89	ACACTGACGACATGGTTCTACACGACAATTCCGGAGAAGATG	TACGGTAGCAGAGACTTGGTCTTCTCCCCCATTTTTGCTTTC
328	12_30545	7H	56.83	ACACTGACGACATGGTTCTACACTGACTACTGAGTGCCTAAC	TACGGTAGCAGAGACTTGGTCTCGAAACCCCCTTCAGTTTAG
329	12_10959	7H	61.04	ACACTGACGACATGGTTCTACAGATTTCATCCACCTCGCGAC	TACGGTAGCAGAGACTTGGTCTCAATTCAGAGCTGCCACTTC
330	12_30880	7H	63.28	ACACTGACGACATGGTTCTACACAGTCTCGGTATATGGGAAG	TACGGTAGCAGAGACTTGGTCTCCATGGGATTGACGTGTTTG
331	11_10050	7H	66.61	ACACTGACGACATGGTTCTACAATTCGTGAGGCAGATGGGTG	TACGGTAGCAGAGACTTGGTCTACTCGTTATGATCGTGAAGC
332	12_30149	7H	67.99	ACACTGACGACATGGTTCTACATTACAAGCACGATCAGGGAG	TACGGTAGCAGAGACTTGGTCTGGTAAAGTAAAAGGTGGAGG
333	11_11348	7H	71.76	ACACTGACGACATGGTTCTACATAGCTAGCTAGAGCTACCTG	TACGGTAGCAGAGACTTGGTCTAGCTTAGGACAATCAGCTGG
334	12_30997	7H	74.84	ACACTGACGACATGGTTCTACACTCGCCGGAGAGAGAAGAA	TACGGTAGCAGAGACTTGGTCTTTGCCCAGCCCTTTCCCTC
335	12_30344	7H	76.06	ACACTGACGACATGGTTCTACAACCAAGGAAGGAACAGTGCG	TACGGTAGCAGAGACTTGGTCTATCTTCCTCATCTCCTCTCC
336	11_10700	7H	78.07	ACACTGACGACATGGTTCTACAGATGGTAACTCATGTCCAAC	TACGGTAGCAGAGACTTGGTCTAGGTGCCCCTTTGTCTATGG
337	12_10982	7H	80.47	ACACTGACGACATGGTTCTACAAGATGAGGGTGGAGATGAAC	TACGGTAGCAGAGACTTGGTCTGTCGCTTCGATGACTCCTTC
338	12_30565	7H	81.07	ACACTGACGACATGGTTCTACATTCTACAACCAATTGATGCC	TACGGTAGCAGAGACTTGGTCTCTGAGAGATGAGGCATAATAC
339	12_30998	7H	82.16	ACACTGACGACATGGTTCTACACCACGACTACATGCTGAAAC	TACGGTAGCAGAGACTTGGTCTGCAGTGGCTTTGATCATGAG
340	11_20083	7H	84.09	ACACTGACGACATGGTTCTACATCTGATTCTGAGGGCATGTC	TACGGTAGCAGAGACTTGGTCTTTCCGCGTTGAGAATGAGTG
341	12_30996	7H	87.29	ACACTGACGACATGGTTCTACAAAGGATCAACTATGGCGGTC	TACGGTAGCAGAGACTTGGTCTCTGAACCCAGCAGGAGAATC
342	12_30026	7H	89.15	ACACTGACGACATGGTTCTACACCGTTGGTGAAGGAGAAAAC	TACGGTAGCAGAGACTTGGTCTCTTTTCAGCTAACTTTGGCG
343	11_21448	7H	92.00	ACACTGACGACATGGTTCTACATAACAGACGACGACGCTTAC	TACGGTAGCAGAGACTTGGTCTTGTACTGCTGTTTCCTCCTG
344	11_21201	7H	95.02	ACACTGACGACATGGTTCTACAAATGTGGAGAGCATTGTGCC	TACGGTAGCAGAGACTTGGTCTATCCTTCCCACCTTTCTTGC
345	11_20808	7H	99.90	ACACTGACGACATGGTTCTACAATTCCTGCCACGACCATAAG	TACGGTAGCAGAGACTTGGTCTCCCTCGATCCATATCGATAG
346	12_31440	7H	102.19	ACACTGACGACATGGTTCTACAAGCCACGGGTCTTCGACAT	TACGGTAGCAGAGACTTGGTCTATGACGACGCTCCTTCATCC
347	11_20824	7H	107.49	ACACTGACGACATGGTTCTACAACTCGTAAGTAACGTCGTGC	TACGGTAGCAGAGACTTGGTCTTGACCTTTGGAAACTCCAAG
348	SCRI_RS_112204	7H	109.00	ACACTGACGACATGGTTCTACAAAGCAATGGCTCAATTGCCG	TACGGTAGCAGAGACTTGGTCTACACCATCCATGATCCATCC

Table C1. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Tradition and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Markers	Chr	cM	Forward Primers	Reverse Primers
349	11_10853	7H	110.40	ACACTGACGACATGGTTCTACAGCTAACCTAAGGGAGCATTG	TACGGTAGCAGAGACTTGGTCTGCTGTGTACCTTAAAATTGC
350	11_20247	7H	117.10	ACACTGACGACATGGTTCTACAGATATCACTCCATTTCAAGG	TACGGTAGCAGAGACTTGGTCTTGCGTGGATCCTATTTTTGG
351	12_30368	7H	119.33	ACACTGACGACATGGTTCTACAAACCAAGAGAATCACAACCC	TACGGTAGCAGAGACTTGGTCTTCGACGGAGAGGGTTTAATG
352	11_11243	7H	125.55	ACACTGACGACATGGTTCTACAGGGCTTTTTGTGTACACAAT	TACGGTAGCAGAGACTTGGTCTACGTTTGGTGGGCATGTGTA
353	12_10543	7H	132.76	ACACTGACGACATGGTTCTACAAAAGGATGAATCGCCAAAGG	TACGGTAGCAGAGACTTGGTCTTTCTCTGCCACAAAGTAAGG
354	SCRI_RS_202130	7H	133.84	ACACTGACGACATGGTTCTACACTCTCCATTTCCATTTTGGG	TACGGTAGCAGAGACTTGGTCTGTGTTCCATGATGGTGTTCG
355	11_10182	7H	133.92	ACACTGACGACATGGTTCTACACACCAAATGCTGTGAACGAC	TACGGTAGCAGAGACTTGGTCTCCTGAAACGTAATTCGCATC
356	11_10861	7H	138.76	ACACTGACGACATGGTTCTACACATGTACAGAGTACCGTAGG	TACGGTAGCAGAGACTTGGTCTCTACCAGTACATCACTAGCG
357	11_10797	7H	141.37	ACACTGACGACATGGTTCTACATGAACACCGGCCTAATTACC	TACGGTAGCAGAGACTTGGTCTTGCGCTTTCAGGAACAACAG
358	11_20847	7H	145.68	ACACTGACGACATGGTTCTACAGCGAAGAAGAACTTGTCCTC	TACGGTAGCAGAGACTTGGTCTAAGGTGCAATCCTAGTAGGG
359	12_30761	7H	147.63	ACACTGACGACATGGTTCTACACTATCGATGACCTTGAGAATG	TACGGTAGCAGAGACTTGGTCTCTTGACCTCAATTTGGTTAGC
360	12_20640	7H	152.51	ACACTGACGACATGGTTCTACATAGCAATAAGGGCCGTGTAG	TACGGTAGCAGAGACTTGGTCTCTAAGTGGCACACCTTTATG
361	11_11275	7H	156.69	ACACTGACGACATGGTTCTACACGGCGACGATGGAGGTCAT	TACGGTAGCAGAGACTTGGTCTGCAGCTCAAAGAACGGATTC
362	11_11012	7H	157.08	ACACTGACGACATGGTTCTACATGGAGGAGGAAGAGGAGGT	TACGGTAGCAGAGACTTGGTCTTTGTCGTACTGCTCGTGCTC
363	11_20586	7H	161.09	ACACTGACGACATGGTTCTACATCAGTTTCGACAGGATCTGG	TACGGTAGCAGAGACTTGGTCTACAAGAAGCCCGGAATCATC
364	11_20185	7H	166.55	ACACTGACGACATGGTTCTACAGGAAAGAGTGACCATCTAGG	TACGGTAGCAGAGACTTGGTCTGTGCTCCTTGCGGTGTTAG
365	12_10378	7H	167.58	ACACTGACGACATGGTTCTACATGGTGAACTCGGCCAGCGTT	TACGGTAGCAGAGACTTGGTCTGTAGCGGGAGCTGCCAGAC

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information.

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
1	12_30969	1H	1.36	ACACTGACGACATGGTTCTACAAAGTTGCCTTAATGACTGGG	TACGGTAGCAGAGACTTGGTCTGCTTGAAGCTGAAGACAGAC
2	12_31144	1H	4.98	ACACTGACGACATGGTTCTACAGAAAAGAAGTGATTCGCCCG	TACGGTAGCAGAGACTTGGTCTTGCAGAAGTTCAAGACCCTG
3	SCRI_RS_66630	1H	5.68	ACACTGACGACATGGTTCTACACATGATATCTGCTTGAGTGG	TACGGTAGCAGAGACTTGGTCTCAGTATATATATCCAGTTCAC
4	11_21226	1H	10.35	ACACTGACGACATGGTTCTACAATCCTCATCAGGTATTCGGC	TACGGTAGCAGAGACTTGGTCTTCCCCTGCTCATTCAGTTTG
5	SCRI_RS_130592	1H	17.20	ACACTGACGACATGGTTCTACATGACGTCCACGTACTACTAC	TACGGTAGCAGAGACTTGGTCTCTTGGCCGCCACTGAACAT
6	11_10030	1H	21.99	ACACTGACGACATGGTTCTACAGATGAACCGAAGTATGCACC	TACGGTAGCAGAGACTTGGTCTTTTCCTCCGCTTTGAAGGTG
7	SCRI_RS_124926	1H	26.32	ACACTGACGACATGGTTCTACAGTCGTATGATCCACGTGATG	TACGGTAGCAGAGACTTGGTCTGCAACCTTTGCGATCATCTC
8	11_10757	1H	28.78	ACACTGACGACATGGTTCTACACTTGTCGTATGAGGCTCTTG	TACGGTAGCAGAGACTTGGTCTTGCAGGGCGTCGTCGACTA
9	11_21048	1H	31.24	ACACTGACGACATGGTTCTACAAGGAGAAGAAGGCGGAGGTG	TACGGTAGCAGAGACTTGGTCTTTCCAGAGCTGATCCATGTC
10	12_31177	1H	37.10	ACACTGACGACATGGTTCTACATAATGAGGATGCAGCCAGAG	TACGGTAGCAGAGACTTGGTCTCATGCCCAGGTTGGAACAC
11	12_30336	1H	42.17	ACACTGACGACATGGTTCTACAGCAGTTTATATTCCGGTGTG	TACGGTAGCAGAGACTTGGTCTACACTGAGTCAACGTAGTGC
12	12_10314	1H	46.53	ACACTGACGACATGGTTCTACATGGCTTGTGGAATTGAGGAC	TACGGTAGCAGAGACTTGGTCTTATAACTTCGGTGGACGCTG
13	11_21095	1H	47.92	ACACTGACGACATGGTTCTACAAGACGTCCACCAAGAAGAAC	TACGGTAGCAGAGACTTGGTCTTGTCACTTCGGCAGAAATGG
14	12_30710	1H	51.86	ACACTGACGACATGGTTCTACACGAACTTATCGATGAGGCTG	TACGGTAGCAGAGACTTGGTCTCATCACAAAGCACCACTGAC
15	11_10176	1H	59.01	ACACTGACGACATGGTTCTACATTGGCCTGCCATTCTCTTTC	TACGGTAGCAGAGACTTGGTCTTTACAGAGAGCCTTGGCATC
16	11_10002	1H	64.93	ACACTGACGACATGGTTCTACACGACGACAATACACAACACC	TACGGTAGCAGAGACTTGGTCTGTTCGGTGCGTCTGTTTTTG
17	12_31464	1H	64.93	ACACTGACGACATGGTTCTACATGGCCCCACATATGCATCAG	TACGGTAGCAGAGACTTGGTCTCCTGAGGTGACCATGATTTG
18	12_10166	1H	69.73	ACACTGACGACATGGTTCTACAAGTGGGCGTTGATGTGATTC	TACGGTAGCAGAGACTTGGTCTAACGGTCGACAAACCGTAAG
19	11_20229	1H	71.70	ACACTGACGACATGGTTCTACAATGAGGCCAAGATTGAGGTG	TACGGTAGCAGAGACTTGGTCTGTCCCTGATGTGAAGCAATG
20	11_20990	1H	74.12	ACACTGACGACATGGTTCTACAAGTAACACTGGACACACACC	TACGGTAGCAGAGACTTGGTCTGCCATAATTGAGAGGCTGTG
21	SCRI_RS_181353	1H	78.25	ACACTGACGACATGGTTCTACAGCTGAAATTCAAAGAGCAGAG	TACGGTAGCAGAGACTTGGTCTCACCTTGAGTGGAAAGATCG
22	SCRI_RS_9158	1H	82.45	ACACTGACGACATGGTTCTACACACCCTCGCTTTCTGCATC	TACGGTAGCAGAGACTTGGTCTAAATCGAGTGGTCCGGATTC
23	11_20792	1H	88.25	ACACTGACGACATGGTTCTACAAAACAGTTGCCAGCTTCCAC	TACGGTAGCAGAGACTTGGTCTAGTGCTACTGCATAATCAGG
24	11_21373	1H	90.98	ACACTGACGACATGGTTCTACACCTTCTCCAAGTCATCCATC	TACGGTAGCAGAGACTTGGTCTGGCAATGTCCGAAAGAAACC
25	11_20149	1H	94.48	ACACTGACGACATGGTTCTACATAATGTGAGAAATTTTGATAT	TACGGTAGCAGAGACTTGGTCTTCAAGACCTTGTGGTTTGGC
26	SCRI_RS_188909	1H	98.45	ACACTGACGACATGGTTCTACACAGCACGTAACATCTGCATC	TACGGTAGCAGAGACTTGGTCTATCCAGCTACTAGAGTGGAC
27	12_31152	1H	101.05	ACACTGACGACATGGTTCTACACTCCAGTAACAATCGACGTG	TACGGTAGCAGAGACTTGGTCTATTTGTCCGGTCGTCCAAAC

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
28	11_20267	1H	105.52	ACACTGACGACATGGTTCTACAAAGCAAAGCAGGAAGCTGAG	TACGGTAGCAGAGACTTGGTCTGAGAGCCTCTCTCTAGCTTC
29	12_30532	1H	109.53	ACACTGACGACATGGTTCTACACTGCACGTCATCATGATGTC	TACGGTAGCAGAGACTTGGTCTCAACAACAGGTTCAGAGTCG
30	12_30014	1H	114.98	ACACTGACGACATGGTTCTACAATATATCCAAAGTGCTGTCG	TACGGTAGCAGAGACTTGGTCTATCAGCTCTGGGACGGCTTG
31	11_20908	1H	126.60	ACACTGACGACATGGTTCTACAGATTGACGAGGCGGTGATAC	TACGGTAGCAGAGACTTGGTCTCAAAGGAAGGAACCGAATGC
32	SCRI_RS_189967	1H	129.42	ACACTGACGACATGGTTCTACAGCACCTTCTTCAGCATCTTC	TACGGTAGCAGAGACTTGGTCTACAGACACTCTCGTACCCTG
33	12_10808	1H	131.46	ACACTGACGACATGGTTCTACATTATACCCTTCAAGCAGCGG	TACGGTAGCAGAGACTTGGTCTGCAGAGGCTAATAAAGCAAC
34	12_10693	1H	132.54	ACACTGACGACATGGTTCTACAAAGTCCCATGGGAGAATCAG	TACGGTAGCAGAGACTTGGTCTTCTTGCCACCAATGGTGAAC
35	SCRI_RS_196025	1H	136.75	ACACTGACGACATGGTTCTACATAGCTACCTTGTTAGGCTCC	TACGGTAGCAGAGACTTGGTCTCCTAACACTACATAGGGTGC
36	11_10590	1H	142.74	ACACTGACGACATGGTTCTACAACAACACAGCGAAAACGAAC	TACGGTAGCAGAGACTTGGTCTTGACGAGACTGCACTAGGTA
37	12_31081	1H	145.82	ACACTGACGACATGGTTCTACAAACTGCTTGGATGGAACCAC	TACGGTAGCAGAGACTTGGTCTGCCTCATTTCTGAGATAGTG
38	12_21415	2H	5.39	ACACTGACGACATGGTTCTACATTTCTCCAAACGGTCCTTCG	TACGGTAGCAGAGACTTGGTCTTTTTGGCTCTACAGACATAG
39	11_10326	2H	7.29	ACACTGACGACATGGTTCTACACTCCATGGGATACCCATGTC	TACGGTAGCAGAGACTTGGTCTTCAAGAAACGGTGATGGTGC
40	SCRI_RS_168604	2H	11.49	ACACTGACGACATGGTTCTACATGGACCTCGTCCGCTTCTAC	TACGGTAGCAGAGACTTGGTCTTCAGGGTTGACCTCGATGAC
41	11_21377	2H	13.19	ACACTGACGACATGGTTCTACAAGCAGCAGCTACTTGCAAAC	TACGGTAGCAGAGACTTGGTCTACTGCCAAAGAGACGATTGC
42	SCRI_RS_152744	2H	23.76	ACACTGACGACATGGTTCTACACATCAAGAAAGAAGCCGGAG	TACGGTAGCAGAGACTTGGTCTACGTACTCGGCGTCCACCA
43	11_10943	2H	25.53	ACACTGACGACATGGTTCTACACCAACACTAACGGTAACAGC	TACGGTAGCAGAGACTTGGTCTTCGCCGTTTTCACTCTTCAG
44	SCRI_RS_153798	2H	31.74	ACACTGACGACATGGTTCTACACACGATCGAGTTCATCATCC	TACGGTAGCAGAGACTTGGTCTAATGCAGGTCGATCGATCGG
45	SCRI_RS_131218	2H	35.04	ACACTGACGACATGGTTCTACAAAGATCAACAGCAGCAGCCC	TACGGTAGCAGAGACTTGGTCTTCACTTCCGTCGTGGCCTCT
46	11_10216	2H	40.73	ACACTGACGACATGGTTCTACAAAGTCTTGATCCAGCCTTGC	TACGGTAGCAGAGACTTGGTCTCGCCTAGTCTTAATGTCTGG
47	SCRI_RS_182371	2H	44.54	ACACTGACGACATGGTTCTACAACTTGCTTGGGACTAAAGGC	TACGGTAGCAGAGACTTGGTCTGGCAATTACCACACTGCAAC
48	SCRI_RS_174935	2H	51.96	ACACTGACGACATGGTTCTACACATGTACCAGTTCGTCAGTC	TACGGTAGCAGAGACTTGGTCTACTGGACTGATGCTAGACTG
49	11_10919	2H	53.26	ACACTGACGACATGGTTCTACATAAAGGGCAAGGAAAAGCGG	TACGGTAGCAGAGACTTGGTCTCCTGATAAGCTACAGCATGA
50	12_30432	2H	54.31	ACACTGACGACATGGTTCTACAAGAACAACCGAGTCATGTGC	TACGGTAGCAGAGACTTGGTCTGTTCCATGCACCCATGATGA
51	SCRI_RS_221843	2H	62.91	ACACTGACGACATGGTTCTACAAAACATGGACGAGGCGGT	TACGGTAGCAGAGACTTGGTCTCCAGAAGCTAGCTGCCTTTC
52	SCRI_RS_151535	2H	65.71	ACACTGACGACATGGTTCTACAGACCTTGACACAGTAAAAGC	TACGGTAGCAGAGACTTGGTCTAGGTCATCTGCTGTTGCATC
53	SCRI_RS_175065	2H	65.81	ACACTGACGACATGGTTCTACACACATCATCACAAGCTAACG	TACGGTAGCAGAGACTTGGTCTTTCCCTTAGCTATGGGAGTG
54	11_10733	2H	66.11	ACACTGACGACATGGTTCTACACACAGAGACGCACACAAATC	TACGGTAGCAGAGACTTGGTCTGATGCCACAGATGAGCTATG

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
55	12_10927	2H	67.89	ACACTGACGACATGGTTCTACATCATGAAGGTATGGCTTCCG	TACGGTAGCAGAGACTTGGTCTCAATTCTTAGGAGGAGACAG
56	11_20532	2H	69.55	ACACTGACGACATGGTTCTACAGCAGATCCCAACAAAAGACG	TACGGTAGCAGAGACTTGGTCTATCCCTCGATGTAGTGTCTG
57	11_21144	2H	72.44	ACACTGACGACATGGTTCTACAAGCAATCGCTGGGCTTTTTG	TACGGTAGCAGAGACTTGGTCTTTCACGATCCTTACCCTTTC
58	11_10265	2H	75.18	ACACTGACGACATGGTTCTACACCACGGTACAGCGAAAATTC	TACGGTAGCAGAGACTTGGTCTCTCTCGATCCTTCTAGGTTG
59	SCRI_RS_150	2H	78.02	ACACTGACGACATGGTTCTACAAGATCGCAGATGGTCTGTTG	TACGGTAGCAGAGACTTGGTCTAACACCTTGCTGTGGCAAAC
60	11_10287	2H	90.99	ACACTGACGACATGGTTCTACAGGTTCATCGTCACTAGGAAG	TACGGTAGCAGAGACTTGGTCTTAGACCTGCCACCAATGAAG
61	SCRI_RS_219074	2H	95.24	ACACTGACGACATGGTTCTACATGCCTTTCTGTTTGCCTCTG	TACGGTAGCAGAGACTTGGTCTTGCTAAAAGAAGGCCGGATG
62	11_10214	2H	99.04	ACACTGACGACATGGTTCTACACATGGCAGCTAAGCCCTAAG	TACGGTAGCAGAGACTTGGTCTGGACGACGAGGAGTAAATAG
63	12_30216	2H	101.98	ACACTGACGACATGGTTCTACACGAGACACTAGAGACACTTC	TACGGTAGCAGAGACTTGGTCTCAGATGACCAGGCATGGTTT
64	11_10398	2H	106.90	ACACTGACGACATGGTTCTACATAGACGGCTTTATTTGGCAG	TACGGTAGCAGAGACTTGGTCTAAGCCACGGTGATTGATGAC
65	12_30480	2H	108.14	ACACTGACGACATGGTTCTACAATGCGGCTCGGATGTGTATC	TACGGTAGCAGAGACTTGGTCTACGAACTTACCTTGATGCGG
66	12_30555	2H	112.22	ACACTGACGACATGGTTCTACATTCCGACCATCACTTGAGAG	TACGGTAGCAGAGACTTGGTCTTCAATCCTGAGAACAAGGAC
67	11_20099	2H	121.76	ACACTGACGACATGGTTCTACACGTTTTCGACGCAGAGTTGT	TACGGTAGCAGAGACTTGGTCTCGTGCAAGTCGCAGAAGAG
68	11_10128	2H	124.50	ACACTGACGACATGGTTCTACATTCTGGACGGTGAGCTTGAC	TACGGTAGCAGAGACTTGGTCTCCAAGAAGATCGGTGAGGAC
69	11_10429	2H	126.63	ACACTGACGACATGGTTCTACATGATGGGCGCATTGACTATG	TACGGTAGCAGAGACTTGGTCTTTCTCATTGTTCTTCGCCCG
70	12_10739	2H	135.51	ACACTGACGACATGGTTCTACACTGAAACACCCAACACTTGC	TACGGTAGCAGAGACTTGGTCTTTTGACTAGCAACCCATGCC
71	11_21220	2H	136.66	ACACTGACGACATGGTTCTACAGCCGCCTAAACTTCTGAATC	TACGGTAGCAGAGACTTGGTCTTTACAAGGGTCAAGCTGCTG
72	SCRI_RS_155161	2H	137.44	ACACTGACGACATGGTTCTACATGCTGTCCAATCTGAAGCTG	TACGGTAGCAGAGACTTGGTCTTCTTGATGCTCAATGTGCTG
73	11_20141	2H	141.70	ACACTGACGACATGGTTCTACAAGCAGCAGTGATGAAGTTGG	TACGGTAGCAGAGACTTGGTCTAGAGACGATGCAGTCGTTGG
74	12_31100	2H	143.07	ACACTGACGACATGGTTCTACAATGGCCTTAGTGTCTCTTCC	TACGGTAGCAGAGACTTGGTCTAGAAAAGGCTGTCCCTTTGG
75	11_21088	2H	144.20	ACACTGACGACATGGTTCTACATATGGAGCATATGGATGCAG	TACGGTAGCAGAGACTTGGTCTCTATCACCCATGGAAACACC
76	11_21125	2H	145.20	ACACTGACGACATGGTTCTACAGCCTCAGCCAGAATAGTAAG	TACGGTAGCAGAGACTTGGTCTAAAGTCTTCCATGGAAGCCC
77	12_10579	2H	149.60	ACACTGACGACATGGTTCTACATCATCTCGTTGCATATGCCC	TACGGTAGCAGAGACTTGGTCTTCCGAACACCCTTCTTCATC
78	12_10579	2H	149.60	ACACTGACGACATGGTTCTACATCATCTCGTTGCATATGCCC	TACGGTAGCAGAGACTTGGTCTTCCGAACACCCTTCTTCATC
79	11_20715	2H	152.83	ACACTGACGACATGGTTCTACACAGCCAGTGGACTTAATGTG	TACGGTAGCAGAGACTTGGTCTTTTGCTCCACACAACGGTAG
80	11_21181	2H	155.66	ACACTGACGACATGGTTCTACATCTATACTGGAGGCAGGTAG	TACGGTAGCAGAGACTTGGTCTATGGAAAGGAGGTTTTCCGC
81	11_11023	2H	160.19	ACACTGACGACATGGTTCTACAGACATGGCAAGAGTACATTC	TACGGTAGCAGAGACTTGGTCTCGTGACGAAGTGAAATGGAC

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
82	SCRI_RS_155544	2H	163.96	ACACTGACGACATGGTTCTACATTTCGCAATGCGGTGATTGG	TACGGTAGCAGAGACTTGGTCTGGAATGTGGGTTAGGCCTTG
83	SCRI_RS_230497	2H	164.26	ACACTGACGACATGGTTCTACATGCTTTTGCCCCACTCACTC	TACGGTAGCAGAGACTTGGTCTCACATCCTCAAAATAGTTTCG
86	12_31180	2H	175.48	ACACTGACGACATGGTTCTACAACGGATGAGATTCAGGAAGC	TACGGTAGCAGAGACTTGGTCTAACCATGTGCATGCATGTCC
87	11_10085	2H	177.38	ACACTGACGACATGGTTCTACACACATAGTCTCGGCTATTCC	TACGGTAGCAGAGACTTGGTCTCAGTAGCATGTTGGTGGTTG
88	SCRI_RS_206020	2H	179.38	ACACTGACGACATGGTTCTACATGCTGTGCCACCGATCGAGA	TACGGTAGCAGAGACTTGGTCTTACCTTCTCTTTGGCAGCTC
89	12_31428	3H	0.00	ACACTGACGACATGGTTCTACAGGCGGTGAGGAGGTAGGTG	TACGGTAGCAGAGACTTGGTCTGCATCCTCTCCGGATTCTTC
90	SCRI_RS_1804	3H	3.89	ACACTGACGACATGGTTCTACAGCCTCTTCTTCAAGTAGACG	TACGGTAGCAGAGACTTGGTCTAGCCCTACATACACTCGAAG
91	11_20252	3H	6.46	ACACTGACGACATGGTTCTACATTCCTGAATGTCTCCTATGC	TACGGTAGCAGAGACTTGGTCTAAACGCCCTGCATTAGCGAC
92	11_20529	3H	8.33	ACACTGACGACATGGTTCTACATCTGGAACATGCCCTTCTTG	TACGGTAGCAGAGACTTGGTCTGCGCCAAGGCCAACTCGTT
93	11_21398	3H	8.86	ACACTGACGACATGGTTCTACAGCTCACTTGTTCAATGCACC	TACGGTAGCAGAGACTTGGTCTATGATTGCTTCCGAAGACCC
94	12_30818	3H	12.23	ACACTGACGACATGGTTCTACACTACTTCTTGGCCGCGGAC	TACGGTAGCAGAGACTTGGTCTATCGTCACGCCCCTCTTCTC
95	SCRI_RS_97417	3H	18.72	ACACTGACGACATGGTTCTACAGTTGCTGAATGAAAGCGATG	TACGGTAGCAGAGACTTGGTCTGCAACAAGTACAGGCTTCAG
96	11_20742	3H	24.63	ACACTGACGACATGGTTCTACAATCAGCACCGTGTACTTGGG	TACGGTAGCAGAGACTTGGTCTCTTGGTCTCGACCACATTGC
97	SCRI_RS_161041	3H	26.28	ACACTGACGACATGGTTCTACACACAAAGTGTGGACATGGAG	TACGGTAGCAGAGACTTGGTCTACGTTTCTGTGGGATGCAAG
98	SCRI_RS_189757	3H	31.88	ACACTGACGACATGGTTCTACACCACCTTTGACGCCAAGAAG	TACGGTAGCAGAGACTTGGTCTTCTCCTGAACTCCAAGCAAC
99	SCRI_RS_144410	3H	33.28	ACACTGACGACATGGTTCTACATGAAGCTGGTGAGAGCCGC	TACGGTAGCAGAGACTTGGTCTTCTGCTTCCCAAACTTGCTG
100	12_30431	3H	40.48	ACACTGACGACATGGTTCTACACTTCCCTTGGAGGTCTTTTG	TACGGTAGCAGAGACTTGGTCTCGCTGCATATAATCGGAACC
101	12_10968	3H	44.82	ACACTGACGACATGGTTCTACACCGAGATGCTTCTACTGTTC	TACGGTAGCAGAGACTTGGTCTGAATCCCGCCCTGCTGAAC
102	12_31298	3H	46.64	ACACTGACGACATGGTTCTACAGCAGCGTTCGGGAAAAAATG	TACGGTAGCAGAGACTTGGTCTACAATTGCCTGTTTCAGCCG
103	11_20356	3H	55.57	ACACTGACGACATGGTTCTACATAGAAACAGGAGCCAGTTGC	TACGGTAGCAGAGACTTGGTCTACTGTAGCTCATGAGCTCAC
104	12_30467	3H	56.79	ACACTGACGACATGGTTCTACAATTCTTCGATGATGGCGGAC	TACGGTAGCAGAGACTTGGTCTGCAAAATGGCCTCAACTTCG
105	11_10373	3H	61.94	ACACTGACGACATGGTTCTACACCGAATCACCGAGAAATAGG	TACGGTAGCAGAGACTTGGTCTCTGCAAGCAAATAAAGTGGC
106	11_21305	3H	65.16	ACACTGACGACATGGTTCTACAGGTATTCCAAGGATCCTCAG	TACGGTAGCAGAGACTTGGTCTGTCCCTGGTTATGCTTGATG
107	12_30399	3H	67.53	ACACTGACGACATGGTTCTACAACTCTTGTCGTGTGGTACTC	TACGGTAGCAGAGACTTGGTCTGCTTTAGAGATATTGTACAG
108	11_20115	3H	71.74	ACACTGACGACATGGTTCTACAGTGAACCTTGTATATTCTG	TACGGTAGCAGAGACTTGGTCTCAGAACCACAAGCTGTAGAC
109	SCRI_RS_153148	3H	75.71	ACACTGACGACATGGTTCTACAGATCCTAAAGGAGGACTAGC	TACGGTAGCAGAGACTTGGTCTATGCCTGCCATAGCATACTC
110	11_10444	3H	78.93	ACACTGACGACATGGTTCTACATTCTCGTAGTTCCTGAGCTG	TACGGTAGCAGAGACTTGGTCTTAGGGTGGATCTCCTCCTAC

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
111	12_31018	3H	81.11	ACACTGACGACATGGTTCTACATGGCTATGGCGTTGTTGAAG	TACGGTAGCAGAGACTTGGTCTTCCTGAAAACTTGAGGCCAG
112	12_31367	3H	85.85	ACACTGACGACATGGTTCTACAGCTTTATCGACCATTCCAGC	TACGGTAGCAGAGACTTGGTCTCAAGGGTCGAATTGTGCAAG
113	SCRI_RS_159340	3H	88.17	ACACTGACGACATGGTTCTACAGACTGCCTACGTTTCTTTGG	TACGGTAGCAGAGACTTGGTCTCATTGGTGGCTTAGTAGCAG
114	SCRI_RS_120503	3H	92.98	ACACTGACGACATGGTTCTACATCAACCGCAAGTTCGCCTTC	TACGGTAGCAGAGACTTGGTCTTTGATCCAGTCCTCCTTCTC
115	12_10344	3H	94.03	ACACTGACGACATGGTTCTACACAGAAGGTCAGAGAGATTGC	TACGGTAGCAGAGACTTGGTCTAGTATCGCCATGAGTGCAAG
116	11_21495	3H	94.03	ACACTGACGACATGGTTCTACAGCACAAGGGCTGAACATAAC	TACGGTAGCAGAGACTTGGTCTTGCTGAGAAACAACCTAGTG
117	12_30342	3H	94.03	ACACTGACGACATGGTTCTACATGAGAGTCGAGACTTGAGAG	TACGGTAGCAGAGACTTGGTCTTCTTGCCAGGTAAGAGTGTC
118	11_21493	3H	95.25	ACACTGACGACATGGTTCTACACGGGCCTTGATTGATTTTGC	TACGGTAGCAGAGACTTGGTCTCCAGGTGGCAGTGAAAAAAC
119	SCRI_RS_221787	3H	99.26	ACACTGACGACATGGTTCTACAGAGTGGAGTGGTTGCTGCTT	TACGGTAGCAGAGACTTGGTCTACCCGCGCGTGGGTGAGTC
120	SCRI_RS_164704	3H	99.26	ACACTGACGACATGGTTCTACATTTCGTAGAGGCCATCGTAG	TACGGTAGCAGAGACTTGGTCTCACATGGTATTATTATTTCCC
121	SCRI_RS_225641	3H	99.46	ACACTGACGACATGGTTCTACAAAGTGATGATGATGCTGCCG	TACGGTAGCAGAGACTTGGTCTCCCCTTCACCAGATCTGCAT
122	SCRI_RS_133339	3H	99.56	ACACTGACGACATGGTTCTACATGACAGAGAGAACGAACTCC	TACGGTAGCAGAGACTTGGTCTTTTGGCCCATGGATCAAGTG
123	SCRI_RS_211929	3H	99.66	ACACTGACGACATGGTTCTACAAAGTACTACGCCGACGACTG	TACGGTAGCAGAGACTTGGTCTTGGTTTTCTGGGTCTTCACG
124	12_30423	3H	99.66	ACACTGACGACATGGTTCTACAGAAGTCAATACCGATTGTAG	TACGGTAGCAGAGACTTGGTCTTGTTGGCAAGAGTTGCCTCC
125	SCRI_RS_235791	3H	99.66	ACACTGACGACATGGTTCTACATGGCGACCATCAAAGCAAAG	TACGGTAGCAGAGACTTGGTCTGCACATGAGGATGTGTGTTG
126	12_31329	3H	103.83	ACACTGACGACATGGTTCTACAAATGAAGTGTTCGGCGACAG	TACGGTAGCAGAGACTTGGTCTTCAGAGTTCATGTTCTGGG
127	11_10584	3H	105.98	ACACTGACGACATGGTTCTACATGCTCGGACAGAGACGTGA	TACGGTAGCAGAGACTTGGTCTTGCTGGGCTTTCTCGACGAT
128	11_20944	3H	109.12	ACACTGACGACATGGTTCTACAGGAGAACATTCCACATTAG	TACGGTAGCAGAGACTTGGTCTATACAACGAGGCATCTGAGC
129	11_21405	3H	111.70	ACACTGACGACATGGTTCTACAAATTGATCCCCATGACTCCC	TACGGTAGCAGAGACTTGGTCTTGCAGTCATCAAATTCAGCG
130	12_30081	3H	115.86	ACACTGACGACATGGTTCTACATGAATGGCCATTGCCATGAG	TACGGTAGCAGAGACTTGGTCTCACACTTGCAAGTACCACAC
131	11_10842	3H	118.71	ACACTGACGACATGGTTCTACAGTCTCCTTTATCCTTGTAAG	TACGGTAGCAGAGACTTGGTCTCAGGACCAGCTAGATTGTTG
132	12_30973	3H	121.02	ACACTGACGACATGGTTCTACACTGAAAACAGATGGTGTAA	TACGGTAGCAGAGACTTGGTCTCCACATATCATTGTTACCTTG
133	11_10381	3H	125.23	ACACTGACGACATGGTTCTACAAAGCACTCTCTGCATCCTTC	TACGGTAGCAGAGACTTGGTCTCAGAAAGCCATCTCTTCGTC
134	SCRI_RS_10016	3H	128.53	ACACTGACGACATGGTTCTACACATTAAGGAGATGCTGCCAC	TACGGTAGCAGAGACTTGGTCTGAACTCTACACAGCATTCAAC
135	12_21386	3H	133.66	ACACTGACGACATGGTTCTACAACATAGTACAGTAGCAAGGG	TACGGTAGCAGAGACTTGGTCTTTCGCTGAAGAACCCACTTG
136	SCRI_RS_183550	3H	133.78	ACACTGACGACATGGTTCTACACTGATTGTTTCTGGACTGGG	TACGGTAGCAGAGACTTGGTCTCACCTAATGGTATTTCAGGAC
137	11_21272	3H	137.48	ACACTGACGACATGGTTCTACATTCCAAACACACAAGCCAGC	TACGGTAGCAGAGACTTGGTCTACCCTGTCATGCTTCACATC

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
138	12_31500	3H	141.20	ACACTGACGACATGGTTCTACATTCTCTATTGCCACCCTGAC	TACGGTAGCAGAGACTTGGTCTTGCTTCATTTTTGCACCCCC
139	SCRI_RS_157113	3H	145.29	ACACTGACGACATGGTTCTACATCCACAAACGCCGCACAAAC	TACGGTAGCAGAGACTTGGTCTTCGCGATGAACGGGATCAG
140	11_11436	3H	145.65	ACACTGACGACATGGTTCTACATGCATCGGACGGTATACTTC	TACGGTAGCAGAGACTTGGTCTGGATTGCTTGTTCCTAATGG
141	SCRI_RS_229623	3H	150.19	ACACTGACGACATGGTTCTACAAGAGAAGAAGAGGAGCAACC	TACGGTAGCAGAGACTTGGTCTATGAGACCGGGTCGAGAATG
142	11_11411	3H	151.23	ACACTGACGACATGGTTCTACACACTCCCATATGGTGGATCA	TACGGTAGCAGAGACTTGGTCTGCTTGGGGTGAACGTCATTG
143	SCRI_RS_156315	3H	153.39	ACACTGACGACATGGTTCTACACATCATAGATACAGCCTGCC	TACGGTAGCAGAGACTTGGTCTTATCAGCAGTGGATGAAGCC
144	SCRI_RS_178836	3H	160.09	ACACTGACGACATGGTTCTACAAATCCTCTGCTTGAGTCGTC	TACGGTAGCAGAGACTTGGTCTTCACTAAGCAGGAACAAGGC
145	11_20145	4H	1.17	ACACTGACGACATGGTTCTACAAGAGAAGAAGAATCGAGCAG	TACGGTAGCAGAGACTTGGTCTTCTCCTCGAACGGAGTAACC
146	11_10409	4H	4.11	ACACTGACGACATGGTTCTACAAGCACTTTGGAAACAGTGGG	TACGGTAGCAGAGACTTGGTCTGAATTTCCCATGAAGAGGTG
147	12_31458	4H	14.13	ACACTGACGACATGGTTCTACAGCAGTTTCAAAAGCAATCTC	TACGGTAGCAGAGACTTGGTCTCAGCTTTCTGCTGTTATGTTC
148	12_30540	4H	16.13	ACACTGACGACATGGTTCTACATGTATGGAGTGTCCATGGAG	TACGGTAGCAGAGACTTGGTCTCACACCGTTTTATCAAATC
149	12_30150	4H	18.47	ACACTGACGACATGGTTCTACAGGTAAACCACCACTTCTTGC	TACGGTAGCAGAGACTTGGTCTCATCCCATCTATCCTGAAAC
150	11_10223	4H	22.43	ACACTGACGACATGGTTCTACACGTCGGTAGACGAGAGAAG	TACGGTAGCAGAGACTTGGTCTTTTCGCTCGGTGTCCAAAAC
151	SCRI_RS_98443	4H	27.64	ACACTGACGACATGGTTCTACACATTCAAGCCCATCAATGCG	TACGGTAGCAGAGACTTGGTCTATTCCAGGAGCTCACTCAAG
152	11_21374	4H	32.68	ACACTGACGACATGGTTCTACAGGAGCAAACGTGTTAGTTGG	TACGGTAGCAGAGACTTGGTCTATACCCAAGATTGTCGGCAC
153	11_20012	4H	46.19	ACACTGACGACATGGTTCTACAGAGAGCATCGCCGAGAAGAT	TACGGTAGCAGAGACTTGGTCTGAAACGAAGGGCATACAGAG
154	SCRI_RS_167844	4H	51.77	ACACTGACGACATGGTTCTACAATGATCAGGCTGGTGAGTTG	TACGGTAGCAGAGACTTGGTCTAAGTCTTTGATCTGGGCCTC
155	11_20472	4H	54.95	ACACTGACGACATGGTTCTACAACGAAATGGACGCGTCAAAG	TACGGTAGCAGAGACTTGGTCTCCTTCCTGTTTGGCTCAAAG
156	SCRI_RS_168496	4H	54.95	ACACTGACGACATGGTTCTACACCACGACTCCATTAGGGATG	TACGGTAGCAGAGACTTGGTCTTATGCGTACAAGTCACCACC
157	SCRI_RS_228477	4H	54.95	ACACTGACGACATGGTTCTACAGGGAGGAAGAGATGTGTTTG	TACGGTAGCAGAGACTTGGTCTACTGCTACGTAATGAACTCC
158	12_30839	4H	54.95	ACACTGACGACATGGTTCTACAGTCAGTGAAGCCTTTGAGTC	TACGGTAGCAGAGACTTGGTCTGAAAGTCACATAAGCATGCC
159	11_20412	4H	54.95	ACACTGACGACATGGTTCTACATCTGCTTCGAGACCCTGAAC	TACGGTAGCAGAGACTTGGTCTTGCCTAACCCGGCGAAGTG
160	11_10509	4H	55.64	ACACTGACGACATGGTTCTACATGGCCCTCTTATATATCCCC	TACGGTAGCAGAGACTTGGTCTGCAAACTGCTTGGCAAAACC
161	SCRI_RS_189180	4H	57.32	ACACTGACGACATGGTTCTACAGTCGAATTTCTTGTGTGGGC	TACGGTAGCAGAGACTTGGTCTTGCTAGCTATCCAGCTCAAG
162	11_20924	4H	58.82	ACACTGACGACATGGTTCTACATCAGTGGAGTGTGATACCAG	TACGGTAGCAGAGACTTGGTCTTCAGTGGACCTTTCAGAAGC
163	11_10606	4H	60.28	ACACTGACGACATGGTTCTACAGGGCACAACTTACTTGCTTA	TACGGTAGCAGAGACTTGGTCTATGTTCATTGCTCCCATCTC
164	11_11513	4H	64.45	ACACTGACGACATGGTTCTACAGTTCAACCACCATCATCCAC	TACGGTAGCAGAGACTTGGTCTGGACAGAGCACCCAAGTTTG

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
165	11_10467	4H	67.22	ACACTGACGACATGGTTCTACAATACACCAGCTACAGCAAGG	TACGGTAGCAGAGACTTGGTCTGGGTCTGTTGTTCAGTCTAC
166	11_11004	4H	70.33	ACACTGACGACATGGTTCTACATGTCGAGCTAGACATGTCTG	TACGGTAGCAGAGACTTGGTCTGCAAATGAAAGAGACGCTCC
167	12_31148	4H	72.70	ACACTGACGACATGGTTCTACAGGAAAAGAGTCCCACAATCG	TACGGTAGCAGAGACTTGGTCTTGAGGTTTCCTATCCATGCG
168	11_20718	4H	76.11	ACACTGACGACATGGTTCTACATTGGCGGTGCCGGCTTTCT	TACGGTAGCAGAGACTTGGTCTAAGCAATACAACGACGTCGC
169	11_20178	4H	80.52	ACACTGACGACATGGTTCTACAGCATGAACGTACAACATCCC	TACGGTAGCAGAGACTTGGTCTTGACGAATGTGTCACCACTG
170	11_10751	4H	82.99	ACACTGACGACATGGTTCTACATCAGATGCTGCGATATCAGG	TACGGTAGCAGAGACTTGGTCTATGACCAGGATCAGGATTTC
171	11_10588	4H	83.34	ACACTGACGACATGGTTCTACAGACCTTGGTGTCTTCACAAC	TACGGTAGCAGAGACTTGGTCTGCTAGCCAAGTTGTTTCCAG
172	SCRI_RS_129218	4H	89.24	ACACTGACGACATGGTTCTACAGATTGTAATGAGGTCCGGTG	TACGGTAGCAGAGACTTGGTCTTACCAGCAGTACACTCCCTC
173	12_30718	4H	94.74	ACACTGACGACATGGTTCTACAAAGGGCACAATGTCAACCTG	TACGGTAGCAGAGACTTGGTCTTAAGGCCGCATTGATCACCG
174	SCRI_RS_189881	4H	94.74	ACACTGACGACATGGTTCTACAGACGTCTCCTCAGAGGTTTC	TACGGTAGCAGAGACTTGGTCTAGGGAGCTGCTCGCCATTG
175	11_20762	4H	96.60	ACACTGACGACATGGTTCTACAGTTATGGAAAGTAGAGGGAC	TACGGTAGCAGAGACTTGGTCTGGCAAAGTTGACGAAATCTG
176	SCRI_RS_131671	4H	99.68	ACACTGACGACATGGTTCTACACAGTGAAACTCATGATCCCC	TACGGTAGCAGAGACTTGGTCTGCTGCCAACATAAACTCTTC
177	11_10510	4H	100.38	ACACTGACGACATGGTTCTACAAGCCGGAAAATTTCCTCCTC	TACGGTAGCAGAGACTTGGTCTCTTGAAGTAGACGGATAGGC
178	11_21111	4H	102.18	ACACTGACGACATGGTTCTACAACATGAGCATGGAGGAGAAC	TACGGTAGCAGAGACTTGGTCTTTGGCGAGCAAGGGTCAAGA
179	SCRI_RS_192689	4H	103.58	ACACTGACGACATGGTTCTACAATTTCGTGTGCTCCAGAACC	TACGGTAGCAGAGACTTGGTCTTACCACGAGCACGACGAAG
180	12_11194	4H	107.77	ACACTGACGACATGGTTCTACACAAGCTTCGAGTGAGCTAAG	TACGGTAGCAGAGACTTGGTCTCGTCTGAAGAGCTCTCGATG
181	SCRI_RS_196076	4H	111.65	ACACTGACGACATGGTTCTACATCTTGTTAACTTTGGAGCCG	TACGGTAGCAGAGACTTGGTCTAGGCCAGGTAAGGTTTAGTC
182	11_20701	4H	114.98	ACACTGACGACATGGTTCTACATTGTTGGCCTGCCTTTTCTC	TACGGTAGCAGAGACTTGGTCTGTACAGGTGCTGAGAACACG
183	11_10697	4H	117.33	ACACTGACGACATGGTTCTACACTCTAACCACTTCTACGTGC	TACGGTAGCAGAGACTTGGTCTCCCGTTCGATGTCGATTTAC
184	11_10269	4H	120.53	ACACTGACGACATGGTTCTACATGATCTCCTTGGTCGGCTC	TACGGTAGCAGAGACTTGGTCTACGCGCCCTACATCTGCAT
185	11_20272	4H	125.11	ACACTGACGACATGGTTCTACATCCATGCCCGGAGAAATAAG	TACGGTAGCAGAGACTTGGTCTCGAAGAAGACGACGACTAAT
186	12_31422	4H	127.26	ACACTGACGACATGGTTCTACACAGTGAGTACATTAGCTCTAC	TACGGTAGCAGAGACTTGGTCTAAAAGGAAACAAACTGCTC
187	12_30824	4H	129.68	ACACTGACGACATGGTTCTACAGACCGTCGGTGTAGAAAATG	TACGGTAGCAGAGACTTGGTCTTCAACATCCCAATCCCACAG
188	12_30975	5H	3.66	ACACTGACGACATGGTTCTACACATACAATGAGTAATGACGTG	TACGGTAGCAGAGACTTGGTCTGGTACAATACAATACCAAAAG
189	11_20206	5H	6.55	ACACTGACGACATGGTTCTACACCGTCTTGGTTGGTTTCGAC	TACGGTAGCAGAGACTTGGTCTAGGTCCATATCACCTCTTCC
190	12_31094	5H	10.10	ACACTGACGACATGGTTCTACACCTCAAATCCTACGAGCTTC	TACGGTAGCAGAGACTTGGTCTCCAGGTTTTTGCGAAAACCG
191	SCRI_RS_149877	5H	18.14	ACACTGACGACATGGTTCTACATGAGCTCCATCGTTCTCCAG	TACGGTAGCAGAGACTTGGTCTTGGTCGTCGTTCATCACAGG

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
192	11_21065	5H	21.24	ACACTGACGACATGGTTCTACACCATGGTGGTGATCAGCAG	TACGGTAGCAGAGACTTGGTCTTCTGGTTCGTCGGCCTCGGT
193	11_21426	5H	21.24	ACACTGACGACATGGTTCTACATTCACGCTGATTGTTGAGCC	TACGGTAGCAGAGACTTGGTCTTGGTCCTTGTCTTTCTTGGG
194	11_11048	5H	27.80	ACACTGACGACATGGTTCTACAGCGTGCTGTTGGTAAAAAGG	TACGGTAGCAGAGACTTGGTCTCTTGAGCTCATATCCTCTGC
195	11_21324	5H	27.88	ACACTGACGACATGGTTCTACACTCTCTACCACAAGGATCTG	TACGGTAGCAGAGACTTGGTCTATCTTGTGTCTTGGCGCAAC
196	11_10580	5H	33.22	ACACTGACGACATGGTTCTACACAGAGCACATGCTACTAAAC	TACGGTAGCAGAGACTTGGTCTGCCGATGGTCAGATTTGCTC
197	11_10621	5H	35.35	ACACTGACGACATGGTTCTACACCTTTCCAACCTTAAGAAGC	TACGGTAGCAGAGACTTGGTCTTGTCAGGAACTTGATCAGGG
198	11_20845	5H	37.62	ACACTGACGACATGGTTCTACACGATCGGCTTTATGATAGGC	TACGGTAGCAGAGACTTGGTCTTCTGCTCCGAAGCAGGAAAG
199	11_20729	5H	41.33	ACACTGACGACATGGTTCTACACAAGCATTGGATTGTTGCCG	TACGGTAGCAGAGACTTGGTCTCACCAGAAGCTTTTGGTGC
200	12_30538	5H	44.99	ACACTGACGACATGGTTCTACATTCGATCAAACCCCTCATGC	TACGGTAGCAGAGACTTGGTCTTGGAGGGTGATTGATCTTTG
201	12_30745	5H	46.21	ACACTGACGACATGGTTCTACATGTTAAGCAAGCCGGTGAAC	TACGGTAGCAGAGACTTGGTCTTGAAGGCCTAGTACCTTCTG
202	11_10641	5H	50.88	ACACTGACGACATGGTTCTACAACTCCTACTTCAACAAGGTC	TACGGTAGCAGAGACTTGGTCTGAAGAGGCCCAACAATCTTG
203	12_20350	5H	53.77	ACACTGACGACATGGTTCTACAACTAGCTTTCTTGCCGACAC	TACGGTAGCAGAGACTTGGTCTTACATGTCCAGATGTCCTAC
204	11_21001	5H	55.83	ACACTGACGACATGGTTCTACACAGAGCAAAGTTTGACGTGG	TACGGTAGCAGAGACTTGGTCTTCCGGAATTCCTGCTGATTG
205	SCRI_RS_160332	5H	67.23	ACACTGACGACATGGTTCTACATAATAAGACGGCGGCACAAC	TACGGTAGCAGAGACTTGGTCTGGACAGTGCAAACTAAGCAG
206	12_10674	5H	68.83	ACACTGACGACATGGTTCTACATAATAAGGCTTCCGACGGAG	TACGGTAGCAGAGACTTGGTCTGCCACCTGCTTGAATGGATG
207	11_20497	5H	73.15	ACACTGACGACATGGTTCTACATCGGATACAACCATGAGAGC	TACGGTAGCAGAGACTTGGTCTCGGTCTGGTTGATCTTCTTG
208	11_10518	5H	76.34	ACACTGACGACATGGTTCTACAAAGACAGCCTCGACATCATC	TACGGTAGCAGAGACTTGGTCTAGTAGTTTCCTCAACACGTC
209	SCRI_RS_158235	5H	82.31	ACACTGACGACATGGTTCTACACATGCCAATACTTTCCTGCC	TACGGTAGCAGAGACTTGGTCTAAATTGGCGTGACACTTGGC
210	11_21421	5H	83.08	ACACTGACGACATGGTTCTACATGCTGCTGCTGTTTATGGTG	TACGGTAGCAGAGACTTGGTCTGATTGACTAGATACTGTGGC
211	11_11350	5H	84.96	ACACTGACGACATGGTTCTACAGAATCTCTTCGTCGTCGATG	TACGGTAGCAGAGACTTGGTCTCGCGAGGGTAGAACATCATT
212	11_20549	5H	87.71	ACACTGACGACATGGTTCTACAAGTTGGAGATGCAGATGCAG	TACGGTAGCAGAGACTTGGTCTCATGAGGAGATGAGAAGAAC
213	11_21061	5H	88.80	ACACTGACGACATGGTTCTACATTCTTGGTTGTTGCCGAGAG	TACGGTAGCAGAGACTTGGTCTTATATGCCTCCCGCTCAAAC
214	12_10752	5H	90.68	ACACTGACGACATGGTTCTACAGGTCAAGTGCTCACAACAAC	TACGGTAGCAGAGACTTGGTCTTCTCGCTCTTTCTTCCTTCC
215	11_11273	5H	93.16	ACACTGACGACATGGTTCTACAATTTAGCCCGGCCACTAAGG	TACGGTAGCAGAGACTTGGTCTGTAAATTGCTTCGGTTGCTG
216	12_30456	5H	95.65	ACACTGACGACATGGTTCTACACTTCTGCAGGAGTGACATTG	TACGGTAGCAGAGACTTGGTCTTCATTGCAGTGTCTGCTCTC
217	11_11200	5H	99.58	ACACTGACGACATGGTTCTACAACCTTTGTTTTGCTTGCAGG	TACGGTAGCAGAGACTTGGTCTGAAGCGCTCATCAACCATAC
218	SCRI_RS_149088	5H	103.35	ACACTGACGACATGGTTCTACAATCGATTCTTTCGGCTCTGG	TACGGTAGCAGAGACTTGGTCTTCTTGAATGGGTTGTCGTGC

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
219	SCRI_RS_234720	5H	106.15	ACACTGACGACATGGTTCTACATCTTCCATCAGATTACAAGG	TACGGTAGCAGAGACTTGGTCTTGTCTGATTTAAGAGCAGCG
220	SCRI_RS_2831	5H	109.35	ACACTGACGACATGGTTCTACAAGCTCGCGGAGTTCTTGCAC	TACGGTAGCAGAGACTTGGTCTACGATTCGACTGTCTTCAGC
221	11_11507	5H	111.56	ACACTGACGACATGGTTCTACAGGGCACAATTTGTTACATAG	TACGGTAGCAGAGACTTGGTCTTAGCCTTCCTTCATTGTGCC
222	11_21422	5H	112.78	ACACTGACGACATGGTTCTACACTGAAAATGACCTCCAAGGG	TACGGTAGCAGAGACTTGGTCTAGCAGGGCTATGATTCCTCT
223	11_20653	5H	115.53	ACACTGACGACATGGTTCTACAATACCACTTGTGATCCGAGG	TACGGTAGCAGAGACTTGGTCTGACCAGGAATTGACTGGAAG
224	12_30067	5H	117.63	ACACTGACGACATGGTTCTACAATTGATGTCGAGAACCGGAG	TACGGTAGCAGAGACTTGGTCTCGAGAAGAACAAAGCACCTG
225	11_21247	5H	119.72	ACACTGACGACATGGTTCTACATCCGTTCCCGTTTGTTACAC	TACGGTAGCAGAGACTTGGTCTGGCTCCATTTTTATGTAACTG
226	SCRI_RS_141778	5H	126.19	ACACTGACGACATGGTTCTACAGACGCCATTGCTGTTGAAAG	TACGGTAGCAGAGACTTGGTCTCATTCTCAGTCCCCTAAACC
227	11_10845	5H	128.80	ACACTGACGACATGGTTCTACACAACAGCGATCCAAGCTTCC	TACGGTAGCAGAGACTTGGTCTCATGGACTAGCCTTGACTTC
228	11_20375	5H	132.00	ACACTGACGACATGGTTCTACAAAAGGGCCTCAGACTTCAAG	TACGGTAGCAGAGACTTGGTCTGTACGACAAGGAGAAACTGC
229	SCRI_RS_213086	5H	132.37	ACACTGACGACATGGTTCTACACTTGCTAAAGCTTGGGCAAC	TACGGTAGCAGAGACTTGGTCTACGAGCCACCAGTATGTTAC
230	11_10557	5H	135.42	ACACTGACGACATGGTTCTACATTGCCACATGCAAGTGACTG	TACGGTAGCAGAGACTTGGTCTGCTTTGTCGACATAAAGGAG
231	12_31165	5H	138.44	ACACTGACGACATGGTTCTACAGCCCAACGTCATCGTACGAA	TACGGTAGCAGAGACTTGGTCTTCCAGAAGAAGGCCAAGGAC
232	12_31221	5H	142.71	ACACTGACGACATGGTTCTACACTTTGCGAAGCACGTTTCTC	TACGGTAGCAGAGACTTGGTCTAACTTTGCCATGGAAGGAAG
233	11_11497	5H	147.70	ACACTGACGACATGGTTCTACATGAGCTACTCTGACTCACTC	TACGGTAGCAGAGACTTGGTCTAGAAGCAGCCGGAGGAAGA
234	SCRI_RS_189174	5H	150.10	ACACTGACGACATGGTTCTACAATGAAGGAGATCGTCAGAGC	TACGGTAGCAGAGACTTGGTCTCGATACTTCACCTCCATCTC
235	11_10336	5H	153.47	ACACTGACGACATGGTTCTACAAGCTCACTTATATATCACC	TACGGTAGCAGAGACTTGGTCTACTGTTGAGGAAGGAACAGC
236	11_21018	5H	153.47	ACACTGACGACATGGTTCTACAGCGAATGTTCTAGACCTTAC	TACGGTAGCAGAGACTTGGTCTCTCTGGGACAATGGAAGTAG
237	12_30162	5H	156.70	ACACTGACGACATGGTTCTACAATGTGAAGACGGAGCTGTAG	TACGGTAGCAGAGACTTGGTCTAAACAACACCCAAGGTCCAC
238	11_20829	5H	158.18	ACACTGACGACATGGTTCTACATTCTCCTCCTTGATGTCACC	TACGGTAGCAGAGACTTGGTCTTGTTCCTTGTGATGAGCACG
239	12_30566	5H	160.32	ACACTGACGACATGGTTCTACAGGACTGATGACTCAAAACCG	TACGGTAGCAGAGACTTGGTCTCGATTTGGCTTCGAAACCTG
240	11_10869	5H	163.16	ACACTGACGACATGGTTCTACAGCTGCTACACACATGAATTG	TACGGTAGCAGAGACTTGGTCTCTAAGATGAAGCTCTGGCTC
241	11_10870	5H	168.24	ACACTGACGACATGGTTCTACACGGTGTAACTGGATGAAGAC	TACGGTAGCAGAGACTTGGTCTCAGGTGCAGCTACTGCATTG
242	11_21138	5H	169.97	ACACTGACGACATGGTTCTACATCCAGCTCAGCAATGTTGTC	TACGGTAGCAGAGACTTGGTCTCAGGAGGTCAGTTTAAGTGC
243	12_30504	5H	172.25	ACACTGACGACATGGTTCTACAGCACCATCACTATCATGCAG	TACGGTAGCAGAGACTTGGTCTAATCTGTTGCTCCATGGCTG
244	11_10310	5H	177.50	ACACTGACGACATGGTTCTACATAGGAGAGGGAGCAAAACAG	TACGGTAGCAGAGACTTGGTCTGAGAATCTTTACTTGACCCG
245	12_10322	5H	181.11	ACACTGACGACATGGTTCTACAGCGCCACCATGTTACGACC	TACGGTAGCAGAGACTTGGTCTAGTGAGTGGCAGACACAGAG

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
246	12_31123	5H	184.75	ACACTGACGACATGGTTCTACACACCGTGCCTTTCTTAGAAG	TACGGTAGCAGAGACTTGGTCTTCCATCGACATCCTTAAGGG
247	11_20232	6H	0.00	ACACTGACGACATGGTTCTACATGTGACGAATTTCTCGAGCC	TACGGTAGCAGAGACTTGGTCTCAAGGATGATGATTGAGGGC
248	11_20212	6H	2.86	ACACTGACGACATGGTTCTACACTTGTCGTCAATGGCGTAAG	TACGGTAGCAGAGACTTGGTCTTCATCGACCGAGGCCGAGAA
249	12_30651	6H	5.44	ACACTGACGACATGGTTCTACAAGCTCCATGCTACCTATGAG	TACGGTAGCAGAGACTTGGTCTTGGCCAATTCCTTCATCTCC
250	11_20294	6H	6.49	ACACTGACGACATGGTTCTACAGGATACTATCTAGGTGGGTC	TACGGTAGCAGAGACTTGGTCTTTGCACTTGACCTTTGGAGC
251	11_21204	6H	7.56	ACACTGACGACATGGTTCTACACTCTATCTTCTATTCTCATC	TACGGTAGCAGAGACTTGGTCTACAGATAATCCGCCTCTACC
252	11_21032	6H	11.35	ACACTGACGACATGGTTCTACAAATCTCTGCATAAGAGCAGG	TACGGTAGCAGAGACTTGGTCTCATCAAGGAAGCTGGAGGTG
253	11_20415	6H	15.16	ACACTGACGACATGGTTCTACAGCTGTCATCTTTCTCGAGTC	TACGGTAGCAGAGACTTGGTCTGTAAGAAACTTCTCCACCAG
254	12_10554	6H	19.07	ACACTGACGACATGGTTCTACATCTTCTTCAAGAAGCACCCG	TACGGTAGCAGAGACTTGGTCTAATGCTGGAATGTAGTGCCC
255	12_30843	6H	23.07	ACACTGACGACATGGTTCTACATCATTGGCTGTGTGTTGTGC	TACGGTAGCAGAGACTTGGTCTGTGGTCAAAGTCCTCACCTG
256	11_10868	6H	27.19	ACACTGACGACATGGTTCTACAGATGTTACGTCCAGGACAAC	TACGGTAGCAGAGACTTGGTCTAGTTCCTGGTTGATGTGGTG
257	11_20745	6H	31.59	ACACTGACGACATGGTTCTACACATTCAGATTCATTCCTTGC	TACGGTAGCAGAGACTTGGTCTCGTCGGTCTGTGTAGTTAGC
258	12_30697	6H	33.39	ACACTGACGACATGGTTCTACATAGGACGGTGCATCCATTTG	TACGGTAGCAGAGACTTGGTCTGAAGATAGGGACTGAAGCTG
259	12_31485	6H	35.29	ACACTGACGACATGGTTCTACATCCTGATAAAGGCAGGAGTC	TACGGTAGCAGAGACTTGGTCTAGTTGTGCGGCTGTTTGTCC
260	11_10939	6H	40.52	ACACTGACGACATGGTTCTACACCAGGAGTACTGTACAGTTC	TACGGTAGCAGAGACTTGGTCTCTTGTGTTGCCGTCATAAGG
261	12_30521	6H	44.61	ACACTGACGACATGGTTCTACAGTTCCCATCACAAAACCAGC	TACGGTAGCAGAGACTTGGTCTTTCGCATACCTGCAATGCTG
262	12_30361	6H	47.81	ACACTGACGACATGGTTCTACAAGTTCTGAAGACTCCACGAC	TACGGTAGCAGAGACTTGGTCTTAATCAAGGTCCCCGTCTCC
263	11_10061	6H	50.41	ACACTGACGACATGGTTCTACAACGTCCTTCTGCTCATAACC	TACGGTAGCAGAGACTTGGTCTATCTGCTGTTCGACTGGGAC
264	11_10462	6H	53.84	ACACTGACGACATGGTTCTACATGTCCCAACACCATTCAGAG	TACGGTAGCAGAGACTTGGTCTAGTGGCACAGAAGCAAAGAG
265	11_10962	6H	58.34	ACACTGACGACATGGTTCTACATCTTGCCATCAGGCCTCAAC	TACGGTAGCAGAGACTTGGTCTTCAGCCATGTTCTTGCTACC
266	SCRI_RS_213566	6H	59.01	ACACTGACGACATGGTTCTACACGGTCATTATCATGGTTAGG	TACGGTAGCAGAGACTTGGTCTGGATCCCCAACAAATCAAAC
267	12_11253	6H	62.91	ACACTGACGACATGGTTCTACATAAGCTTTGCTTGCTGTGCC	TACGGTAGCAGAGACTTGGTCTAGTCCACCGTTTAGTGTCTC
268	SCRI_RS_209993	6H	64.78	ACACTGACGACATGGTTCTACAGGGTTGATTCGACAAGCAAG	TACGGTAGCAGAGACTTGGTCTCATGTCAGCAACATCAGCAC
269	SCRI_RS_204148	6H	68.09	ACACTGACGACATGGTTCTACATTCGGCTTCTGGTATGTATC	TACGGTAGCAGAGACTTGGTCTGGTCATGTACAGGATCAGAG
270	11_10455	6H	72.29	ACACTGACGACATGGTTCTACAGCTGTAAGCAATGTCTTCCG	TACGGTAGCAGAGACTTGGTCTGACGACAAAATGGTACAAGG
271	11_10124	6H	73.83	ACACTGACGACATGGTTCTACAAGGACTAAACCCTCTGTCTG	TACGGTAGCAGAGACTTGGTCTCAATAAGCTAAAGCAAGAC
272	11_20892	6H	74.18	ACACTGACGACATGGTTCTACATGAAAACCATTGCCCCGAAG	TACGGTAGCAGAGACTTGGTCTTATGAACATCTCGGGTCCTG

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
273	12_31111	6H	76.62	ACACTGACGACATGGTTCTACAGGCGAAACCACAGATGGTTG	TACGGTAGCAGAGACTTGGTCTAACAGCAACTGGAGGATTGG
274	11_10220	6H	80.31	ACACTGACGACATGGTTCTACAGTGTTTGTGTACATGGTGCG	TACGGTAGCAGAGACTTGGTCTTTTCCCTGCACATGCTTCTC
275	11_11246	6H	81.48	ACACTGACGACATGGTTCTACAGGTGCAATCCATTGTTGTTG	TACGGTAGCAGAGACTTGGTCTAGTTCAGCATCCCCAGTGAC
276	12_30698	6H	88.85	ACACTGACGACATGGTTCTACAAAGTACACACGGCAAGGAAG	TACGGTAGCAGAGACTTGGTCTACTGCTAGCTAACCGGAAAC
277	11_21025	6H	92.12	ACACTGACGACATGGTTCTACAGATGACGTCAAGCACATCAG	TACGGTAGCAGAGACTTGGTCTGAGGCAGAAAGTTCAGAGAG
278	12_30151	6H	97.15	ACACTGACGACATGGTTCTACAACGGCGGCTACTACACGCT	TACGGTAGCAGAGACTTGGTCTTTGCACTCCACGAACGGCTC
279	12_31353	6H	100.53	ACACTGACGACATGGTTCTACAGTTTTGCCACATTGAAAGGAG	TACGGTAGCAGAGACTTGGTCTAGCAACGGTAAAATACGGGC
280	12_31115	6H	102.03	ACACTGACGACATGGTTCTACAAACCACACCAACTGACTTGC	TACGGTAGCAGAGACTTGGTCTGCATGAACAAAACCGACGAG
281	11_20379	6H	105.23	ACACTGACGACATGGTTCTACAATAAGCCACTGCTCCCCTTC	TACGGTAGCAGAGACTTGGTCTCGAGTAGGAGTATGTCACTG
282	11_20036	6H	110.59	ACACTGACGACATGGTTCTACATCCTATCGTTTGGCTTTCGG	TACGGTAGCAGAGACTTGGTCTTAGGAGAGTCAGCGCAGAAG
283	SCRI_RS_3070	6H	110.59	ACACTGACGACATGGTTCTACATCTAGACAAATCAGTGGCGG	TACGGTAGCAGAGACTTGGTCTCGTGCAAGTTGGCGATCAAT
284	11_20355	6H	113.05	ACACTGACGACATGGTTCTACAATCCATGATACAGCCTAGTG	TACGGTAGCAGAGACTTGGTCTCCCCAGTTTCAATTGATTCC
285	SCRI_RS_206207	6H	115.49	ACACTGACGACATGGTTCTACATCAGTGAGGTGGAGAAGAAC	TACGGTAGCAGAGACTTGGTCTCGGACAGAGGGAATGTCTTG
286	11_10645	6H	122.99	ACACTGACGACATGGTTCTACATTGCTCTGCCCAATCTCATC	TACGGTAGCAGAGACTTGGTCTGCACAAGGTGCAATGTGATG
287	SCRI_RS_206827	6H	126.21	ACACTGACGACATGGTTCTACATTGAGAGGGCTGATCTGGTG	TACGGTAGCAGAGACTTGGTCTAAGCAGCAGAGCTTCTCTGG
288	11_10390	6H	130.44	ACACTGACGACATGGTTCTACAAGGACCAGGATGCATTCTTC	TACGGTAGCAGAGACTTGGTCTATCCCTCAGGAGGATCAAAC
289	SCRI_RS_10811	6H	136.62	ACACTGACGACATGGTTCTACAGCCAGTACTGGATACAAGAG	TACGGTAGCAGAGACTTGGTCTCTGCTCATAGCCTTCTTACC
290	12_30956	6H	139.39	ACACTGACGACATGGTTCTACATCATTTGGATGACCTGGTGG	TACGGTAGCAGAGACTTGGTCTGTTGATGACGCCGGTGTATG
291	11_21419	7H	0.00	ACACTGACGACATGGTTCTACACATGGTCTTGCAGACATTCC	TACGGTAGCAGAGACTTGGTCTCTTGATCGTCAAGAGAAGTG
292	11_10682	7H	1.08	ACACTGACGACATGGTTCTACACGCCTAGTTTAGTGGCTGGT	TACGGTAGCAGAGACTTGGTCTCTGGAAATCTAAGATGAACC
293	11_10894	7H	1.78	ACACTGACGACATGGTTCTACAGGCCACATCATCAGCAATAC	TACGGTAGCAGAGACTTGGTCTTTCAGCAGCATCAGGGACG
294	11_21307	7H	1.78	ACACTGACGACATGGTTCTACATTGGGAAGCTCCATTTGGTC	TACGGTAGCAGAGACTTGGTCTTCCATAGGCCTTAGAGCGTC
295	SCRI_RS_237689	7H	1.80	ACACTGACGACATGGTTCTACATTGGATCCCTGGGTGATGGC	TACGGTAGCAGAGACTTGGTCTTGTAGGGCTTCCGCACCCTC
296	11_20710	7H	2.47	ACACTGACGACATGGTTCTACATGCCATTGCTGCAAGGATAG	TACGGTAGCAGAGACTTGGTCTACAATTCATCCCCACCTCTC
297	11_20242	7H	4.52	ACACTGACGACATGGTTCTACACCTGGTCATCCTTGATGCTG	TACGGTAGCAGAGACTTGGTCTTTTTCGCCGAGTGGAACAAG
298	12_11035	7H	6.29	ACACTGACGACATGGTTCTACACCAGGTGTATCTGAATAAGAC	TACGGTAGCAGAGACTTGGTCTTGCAGCTGAAAATCCAATAG
299	11_10841	7H	12.97	ACACTGACGACATGGTTCTACAGCTTTGAAGCATGATCGGAC	TACGGTAGCAGAGACTTGGTCTATTCGGTTGACCACAAGCTC

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
300	11_21437	7H	15.44	ACACTGACGACATGGTTCTACACCAGACAATGTTCAAGGTCC	TACGGTAGCAGAGACTTGGTCTTATCTCATTCTCGCACCCTC
301	11_10025	7H	16.78	ACACTGACGACATGGTTCTACACTACTTCAGAACCAGTGCTC	TACGGTAGCAGAGACTTGGTCTAAGGGTGGATACACCATCTC
302	11_20722	7H	19.22	ACACTGACGACATGGTTCTACAGGAGGCAACATCTCTGATAG	TACGGTAGCAGAGACTTGGTCTAAATGCCTGATGTCGCGATG
303	11_20495	7H	22.12	ACACTGACGACATGGTTCTACACGCATTGACGCTTCCTTTTG	TACGGTAGCAGAGACTTGGTCTAGTCTAAGGACAACGGACAC
304	12_30530	7H	26.35	ACACTGACGACATGGTTCTACATACGAGGTGCACGGCCTCAT	TACGGTAGCAGAGACTTGGTCTGGTCGTGGTAGATGTGGTC
305	SCRI_RS_127224	7H	26.72	ACACTGACGACATGGTTCTACAAGGAGATTGCGGATAGAGTG	TACGGTAGCAGAGACTTGGTCTATAAGTCCACACGGTGTGAG
306	12_30780	7H	29.62	ACACTGACGACATGGTTCTACATTGGTGAAGGCCGCAGCAAC	TACGGTAGCAGAGACTTGGTCTGGTGGGGTTCTACCAGAAG
307	11_20993	7H	34.74	ACACTGACGACATGGTTCTACATACAGCGAATGGATCGATGA	TACGGTAGCAGAGACTTGGTCTTTTGGAAGCGGGACATGCTC
308	12_30895	7H	38.31	ACACTGACGACATGGTTCTACACACCTGCAGGCAGTATAAAG	TACGGTAGCAGAGACTTGGTCTCAGCTTATGTACGTACTCTC
309	11_10838	7H	40.46	ACACTGACGACATGGTTCTACACGTTGAGAATTGCGAAGGAC	TACGGTAGCAGAGACTTGGTCTCAGTCATCAGCATAAGTTCC
310	SCRI_RS_179937	7H	43.38	ACACTGACGACATGGTTCTACATCAGCTAAGGACAAGATAAC	TACGGTAGCAGAGACTTGGTCTCAGAAACCAACTGGTGTCTC
311	12_31305	7H	44.58	ACACTGACGACATGGTTCTACATGGAAGCTGGTGAACAACTG	TACGGTAGCAGAGACTTGGTCTAGACCTCGATGGAAGAGCTG
312	11_10576	7H	46.89	ACACTGACGACATGGTTCTACACGACAATTCCGGAGAAGATG	TACGGTAGCAGAGACTTGGTCTTCTCCCCCATTTTTGCTTTC
313	SCRI_RS_8200	7H	49.46	ACACTGACGACATGGTTCTACATCCATACACTCCTCCCTAC	TACGGTAGCAGAGACTTGGTCTACGTACGGCGGCGTGTATGG
314	SCRI_RS_169904	7H	55.53	ACACTGACGACATGGTTCTACAATTGTGTCCATATCCCTCGC	TACGGTAGCAGAGACTTGGTCTTAAAACCTAACGCCTGGCCC
315	SCRI_RS_219349	7H	61.13	ACACTGACGACATGGTTCTACATGGGCTGGGCCGGGTTCCAC	TACGGTAGCAGAGACTTGGTCTAGTCGTCGCCGTAAATGAAC
316	SCRI_RS_213842	7H	65.05	ACACTGACGACATGGTTCTACAGGAGAATGCAGAGCTGAAAG	TACGGTAGCAGAGACTTGGTCTACGATGGAGACATGCTTCGG
317	11_10050	7H	66.61	ACACTGACGACATGGTTCTACAATTCGTGAGGCAGATGGGTG	TACGGTAGCAGAGACTTGGTCTACTCGTTATGATCGTGAAGC
318	12_30149	7H	67.99	ACACTGACGACATGGTTCTACATTACAAGCACGATCAGGGAG	TACGGTAGCAGAGACTTGGTCTGGTAAAGTAAAAGGTGGAGG
319	11_11348	7H	71.76	ACACTGACGACATGGTTCTACATAGCTAGCTAGAGCTACCTG	TACGGTAGCAGAGACTTGGTCTAGCTTAGGACAATCAGCTGG
320	12_30997	7H	74.84	ACACTGACGACATGGTTCTACACTCGCCGGAGAGAGAAGAA	TACGGTAGCAGAGACTTGGTCTTTGCCCAGCCCTTTCCCTC
321	12_30344	7H	76.06	ACACTGACGACATGGTTCTACAACCAAGGAAGGAACAGTGCG	TACGGTAGCAGAGACTTGGTCTATCTTCCTCATCTCCTCTCC
322	12_10982	7H	80.47	ACACTGACGACATGGTTCTACAAGATGAGGGTGGAGATGAAC	TACGGTAGCAGAGACTTGGTCTGTCGCTTCGATGACTCCTTC
323	12_30565	7H	81.07	ACACTGACGACATGGTTCTACATTCTACAACCAATTGATGCC	TACGGTAGCAGAGACTTGGTCTCTGAGAGATGAGGCATAATAC
324	12_30998	7H	82.16	ACACTGACGACATGGTTCTACACCACGACTACATGCTGAAAC	TACGGTAGCAGAGACTTGGTCTGCAGTGGCTTTGATCATGAG
325	SCRI_RS_104566	7H	86.89	ACACTGACGACATGGTTCTACAGGGAGGTTCGGATGACAGG	TACGGTAGCAGAGACTTGGTCTACATCTCCATCCCCTTCAAC
326	11_21448	7H	92.00	ACACTGACGACATGGTTCTACATAACAGACGACGACGCTTAC	TACGGTAGCAGAGACTTGGTCTTGTACTGCTGTTTCCTCCTG

Table C2. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI67381 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	Chr	cM	Forward Primers	Reverse Primers
327	11_21201	7H	95.02	ACACTGACGACATGGTTCTACAAATGTGGAGAGCATTGTGCC	TACGGTAGCAGAGACTTGGTCTATCCTTCCCACCTTTCTTGC
328	12_30806	7H	95.70	ACACTGACGACATGGTTCTACACTGTCGCCGACGCACGCTC	TACGGTAGCAGAGACTTGGTCTTCCCCAAATCCATCGTCCAG
329	11_10301	7H	98.14	ACACTGACGACATGGTTCTACACGTGCAATAGGGTGAAGAAG	TACGGTAGCAGAGACTTGGTCTAGAAGGAAGAGGAGAAGGAG
330	11_20808	7H	99.90	ACACTGACGACATGGTTCTACAATTCCTGCCACGACCATAAG	TACGGTAGCAGAGACTTGGTCTCCCTCGATCCATATCGATAG
331	11_20103	7H	101.99	ACACTGACGACATGGTTCTACATGGCTTGCCGATGATCCTG	TACGGTAGCAGAGACTTGGTCTAGCTGAGCCTGAGCACCAAC
332	12_31440	7H	102.19	ACACTGACGACATGGTTCTACAAGCCACGGGTCTTCGACAT	TACGGTAGCAGAGACTTGGTCTATGACGACGCTCCTTCATCC
333	SCRI_RS_141732	7H	107.80	ACACTGACGACATGGTTCTACATGGAATAATTTCTCCGGTGC	TACGGTAGCAGAGACTTGGTCTGTGGGTTGTATGAAACGTTG
334	SCRI_RS_112204	7H	109.00	ACACTGACGACATGGTTCTACAAAGCAATGGCTCAATTGCCG	TACGGTAGCAGAGACTTGGTCTACACCATCCATGATCCATCC
335	11_10853	7H	110.40	ACACTGACGACATGGTTCTACAGCTAACCTAAGGGAGCATTG	TACGGTAGCAGAGACTTGGTCTGCTGTGTACCTTAAAATTGC
336	SCRI_RS_172243	7H	113.60	ACACTGACGACATGGTTCTACACTGTCTACTAAGGTACATTC	TACGGTAGCAGAGACTTGGTCTTCCATTTCTTCCTGCTGCTG
337	11_20247	7H	117.10	ACACTGACGACATGGTTCTACAGATATCACTCCATTTCAAGG	TACGGTAGCAGAGACTTGGTCTTGCGTGGATCCTATTTTTGG
338	12_30368	7H	119.33	ACACTGACGACATGGTTCTACAAACCAAGAGAATCACAACCC	TACGGTAGCAGAGACTTGGTCTTCGACGGAGAGGGTTTAATG
339	11_20354	7H	127.41	ACACTGACGACATGGTTCTACAGCTGTGCAACATCTGAGAAG	TACGGTAGCAGAGACTTGGTCTTCATTGAGGATGCTAGGCAC
340	SCRI_RS_202130	7H	133.84	ACACTGACGACATGGTTCTACACTCTCCATTTCCATTTTGGG	TACGGTAGCAGAGACTTGGTCTGTGTTCCATGATGGTGTTCG
341	SCRI_RS_196031	7H	135.99	ACACTGACGACATGGTTCTACAATTCCTTCGGTTCCGTTGTG	TACGGTAGCAGAGACTTGGTCTGGGCTGTTGTACTAGTTAG
342	SCRI_RS_225155	7H	137.76	ACACTGACGACATGGTTCTACAGTTTTCGCAGCATGATGCGG	TACGGTAGCAGAGACTTGGTCTGACAATTAGGGTGGACCTTC
343	11_10797	7H	141.37	ACACTGACGACATGGTTCTACATGAACACCGGCCTAATTACC	TACGGTAGCAGAGACTTGGTCTTGCGCTTTCAGGAACAACAG
344	11_20847	7H	145.68	ACACTGACGACATGGTTCTACAGCGAAGAAGAACTTGTCCTC	TACGGTAGCAGAGACTTGGTCTAAGGTGCAATCCTAGTAGGG
345	11_10687	7H	146.03	ACACTGACGACATGGTTCTACATCCCAACTCCTTGCATATCC	TACGGTAGCAGAGACTTGGTCTGATGGGTGCTGATGCAGTTC
346	SCRI_RS_171456	7H	148.45	ACACTGACGACATGGTTCTACACGGATGACATCAGCATTTCC	TACGGTAGCAGAGACTTGGTCTCCAATTGGCAATTGCACAGC
347	12_20640	7H	152.51	ACACTGACGACATGGTTCTACATAGCAATAAGGGCCGTGTAG	TACGGTAGCAGAGACTTGGTCTCTAAGTGGCACACCTTTATG
348	11_11275	7H	156.69	ACACTGACGACATGGTTCTACACGGCGACGATGGAGGTCAT	TACGGTAGCAGAGACTTGGTCTGCAGCTCAAAGAACGGATTC
349	11_11012	7H	157.08	ACACTGACGACATGGTTCTACATGGAGGAGGAAGAGGAGGT	TACGGTAGCAGAGACTTGGTCTTTGTCGTACTGCTCGTGCTC
350	11_20185	7H	166.55	ACACTGACGACATGGTTCTACAGGAAAGAGTGACCATCTAGG	TACGGTAGCAGAGACTTGGTCTGTGCTCCTTGCGGTGTTAG
351	12_10378	7H	167.58	ACACTGACGACATGGTTCTACATGGTGAACTCGGCCAGCGTT	TACGGTAGCAGAGACTTGGTCTGTAGCGGGAGCTGCCAGAC

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information.

SN	Marker	chr	cM	Forward Primers	Reverse Primers
1	12_30969	1H	1.36	ACACTGACGACATGGTTCTACAAAGTTGCCTTAATGACTGGG	ACACTGACGACATGGTTCTACAAAGTTGCCTTAATGACTGGG
2	SCRI_RS_66630	1H	5.68	ACACTGACGACATGGTTCTACACATGATATCTGCTTGAGTGG	ACACTGACGACATGGTTCTACACATGATATCTGCTTGAGTGG
3	11_21174	1H	9.34	ACACTGACGACATGGTTCTACAAGAAAGGACGTCGAAACGAG	ACACTGACGACATGGTTCTACAAGAAAGGACGTCGAAACGAG
4	11_21226	1H	10.35	ACACTGACGACATGGTTCTACAATCCTCATCAGGTATTCGGC	ACACTGACGACATGGTTCTACAATCCTCATCAGGTATTCGGC
5	12_30919	1H	12.50	ACACTGACGACATGGTTCTACAAAGATCAGTGCGCAATAACG	ACACTGACGACATGGTTCTACAAAGATCAGTGCGCAATAACG
6	SCRI_RS_130592	1H	17.20	ACACTGACGACATGGTTCTACATGACGTCCACGTACTACTAC	ACACTGACGACATGGTTCTACATGACGTCCACGTACTACTAC
7	12_30948	1H	20.31	ACACTGACGACATGGTTCTACAATGGCGAGCTCGACTTGTTC	ACACTGACGACATGGTTCTACAATGGCGAGCTCGACTTGTTC
8	11_10030	1H	21.99	ACACTGACGACATGGTTCTACAGATGAACCGAAGTATGCACC	ACACTGACGACATGGTTCTACAGATGAACCGAAGTATGCACC
9	SCRI_RS_124926	1H	26.32	ACACTGACGACATGGTTCTACAGTCGTATGATCCACGTGATG	ACACTGACGACATGGTTCTACAGTCGTATGATCCACGTGATG
10	11_10757	1H	28.78	ACACTGACGACATGGTTCTACACTTGTCGTATGAGGCTCTTG	ACACTGACGACATGGTTCTACACTTGTCGTATGAGGCTCTTG
11	12_31276	1H	32.09	ACACTGACGACATGGTTCTACATGGCACTGGTGCAATTGTTC	ACACTGACGACATGGTTCTACATGGCACTGGTGCAATTGTTC
12	12_31177	1H	37.10	ACACTGACGACATGGTTCTACATAATGAGGATGCAGCCAGAG	ACACTGACGACATGGTTCTACATAATGAGGATGCAGCCAGAG
13	11_21095	1H	47.92	ACACTGACGACATGGTTCTACAAGACGTCCACCAAGAAGAAC	ACACTGACGACATGGTTCTACAAGACGTCCACCAAGAAGAAC
14	12_30683	1H	49.10	ACACTGACGACATGGTTCTACAAGAGCCCACTGTACACTATC	ACACTGACGACATGGTTCTACAAGAGCCCACTGTACACTATC
15	12_30110	1H	50.30	ACACTGACGACATGGTTCTACAGTTCTCCTCTACCTCTAGTG	ACACTGACGACATGGTTCTACAGTTCTCCTCTACCTCTAGTG
16	12_30710	1H	51.86	ACACTGACGACATGGTTCTACACGAACTTATCGATGAGGCTG	ACACTGACGACATGGTTCTACACGAACTTATCGATGAGGCTG
17	11_20997	1H	54.14	ACACTGACGACATGGTTCTACACTTATCGTTGGTGGGATTGC	ACACTGACGACATGGTTCTACACTTATCGTTGGTGGGATTGC
18	11_10176	1H	59.01	ACACTGACGACATGGTTCTACATTGGCCTGCCATTCTCTTTC	ACACTGACGACATGGTTCTACATTGGCCTGCCATTCTCTTTC
19	12_31464	1H	64.93	ACACTGACGACATGGTTCTACATGGCCCCACATATGCATCAG	ACACTGACGACATGGTTCTACATGGCCCCACATATGCATCAG
20	11_20290	1H	69.73	ACACTGACGACATGGTTCTACAAGGGCAAGTACAACTACAAC	ACACTGACGACATGGTTCTACAAGGGCAAGTACAACTACAAC
21	11_20229	1H	71.70	ACACTGACGACATGGTTCTACAATGAGGCCAAGATTGAGGTG	ACACTGACGACATGGTTCTACAATGAGGCCAAGATTGAGGTG
22	11_10279	1H	72.86	ACACTGACGACATGGTTCTACATGTACTCTTTTGCGACGCTG	ACACTGACGACATGGTTCTACATGTACTCTTTTGCGACGCTG
23	11_20990	1H	74.12	ACACTGACGACATGGTTCTACAAGTAACACTGGACACACACC	ACACTGACGACATGGTTCTACAAGTAACACTGGACACACACC
24	SCRI_RS_181353	1H	78.25	ACACTGACGACATGGTTCTACAGCTGAAATTCAAAGAGCAGAG	ACACTGACGACATGGTTCTACAGCTGAAATTCAAAGAGCAGAG
25	SCRI_RS_9158	1H	82.45	ACACTGACGACATGGTTCTACACACCCTCGCTTTCTGCATC	ACACTGACGACATGGTTCTACACACCCTCGCTTTCTGCATC
26	11_10471	1H	83.15	ACACTGACGACATGGTTCTACAGACAGGAGGTTCAACATAGC	ACACTGACGACATGGTTCTACAGACAGGAGGTTCAACATAGC
27	SCRI_RS_135092	1H	85.05	ACACTGACGACATGGTTCTACAGAGTGCATTACTTGCATCGG	ACACTGACGACATGGTTCTACAGAGTGCATTACTTGCATCGG

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
28	11_21373	1H	90.98	ACACTGACGACATGGTTCTACACCTTCTCCAAGTCATCCATC	ACACTGACGACATGGTTCTACACCTTCTCCAAGTCATCCATC
29	11_20149	1H	94.48	ACACTGACGACATGGTTCTACATAATGTGAGAAATTTTGATAT	ACACTGACGACATGGTTCTACATAATGTGAGAAATTTTGATAT
30	SCRI_RS_188909	1H	98.45	ACACTGACGACATGGTTCTACACAGCACGTAACATCTGCATC	ACACTGACGACATGGTTCTACACAGCACGTAACATCTGCATC
31	12_20187	1H	101.05	ACACTGACGACATGGTTCTACAAAGGACCTCGACAAGGAGGA	ACACTGACGACATGGTTCTACAAAGGACCTCGACAAGGAGGA
32	12_31152	1H	101.05	ACACTGACGACATGGTTCTACACTCCAGTAACAATCGACGTG	ACACTGACGACATGGTTCTACACTCCAGTAACAATCGACGTG
33	11_20909	1H	104.10	ACACTGACGACATGGTTCTACATCGGCGGCGGCGATGTCCT	ACACTGACGACATGGTTCTACATCGGCGGCGGCGATGTCCT
34	11_20267	1H	105.52	ACACTGACGACATGGTTCTACAAAGCAAAGCAGGAAGCTGAG	ACACTGACGACATGGTTCTACAAAGCAAAGCAGGAAGCTGAG
35	12_30532	1H	109.53	ACACTGACGACATGGTTCTACACTGCACGTCATCATGATGTC	ACACTGACGACATGGTTCTACACTGCACGTCATCATGATGTC
36	SCRI_RS_238125	1H	114.30	ACACTGACGACATGGTTCTACACAAATTCTTCCTGCCGCGTC	ACACTGACGACATGGTTCTACACAAATTCTTCCTGCCGCGTC
37	SCRI_RS_224392	1H	122.40	ACACTGACGACATGGTTCTACAACTGTTGGCCTAGCTGCTG	ACACTGACGACATGGTTCTACAACTGTTGGCCTAGCTGCTG
38	11_20908	1H	126.60	ACACTGACGACATGGTTCTACAGATTGACGAGGCGGTGATAC	ACACTGACGACATGGTTCTACAGATTGACGAGGCGGTGATAC
39	SCRI_RS_189967	1H	129.42	ACACTGACGACATGGTTCTACAGCACCTTCTTCAGCATCTTC	ACACTGACGACATGGTTCTACAGCACCTTCTTCAGCATCTTC
40	12_10808	1H	131.46	ACACTGACGACATGGTTCTACATTATACCCTTCAAGCAGCGG	ACACTGACGACATGGTTCTACATTATACCCTTCAAGCAGCGG
41	12_10693	1H	132.54	ACACTGACGACATGGTTCTACAAAGTCCCATGGGAGAATCAG	ACACTGACGACATGGTTCTACAAAGTCCCATGGGAGAATCAG
42	SCRI_RS_196025	1H	136.75	ACACTGACGACATGGTTCTACATAGCTACCTTGTTAGGCTCC	ACACTGACGACATGGTTCTACATAGCTACCTTGTTAGGCTCC
43	11_11105	1H	142.16	ACACTGACGACATGGTTCTACAGAGATCTGGGAAGCTTAGAC	ACACTGACGACATGGTTCTACAGAGATCTGGGAAGCTTAGAC
44	12_31446	2H	1.08	ACACTGACGACATGGTTCTACAGGGAGTGTTTGTCCTTCTAC	ACACTGACGACATGGTTCTACAGGGAGTGTTTGTCCTTCTAC
45	11_10326	2H	7.29	ACACTGACGACATGGTTCTACACTCCATGGGATACCCATGTC	ACACTGACGACATGGTTCTACACTCCATGGGATACCCATGTC
46	SCRI_RS_168604	2H	11.49	ACACTGACGACATGGTTCTACATGGACCTCGTCCGCTTCTAC	ACACTGACGACATGGTTCTACATGGACCTCGTCCGCTTCTAC
47	11_21377	2H	13.19	ACACTGACGACATGGTTCTACAAGCAGCAGCTACTTGCAAAC	ACACTGACGACATGGTTCTACAAGCAGCAGCTACTTGCAAAC
48	SCRI_RS_141771	2H	15.17	ACACTGACGACATGGTTCTACATCGTCTACTTCGCCGACGAG	ACACTGACGACATGGTTCTACATCGTCTACTTCGCCGACGAG
49	12_30631	2H	19.42	ACACTGACGACATGGTTCTACAATTTATGGACGAGGCAACTG	ACACTGACGACATGGTTCTACAATTTATGGACGAGGCAACTG
50	SCRI_RS_152744	2H	23.76	ACACTGACGACATGGTTCTACACATCAAGAAAGAAGCCGGAG	ACACTGACGACATGGTTCTACACATCAAGAAAGAAGCCGGAG
51	11_10943	2H	25.53	ACACTGACGACATGGTTCTACACCAACACTAACGGTAACAGC	ACACTGACGACATGGTTCTACACCAACACTAACGGTAACAGC
52	SCRI_RS_153798	2H	31.74	ACACTGACGACATGGTTCTACACACGATCGAGTTCATCATCC	ACACTGACGACATGGTTCTACACACGATCGAGTTCATCATCC
53	12_30871	2H	38.60	ACACTGACGACATGGTTCTACATGTTGTCAATCCTTCGGGTC	ACACTGACGACATGGTTCTACATGTTGTCAATCCTTCGGGTC
54	SCRI_RS_182371	2H	44.54	ACACTGACGACATGGTTCTACAACTTGCTTGGGACTAAAGGC	ACACTGACGACATGGTTCTACAACTTGCTTGGGACTAAAGGC

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
55	11_21304	2H	47.35	ACACTGACGACATGGTTCTACATCAAGGACATGCGCTTCAG	ACACTGACGACATGGTTCTACATCAAGGACATGCGCTTCAG
56	11_10919	2H	53.26	ACACTGACGACATGGTTCTACATAAAGGGCAAGGAAAAGCGG	ACACTGACGACATGGTTCTACATAAAGGGCAAGGAAAAGCGG
57	11_10325	2H	66.11	ACACTGACGACATGGTTCTACAAATGGAGTCGAATGGACGAG	ACACTGACGACATGGTTCTACAAATGGAGTCGAATGGACGAG
58	SCRI_RS_154617	2H	66.11	ACACTGACGACATGGTTCTACAAGTTCGTGACGCACGAGATG	ACACTGACGACATGGTTCTACAAGTTCGTGACGCACGAGATG
59	11_20532	2H	69.55	ACACTGACGACATGGTTCTACAGCAGATCCCAACAAAAGACG	ACACTGACGACATGGTTCTACAGCAGATCCCAACAAAAGACG
60	11_21144	2H	72.44	ACACTGACGACATGGTTCTACAAGCAATCGCTGGGCTTTTTG	ACACTGACGACATGGTTCTACAAGCAATCGCTGGGCTTTTTG
61	11_10265	2H	75.18	ACACTGACGACATGGTTCTACACCACGGTACAGCGAAAATTC	ACACTGACGACATGGTTCTACACCACGGTACAGCGAAAATTC
62	SCRI_RS_150	2H	78.02	ACACTGACGACATGGTTCTACAAGATCGCAGATGGTCTGTTG	ACACTGACGACATGGTTCTACAAGATCGCAGATGGTCTGTTG
63	11_10196	2H	81.26	ACACTGACGACATGGTTCTACACAACTGCATTCCCTGGTTAC	ACACTGACGACATGGTTCTACACAACTGCATTCCCTGGTTAC
64	11_21242	2H	86.84	ACACTGACGACATGGTTCTACATCGGGAAAAAGGTCCAATAG	ACACTGACGACATGGTTCTACATCGGGAAAAAGGTCCAATAG
65	11_10287	2H	90.99	ACACTGACGACATGGTTCTACAGGTTCATCGTCACTAGGAAG	ACACTGACGACATGGTTCTACAGGTTCATCGTCACTAGGAAG
66	SCRI_RS_219074	2H	95.24	ACACTGACGACATGGTTCTACATGCCTTTCTGTTTGCCTCTG	ACACTGACGACATGGTTCTACATGCCTTTCTGTTTGCCTCTG
67	11_10214	2H	99.04	ACACTGACGACATGGTTCTACACATGGCAGCTAAGCCCTAAG	ACACTGACGACATGGTTCTACACATGGCAGCTAAGCCCTAAG
68	12_30216	2H	101.98	ACACTGACGACATGGTTCTACACGAGACACTAGAGACACTTC	ACACTGACGACATGGTTCTACACGAGACACTAGAGACACTTC
69	11_10398	2H	106.90	ACACTGACGACATGGTTCTACATAGACGGCTTTATTTGGCAG	ACACTGACGACATGGTTCTACATAGACGGCTTTATTTGGCAG
70	12_30480	2H	108.14	ACACTGACGACATGGTTCTACAATGCGGCTCGGATGTGTATC	ACACTGACGACATGGTTCTACAATGCGGCTCGGATGTGTATC
71	12_30555	2H	112.22	ACACTGACGACATGGTTCTACATTCCGACCATCACTTGAGAG	ACACTGACGACATGGTTCTACATTCCGACCATCACTTGAGAG
72	11_20099	2H	121.76	ACACTGACGACATGGTTCTACACGTTTTCGACGCAGAGTTGT	ACACTGACGACATGGTTCTACACGTTTTCGACGCAGAGTTGT
73	11_10128	2H	124.50	ACACTGACGACATGGTTCTACATTCTGGACGGTGAGCTTGAC	ACACTGACGACATGGTTCTACATTCTGGACGGTGAGCTTGAC
74	12_31095	2H	131.66	ACACTGACGACATGGTTCTACAAGTGGGCGTTCTTCTTGATG	ACACTGACGACATGGTTCTACAAGTGGGCGTTCTTCTTGATG
75	11_21220	2H	136.66	ACACTGACGACATGGTTCTACAGCCGCCTAAACTTCTGAATC	ACACTGACGACATGGTTCTACAGCCGCCTAAACTTCTGAATC
76	11_20141	2H	141.70	ACACTGACGACATGGTTCTACAAGCAGCAGTGATGAAGTTGG	ACACTGACGACATGGTTCTACAAGCAGCAGTGATGAAGTTGG
77	11_21088	2H	144.20	ACACTGACGACATGGTTCTACATATGGAGCATATGGATGCAG	ACACTGACGACATGGTTCTACATATGGAGCATATGGATGCAG
78	12_10579	2H	149.60	ACACTGACGACATGGTTCTACATCATCTCGTTGCATATGCCC	ACACTGACGACATGGTTCTACATCATCTCGTTGCATATGCCC
79	11_20715	2H	152.83	ACACTGACGACATGGTTCTACACAGCCAGTGGACTTAATGTG	ACACTGACGACATGGTTCTACACAGCCAGTGGACTTAATGTG
80	11_11023	2H	160.19	ACACTGACGACATGGTTCTACAGACATGGCAAGAGTACATTC	ACACTGACGACATGGTTCTACAGACATGGCAAGAGTACATTC
81	SCRI_RS_230497	2H	164.26	ACACTGACGACATGGTTCTACATGCTTTTGCCCCACTCACTC	ACACTGACGACATGGTTCTACATGCTTTTGCCCCACTCACTC

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
82	12_30823	2H	168.93	ACACTGACGACATGGTTCTACAACCATCACAACCAAGGTGAG	ACACTGACGACATGGTTCTACAACCATCACAACCAAGGTGAG
83	SCRI_RS_109266	2H	172.15	ACACTGACGACATGGTTCTACAATTTCCTCTTCTTCCTCGCC	ACACTGACGACATGGTTCTACAATTTCCTCTTCTTCCTCGCC
84	12_31180	2H	175.48	ACACTGACGACATGGTTCTACAACGGATGAGATTCAGGAAGC	ACACTGACGACATGGTTCTACAACGGATGAGATTCAGGAAGC
85	11_10085	2H	177.38	ACACTGACGACATGGTTCTACACACATAGTCTCGGCTATTCC	ACACTGACGACATGGTTCTACACACATAGTCTCGGCTATTCC
86	12_31428	3H	0.00	ACACTGACGACATGGTTCTACAGGCGGTGAGGAGGTAGGTG	ACACTGACGACATGGTTCTACAGGCGGTGAGGAGGTAGGTG
87	SCRI_RS_1804	3H	3.89	ACACTGACGACATGGTTCTACAGCCTCTTCTTCAAGTAGACG	ACACTGACGACATGGTTCTACAGCCTCTTCTTCAAGTAGACG
88	11_20252	3H	6.46	ACACTGACGACATGGTTCTACATTCCTGAATGTCTCCTATGC	ACACTGACGACATGGTTCTACATTCCTGAATGTCTCCTATGC
89	11_20529	3H	8.33	ACACTGACGACATGGTTCTACATCTGGAACATGCCCTTCTTG	ACACTGACGACATGGTTCTACATCTGGAACATGCCCTTCTTG
90	12_30818	3H	12.23	ACACTGACGACATGGTTCTACACTACTTCTTGGCCGCGGAC	ACACTGACGACATGGTTCTACACTACTTCTTGGCCGCGGAC
91	SCRI_RS_97417	3H	18.72	ACACTGACGACATGGTTCTACAGTTGCTGAATGAAAGCGATG	ACACTGACGACATGGTTCTACAGTTGCTGAATGAAAGCGATG
92	11_20742	3H	24.63	ACACTGACGACATGGTTCTACAATCAGCACCGTGTACTTGGG	ACACTGACGACATGGTTCTACAATCAGCACCGTGTACTTGGG
93	SCRI_RS_161041	3H	26.28	ACACTGACGACATGGTTCTACACACAAAGTGTGGACATGGAG	ACACTGACGACATGGTTCTACACACAAAGTGTGGACATGGAG
94	SCRI_RS_144410	3H	33.28	ACACTGACGACATGGTTCTACATGAAGCTGGTGAGAGCCGC	ACACTGACGACATGGTTCTACATGAAGCTGGTGAGAGCCGC
95	12_30431	3H	40.48	ACACTGACGACATGGTTCTACACTTCCCTTGGAGGTCTTTTG	ACACTGACGACATGGTTCTACACTTCCCTTGGAGGTCTTTTG
96	11_20356	3H	55.57	ACACTGACGACATGGTTCTACATAGAAACAGGAGCCAGTTGC	ACACTGACGACATGGTTCTACATAGAAACAGGAGCCAGTTGC
97	12_30467	3H	56.79	ACACTGACGACATGGTTCTACAATTCTTCGATGATGGCGGAC	ACACTGACGACATGGTTCTACAATTCTTCGATGATGGCGGAC
98	11_20583	3H	58.31	ACACTGACGACATGGTTCTACATTCAATCGCTGACCCGCAC	ACACTGACGACATGGTTCTACATTCAATCGCTGACCCGCAC
99	11_10373	3H	61.94	ACACTGACGACATGGTTCTACACCGAATCACCGAGAAATAGG	ACACTGACGACATGGTTCTACACCGAATCACCGAGAAATAGG
100	11_21305	3H	65.16	ACACTGACGACATGGTTCTACAGGTATTCCAAGGATCCTCAG	ACACTGACGACATGGTTCTACAGGTATTCCAAGGATCCTCAG
101	12_30399	3H	67.53	ACACTGACGACATGGTTCTACAACTCTTGTCGTGTGGTACTC	ACACTGACGACATGGTTCTACAACTCTTGTCGTGTGGTACTC
102	11_20115	3H	71.74	ACACTGACGACATGGTTCTACAGTGAACCTTGTATATTCTG	ACACTGACGACATGGTTCTACAGTGAACCTTGTATATTCTG
103	SCRI_RS_153148	3H	75.71	ACACTGACGACATGGTTCTACAGATCCTAAAGGAGGACTAGC	ACACTGACGACATGGTTCTACAGATCCTAAAGGAGGACTAGC
104	11_10444	3H	78.93	ACACTGACGACATGGTTCTACATTCTCGTAGTTCCTGAGCTG	ACACTGACGACATGGTTCTACATTCTCGTAGTTCCTGAGCTG
105	12_10134	3H	81.31	ACACTGACGACATGGTTCTACAGCTTCCTGTACATTGCTGTC	ACACTGACGACATGGTTCTACAGCTTCCTGTACATTGCTGTC
106	12_30250	3H	88.17	ACACTGACGACATGGTTCTACAACAGATCCAAGGTGAAGAGC	ACACTGACGACATGGTTCTACAACAGATCCAAGGTGAAGAGC
107	SCRI_RS_120503	3H	92.98	ACACTGACGACATGGTTCTACATCAACCGCAAGTTCGCCTTC	ACACTGACGACATGGTTCTACATCAACCGCAAGTTCGCCTTC
108	12_10344	3H	94.03	ACACTGACGACATGGTTCTACACAGAAGGTCAGAGAGATTGC	ACACTGACGACATGGTTCTACACAGAAGGTCAGAGAGATTGC

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
109	11_21495	3H	94.03	ACACTGACGACATGGTTCTACAGCACAAGGGCTGAACATAAC	ACACTGACGACATGGTTCTACAGCACAAGGGCTGAACATAAC
110	12_30342	3H	94.03	ACACTGACGACATGGTTCTACATGAGAGTCGAGACTTGAGAG	ACACTGACGACATGGTTCTACATGAGAGTCGAGACTTGAGAG
111	11_21493	3H	95.25	ACACTGACGACATGGTTCTACACGGGCCTTGATTGATTTTGC	ACACTGACGACATGGTTCTACACGGGCCTTGATTGATTTTGC
112	SCRI_RS_221787	3H	99.26	ACACTGACGACATGGTTCTACAGAGTGGAGTGGTTGCTGCTT	ACACTGACGACATGGTTCTACAGAGTGGAGTGGTTGCTGCTT
113	SCRI_RS_164704	3H	99.26	ACACTGACGACATGGTTCTACATTTCGTAGAGGCCATCGTAG	ACACTGACGACATGGTTCTACATTTCGTAGAGGCCATCGTAG
114	SCRI_RS_225641	3H	99.46	ACACTGACGACATGGTTCTACAAAGTGATGATGATGCTGCCG	ACACTGACGACATGGTTCTACAAAGTGATGATGATGCTGCCG
115	SCRI_RS_133339	3H	99.56	ACACTGACGACATGGTTCTACATGACAGAGAGAACGAACTCC	ACACTGACGACATGGTTCTACATGACAGAGAGAACGAACTCC
116	SCRI_RS_211929	3H	99.66	ACACTGACGACATGGTTCTACAAAGTACTACGCCGACGACTG	ACACTGACGACATGGTTCTACAAAGTACTACGCCGACGACTG
117	12_30423	3H	99.66	ACACTGACGACATGGTTCTACAGAAGTCAATACCGATTGTAG	ACACTGACGACATGGTTCTACAGAAGTCAATACCGATTGTAG
118	SCRI_RS_235791	3H	99.66	ACACTGACGACATGGTTCTACATGGCGACCATCAAAGCAAAG	ACACTGACGACATGGTTCTACATGGCGACCATCAAAGCAAAG
119	12_31329	3H	103.83	ACACTGACGACATGGTTCTACAAATGAAGTGTTCGGCGACAG	ACACTGACGACATGGTTCTACAAATGAAGTGTTCGGCGACAG
120	11_10584	3H	105.98	ACACTGACGACATGGTTCTACATGCTCGGACAGAGACGTGA	ACACTGACGACATGGTTCTACATGCTCGGACAGAGACGTGA
121	11_20944	3H	109.12	ACACTGACGACATGGTTCTACAGGAGAACATTCCACATTAG	ACACTGACGACATGGTTCTACAGGAGAACATTCCACATTAG
122	12_30081	3H	115.86	ACACTGACGACATGGTTCTACATGAATGGCCATTGCCATGAG	ACACTGACGACATGGTTCTACATGAATGGCCATTGCCATGAG
123	11_10842	3H	118.71	ACACTGACGACATGGTTCTACAGTCTCCTTTATCCTTGTAAG	ACACTGACGACATGGTTCTACAGTCTCCTTTATCCTTGTAAG
124	12_30973	3H	121.02	ACACTGACGACATGGTTCTACACTGAAAACAGATGGTGTAA	ACACTGACGACATGGTTCTACACTGAAAACAGATGGTGTAA
125	11_10381	3H	125.23	ACACTGACGACATGGTTCTACAAAGCACTCTCTGCATCCTTC	ACACTGACGACATGGTTCTACAAAGCACTCTCTGCATCCTTC
126	11_20085	3H	126.41	ACACTGACGACATGGTTCTACAATGGAGGACGAATAGGGAGG	ACACTGACGACATGGTTCTACAATGGAGGACGAATAGGGAGG
127	SCRI_RS_10016	3H	128.53	ACACTGACGACATGGTTCTACACATTAAGGAGATGCTGCCAC	ACACTGACGACATGGTTCTACACATTAAGGAGATGCTGCCAC
128	SCRI_RS_183550	3H	133.78	ACACTGACGACATGGTTCTACACTGATTGTTTCTGGACTGGG	ACACTGACGACATGGTTCTACACTGATTGTTTCTGGACTGGG
129	11_21272	3H	137.48	ACACTGACGACATGGTTCTACATTCCAAACACACAAGCCAGC	ACACTGACGACATGGTTCTACATTCCAAACACACAAGCCAGC
130	12_31500	3H	141.20	ACACTGACGACATGGTTCTACATTCTCTATTGCCACCCTGAC	ACACTGACGACATGGTTCTACATTCTCTATTGCCACCCTGAC
131	SCRI_RS_157113	3H	145.29	ACACTGACGACATGGTTCTACATCCACAAACGCCGCACAAAC	ACACTGACGACATGGTTCTACATCCACAAACGCCGCACAAAC
132	11_11436	3H	145.65	ACACTGACGACATGGTTCTACATGCATCGGACGGTATACTTC	ACACTGACGACATGGTTCTACATGCATCGGACGGTATACTTC
133	SCRI_RS_229623	3H	150.19	ACACTGACGACATGGTTCTACAAGAGAAGAAGAGGAGCAACC	ACACTGACGACATGGTTCTACAAGAGAAGAAGAGGAGCAACC
134	11_11411	3H	151.23	ACACTGACGACATGGTTCTACACACTCCCATATGGTGGATCA	ACACTGACGACATGGTTCTACACACTCCCATATGGTGGATCA
135	SCRI_RS_156315	3H	153.39	ACACTGACGACATGGTTCTACACATCATAGATACAGCCTGCC	ACACTGACGACATGGTTCTACACATCATAGATACAGCCTGCC

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
136	SCRI_RS_178836	3H	160.09	ACACTGACGACATGGTTCTACAAATCCTCTGCTTGAGTCGTC	ACACTGACGACATGGTTCTACAAATCCTCTGCTTGAGTCGTC
137	12_30135	3H	164.42	ACACTGACGACATGGTTCTACACAGGGTCCCAGAATTTATAG	ACACTGACGACATGGTTCTACACAGGGTCCCAGAATTTATAG
138	11_20145	4H	1.17	ACACTGACGACATGGTTCTACAAGAGAAGAAGAATCGAGCAG	ACACTGACGACATGGTTCTACAAGAGAAGAAGAATCGAGCAG
139	11_10409	4H	4.11	ACACTGACGACATGGTTCTACAAGCACTTTGGAAACAGTGGG	ACACTGACGACATGGTTCTACAAGCACTTTGGAAACAGTGGG
140	12_31486	4H	8.28	ACACTGACGACATGGTTCTACAATTGTCATGGAGTCTGGTGC	ACACTGACGACATGGTTCTACAATTGTCATGGAGTCTGGTGC
141	12_31458	4H	14.13	ACACTGACGACATGGTTCTACAGCAGTTTCAAAAGCAATCTC	ACACTGACGACATGGTTCTACAGCAGTTTCAAAAGCAATCTC
142	12_30540	4H	16.13	ACACTGACGACATGGTTCTACATGTATGGAGTGTCCATGGAG	ACACTGACGACATGGTTCTACATGTATGGAGTGTCCATGGAG
143	12_30150	4H	18.47	ACACTGACGACATGGTTCTACAGGTAAACCACCACTTCTTGC	ACACTGACGACATGGTTCTACAGGTAAACCACCACTTCTTGC
144	11_10223	4H	22.43	ACACTGACGACATGGTTCTACACGTCGGTAGACGAGAGAAG	ACACTGACGACATGGTTCTACACGTCGGTAGACGAGAGAAG
145	SCRI_RS_98443	4H	27.64	ACACTGACGACATGGTTCTACACATTCAAGCCCATCAATGCG	ACACTGACGACATGGTTCTACACATTCAAGCCCATCAATGCG
146	11_20777	4H	31.64	ACACTGACGACATGGTTCTACAGATGAGGATGTCCTCCATTG	ACACTGACGACATGGTTCTACAGATGAGGATGTCCTCCATTG
147	11_21374	4H	32.68	ACACTGACGACATGGTTCTACAGGAGCAAACGTGTTAGTTGG	ACACTGACGACATGGTTCTACAGGAGCAAACGTGTTAGTTGG
148	SCRI_RS_145412	4H	42.89	ACACTGACGACATGGTTCTACACCATCAAGGCCAAGATCATC	ACACTGACGACATGGTTCTACACCATCAAGGCCAAGATCATC
149	11_20012	4H	46.19	ACACTGACGACATGGTTCTACAGAGAGCATCGCCGAGAAGAT	ACACTGACGACATGGTTCTACAGAGAGCATCGCCGAGAAGAT
150	11_11180	4H	50.70	ACACTGACGACATGGTTCTACATGTTAGGAGGTGAGTTGTCG	ACACTGACGACATGGTTCTACATGTTAGGAGGTGAGTTGTCG
151	SCRI_RS_167844	4H	51.77	ACACTGACGACATGGTTCTACAATGATCAGGCTGGTGAGTTG	ACACTGACGACATGGTTCTACAATGATCAGGCTGGTGAGTTG
152	SCRI_RS_157310	4H	53.67	ACACTGACGACATGGTTCTACAATGTCCTCCCCGTCCTTCAC	ACACTGACGACATGGTTCTACAATGTCCTCCCCGTCCTTCAC
153	11_10093	4H	53.87	ACACTGACGACATGGTTCTACAAGTTTCATGAGGACTGCATC	ACACTGACGACATGGTTCTACAAGTTTCATGAGGACTGCATC
154	SCRI_RS_155554	4H	54.66	ACACTGACGACATGGTTCTACACGCAAATCGGTTTCAAGCAC	ACACTGACGACATGGTTCTACACGCAAATCGGTTTCAAGCAC
155	11_20472	4H	54.95	ACACTGACGACATGGTTCTACAACGAAATGGACGCGTCAAAG	ACACTGACGACATGGTTCTACAACGAAATGGACGCGTCAAAG
156	SCRI_RS_168496	4H	54.95	ACACTGACGACATGGTTCTACACCACGACTCCATTAGGGATG	ACACTGACGACATGGTTCTACACCACGACTCCATTAGGGATG
157	SCRI_RS_228477	4H	54.95	ACACTGACGACATGGTTCTACAGGGAGGAAGAGATGTGTTTG	ACACTGACGACATGGTTCTACAGGGAGGAAGAGATGTGTTTG
158	12_30839	4H	54.95	ACACTGACGACATGGTTCTACAGTCAGTGAAGCCTTTGAGTC	ACACTGACGACATGGTTCTACAGTCAGTGAAGCCTTTGAGTC
159	11_20412	4H	54.95	ACACTGACGACATGGTTCTACATCTGCTTCGAGACCCTGAAC	ACACTGACGACATGGTTCTACATCTGCTTCGAGACCCTGAAC
160	11_10509	4H	55.64	ACACTGACGACATGGTTCTACATGGCCCTCTTATATATCCCC	ACACTGACGACATGGTTCTACATGGCCCTCTTATATATCCCC
161	SCRI_RS_189180	4H	57.32	ACACTGACGACATGGTTCTACAGTCGAATTTCTTGTGTGGGC	ACACTGACGACATGGTTCTACAGTCGAATTTCTTGTGTGGGC
162	11_10606	4H	60.28	ACACTGACGACATGGTTCTACAGGGCACAACTTACTTGCTTA	ACACTGACGACATGGTTCTACAGGGCACAACTTACTTGCTTA

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
163	11_11513	4H	64.45	ACACTGACGACATGGTTCTACAGTTCAACCACCATCATCCAC	ACACTGACGACATGGTTCTACAGTTCAACCACCATCATCCAC
164	11_10467	4H	67.22	ACACTGACGACATGGTTCTACAATACACCAGCTACAGCAAGG	ACACTGACGACATGGTTCTACAATACACCAGCTACAGCAAGG
165	11_10309	4H	67.91	ACACTGACGACATGGTTCTACAACACACACAACACACAAGGG	ACACTGACGACATGGTTCTACAACACACACAACACACAAGGG
166	12_31148	4H	72.70	ACACTGACGACATGGTTCTACAGGAAAAGAGTCCCACAATCG	ACACTGACGACATGGTTCTACAGGAAAAGAGTCCCACAATCG
167	11_20718	4H	76.11	ACACTGACGACATGGTTCTACATTGGCGGTGCCGGCTTTCT	ACACTGACGACATGGTTCTACATTGGCGGTGCCGGCTTTCT
168	12_10022	4H	80.62	ACACTGACGACATGGTTCTACAACCATCTCTTTCAGGAGGAC	ACACTGACGACATGGTTCTACAACCATCTCTTTCAGGAGGAC
169	11_10751	4H	82.99	ACACTGACGACATGGTTCTACATCAGATGCTGCGATATCAGG	ACACTGACGACATGGTTCTACATCAGATGCTGCGATATCAGG
170	SCRI_RS_129218	4H	89.24	ACACTGACGACATGGTTCTACAGATTGTAATGAGGTCCGGTG	ACACTGACGACATGGTTCTACAGATTGTAATGAGGTCCGGTG
171	SCRI_RS_189881	4H	94.74	ACACTGACGACATGGTTCTACAGACGTCTCCTCAGAGGTTTC	ACACTGACGACATGGTTCTACAGACGTCTCCTCAGAGGTTTC
172	11_20762	4H	96.60	ACACTGACGACATGGTTCTACAGTTATGGAAAGTAGAGGGAC	ACACTGACGACATGGTTCTACAGTTATGGAAAGTAGAGGGAC
173	11_21111	4H	102.18	ACACTGACGACATGGTTCTACAACATGAGCATGGAGGAGAAC	ACACTGACGACATGGTTCTACAACATGAGCATGGAGGAGAAC
174	12_11194	4H	107.77	ACACTGACGACATGGTTCTACACAAGCTTCGAGTGAGCTAAG	ACACTGACGACATGGTTCTACACAAGCTTCGAGTGAGCTAAG
175	SCRI_RS_196076	4H	111.65	ACACTGACGACATGGTTCTACATCTTGTTAACTTTGGAGCCG	ACACTGACGACATGGTTCTACATCTTGTTAACTTTGGAGCCG
176	11_20701	4H	114.98	ACACTGACGACATGGTTCTACATTGTTGGCCTGCCTTTTCTC	ACACTGACGACATGGTTCTACATTGTTGGCCTGCCTTTTCTC
177	11_10697	4H	117.33	ACACTGACGACATGGTTCTACACTCTAACCACTTCTACGTGC	ACACTGACGACATGGTTCTACACTCTAACCACTTCTACGTGC
178	11_10269	4H	120.53	ACACTGACGACATGGTTCTACATGATCTCCTTGGTCGGCTC	ACACTGACGACATGGTTCTACATGATCTCCTTGGTCGGCTC
179	11_20272	4H	125.11	ACACTGACGACATGGTTCTACATCCATGCCCGGAGAAATAAG	ACACTGACGACATGGTTCTACATCCATGCCCGGAGAAATAAG
180	12_31422	4H	127.26	ACACTGACGACATGGTTCTACACAGTGAGTACATTAGCTCTAC	ACACTGACGACATGGTTCTACACAGTGAGTACATTAGCTCTAC
181	12_30975	5H	3.66	ACACTGACGACATGGTTCTACACATACAATGAGTAATGACGTG	ACACTGACGACATGGTTCTACACATACAATGAGTAATGACGTG
182	11_20206	5H	6.55	ACACTGACGACATGGTTCTACACCGTCTTGGTTGGTTTCGAC	ACACTGACGACATGGTTCTACACCGTCTTGGTTGGTTTCGAC
183	12_30714	5H	13.47	ACACTGACGACATGGTTCTACATCCAGCTTAGGTCCTTGAAC	ACACTGACGACATGGTTCTACATCCAGCTTAGGTCCTTGAAC
184	SCRI_RS_149877	5H	18.14	ACACTGACGACATGGTTCTACATGAGCTCCATCGTTCTCCAG	ACACTGACGACATGGTTCTACATGAGCTCCATCGTTCTCCAG
185	11_21426	5H	21.24	ACACTGACGACATGGTTCTACATTCACGCTGATTGTTGAGCC	ACACTGACGACATGGTTCTACATTCACGCTGATTGTTGAGCC
186	11_11048	5H	27.80	ACACTGACGACATGGTTCTACAGCGTGCTGTTGGTAAAAAGG	ACACTGACGACATGGTTCTACAGCGTGCTGTTGGTAAAAAGG
187	11_21324	5H	27.88	ACACTGACGACATGGTTCTACACTCTCTACCACAAGGATCTG	ACACTGACGACATGGTTCTACACTCTCTACCACAAGGATCTG
188	11_10580	5H	33.22	ACACTGACGACATGGTTCTACACAGAGCACATGCTACTAAAC	ACACTGACGACATGGTTCTACACAGAGCACATGCTACTAAAC
189	11_10621	5H	35.35	ACACTGACGACATGGTTCTACACCTTTCCAACCTTAAGAAGC	ACACTGACGACATGGTTCTACACCTTTCCAACCTTAAGAAGC

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
190	11_20845	5H	37.62	ACACTGACGACATGGTTCTACACGATCGGCTTTATGATAGGC	ACACTGACGACATGGTTCTACACGATCGGCTTTATGATAGGC
191	11_20729	5H	41.33	ACACTGACGACATGGTTCTACACAAGCATTGGATTGTTGCCG	ACACTGACGACATGGTTCTACACAAGCATTGGATTGTTGCCG
192	12_30538	5H	44.99	ACACTGACGACATGGTTCTACATTCGATCAAACCCCTCATGC	ACACTGACGACATGGTTCTACATTCGATCAAACCCCTCATGC
193	12_30745	5H	46.21	ACACTGACGACATGGTTCTACATGTTAAGCAAGCCGGTGAAC	ACACTGACGACATGGTTCTACATGTTAAGCAAGCCGGTGAAC
194	11_10641	5H	50.88	ACACTGACGACATGGTTCTACAACTCCTACTTCAACAAGGTC	ACACTGACGACATGGTTCTACAACTCCTACTTCAACAAGGTC
195	12_20350	5H	53.77	ACACTGACGACATGGTTCTACAACTAGCTTTCTTGCCGACAC	ACACTGACGACATGGTTCTACAACTAGCTTTCTTGCCGACAC
196	11_21001	5H	55.83	ACACTGACGACATGGTTCTACACAGAGCAAAGTTTGACGTGG	ACACTGACGACATGGTTCTACACAGAGCAAAGTTTGACGTGG
197	11_20236	5H	59.03	ACACTGACGACATGGTTCTACATGCCGATGAGGCGATTATTC	ACACTGACGACATGGTTCTACATGCCGATGAGGCGATTATTC
198	11_21445	5H	64.65	ACACTGACGACATGGTTCTACAACGACGGTTTCTTAGGTGAG	ACACTGACGACATGGTTCTACAACGACGGTTTCTTAGGTGAG
199	SCRI_RS_160332	5H	67.23	ACACTGACGACATGGTTCTACATAATAAGACGGCGGCACAAC	ACACTGACGACATGGTTCTACATAATAAGACGGCGGCACAAC
200	12_10674	5H	68.83	ACACTGACGACATGGTTCTACATAATAAGGCTTCCGACGGAG	ACACTGACGACATGGTTCTACATAATAAGGCTTCCGACGGAG
201	11_20497	5H	73.15	ACACTGACGACATGGTTCTACATCGGATACAACCATGAGAGC	ACACTGACGACATGGTTCTACATCGGATACAACCATGAGAGC
202	11_10518	5H	76.34	ACACTGACGACATGGTTCTACAAAGACAGCCTCGACATCATC	ACACTGACGACATGGTTCTACAAAGACAGCCTCGACATCATC
203	SCRI_RS_158235	5H	82.31	ACACTGACGACATGGTTCTACACATGCCAATACTTTCCTGCC	ACACTGACGACATGGTTCTACACATGCCAATACTTTCCTGCC
204	11_21061	5H	88.80	ACACTGACGACATGGTTCTACATTCTTGGTTGTTGCCGAGAG	ACACTGACGACATGGTTCTACATTCTTGGTTGTTGCCGAGAG
205	11_11273	5H	93.16	ACACTGACGACATGGTTCTACAATTTAGCCCGGCCACTAAGG	ACACTGACGACATGGTTCTACAATTTAGCCCGGCCACTAAGG
206	11_11200	5H	99.58	ACACTGACGACATGGTTCTACAACCTTTGTTTTGCTTGCAGG	ACACTGACGACATGGTTCTACAACCTTTGTTTTGCTTGCAGG
207	SCRI_RS_149088	5H	103.35	ACACTGACGACATGGTTCTACAATCGATTCTTTCGGCTCTGG	ACACTGACGACATGGTTCTACAATCGATTCTTTCGGCTCTGG
208	SCRI_RS_234720	5H	106.15	ACACTGACGACATGGTTCTACATCTTCCATCAGATTACAAGG	ACACTGACGACATGGTTCTACATCTTCCATCAGATTACAAGG
209	SCRI_RS_2831	5H	109.35	ACACTGACGACATGGTTCTACAAGCTCGCGGAGTTCTTGCAC	ACACTGACGACATGGTTCTACAAGCTCGCGGAGTTCTTGCAC
210	11_11507	5H	111.56	ACACTGACGACATGGTTCTACAGGGCACAATTTGTTACATAG	ACACTGACGACATGGTTCTACAGGGCACAATTTGTTACATAG
211	11_21422	5H	112.78	ACACTGACGACATGGTTCTACACTGAAAATGACCTCCAAGGG	ACACTGACGACATGGTTCTACACTGAAAATGACCTCCAAGGG
212	11_20653	5H	115.53	ACACTGACGACATGGTTCTACAATACCACTTGTGATCCGAGG	ACACTGACGACATGGTTCTACAATACCACTTGTGATCCGAGG
213	12_30067	5H	117.63	ACACTGACGACATGGTTCTACAATTGATGTCGAGAACCGGAG	ACACTGACGACATGGTTCTACAATTGATGTCGAGAACCGGAG
214	11_21247	5H	119.72	ACACTGACGACATGGTTCTACATCCGTTCCCGTTTGTTACAC	ACACTGACGACATGGTTCTACATCCGTTCCCGTTTGTTACAC
215	SCRI_RS_141778	5H	126.19	ACACTGACGACATGGTTCTACAGACGCCATTGCTGTTGAAAG	ACACTGACGACATGGTTCTACAGACGCCATTGCTGTTGAAAG
216	11_10845	5H	128.80	ACACTGACGACATGGTTCTACACAACAGCGATCCAAGCTTCC	ACACTGACGACATGGTTCTACACAACAGCGATCCAAGCTTCC

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
217	SCRI_RS_213086	5H	132.37	ACACTGACGACATGGTTCTACACTTGCTAAAGCTTGGGCAAC	ACACTGACGACATGGTTCTACACTTGCTAAAGCTTGGGCAAC
218	11_20100	5H	135.42	ACACTGACGACATGGTTCTACATGGTGAAGAGGGCCGAGAAG	ACACTGACGACATGGTTCTACATGGTGAAGAGGGCCGAGAAG
219	11_10557	5H	135.42	ACACTGACGACATGGTTCTACATTGCCACATGCAAGTGACTG	ACACTGACGACATGGTTCTACATTGCCACATGCAAGTGACTG
220	12_31050	5H	137.22	ACACTGACGACATGGTTCTACATTGTCGTGCTGCCCTTGGAA	ACACTGACGACATGGTTCTACATTGTCGTGCTGCCCTTGGAA
221	12_31165	5H	138.44	ACACTGACGACATGGTTCTACAGCCCAACGTCATCGTACGAA	ACACTGACGACATGGTTCTACAGCCCAACGTCATCGTACGAA
222	12_31221	5H	142.71	ACACTGACGACATGGTTCTACACTTTGCGAAGCACGTTTCTC	ACACTGACGACATGGTTCTACACTTTGCGAAGCACGTTTCTC
223	11_11497	5H	147.70	ACACTGACGACATGGTTCTACATGAGCTACTCTGACTCACTC	ACACTGACGACATGGTTCTACATGAGCTACTCTGACTCACTC
224	SCRI_RS_189174	5H	150.10	ACACTGACGACATGGTTCTACAATGAAGGAGATCGTCAGAGC	ACACTGACGACATGGTTCTACAATGAAGGAGATCGTCAGAGC
225	11_10336	5H	153.47	ACACTGACGACATGGTTCTACAAGCTCACTTATATATCACC	ACACTGACGACATGGTTCTACAAGCTCACTTATATATCACC
226	12_30162	5H	156.70	ACACTGACGACATGGTTCTACAATGTGAAGACGGAGCTGTAG	ACACTGACGACATGGTTCTACAATGTGAAGACGGAGCTGTAG
227	12_30566	5H	160.32	ACACTGACGACATGGTTCTACAGGACTGATGACTCAAAACCG	ACACTGACGACATGGTTCTACAGGACTGATGACTCAAAACCG
228	11_10869	5H	163.16	ACACTGACGACATGGTTCTACAGCTGCTACACACATGAATTG	ACACTGACGACATGGTTCTACAGCTGCTACACACATGAATTG
229	11_10870	5H	168.24	ACACTGACGACATGGTTCTACACGGTGTAACTGGATGAAGAC	ACACTGACGACATGGTTCTACACGGTGTAACTGGATGAAGAC
230	12_30494	5H	171.16	ACACTGACGACATGGTTCTACATCATCCAGTTCAGCGCCTTC	ACACTGACGACATGGTTCTACATCATCCAGTTCAGCGCCTTC
231	12_30504	5H	172.25	ACACTGACGACATGGTTCTACAGCACCATCACTATCATGCAG	ACACTGACGACATGGTTCTACAGCACCATCACTATCATGCAG
232	SCRI_RS_178615	5H	177.03	ACACTGACGACATGGTTCTACATAGCTGACGCCGCAAGAAAC	ACACTGACGACATGGTTCTACATAGCTGACGCCGCAAGAAAC
233	11_11364	5H	179.67	ACACTGACGACATGGTTCTACAGTTCTCCAGGAAACAACCAG	ACACTGACGACATGGTTCTACAGTTCTCCAGGAAACAACCAG
234	12_10322	5H	181.11	ACACTGACGACATGGTTCTACAGCGCCACCATGTTACGACC	ACACTGACGACATGGTTCTACAGCGCCACCATGTTACGACC
235	12_31123	5H	184.75	ACACTGACGACATGGTTCTACACACCGTGCCTTTCTTAGAAG	ACACTGACGACATGGTTCTACACACCGTGCCTTTCTTAGAAG
236	11_20232	6H	0.00	ACACTGACGACATGGTTCTACATGTGACGAATTTCTCGAGCC	ACACTGACGACATGGTTCTACATGTGACGAATTTCTCGAGCC
237	11_20294	6H	6.49	ACACTGACGACATGGTTCTACAGGATACTATCTAGGTGGGTC	ACACTGACGACATGGTTCTACAGGATACTATCTAGGTGGGTC
238	11_21032	6H	11.35	ACACTGACGACATGGTTCTACAAATCTCTGCATAAGAGCAGG	ACACTGACGACATGGTTCTACAAATCTCTGCATAAGAGCAGG
239	11_20415	6H	15.16	ACACTGACGACATGGTTCTACAGCTGTCATCTTTCTCGAGTC	ACACTGACGACATGGTTCTACAGCTGTCATCTTTCTCGAGTC
240	12_10554	6H	19.07	ACACTGACGACATGGTTCTACATCTTCTTCAAGAAGCACCCG	ACACTGACGACATGGTTCTACATCTTCTTCAAGAAGCACCCG
241	12_30843	6H	23.07	ACACTGACGACATGGTTCTACATCATTGGCTGTGTGTTGTGC	ACACTGACGACATGGTTCTACATCATTGGCTGTGTGTTGTGC
242	11_10868	6H	27.19	ACACTGACGACATGGTTCTACAGATGTTACGTCCAGGACAAC	ACACTGACGACATGGTTCTACAGATGTTACGTCCAGGACAAC
243	12_30697	6H	33.39	ACACTGACGACATGGTTCTACATAGGACGGTGCATCCATTTG	ACACTGACGACATGGTTCTACATAGGACGGTGCATCCATTTG
244	12_31485	6H	35.29	ACACTGACGACATGGTTCTACATCCTGATAAAGGCAGGAGTC	ACACTGACGACATGGTTCTACATCCTGATAAAGGCAGGAGTC

Table C3. Polymorphic single nucleotide polymorphism (SNP) markers evenly spread throughout the genome between barley lines Pinnacle and PI84314 along with the forward and reverse primers sequences. The marker position (cM) and chromosome were based on 9k Illuminum ISelect chip information (continued).

SN	Marker	chr	cM	Forward Primers	Reverse Primers
245	11_10799	6H	38.12	ACACTGACGACATGGTTCTACACCATTTCCCCTAGGAATCAG	ACACTGACGACATGGTTCTACACCATTTCCCCTAGGAATCAG
246	11_10939	6H	40.52	ACACTGACGACATGGTTCTACACCAGGAGTACTGTACAGTTC	ACACTGACGACATGGTTCTACACCAGGAGTACTGTACAGTTC
247	11_10427	6H	41.35	ACACTGACGACATGGTTCTACAGAACAGGTACCACAAATGGG	ACACTGACGACATGGTTCTACAGAACAGGTACCACAAATGGG
248	SCRI_RS_154121	6H	44.91	ACACTGACGACATGGTTCTACACACTGGGGTTCTTTGCAATC	ACACTGACGACATGGTTCTACACACTGGGGTTCTTTGCAATC
249	12_30361	6H	47.81	ACACTGACGACATGGTTCTACAAGTTCTGAAGACTCCACGAC	ACACTGACGACATGGTTCTACAAGTTCTGAAGACTCCACGAC
250	11_10061	6H	50.41	ACACTGACGACATGGTTCTACAACGTCCTTCTGCTCATAACC	ACACTGACGACATGGTTCTACAACGTCCTTCTGCTCATAACC
251	11_20675	6H	55.90	ACACTGACGACATGGTTCTACATTGGGCGTACGAGGAGTATG	ACACTGACGACATGGTTCTACATTGGGCGTACGAGGAGTATG