

Characterization of QTLs for disease resistance and rolled leaf traits in a mutant derived wheat
RIL population

by

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B.S., China Agricultural University, 2015

M.S., China Agricultural University, 2017

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Abstract

Both biotic and abiotic stresses are major constraints for global wheat production. Abiotic stress such as drought and heat and biotic stress such as fungal and viral diseases can significantly reduce grain yield and quality of wheat. In this study, a mutant, named JagMut1095, identified from EMS treated Jagger cultivar, showed significant higher Type II resistance to Fusarium head blight (FHB) than Jagger in the greenhouse experiments. This mutant also showed obvious rolled leaf (RL) and BYDV susceptibility in contrast to Jagger in the field experiments. A population of 154 recombinant inbred lines (RILs) was developed from the cross of JagMut1095 x Jagger and was used for QTL analysis on these three traits. For Type II FHB resistance, 12 QTLs were identified on chromosomes 1B, 2B, 3A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 7A and 7B. One major QTL *QFhb.hwwg-4BS* was mapped in a 1.5 Mb interval in chromosome arm 4BS and explained up to 32.8% of the phenotypic variation. The *QFhb.hwwg-4BS* interval has 17 high confidence genes including *Rht1*, a ‘Green Revolution’ gene, and overlaps with the major QTL *QPh.hwwg-4BS* for plant height, thus, *QPh.hwwg-4BS* is most likely *Rht-1B*. QTL analysis on barley yellow dwarf (BYDV) data from the population identified one major QTL, *QByd.hwwg-2DL*, in a physical interval between 642,415,923 bp and 642,998,925 bp on chromosome arm 2DL and Jagger contributed the resistance allele. Two major QTLs (*QRI.hwwg-1AS* and *QRI.hwwg-5AL*) for RL were detected and together explained up to 61% of the phenotypic variation. The mutant contributed RL alleles at both QTLs. These major QTLs for RL and BYDV resistance in this study are novel and they provide the new genetic resources and insight for further characterization and utilization of these genes in breeding. Fine-mapping of *QFhb.hwwg-4BS* will facilitate further understanding of the genetic relationship between FHB resistance and plant height to improve wheat FHB resistance in semidwarf wheat cultivars.

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Chapter 1 - Literature review: Wheat resistance to abiotic and biotic stresses

1.1 Origin and cultivation of wheat

Wheat is one of the most important staple foods in the world. During the wheat domestication and evolution processes, *Triticum* species have been through three ploidy levels from diploid ($2n = 2x = 14$) to tetraploid ($2n = 4x = 28$), and then to hexaploid ($2n = 6x = 42$) (Levy and Feldman 2022). Today's common wheat (*Triticum aestivum*), also known as bread wheat, originated in the fertile crescent by one or several rare independent hybridizations. Firstly, the wild species *Triticum urartu* (AA) hybridized naturally with *Aegilops speltoides* (the B genome ancestor), producing the wild tetraploid *Triticum turgidum* with AABB genome, which was later domesticated as cultivated emmer wheat (Matsuoka, 2011). Then, an initial sterile triploid (ABD) generated by the hybridization between emmer wheat and *Aegilops tauschii* (DD) about 10,000 years ago in the Fertile Crescent region, following a chromosome doubling event led to the hexaploid common wheat (AABBDD) (Feldman 2001).

Since the domestication of common wheat, it has spread across the world (Dubcovsky and Dvorak, 2007) and is cultivated in six major regions of the world, including North America, Europe, Asia, eastern Africa, Australia, and South America. In the United States (U.S.), wheat has been ranked the third largest crop after corn and soybean, and has been categorized into six major classes based on seed color, hardness and plant growing season including durum, hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), soft durum white (SW) and hard white (HW) wheat (Vocke and Ali 2013). Durum wheat is tetraploid (AABB) and is mainly used for pasta, while the five other classes are hexaploid (AABBDD), and mainly used for bread and other food products (Sissons 2008). Kansas is in the central U.S. and has suitable natural

resources for wheat growing (such as climate, soil and rainfall), thus it is the leading wheat producer in U.S. and grows predominantly hard red winter wheat (https://agriculture.ks.gov/docs/default-source/ag-growth-summit/january-2018-documents/wheat-sector.pdf?sfvrsn=970380c1_16).

Due to being grown over a wide range of environments, wheat continuously encounters various stresses in the field. New varieties of wheat have been continuously selected and released from breeding programs to not only improve the quality and yield of wheat in past decades, but also to battle those biotic and abiotic stresses. Understanding genetic mechanisms underlying wheat resistance or tolerance to those stresses is critical to provide information for breeders to select resistant cultivars that adapt to a wide range environments.

1.2 Rolled leaf trait in wheat

Rolled leaf (RL) has been reported as a typical morphological response to water deficit in numerous species such as rice, maize, wheat and sorghum (Corlett et al. 1994; Peleg et al. 2009; Zou et al. 2014; Saglam et al. 2014; Tahmasebi et al. 2016). RL can also result from other abiotic stress factors such as salt, high temperature, heavy metals and radiation (Kadioglu et al. 2012). Additionally, biotic stresses from herbivores, viruses, bacteria and fungi are also factors of RL (Linscott et al. 2001; Font et al. 2007; Yang 2010; Kadioglu et al. 2012). To understand the rolling mechanism and the potential use of RL in wheat breeding, characterizing the physiological and molecular mechanisms in plant RL underlying stress tolerance is an important area of future research.

1.2.1 RL under abiotic stress factors

The three most typical reasons of RL are water deficit, temperature and irradiation in various plants including rice, maize, wheat and sorghum (Kadioglu and Terzi 2007). As one of

the most observed responses to drought stress, RL increases stress tolerance in many plants (Turner and Begg 1981). In the *Gramineae*, RL is an indication of water deficit (O'Toole et al. 1979). In *Ctenanthe setosa*, RL can protect PSII functionality from damage induced by drought stress (Nar et al. 2009). RL in rice was related to osmotic adjustment under drought stress (Hsiao et al. 1984). Additionally, factors causing water deficit such as salt stress can also induce RL. For example, RL is used as one visual symptom of salt stress in rice (Tatar et al. 2010).

High temperature is also a factor affecting the degree of RL in many plants. The leaf osmotic potential and the leaf temperature were linearly correlated with RL in rice (Kadioglu and Terzi 2007). One physiological role of RL is to maintain the adaptation potential of photosynthesis by increasing the efficiency of water metabolism in wheat flag leaves under high temperature (Sarieva et al. 2010). In plants such as *Rhododendron* species, low temperature above the tissue freezing points can also cause RL (Nilsen 1991) and chilling treatment also results in RL in rice (Yan et al. 2006).

RL is also a response to solar radiation in plants. In *Triticum aestivum*, RL occurs between 11 am and 2 pm in a sunny day in some soft-winter wheat (Kadioglu and Terzi 2007). As an obligate shade plant, *Amomum villosum* rolls its leaves when exposed to bright sun (Feng et al. 2002). Plants can prevent photodamage through the mechanisms of RL, thermal dissipation and reversible inactivation of PSII reaction centers.

Apart from above-mentioned factors, herbicides, heavy metals, and ozone have been found to cause RL in plants. For example, application of sulfonylurea herbicides caused RL in cherry trees (Putnam 1999). Cadmium toxicity in plants causes RL and also chlorosis and stunting in tomatoes (Djebali et al. 2010). Mineral such as copper and sulphur deficiency results

in RL in wheat (Owuochel et al. 1995). Moreover, ozone can induce RL and reduced growth in *Arabidopsis thaliana* (Sharma and Davis 1994).

1.2.2 RL benefits plant under drought stress

Plants under water deficit may face photodamage due to stomata closed (Baker and Rosenqvist 2004) and RL can help a plant keep the stomata open to reduce the exposed leaf area under radiation and create a microclimate near the leaf surfaces to lower transpiration (Oppenheimer 1960; Turner and Begg 1981; Saglam et al. 2014). Additionally, RL is flexible to revise the change under hydrated conditions, which provides flexibility to reduce the radiation load on the canopy only when necessary. Moderate RL benefits the erectness of leaves in rice to minimize shadow among leaves and improve photosynthetic efficiency and grain yield (Govaerts et al. 1996; Liu et al. 2016). Also, RL enhances plant density tolerance by reducing the mass shading factors in maize (JunFeng et al. 2010; Gao et al. 2019).

1.2.3 Cytological mechanisms of RL

Changes of various cells of leaf blades can cause RL. Zou et al. (2014) used 46 stable mutants to elucidate the cytological mechanisms of inward (adaxial) or outward (abaxial) RL phenotypes. Histological investigation of those mutants showed that RL related to the changes in number, size, and pattern of bulliform cells, sclerenchyma cells, parenchyma cells, and mesophyll cells as well as vascular bundles in the plant leaves. In the abaxial RL mutants, the changes in leaf cells include increased bulliform cell number, cell size and even cell area. Phloem expansion of vascular system was also observed in several abaxial rolling mutant lines. In the adaxial RL mutants, leaf cell changes include decreased bulliform cell number and size, and defective sclerenchyma cells on the abaxial side of small veins. Also, bulliform-like cells are formed on the adaxial cell layers of small veins in some mutant lines and some mesophyll cells

become parenchyma-like cells in several mutants. In generally, the same RL phenotype can be resulted from one or multiple alteration of a particular cell type or even from multiple cell types of plant leaves.

1.2.4 Genetical basis of RL

RL can be controlled by either a single gene with a major effect (Singh and Mackill, 2008) or multiple genes with minor effects (Price et al. 1997). In rice, more than 70 genes or quantitative trait loci (QTLs) have been reported for RL and at least 17 mutants have been characterized for adaxially or abaxially RL (Liu et al. 2016; Qiang et al. 2016). At least 18 rice genes for RL were cloned and related to formation of bulliform cells, a group of large, bubble-shaped epidermal cells on the upper surface of the leaves of many monocots (Grigore and Toma 2017). For example, rice outermost cell-specific gene 5 (*Roc5*) is a member of the class IV HD-Zip genes and controls the number and size of bulliform cells, while the overexpression of *Roc5* causes adaxially RL (Zou et al. 2011). *Semi-rolled leaf1* (*SRL1*), encoding a putative glycosylphosphatidylinositol-anchored protein in rice, regulates the formation of bulliform cells at the adaxial cell layers to modulate RL (Xiang et al. 2012). A zinc finger homeodomain class homeobox transcription factor (*OsZHD1*) in rice increases the number and the abnormal arrangement of the bulliform cells to cause abaxially RL (Xu et al. 2014).

In maize, RL enhanced plant density tolerance by reducing the mass shading factors. Maize RL mutants have been identified including *leaf bladeless1* (*Lbl1*) and *rolled leaf1* (*rld1*) (Timmermans et al. 1998; Juarez et al. 2004). The leaves of *Lbl1* mutants rolled abaxially due to a complete loss of adaxial cell types. Gene *Rld1* encodes a class III homeodomain-leucine zipper (HD-ZIP III) protein that specifies adaxial cell fate and controls upward curling of leaf blades (Juarez et al. 2004). Beside bulliform cells (number and size) and leaf polarity

(Adaxial/Abaxial), genes for RL may also affect the development of sclerenchymatous cells and cuticle, but the physiological and molecular mechanisms underlying these genes remain unknown (Xu et al. 2018).

In wheat, many studies have demonstrated that wheat RL may contribute significantly to drought tolerance, but only a few studies have been conducted on mapping QTLs or genes for RL in wheat. Tahmasebi et al. (2016) mapped 19 putative QTLs for RL using a wheat recombinant inbred line (RIL) population from ‘SeriM82’ × ‘Babax’. Verma et al. (2020) identified 12 QTLs for RL using RIL population derived from the cross between ‘NI5439’ × ‘HD2012’. QTLs for RL were also identified from several wheat relatives with four stable RL QTLs detected on chromosomes 3R, 5R, and 7R of Rye (*Secale cereale*), and three on chromosomes 2H and 3H of barley (*Hordeum vulgare*) (Obsa et al. 2016). In tetraploid wheat, Peleg et al. (2009) mapped 14 QTLs for flag RL using a RIL population derived from durum wheat x wild emmer wheat, but none of them showed a major effect on RL.

1.2.5 Rolled leaf phenotyping

RL trait is usually scored using a 1 to 5 scale, where 1 is slightly rolled and 5 is tightly rolled (O’Toole and Cruz 1980). The degree of RL could be also estimated by the percentage reduction in leaf width resulting from rolling (Premachandra et al. 1993) or by the ratio of RL width to unrolled leaf width (Matthews et al. 1990). Sirault et al. (2015) used a method applying smoothing splines to skeletonized images of transverse wheat leaf sections to measure RL in wheat. Also, some studies also score additional traits as complements of RL to characterize the drought tolerance, such as the specific leaf weight and relative water content (RWC) (Ben-Amar et al. 2020). Some studies measured transpiration rate, water use efficiency and photosynthetic rate in RL plants to understand the mechanism under drought stress causing RL (Dingkuhn et al.

1989; Nar et al. 2009; Saglam et al. 2014). Although RL was reported in some studies to be related to drought or heat tolerance, RL was more as sensitivity to stress rather than tolerance and should be treated carefully in a breeding program.

1.3 FHB resistance in wheat

Fusarium head blight (FHB or scab) is a devastating fungal disease of small grain crops, and it can be incited by a range of different *Fusarium* species. *Fusarium graminearum* (teleomorph *Gibberella zeae*), *Fusarium culmorum* and *Fusarium avenaceum* (teleomorph *Gibberella avenaceae*) appear to be predominant pathogens for FHB in different climate conditions (Parry et al. 1995). FHB severely lowers grain yield and quality, and the grain contamination with toxic fungal secondary metabolites, known as mycotoxins, causes major health risk for human and animals (Buerstmayr et al. 2002). Weather and environmental factors heavily affect FHB epidemics, and FHB epidemics occur when the susceptible plants are exposed to abundant natural inoculum in warm and humid weather at flowering time. From 1991-1997, severe FHB outbreaks resulted in about \$1.3 billion of total direct losses on wheat and barley in the United States with \$4.8 billion of total losses from the accumulative economic impact of FHB (Bai and Shaner 2004). Severe and frequent FHB epidemics have become a growing threat to wheat production in North America, Europe, China and other wheat growing areas (Figuerola et al. 2018) and mycotoxin contamination in the FHB infected grain is viewed as a serious risk to food safety (Placinta et al. 1999).

To reduce the losses caused by FHB, several strategies including chemical, agronomic and genetic measures have been used to minimize FHB damage (Figuerola et al. 2018), but none of them can fully control FHB. Growing FHB tolerant cultivars is the most promising strategy to control FHB (Petersen et al. 2017; Bai et al. 2018).

1.3.1 Sources for FHB resistance

To date, FHB resistant sources have been identified in Asian, South American and European wheat cultivars and germplasm. However, most of the resistant accessions with high levels of resistance are either landraces or alien species, and are hard to incorporate into breeding programs due to the association with undesired agronomic traits (Bai and Shaner 2004). In 1980, Chinese FHB Collaboration Group screened a collection of more than 10,000 wheat germplasm from both China and abroad and identified many Chinese landraces and some cultivars with high or moderate FHB resistance. ‘Sumai 3’, ‘Ning 7840’, ‘Wangshuibai’ and some other ‘Sumai 3’ derived lines are among these resistant accessions and have been used as FHB resistance parents in breeding programs and as the major genetic materials for research to understand the mechanisms of wheat FHB resistance worldwide. Then, the first FHB resistance QTL was identified in ‘Sumai 3’ (Waldron et al. 1999) and ‘Ning 7840’ (Bai et al. 1999), which was named as *Fhb1* and validated to be the QTL with the largest and most stable effect on FHB resistance identified from wheat to date.

The International Maize and Wheat Improvement Center (CIMMYT) started an FHB breeding program to identify and incorporate new resistance genes from Chinese sources into their elite CIMMYT germplasm since 1980’s. Since then, CIMMYT continuously distributed promising lines with good FHB resistance through the Fusarium Head Blight Screening Nursery (FHBSN) worldwide (He et al. 2013). In the U.S., after many years of breeding effort to incorporate FHB resistance genes from different sources to US wheat, locally adapted FHB resistant cultivars have been released from SRW (such as ‘Jamestown’ and ‘Tribute’ from Virginia), HSW (such as ‘Glenn’ and ‘Driver’) and HRW wheat materials (such as ‘Everest’, ‘Overland’ and ‘Lyman’) were used in USA wheat production (Brown-Guedira et al. 2008;

Griffey et al. 2010; Bai et al. 2018; <https://Sscabsmart.org>). ‘Jamestown’ has been used as the main native source of FHB resistance in SRW wheat and serves as a moderately resistance check in the Southern Uniform Winter Wheat Scab Nursery (SUWWSN) (Zhu et al. 2019). Everest was the leading cultivar in Kansas from 2013 to 2018, occupying 9.3%–15.8% of the wheat planted area, while Overland and Lyman were planted on approximately 13.5% of the total winter wheat area in South Dakota and 20% in Nebraska in 2016 (Wilson et al. 2017). FHB resistance has also been tested in large collections of European winter wheat germplasm (Liu et al. 2009; Venske et al. 2019).

1.3.2 FHB resistance types

In wheat, FHB resistance shows quantitative inheritance and multiple genes or QTLs may be involved in resistance in a specific germplasm line. Five resistance types have been reported and they are often highly correlated with each other (Petersen et al. 2017). Type I is resistance to initial infection and Type II is resistance to the spread of infection within a spike (Schroeder and Christensen 1963). Type III is low DON accumulation (Miller et al. 1985) and the other two types including low Fusarium damaged kernel (FDK) and tolerance to FHB (Mesterházy 1995). Among five types of resistance, Type I and Type II are the two major types of FHB resistance. Type II resistance has been especially well characterized. Type II resistance is easier to assess in the greenhouse by single-floret inoculation (SFI) and measuring FHB severity as percentage of symptomatic spikelets in a spike (PSS), where Type I is harder to evaluate and is more affected by the environment than Type II. For this reason, most genetic studies focus on Type II resistance (Bai and Shaner 2004). FDK and DON content are the other two traits to measure FHB resistance (Buerstmayr et al. 2019). FDK are usually decolorized, shriveled, light-weighted, and produce significantly lower grain and flour quality than healthy kernels (Wiwart et al. 2001;

Hatcher et al. 2011). FDK is usually rated visually by counting percent FDK in total kernels from a sample (McCartney et al. 2007) or comparing seed samples with a set of FDK standards to estimate percent FDK in each sample (Jin et al. 2014).

Different types of resistance may share common genes/QTLs or be controlled by different genes (Petersen et al. 2017). However, how these different resistance types and genes for different resistance types work together to conquer FHB remains unclear, therefore continuous research is needed to discover effective FHB resistant sources and their FHB resistance mechanisms.

1.3.3 Quantitative trait loci (QTLs) for FHB resistance in wheat

Researchers have put significant effort into genetic analysis of FHB resistance through chromosomal manipulation (e.g., intervarietal substitutions) or QTL mapping and gene cloning based on high-density genetic maps (Cuthbert et al. 2006). To date, more than 150 QTLs with varied effects were identified on all wheat chromosomes. Fifty QTLs have the unique chromosome locations (Buerstmayr et al. 2009a; Liu et al. 2009; Bai et al. 2018). Among the reported QTLs, seven were given gene designations and were named as *Fhb1* to *Fhb7*. *Fhb1* was the first QTL identified on 3BS from two independent QTL mapping studies using different mapping populations (Bai et al. 1999; Waldron et al. 1999), which shows stable effects on Type II resistance across multiple backgrounds and has been incorporated into wheat breeding programs worldwide (Bai et al. 2018). Recently *Fhb1* has been cloned by several research groups (Rawat et al. 2016; Li et al. 2019; Su et al. 2019). Screening a worldwide collection of wheat germplasm found that *Fhb1* resistance allele is present only in Chinese and Japanese germplasm. In China, it is present only in germplasm from the southern Chinese wheat growing region where FHB epidemics are more frequent and severe, indicating that *Fhb1* most likely originated from

southern China (Su et al. 2019). *Fhb2* is mapped at 2 cM away from the SSR *Xgwm644* on chromosome 6BS (Cuthbert et al. 2007). *Fhb2* has been found in both Chinese, Canadian and European germplasm (Bai et al. 2018), and mainly controls Type II FHB resistance. *Fhb4* was finely mapped within a 1.7 cM interval flanked by *Xhbg226* and *Xgwm149* (Xue et al. 2010). *Fhb4* has a lower percent phenotypic variation explained (PVE) than *Fhb1* and *Fhb2* and is present in both Chinese and US germplasm. *Fhb5* is associated with both Type I and Type II resistance and has been mapped on chromosome 5A in more than 10 populations. Fine mapping work using two backcross populations and a RIL population located *Fhb5* to a 0.3 cM interval flanked by *Xgwm304* and *Xgwm415* on chromosome 5AS (Xue et al. 2011), where the Q gene located. However, a recombination has been identified between Q gene and *Fhb5* thus indicating they are independent genes. *Fhb5* has been reported in wheat cultivars from China ('Wangshuibai'), Japan ('Nyu Bai'), Europe ('F201R'), South America ('Frontana') and North America ('Ernie'). Another three QTLs were all from alien species, including *Fhb3* on chromosome 7AS from *Leymus racemosus* (Qi et al. 2008), *Fhb6* on chromosome 1A from *Elymus tsukushiensis* (Cainong et al. 2015), and *Fhb7* on chromosome 7D from *Thinopyrum ponticum* (Guo et al. 2015; Wang et al. 2020). *Fhb6* and *Fhb7* have been successfully transferred into several wheat cultivars and selected lines show high levels of FHB resistance in different wheat backgrounds (Bai et al. 2018).

1.3.4 Clone FHB resistance gene in wheat

Three groups reported the *Fhb1* cloning work with two different candidate genes. Rawat et al. (2016) first reported a pore forming toxin like protein gene (PFT) as the candidate gene. PFT protein may have similar function with antifungal lectins that may participate in the recognition of fungus-specific carbohydrates and cause toxicity to the pathogen. However,

TaPFT has been found in many highly susceptible accessions without *Fhb1*-mediated resistance, suggesting *TaPFT* isn't likely the candidate for *Fhb1*. Later, two papers simultaneously reported the *TaHRC* encoding the reticulum histidine-rich calcium-binding protein as the candidate of *Fhb1* (Li et al. 2019; Su et al. (2019), but they proposed different functional mechanisms for *TaHRC*. Su et al (2019) reported *TaHRC* as a susceptibility gene that gained FHB resistance through a large deletion in the start codon region that silenced the susceptible allele. The functional *TaHRC* protein with a histidine-rich region was located in nucleus of wheat leaf protoplasts, which may interact with pathogens and trigger susceptibility (Su et al. 2019). Whereas, Li et al (2019) claimed that the deletion in *TaHRC* resulted in a frameshift that forms a new functional protein to control *Fhb1* resistance. The authors suggest the gene is likely involved in the Ca^{2+} signaling pathway or interacts with other defense-associated signaling pathways to regulate the plant defense against pathogens (*TaHRC* suppresses the calcium-mediated immune response and triggers wheat Fusarium head blight susceptibility) (Li et al. 2019; Chen et al. 2022). More research remains needed to determine the mechanisms of *Fhb1* resistance.

More recently, *Fhb7* gene has been cloned to encode a glutathione S-transferase (GST). GST is an FHB resistance gene that was transferred from 7E chromosome of a wheat relative *Thinopyrum* to wheat and it could be conjugated onto trichothecene toxins produced by the pathogen (Wang et al. 2020). Plants with the resistance allele of *Fhb7* can detoxify the toxin produced by the pathogen in the spikes. Additionally, *Fhb7* shared 97% identity with the sequence of *Epichloë* species, an endophytic fungus symbiotic with temperate grasses, suggesting that *Fhb7* might originate from *Epichloë* and was transferred to *Th. elongatum* through horizontal gene transfer during the evolution process.

1.3.5 Relationship between plant height and FHB resistance

Among QTLs identified for FHB resistance in wheat, many overlapped with QTLs for plant height (PH) in both field and greenhouse conditions. Taller alleles have been often associated with FHB-resistance in many studies (Yan et al. 2011; Buerstmayr et al. 2013; He et al. 2016). However, some exceptions have also been observed where taller plants showed more susceptibility, in particular for Type II resistance using single floret inoculation (SFI). For example, lines carrying semi-dwarf allele *Rht-B1b* showed more resistance to fungal spread (Srinivasachary et al. 2009) with lower DON content than their taller sister lines (Saville et al. 2012). Thus, further characterization of the relationship between FHB resistance and plant height is critical to determination of genetic relationship between FHB resistance and plant height in wheat.

1.3.6 Reduced height genes in wheat

Plant height is a major agronomic trait for wheat production under diverse wheat growing environments, and lodging of taller plants can cause large yield losses. In the middle of the 20th century, the “Green Revolution” introduced semi-dwarfing genes into cereal crops resulting in reduced lodging and increased yield. As the most important component of the “Green Revolution”, two reduced-height (*Rht*) genes, *Rht-B1* and *Rht-D1*, were used in wheat to improve yield worldwide. The two dwarfing genes encode DELLA proteins that interfere with the signal transduction pathway of the gibberellin (GA) growth hormone to reduce plant height (Peng et al. 1999). The GA-insensitive locus *Rht-B1* on chromosomes 4B is also designated *Rht1*, and its homolog (*Rht-D1*) on chromosome 4D designated as *Rht2* (Börner et al. 1997). Both wheat *Rht1* and *Rht2* are the orthologs of GAI from *Arabidopsis* and dwarf8 from maize. A truncated N-terminal GID1–GA binding domain resulted in a premature stop codon in the coding regions of

Rht1 and *Rht2*, and shortens plants. Besides *Rht1* and *Rht2*, *Rht8* is another cloned GA-sensitive gene for plant height. This gene was mapped on chromosome 2D (Worland et al. 1998) and cloned as a Ribonuclease H-like gene (Chai et al. 2022). *Rht8* reduces wheat plant height without compromising grain yield, in particular under dry environments such as Mediterranean countries with increased plant adaptability. The utilization of *Rht* genes make it possible for high input of nitrogen fertilizers and irrigation to increase yield of wheat. The GA-insensitive dwarfing alleles significantly increase spikelet fertility, grain number per ear, and grain yields from 8 to 24% depending on genetic backgrounds (Li et al. 2006).

1.3.7 Overlapping QTLs for plant height and FHB resistance

Dwarf allele of *Rht* genes have been widely used in wheat breeding programs to determine plant architecture and contribute grain yield, thus, the *Rht*-associated FHB severity could greatly influence the final yield of wheat production. To date, three possible mechanisms have been proposed for the association between plant height and FHB resistance, including disease escape, pleiotropy of *Rht* genes, and tight linkage (Buerstmayr et al. 2009; He et al. 2016). Several studies showed molecular evidence for the association between QTLs for FHB and plant height including *Rht-B1*, *Rht-D1* and *Rht8* (Gervais et al. 2003; Somers et al. 2003; Steiner et al. 2004; Schmolke et al. 2005; Draeger et al. 2007; Srinivasachary et al. 2008; Mao et al. 2010). FHB Type I resistance showed higher association with plant morphological traits and was influenced more by microclimate that is created by plant height in the field. Taller plants often have better FHB resistance in the field due to disease escape in the field, and shorter plants have more severe FHB (Mesterházy 1995; Hilton et al. 1999; Buerstmayr et al. 2009; Mao et al. 2010). Draeger et al. (2007) identified a significant FHB resistance QTL at the *Rht-D1* locus, which moderately correlated with the taller allele of *Rht-D1*. Later Srinivasachary et al. (2008)

verified the association between *Rht-D1b* and FHB susceptibility in another independent mapping study using the population derived from ‘Rialto’ x ‘Spark’. Yan et al. (2011) inoculated NILs for Type I resistance then adjusted spikes of both tall and short plants of nine *Rht* dwarf isolines to the same height for disease development. The FHB resistance between the isolines were similar in most pairs of NILs, supporting that the resistance responses of the tall *Rht* alleles is due to the micro-environmental conditions around spikes created by plant height. However, four NIL pairs showed significant difference in Type II resistance, in which the dwarf isolines gave better Type II resistant performance. Their results strongly supported that the association between plant height and Type II FHB resistance is either due to pleiotropic effect or tightly linkage. To validate this hypothesis, fine-mapping of the overlapped QTL region is essential to dissect the QTL effect to determine the relation between plant height and the FHB resistance in the QTL region.

1.4 Barley Yellow Dwarf Virus disease in wheat

Plant viruses hurt the host fitness by reducing the survivorship, fecundity or competitive ability of hosts relative to uninfected individuals in the population (Malmstrom et al. 2005). Barley yellow dwarf virus (BYDV) is one of the most economically important virus disease of cereals, and can be found in most of the crop growing season in the world. Although widespread outbreaks of BYDV in cereals have been noted in the United States as early as 1907 and 1949, BYDV has not been recognized as the cause of the disease in barley (*Horedum vulgare* L.) until 1951 (Oswald and Houston, 1951). Later, the disease has been found in oat (*Avena sativa* L.) and wheat worldwide (Perry et al. 2000; Jin et al. 2007). BYDVs are phloem-limited and transmitted by aphids in a non-propagative but persistent and circulated manner (Texcoco et al. 2002). BYDV occurs in barley and wheat, rye, oat and maize (Ayala et al. 2001).

In wheat, yield losses due to BYDV have been reported to be 40–50% in severely infected fields (Larkin et al. 2002), and up to 25% even in the fields with minimal or near-invisible BYDV symptoms (Jarošová et al. 2016).

BYDV infected plants usually show leaf discoloration varying from shades of yellow to red and purple, and in some cases stunted plants due to the elongation failure of stem internodes, with the symptoms similar as nutrient deficiency (Miller and Rasochová 1997). Usually, the BYDV symptoms appear at jointing in late spring, which are predominately from fall infections (Nega 2014). Seedling infection can cause lethal or distinct yellowing in older leaves, and post seedling infections cause yellow or red flag leaves. Early infection in fall may predispose the plants to winterkilling, and the disease becomes more severe when new growth starts in the following spring. Generally, the earlier the infection, the more severe the disease with the root system damaged as severely as the tops. The disease symptoms are more severe if infection takes place in the fall than in the spring and high light intensity and cool temperatures (15 to 18° C) favor the expression of BYD symptoms (Nass et al. 1995). BYDV can also cause stiff leaves, underdeveloped root systems, reduced tillering, and restrained spike development and grain filling.

1.4.1 BYDV pathosystem

BYDV pathosystem has three biotic elements: the BYDV complex formed by the luteoviruses, the aphid species as virus vectors, the plant species as the hosts of both the virus and the vectors. The transmission efficiency of various BYDV viral strains can be influenced by the aphid species, length of feeding period, and the physiological age of plant tissues (Gray et al. 1991). Many factors may affect BYDV symptoms, including host crops or varieties, aphid

infestation time, host developmental stage when the infestation occurs, infestation pressure and environments (Shah et al. 2012; Choudhury et al. 2019a).

1.4.1.1 BYDV strains

The classification of BYDVs is a challenge because a group of related single-stranded RNA viruses from different genera can cause the same disease symptoms. BYDV-PAV, PAS, MAV and Kerll belong to the genus *Luteovirus*, while cereal yellow dwarf virus (CYDV)-RPV, RPS and Maize yellow dwarf virus- RMV belong to the genus *Polerovirus* (Miller and Rasochová 1997; Miller et al. 2002; Gray and Gildow 2003). Other two strains including BYDV-SGV and GPV in the family *Luteovirida* have not been assigned to a specific genus. Among them, BYDV-PAV is the most prevalent virus species worldwide, probably because of the wide distribution of its primary vector *Rhopalosiphum padi* and its high vectoring efficiency (Aradottir and Crespo-Herrera 2021).

1.4.1.2 BYDV vectors

Aphids feed on the phloem sieve tubes to acquire and transmit BYDVs to host plants. BYDVs are phloem restricted and can be transmitted more efficiently in a non-propagative but persistent and circulate manner by aphid species (Miller and Rasochová 1997). The different viral isolates are transmitted by different major vectors: RPV transmitted by *Rhopalosiphum padi*, RMV by *Rhopalosiphum maidis*, MAV by *Sitobion avenae*, SGV by *Schizaphis graminum*, PAV by *R. padi*, *S. avenae*, and others, PAS by *R. maidis* and *Metopolophium dirhodum*, GAV by *S. graminum* and *S. avenae*, GPV by *R. padi* and *S. graminum*, and RPS by *R. padi* (Aradottir and Crespo-Herrera 2021). The viral transmission by insect vectors and the plant infection are dependent on a range of abiotic (such as rainfall, temperature, and nutrient supply) and biotic (such as natural enemy of vectors, coinfecting pathogens, and relative abundance of non-hosts)

factors that affect the virus, the insect vectors and the plant hosts (Seabloom et al. 2010). These factors vary across space and time, causing the regional variation in disease incidence to impact crops (Smith and Plumb 1981). Competition between vectors and virus species may determine the prevalence of a virus in a given region.

1.4.1.3 BYDV hosts

Among a wide range of species that have been tested as the experimental hosts of BYDVs, only a few are found naturally in the field although discrepancy exists among the reports (Panayotou 1982; Oswald and Houston 1953; Watson and Mulligan 1960). Oat and barley have the most severe symptoms of BYDV in the field. Although wheat and rye are also susceptible to BYDV, they have more tolerance to BYDV infection with less readily developed symptoms than oats and barley. Other crops including maize (Stoner 1977) and rice (Jorda et al. 1987) are also susceptible to BYDVs.

1.4.2 Genes or QTLs for BYDV resistance

Using wheat resistance or tolerance to BYDVs or their vectors is one of the most promising approaches to control the disease (Cooper and Jones, 1983). To date, several BYDV tolerance genes have been reported in barley including *Ryd1*, *Ryd2*, *Ryd3* and *Ryd4*. *Ryd1* from cultivar ‘Rojo’ was first reported in 1955 but it was rarely used in breeding because of its low level of resistance (Suneson 1955; Rasmusson and Schaller 1959). *Ryd2* on chromosome 3HL of ‘Ethiopian’ (Collins et al. 1996) has been successfully incorporated to different spring and winter barley cultivars and is used in commercial production (Delogu et al. 1995; Ovesná et al. 2000). *Ryd3*, derived from ‘Ethiopian’, was mapped at the centromeric region of chromosome 6H and explained 75% of the phenotypic variance for BYDV tolerance under severe BYDV infection conditions (Niks et al. 2004). *Ryd4^{Hb}* is on a subterminal introgression from a wild relative H.

bulbosum to chromosome arm 3HL of a common barley. The translocation conferred true resistance to BYDV-PAV, BYDV-MAV, and CYDV-RPV (Scholz et al. 2009). However, linkage drag of this gene prevented it from wide deployment in barley breeding (Jarošová et al. 2016). In addition to the four genes, several quantitative trait loci (QTLs) for BYD-PAV resistance have been identified on chromosomes 1H, 2H, 4H and 7H (Toojinda et al. 2000; Scheurer et al. 2001, Friedt et al. 2003). Several QTLs contributing to BYDV tolerance have also been detected in oat (*Avena sativa*) (Jin et al. 1998; Zhu et al. 2003; Kuehn et al. 2009), however, none of the genes or QTLs from barley or oat has been used in wheat breeding.

Reports on BYDV resistance in common wheat are rare. *Bdv1* was the only reported gene originated from a Brazilian spring wheat cultivar ‘Frontana’. This gene was located on the short arm of chromosome 7D, and showed a pleiotropic effect on rust resistance (*Lr34/Yr18*, (Singh et al. 1993). *Bdv1* shows resistance to only some BYDV species or in some environments (Zhang et al. 2009). Another three genes (*Bdv2*, *Bdv3* and *Bdv4*) all originated from the intermediate wheatgrass (*Thinopyrum intermedium*, $2n = 42$, genomes $E1E2X = EaEbSt = JJSS$), syn. *Agropyron intermedium*, *Elytrigia intermedia*. Various wheat-Th. *intermedium* chromosome addition lines, substitution lines, and translocation lines with BYDV resistance have been developed in wheat, including *Bdv2* in 7D-7Ai#1 (Banks et al. 1995), *Bdv3* in 7D-7E (Sharma et al. 1995), and *Bdv4* in 2D-2Ai-2 (Zhang et al. 2009). Among them, *Bdv2* has been widely used in BYDV-PAV resistant wheat breeding in Australia (Zhou et al. 2015). Beside the four named genes available in wheat, Ayala et al. (2002) identified 22 QTLs in ‘Opata’ × ‘Synthetic’ population and 7 QTLs from ‘Frontana’ × ‘INIA66’ population, including one in the similar location to *Bdv1* on 7DS. However, most of the QTLs showed minor effects explaining 6.6% to 13.0% of the phenotypic variation for visual leaf disease scores and 5.6% to 10.5% for dwarfing

and biomass reduction. Choudhury et al. (2019b) identified four QTLs for BYDV resistance on chromosomes 2A, 2B, 6A, and 7A using GWAS and, these QTLs explained 4.4% - 7.4% of the phenotypic variation. Silva et al. (2022) identified significant marker-trait associations for BYDV severity on chromosomes 5AS, 7AL, and 7DL using high-throughput phenotyping and GWAS on 30 wheat cultivars and 351 advanced breeding lines. The major QTL at the 571-637 Mbp on chromosome arm 7DL that reduced about 10% of disease severity was confirmed to be *Bdv2*. Most of the reported genes or QTLs for BYDV resistance show unstable minor effects in wheat, and have not been widely deployed in breeding programs (Silva et al. 2022).

1.4.3 Sources of resistance to BYDV vectors

Since aphids vector BYDV, incorporating aphid resistance in wheat cultivars can also reduce BYDV damage. Actually, resistance to virus transmission is not independent from resistance to aphids (especially by antibiosis) in many cases, as both of them target aphid feeding functions (Jarošová et al. 2016). To date, more than 20 examples from different crop/virus combinations showed that the resistance to the vector decreased viral incidence in the field and/or laboratory experiments (Tanguy and Dedryver 2009).

The resistance to aphids is dependent on both host and aphid genotypes. Three possible mechanisms have been proposed for plant resistance to insects: 1) antixenosis (Insects show non-preference reaction to host plants), 2) antibiosis (Plant inhibits the insect's biotic potential including growth, development, and reproduction) (Dogimont et al. 2010) and 3) tolerance (Plants can withstand or recover from insect damage) (Zhu et al. 2004, 2005).

Resistance to aphid species has been reported in wheat and its wild relatives in the primary and secondary gene pools. Resistance to both *R. padi* and *S. avenae* was found in *Aegilops neglecta* Req. ex Bertol (Smith et al. 2004), and resistance to *R. padi* and *S. graminum*

as found in several wheat germplasm accessions (Hesler et al. 1999; Crespo-Herrera et al. 2014). Some *Triticale* accessions showed antibiosis-type resistance to *R. padi* (Hesler et al. 2007). Wheat–alien substitution and translocation lines derived from *Secale cereale* or *Aegilops speltoides* showed resistance to multiple cereal aphids including *R. padi*, *S. avenae*, and *S. graminum* (Crespo-Herrera et al. 2013).

1.5 Conclusion and future perspective

Wheat production in the field will be consequently facing abiotic and/or biotic stresses, which could induce large yield loss. Drought or heat stresses, and the fungal or viral diseases are all the negative factors that provide a significant challenge to maximize wheat yields, now and into the future. For this reason, the understanding of traits related to abiotic stress and disease resistance are of immense importance. In this review, we summarized the key aspects of RL which is the typical morphological trait related to drought (or heat) stress, and two of the important wheat diseases including FHB and BYDV of wheat. These three traits were furtherly characterized by QTL analysis using a RIL population showing significant variations of the three traits, which could provide the fundamental understanding for further molecular characterization of the wheat stress responses.

Chapter 2 - QTL mapping of rolled leaf trait in the JagMut1095 x

Jagger RIL population

2.1 Introduction

Drought is one of the most harmful environmental stresses for crops in most rain-fed areas worldwide. Drought not only reduces crop yield, but also reduces the availability of land for agriculture. Plant phenology allows the crop demand to synchronize with environmental resource availability and escape from seasonal abiotic stresses (Bogard et al. 2021). Breeding for cultivars with increased tolerance to drought and heat has been the most effective strategy to reduce the negative impact of drought on crop production (Verma et al. 2020). However, the global climate change has been projected to increase air temperature and raise water demand of rainfed and irrigated crops, which creates a major challenge for breeders to improve the adaptation to increased drought and heat stress during flowering and grain filling of winter and spring crops such as wheat and barley (Gouache et al. 2012; Sommer et al. 2013).

Drought tolerant plants use biochemical, physiological, or morphological strategies to cope with drought stress (Turner and Begg 1981; Kadioglu and Terzi 2007). Rolled leaf (RL) in plants, including leaf folding and paraheliotropism, is one of the morphological strategies for dehydration avoidance mechanism to protect plant leaves from photodamage under drought conditions in field (Corlett et al. 1994). RL under drought stress reduces leaf area under solar radiation or lowers transpiration rates through the creation of a microclimate near the leaf surface (Oppenheimer 1960; Turner and Begg 1981). Under drought stress, the closed stomata reduce transpiration, CO₂ uptake, and photosynthetic activity, which generates an imbalance between PSII activity and the Calvin cycle to increase the excitation energy on PSII leading to subsequent

photodamage (Baker and Rosenqvist 2004). RL, however, helps a plant maintain leaf hydration, but keeps stomata open to prevent losses of photosynthetic pigments and sustain the activities of PSII and Rubisco (Saglam et al. 2014). Additionally, RL is reversible, which provides the flexibility to reduce the radiation load on the canopy only when necessary. All the benefits from RL under drought stress make RL an important target trait for drought tolerance breeding in several crops including wheat (*Triticum aestivum* L.) (Myśków et al. 2018).

In plants, several factors including water deficit, high air temperature or high radiation from sunlight can cause RL. Depending on the populations, genetic variation in RL trait may be generated by a single gene with a major effect (Singh and Mackill, 2008) or multiple genes with minor effects (Price et al. 1997). In rice, more than 70 genes or quantitative trait loci (QTLs) have been identified for RL and at least 17 mutants have been characterized for adaxially or abaxially RL (Liu et al. 2016; Qiang et al. 2016). Remarkably, at least 18 cloned genes for RL in rice are related to bulliform cells, a group of thin-walled and highly vacuolated large cells between the vascular bundles on the adaxial epidermis of a rice leaf blade (Gao et al. 2019). For example, the rice outermost cell-specific gene 5 (*Roc5*) gene, a member of the class IV HD-Zip genes, controls the number and size of bulliform cells, while the overexpression of *Roc5* caused adaxially RL (Zou et al. 2011). *Semi-rolled leaf1* (*SRL1*) is another rice RL gene encoding a putative glycosylphosphatidylinositol-anchored protein and modulates RL by regulating the formation of bulliform cells at the adaxial cell layers (Xiang et al. 2012). A zinc finger homeodomain class homeobox transcription factor (*OsZHD1*) has also been reported to increase the number and the abnormal arrangement of the bulliform cells to cause abaxially RL (Xu et al. 2014).

In maize, RL is selected by breeders to enhance plant density tolerance by reducing the mass shading factors (Gao et al. 2019). To date, five maize RL mutants have been reported including *leafbladeless1* (*Lbl1*) and *rolled leaf1* (*rld1*) (Timmermans et al. 1998; Juarez et al. 2004). The *Lbl1* mutant has abaxialized leaves due to a complete loss of adaxial cell types. *Rld1* encodes a class III homeodomain-leucine zipper (HD-ZIP III) protein that is required for specifying adaxial cell fate and controlling upward curling of leaf blades (Juarez et al. 2004). Beside bulliform cells (number and size) and leaf polarity (Adaxial/Abaxial), genes for RL may also contribute to development of sclerenchymatous cells and cuticle, but the physiological and molecular mechanisms underlying these genes remain unknown (Xu et al. 2018).

Wheat is a staple food crop, accounting for 20% of calories and proteins consumed by the world's population (Shiferaw et al. 2013). In developing countries, almost 50% of wheat (50 million ha) is sown under rain-fed areas with less than 600 mm of precipitation annually (Gupta et al. 2017). Drought tolerance is an essential trait to have in most wheat breeding programs in those regions. Many studies have demonstrated that wheat RL may contribute significantly to drought tolerance, but only a few studies have been conducted on mapping QTLs or genes for RL in common wheat (Tahmasebi et al. 2016; Verma et al. 2020) and its relatives including rye (*Secale cereale*), barley (*Hordeum vulgare*) (Obsa et al. 2016), and tetraploid wheat (Peleg et al. 2009).

Single nucleotide polymorphism (SNP) markers generated by genotyping-by-sequencing (GBS) have been frequently used in QTL mapping. Previously, we developed a RIL population from a cross between Jagger and an ethyl methanesulfonate (EMS) induced mutant (JagMut1095) of Jagger. The objectives of this study were to identify QTLs controlling the RL

trait using the RIL population of JagMut1095 x Jagger and provide the foundation for map-based cloning and functional characterization of RL trait in the mutant.

2.2 Materials and Methods

2.2.1 Plant materials

Jagger (PI 593688) is a hard winter wheat cultivar from Kansas and does not show visible RL in the field. JagMut1095 is a mutant that was selected from the mutant population derived by EMS mutagenesis of Jagger as described by Rawat et al. (2019) and showed significant RL in both the field and greenhouse conditions spontaneously. An M5 mutant plant (JagMut1095) derived by single seed descent in the greenhouse was crossed to Jagger and a population of 154 RILs developed from the cross was used for QTL analysis.

2.2.2 Phenotyping of the rolled leaf trait in the field

The RL trait was scored in five field experiments conducted in three Kansas locations (Ashland Bottoms Research Farm and Rocky Ford Research Station, Manhattan, KS and Agricultural Research Center at Hays, KS) for two wheat growing seasons (2019-2020 and 2020-2021) with the loss of the experiment at Hays in the 2020-2021 season due to severe hail damage. The five experiments were designated as combinations of years and locations of the experiments conducted (2020-Ashland, 2020-RockyFord, 2020-Hays, 2021-Ashland and 2021-RockyFord). The F₈ RIL population was planted with two replications and the parents with five replications using a randomized complete block design (RCBD) in each experiment. One gram of seeds per line was sowed in a 1-meter-long single-row plot. RL was visually scored on a 1 (no rolled leaf) to 5 (complete rolled leaf) scale at noon during sunny days after flowering.

2.2.3 Genotyping the parents and RIL population

Leaf tissues were collected at the three-leaf stage from a single plant of each F5 RIL and parents into 96-deepwell plates, dried in a freeze dryer (ThermoFisher, Waltham, MA) for 48 h, and then ground into fine powder at 30 cycles/sec for 3 min in a GenoGrinder 2010 tissue grinder (SPEX SamplePrep, NJ, USA). DNA was isolated using a modified cetyltrimethyl ammonium bromide (CTAB) method (Bai et al, 1999). DNA concentration was estimated using a Quant-iTTM PicoGreen[®] dsDNA Assay kit (ThermoFisher Scientific, Waltham, MA) in a FLUOstar Omega microplate reader (BMG LABTECH, German), and normalized to 20 ng/ μ L for GBS library construction (Poland and Rife, 2012). In brief, normalized genomic DNA was double-digested using restriction enzymes PstI and MspI (New England Biolabs Inc., Ipswich, MA) and ligated to a set of barcode adapters using T4 ligase (New England Biolabs Inc). The GBS library was size-selected for 200-300 bp fragments in an E-gel system (ThermoFisher Scientific). The selected polymerase chain reaction (PCR) fragments were purified with the GenCatch PCR purification kit (Epoch Life Science Inc., Sugar Land, TX), and quantified with the Qubit dsDNA HS assay kit (ThermoFisher Scientific) before sequencing using PI v3 chips and Hi-Q sequencing kits (ThermoFisher Scientific) in an Ion Torrent Proton sequencer (Life Technologies, Carlsbad, CA). The raw sequence reads were assigned to each sample based on attached barcodes, then trimmed to 64 bp DNA sequences including the 5' restriction site. SNPs were detected using a reference-based pipeline in TASSEL 5.0 (Bradbury et al. 2007) using the IWGSC RefSeq v2.1 genome (IWGSC, 2018) to assign the physical positions for each SNP. Exome-capture data were obtained for JagMut1095 and Jagger using an assay described previously (Jordan et al. 2015). The quality of raw sequencing reads was assessed using NGSQC toolkit v.2.3.3 (Patel and Jain 2012). The sequence reads were aligned to the IWGSC wheat

reference genome RefSeq v.1.1 (IWGSC, 2018) using HISAT2 (Kim et al. 2015) retaining only uniquely mapped reads. The resulting alignments were processed using Samtools (Li et al. 2009) and run through the GATK pipeline (McKenna et al. 2010) using Haplotype Caller to generate a variant call file including variants for Jagger and the Jagger mutant. Additional SNPs in the identified QTL intervals were used to increase marker density. The position of each exome-capture SNP on the IWGSC wheat reference genome RefSeq v.2.1 (IWGSC 2018) was decided by blasting the flanking sequence of each SNP.

2.2.4 KASP marker development and validation

In the major QTL intervals for RL, primers for kompetitive allele specific PCR (KASP) were designed based on the SNP sequences generated from both GBS and exome capture using Primer3web version 4.1.0 (<http://primer3.wi.mit.edu/>) (Untergasser et al. 2012). The PCR Allele Competitive Extension (PACE) assays were performed to screen KASP primers in parents and the RIL population. Markers segregating at expected ratios in the RIL population were used to refine the genetic map. Each PACE assay consists of 1.94 μ L of 2X PACE™ reaction mix (3CR Bioscienc, Harlow, England, UK), 0.06 μ L of primer assay mix, and 2 μ L of 20 ng/ μ L DNA. The PCR initially started at 94 °C for 15 min, followed by 10 cycles at 94 °C for 20 s, 65 °C for 1 min with -0.8 °C in each subsequent cycle to 57 °C, then by 30 cycles of 94 °C for 20 s, and 57 °C for 1 min.

2.2.5 Linkage map construction and QTL analysis

The linkage map was constructed using QTL IciMapping V4.1 (Wang et al. 2009; Meng et al. 2015). Individual linkage groups of GBS-SNPs were anchored to 21 corresponding chromosomes based on IWGSC RefSeq v2.1 (IWGSC, 2018). The Kosambi mapping function was used to convert recombination frequency to genetic distance in the linkage map (Kosambi

1943). The TwoOpt algorithm was used to order the SNPs in each linkage group, and SARF (Sum of adjacent recombination frequencies) module was used for fine-tuning of the marker order in each linkage group.

QTL analysis was performed using the composite interval mapping (CIM) module in QTL Cartographer version 2.5 (Wang 2007) with a walking speed of 1.0 cM. The averaged RL score over two replications in each environment and the adjusted mean best linear unbiased estimators (BLUE) of five environments were calculated and included in QTL analysis. The significant QTLs for RL were claimed at the LOD threshold of 4.10 and at 4.0 for HD derived by 1000-time permutations using the BLUE values in QTL Cartographer version 2.5 (Doerge and Churchill 1996). QTL names were designated using standard QTL nomenclature starting with Q representing QTL, followed by the abbreviations of the trait name (RL for rolled leaf) and the institute name (Hwwg for Hard Winter Wheat Genetic Research Unit), then by chromosome (or arm) location where the QTL is located after a dash line. QTLs that mapped at the same or overlapping location were considered as the same QTL and a QTL that was significant in multiple experiments was considered as a stable QTL.

2.3 Results

2.3.1 Rolled leaf scores in parents and RILs

The BLUE of RL score across all five environments was 4.77 for JagMut1095, and 1.05 for Jagger. The differences in RL were significant ($P < 0.0001$, Fig. 2.1) between the parents and among the RILs of JagMut1095 x Jagger across the five experiments. The frequency of RL scores for the RIL population did not follow a normal distribution (Fig. 2.2; Shapiro-Wilk test $P < 0.0001$). The mean RL score over the five experiments was 2.88, ranging from 1.07 to 5.00 and the coefficient of variation (CV) was around 27.5%. High positive correlations (0.661 - 0.836; P

< 0.001) were observed for RL scores between the five experiments (Table 2.1). The broad sense heritability for RL was high, ranging from 0.73 in 2020-RockyFord to 0.92 in 2020-Hays. The effects of genotypes, environments, and genotype-by-environment interaction on RL scores were highly significant ($P < 0.0001$) (Table 2.2). The RL significantly correlated with HD ($r = 0.41$; $P < 0.001$), but the correlations between RL and PH were not significant across the five environments ($P > 0.05$).

2.3.2 SNP genotyping and linkage map construction

Sequencing the GBS libraries of 154 RILs and their parents identified 66,081 SNPs, with 23,206 SNPs in the A genome, 29,535 SNPs in the B genome and 13,340 SNPs in the D genome. Chromosome 1B had the most markers (5,306), and chromosome 4D had the least (1,450). After removal of SNPs with >20% missing data, >10% heterozygotes or < 20% minor allele frequency, 3,703 high quality SNPs were used for construction of a genetic map. After binning the SNPs that mapped at the same location, only 1,003 unique SNPs were mapped to 22 linkage groups corresponding to the 21 chromosomes with a total map length of 3,106 cM and a marker density of 3.1 cM per marker (Fig. 2.3).

2.3.3 QTL analysis and KASP marker development

QTL analysis using CIM module identified two stable QTLs on chromosomes 1A and 5A, designated as *QRI.hwwg-1AS* and *QRI.hwwg-5AL*. These two QTLs were consistently significant in multiple environments with both RL alleles derived from JagMut1095. *QRI.hwwg-1AS* was mapped to the distal end of chromosome arm 1AS between *Exon1A-35* (3,442,958 bp) and *Exon1A-45* (11,012,144 bp), and showed the largest effect that explained 24% to 56% of the phenotypic variation (Table 2.3, Fig. 2.4 b). *QRI.hwwg-5AL* was mapped near the centromere of 5AL between 228,517,222 and 456,003,747 bp in one environment and between 441,373,760

and 491,620,424 bp in other four environments and for BLUE, which explained up to 20% of the phenotypic variation (Table 2.3, Fig 2.4 a). As RL and HD showed a significant positive correlation in the field experiments (0.41; $P < 0.001$), QTL analysis for HD was conducted to check possible overlapped QTLs between RL and HD in these two intervals (Table 2.3). As the result, one HD QTL was detected on chromosome 1A in environment 2020-Ashland and BLUE (LOD = 5.11 - 6.98) with the later heading allele from JagMut1095. This QTL overlapped with *QRL.hwwg-1AS* for RL. In addition, four other HD QTLs were detected on chromosomes 2B, 5B, 5D and 7B with all the later heading alleles from JagMut1095 except 5D QTL from Jagger. Among these HD QTLs, the 2B QTL between 64,462,647 and 75,415,490 bp explained the largest phenotypic variation (PVE).

To increase SNP density in the *QRL.hwwg-1AS* region, additional KASP markers were designed using the flanking sequence of the SNPs identified within or close to the QTL region from exome capture assays. Among the 47 pairs of primers screened, 17 were polymorphic between the parents. Among these polymorphic markers, eight clearly segregated among the RILs (Table 2.4, Fig. 2.5), therefore were added into the chromosome 1A linkage map for QTL analysis. The physical locations of the eight KASP markers range from 3,381,332 to 12,956,070 bp based on the IWGSC RefSeq v2.1 genome. After removal of markers that mapped to the same position, five markers, *Exon1A-17*, *Exon1A-25*, *Exon1A-28*, *Exon1A-35* and *Exon1A-45*, remained in the genetic map. The order of these markers in the genetic map was consistent with their physical positions in the IWGSC RefSeq v2.1 genome (Fig. 2.4 c).

2.3.4 Candidate interval of *QRL.hwwg-1AS*

To further narrow down the candidate interval of *QRL.hwwg-1AS*, one F₄ heterogeneous inbred family (HIF) was identified from JagMut1095 x Jagger population and used to develop

recombinants within the QTL region. One pair of near-isogenic lines (NILs), L73-8-3 and L73-8-4, with a recombination within the QTL region was identified and showed significant contrast in the RL levels (5 and 2, respectively) in all the three replications in year 2022-RockyFord (Fig. 2.4 b and 2.4 c). This moved the right border of the candidate QTL interval to left side of *Exon1A-25* (9,482,836 bp) based on IWGSC RefSeq v2.1. Two RILs (RIL-74 and RIL-76) each showed a recombination at the right site of the marker *Exon1A-35* (Fig. 2.4 c) and phenotypic data of the two RILs defined the *Exon1A-35* as the left flanking of *QRL.hwwg-1AS*. Therefore, *QRL.hwwg-1AS* is delimited in a 6 Mb interval between *Exon1A-35* (3,442,958 Mb) and *Exon1A-25* (9,482,836 bp).

2.4 Discussion

RL has been considered as an important morphological trait involved in drought stress response and has been shown to have either monogenic (Singh and Mackill, 2008) or polygenic inheritance (Price et al. 1997). Although studies on RL have been reported in different crop species (Xu et al. 2018; Gao et al. 2019; Myśków et al. 2018), only a few studies on QTL mapping for RL have been conducted in wheat. Verma et al. (2020) reported 12 QTLs for RL on wheat chromosomes 1B, 2A, 2B, 2D, 3A, 4A, 4B, 5D, and 6B (Verma et al. 2020); and Peleg et al. (2009) found 14 RL QTLs on chromosomes 1A, 2A, 2B (3), 3B, 4B, 5A, 5B, 6A, 6B, 7A, 7B (2) of tetraploid wheat (*T. turgidum* ssp. *Dicoccoides*) (Peleg et al. 2009). The 1A QTL in tetraploid wheat was mapped near *Xgwm691* between 23.3 and 56.5 cM. The physical location of *Xgwm691* was unavailable, but the SSR marker *Xcfa2158a* at 21.7 Mb was located 7.6 cM distal to *Xgwm691*. Therefore, *Xgwm691* is likely located between 21.7 Mb and centromere on 1AS. However, *QRL.hwwg-1AS* in the current study was mapped to the interval between 3.4 and 9.5 Mb in the distal end of 1AS, thus is far from *Xgwm691*, suggesting that *QRL.hwwg-1AS* identified

in this study is most likely a new QTL for RL. *QRL.hwwg-5AL* was the second QTL consistently detected in different experiments in this study. Previously, one QTL for RL was mapped near *Xgwm156* between 21 and 79.6 cM on 5AL of a tetraploid wheat (Peleg et al. 2009). *Xgwm156* is located at 450.4 Mb in IWGSC RefSeq v2.1 (IWGSC, 2018), which is within the *QRL.hwwg-5AL* interval (441.4 and 491.6 Mb). Therefore, it is possible that *QRL.hwwg-5AL* is the same QTL as the one reported by Peleg et al. (2009).

As *QRL.hwwg-1AS* explained the largest portion of the phenotypic variation, it was chosen for initial fine mapping. Phenotypic and genotypic analyses of RILs and the RIL-derived HIFs delimited the *QRL.hwwg-1AS* to a 6.04 Mb interval between the markers *Exon1A-35* (3,442,958 bp) and *Exon1A-25* (9,482,836 bp). Within this interval, 115 high confidence genes were annotated in Chinese Spring reference genome (IWGSC, 2018), including *TraesCS1A03G0023500* and *TraesCS1A03G0027900* that are orthologs of cloned RL genes in rice. *TraesCS1A03G0023500* was annotated as a 2OG-Fe (II) oxygenase family protein, a gene homolog of *RL14* cloned in rice (Fang et al. 2012). *RL14* was found to modulate rice RL by affecting secondary cell wall formation and inducing shrinkage and abnormal shape of bulliform cells. *TraesCS1A03G0027900* was annotated as a cellulose synthase-like protein, and its ortholog *NRL1* in rice encoded a cellulose synthase-like protein (*OsCslD4*) that induces a reduced leaf width and semi-rolled leaf by modulating the development of the vascular tissue and the cell wall biosynthesis (Hu et al. 2010). The current *QRL.hwwg-1AS* interval including 115 annotated genes remains too large for determining the causal candidate gene for the QTL and further fine mapping is needed to clone the gene. However, since the homologs of *TraesCS1A03G0023500* and *TraesCS1A03G0027900* condition RL trait in rice, functional

characterization of the two wheat genes may facilitate understanding of mechanisms of leaf rolling in wheat.

Reports on the effects of RL on drought tolerance remain controversial to date. Many studies demonstrated that RL reduced the leaf area under solar radiation or reduced the transpiration rates through the creation of a microclimate near the leaf surfaces, therefore benefited plants under drought stress (Turner and Begg 1981; Oppenheimer 1960). RL was reported to reduce the leaf surface area by 41 to 65% in wheat (Clarke 1986) and *Ctenanthe setosa* (Turgut and Kadioglu 1998). In rice, RL lost only 18% of initial fresh weight as compared to 38% loss in unrolled leaves after three hours dehydration (Singh and Singh 2000). However, some other studies suggested that RL may not be a morphological trait for drought tolerance because RL reduced the effective leaf area for light interception and increased the diffusive resistance to CO₂ to reduce photosynthesis efficiency (Hsiao et al. 1984). Saneoka and Waichi (1996) reported that the degree of RL in drought tolerant cultivars was lower than drought susceptible cultivars under moderate and high-water stress treatments, and the drought tolerant cultivars maintained a higher osmotic adjustment (OA). As drought tolerance is a complicated trait and can be generated by different mechanisms such as cellular OA, water use efficiency (WUE), or morphological changes to reduce plant dehydration (Blum 2005; Cao et al. 2007), breeding for drought tolerance may need to consider multiple mechanisms together including RL.

In this study, a significantly positive correlation ($r = 0.40$) was observed between RL and HD across the environments. As plants with a longer HD may have higher chance to catch drought and heat stresses in grain filling stage in the field, the significant positive correlation between RL and HD suggests the phenology changes can affect environments for plant growth

and development. In the present study, a QTL for HD was detected on 1A in one environment and overlapped with *QRI.hwwg-1AS*. However, *QRI.hwwg-1AS* was significant in all the environments, even at the seedling stage, thus, the two QTLs are unlikely the same QTL of pleiotropic effect. Further characterization of *QRI.hwwg-1AS* may shed a light on the RL mechanism and the interaction between plant phenology and responses to environmental changes.

2.5 Conclusion

In this study, we analyzed the RL trait in a wheat RILs and found two major QTLs (*QRI.hwwg-1AS* and *QRI.hwwg-5AL*) mainly responsible for the RL phenotype In JagMut1095. *QRI.hwwg-5AL* has been mapped in a 50 Mb interval in this study and it is likely the same QTL reported previously. *QRI.hwwg-1AS* mapped to a 6.04 Mb physical interval on 1AS showed the large effect in all five experiments and is a novel QTL identified in this study. Further fine mapping of *QRI.hwwg-1AS* will facilitate map-based cloning and further understanding of mechanisms of wheat drought tolerance.

Figures and tables

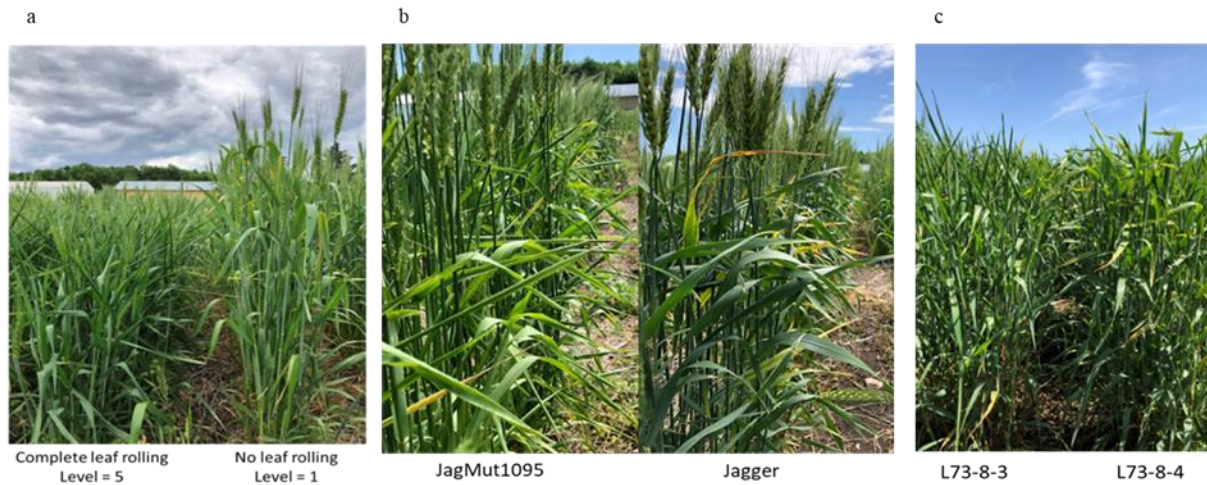


Figure 2.1– Rolled leaf (RL) trait evaluated in field experiments.

a) Segregation of RL trait in the recombinant inbred lines (RILs) of JagMut1095 x Jagger, showing the two RILs with extreme scores of 1 (no rolling, right) and 5 (complete rolling, left); b) Contrast RL trait between the two parents JagMut1095 (left) and Jagger (right); c) Contrast RL trait between two near-isogenic lines L73-8-3 and L73-8-4 evaluated in the 2022 RockyFord field.

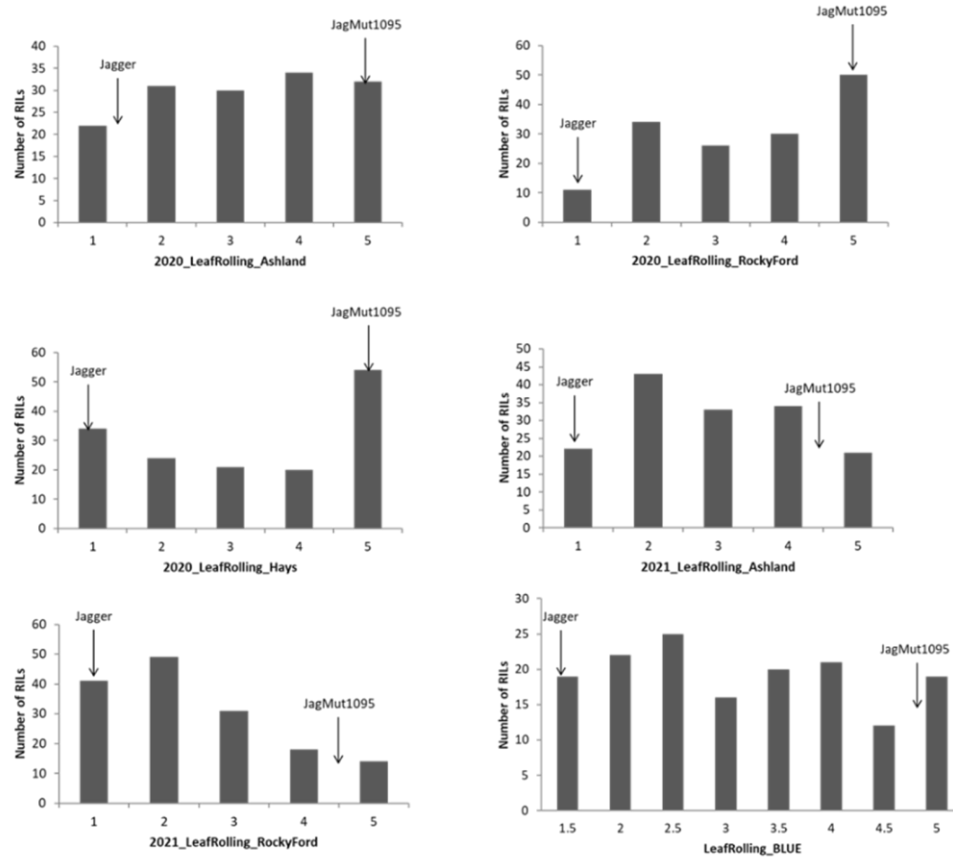


Figure 2.2 – Frequency distribution of rolled leaf scores for the recombinant inbred population of JagMut1095 x Jagger in five experiments and BLUE.

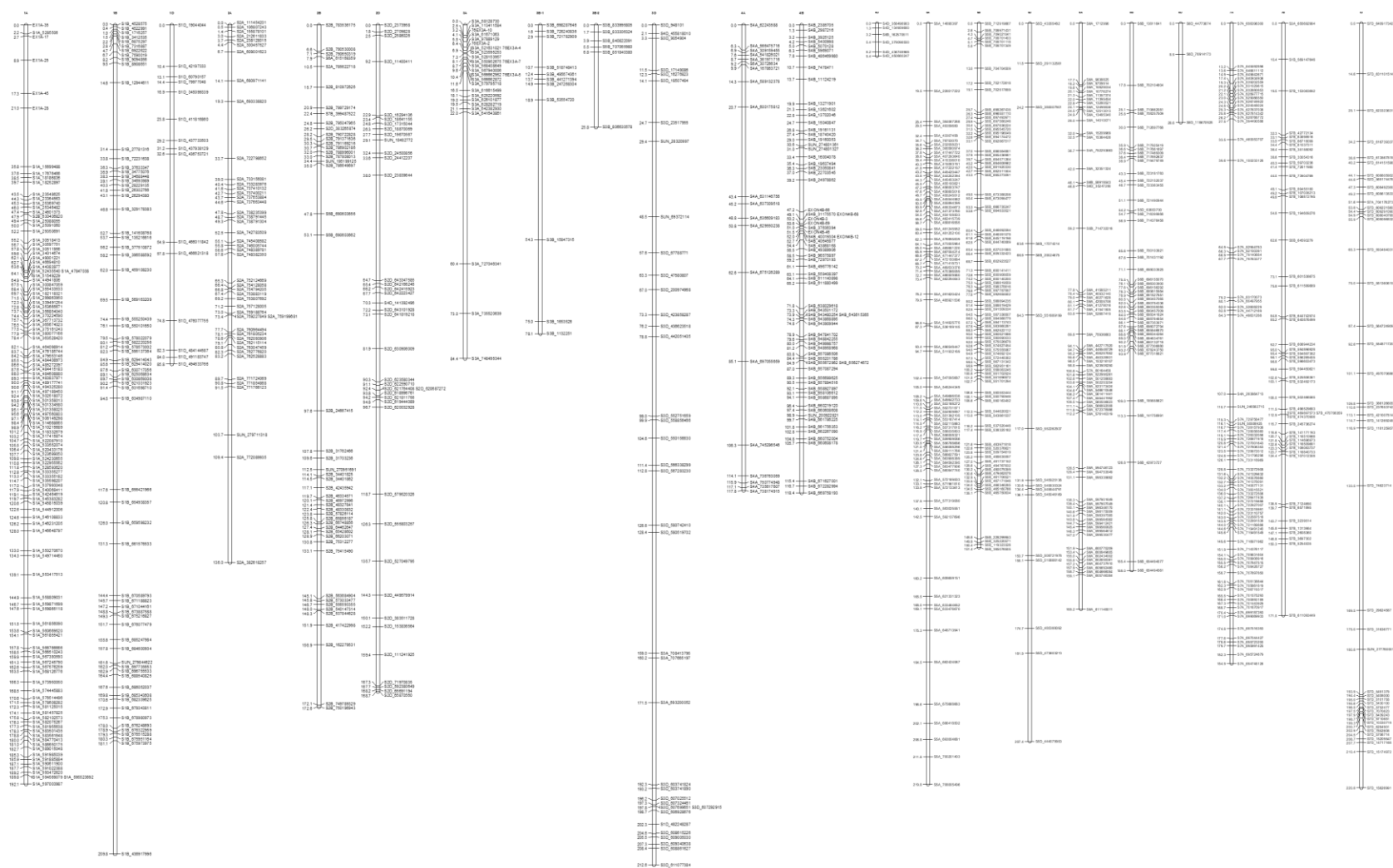


Figure 2.3 – Genetic map constructed using recombinant inbred lines of JagMut1095 x Jagger and single nucleotide polymorphism (SNP) markers.

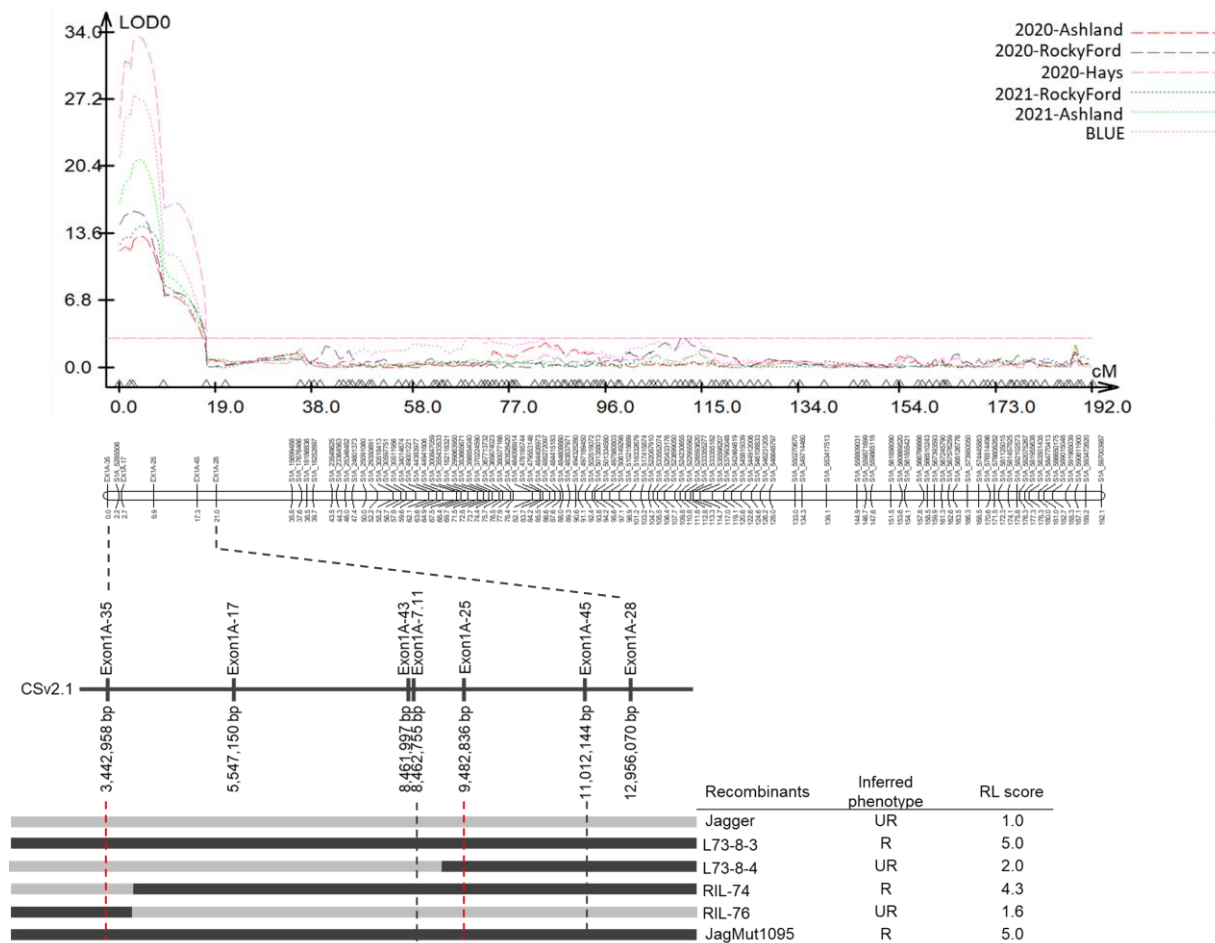


Figure 2.4 – Genetic maps of 5A and 1A and the map locations of *QRI.hwwg-5AL* and *QRI.hwwg-1AS*.

(a) A genetic map of 5A showing *QRI.hwwg-5AL* locations detected in the five field experiments; (b) A genetic map of 1A showing *QRI.hwwg-1AS* locations detected in the five field experiments; (c) a physical map of *QRI.hwwg-1AS* showing newly identified flanking marker positions (red dash lines). Rectangular bars under the physical map represent recombinant lines with a recombination in the QTL region with black bar for JagMut1095 genotype and gray bar for Jagger genotype. The table on the right list the recombinant line name and their phenotypic data.

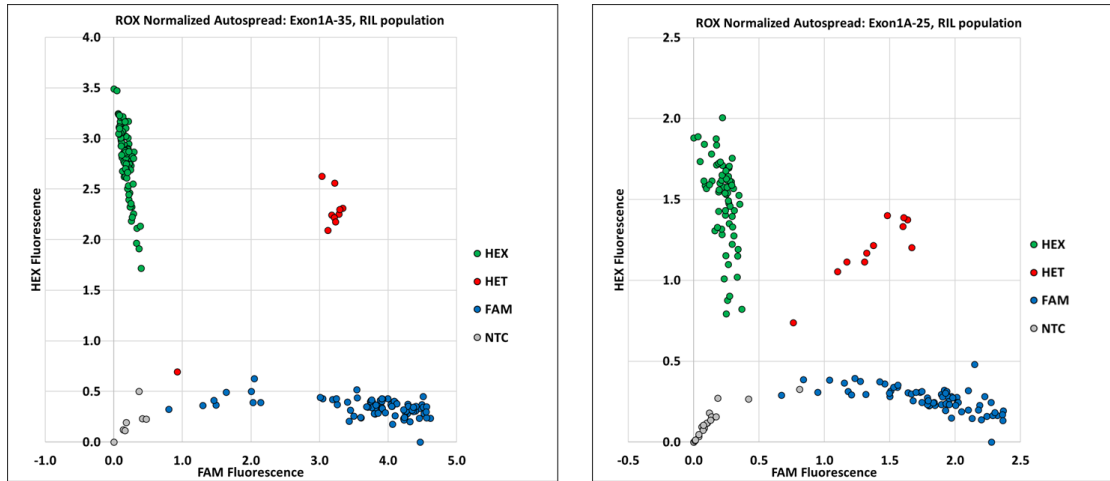


Figure 2.5 – KASP markers segregated in the recombinant inbred lines of JagMut1095 x Jagger.

The X- and Y-axes indicate the FAM and HEX fluorescence, respectively. The green and blue dots represent two parental alleles of each marker, the red dots represent heterozygous genotypes and the grey dots represent non-template controls.

Table 2.1 – The correlation coefficients of rolled leaf scores among the five environments

	2020-Hays	2021-Ashland	2021-Rockyford	2020-Ashland
2021-Ashland	0.836***			
2021-Rockyford	0.704***	0.834***		
2020-Ashland	0.813***	0.799***	0.661***	
2020-Rockyford	0.799***	0.724***	0.692***	0.710***

*Note: *** refers to the highly significant correlation at $p < 0.001$.*

Table 2.2 – The degrees of freedom (*df*) and sums of squares (SS) of genotype, environment and replication effects of rolled leaf trait evaluated over the five environments using analysis of variance

Source	<i>df</i>	SS	F-value	Pr>F
Blocks	5	26.17	9.75	0.00
Genotypes	153	2070.63	25.21	0.00
Environments	4	184.85	86.08	0.00
GxE-interaction	601	505.50	1.57	0.00
Error	694	372.56		
Total	1457	3159.71		

Table 2.3 – The parameters generated from composite interval mapping (CIM) using rolled leaf (RL) scores collected from the RIL population of JagMut1095 x Jagger evaluated in five field experiments

QTL name	Environments	Chromosome	Position(cM)	Peak interval (cM)	LOD	Add.	R ²	Flanking markers	Physical interval in CS RefSeq (v2.1)
<i>QRI.hwwg-1AS</i>	2020-Ashland-RL	1A	3.71	2.2-6.7	13.51	0.84	0.36	<i>Exon1A-35-Exon1A-45</i>	3442958-11012144
	2020-RockyFord-RL	1A	2.71	0-5.9	15.92	0.76	0.3	<i>Exon1A-35-Exon1A-45</i>	3442958-11012144
	2020-Hays-RL	1A	3.71	2.8-5.8	33.9	1.24	0.56	<i>Exon1A-35-Exon1A-45</i>	3442958-11012144
	2021-RockyFord-RL	1A	2.71	0-5.8	14.57	0.6	0.24	<i>Exon1A-35-Exon1A-45</i>	3442958-11012144
	2021-Ashland-RL	1A	3.71	2.3-6.0	21.45	0.74	0.29	<i>Exon1A-35-Exon1A-45</i>	3442958-11012144
	BLUE-RL	1A	2.71	2.3-6.0	27.29	0.8	0.44	<i>Exon1A-35-Exon1A-45</i>	3442958-11012144
<i>QRI.hwwg-5AL</i>	2020-RockyFord-RL	5A	65.01	62.3-68.5	5.93	0.31	0.09	<i>S5A_441373760-S5A_491620424</i>	441373760-491620424
	2020-Ashland-RL	5A	64.11	62.3-65.8	5.34	0.4	0.08	<i>S5A_441373760-S5A_491620424</i>	441373760-491620424
	2020-Hays-RL	5A	65.01	63.3-68.0	4.81	0.34	0.05	<i>S5A_441373760-S5A_491620424</i>	441373760-491620424
	2021-RockyFord-RL	5A	41.81	40.8-43.8	11.65	0.56	0.2	<i>S5A_228517222-S5A_456003747</i>	228517222-456003747
	2021-Ashland-RL	5A	64.11	62.3-65.5	11.86	0.51	0.15	<i>S5A_441373760-S5A_491620424</i>	441373760-491620424
	BLUE-RL	5A	64.11	62.9-65.3	10.4	0.37	0.11	<i>S5A_441373760-S5A_491620424</i>	441373760-491620424
<i>QHd.hwwg-1AS</i>	2020-Ashland-HD	1A	5.71	0-14.0	6.98	1	0.17	<i>Exon1A-35-Exon1A-45</i>	3442958-11012144
	BLUE-HD	1A	3.71	2.0-8.9	5.11	0.58	0.05	<i>Exon1A-35-Exon1A-45</i>	3442958-11012144
<i>QHd.hwwg-2BL</i>	2020-RockyFord-HD	2B	125.81	125.7-127.5	11.49	1.4	0.21	<i>S2B_64462647-S2B_75415490</i>	64462647-75415490

	2021-RockyFord-HD	2B	126.31	125.8-128.1	7.71	1.23	0.14	S2B_64462647-S2B_75415490	64462647-75415490
	2021-Ashland-HD	2B	126.31	125.8-127.9	10.02	1.04	0.2	S2B_64462647-S2B_75415490	64462647-75415490
	BLUE-HD	2B	126.31	125.7-127.4	11.41	1.09	0.2	S2B_64462647-S2B_75415490	64462647-75415490
<i>QHd.hwwg-5BL</i>	2021-RockyFord-HD	5B	126.21	125.2-128.0	4.06	0.67	0.04	S5B_696551110-S5B_702517659	696551110-702517659
	2020-Ashland-HD	5B	129.71	128.4-130.1	4.38	0.45	0.03	S5B_696551110-S5B_702172618	696551110-702172618
	BLUE-HD	5B	126.21	122.6-128.7	5.25	0.64	0.07	S5B_697588246-S5B_702517659	697588246-702517659
<i>QHd.hwwg-5DL</i>	2021-RockyFord-HD	5D	182.91	169.7-196.2	5.7	-	0.11	S5D_510666142-S5D_444079953	444079953-510666142
	BLUE-HD	5D	181.91	180.6-183.3	7.39	-	0.06	S5D_510666142-S5D_444079953	444079953-510666142
<i>QHd.hwwg-7BS</i>	2020-RockyFord-HD	7B	119.61	118.8-123.3	5.29	0.86	0.09	S7B_194609276-S7B_73904769	73904769-194609276
	2021-RockyFord-HD	7B	118.61	116.0-122.6	5.95	1.03	0.11	S7B_194609276-S7B_73904769	73904769-194609276
	2021-Ashland-HD	7B	118.61	113.0-120.7	5.16	0.73	0.1	S7B_194609276-S7B_106813195	106813195-194609276
	BLUE-HD	7B	120.61	116.2-123.3	5.06	0.69	0.09	S7B_194609276-S7B_73904769	73904769-194609276

a LOD = log likelihood ratios

b PVE = the phenotypic variation explained by a QTL

c ADD = additive effect in which a positive value indicates increased RL level contributed by JagMut1095

Table 2.4 – A list of primers for polymorphic kompetitive allele specific PCR-based single nucleotide polymorphisms (KASP–SNPs) that were tightly linked to *QRI.hwwg-1AS* and their putative physical locations in Chinese Spring reference V2.1 genomes

SNP markers	Forward primers	Reverse primers	Physical positions in CSv2.1
<i>Exon1A-11</i>	GAAGGTGACCAAGTTCATGCTTGTCAAAATGTATGGATTCTGCTCc GAAGGTCGGAGTCAACGGATTTGTCAAAATGTATGGATTCTGCTCt	GGTGGACTGCATTCATCGTG	3,969,737
<i>Exon1A-17</i>	GAAGGTGACCAAGTTCATGCTCTGCTCTGGGTTTGTGTCTc GAAGGTCGGAGTCAACGGATTCTGCTCTGGGTTTGTGTCTt	TCAGCCTTTCCCACTTACGT	5,547,150
<i>Exon1A-25</i>	GAAGGTGACCAAGTTCATGCTACACTAGAGGTGCTGGTGTTTAc GAAGGTCGGAGTCAACGGATTACACTAGAGGTGCTGGTGTTTAAt	TTGTGTGGGTTGCTAATCGG	9,482,836
<i>Exon1A-26</i>	GAAGGTGACCAAGTTCATGCTGTTTAGCTACATCATCGATGGCc GAAGGTCGGAGTCAACGGATTGTTTAGCTACATCATCGATGGCt	CCCACCTTTTCCTTGAACCG	10,925,579
<i>Exon1A-28</i>	GAAGGTGACCAAGTTCATGCTTCGTCAAAACAAGGCTGTTGTc GAAGGTCGGAGTCAACGGATTTTCGTCAAAACAAGGCTGTTGTt	TGATTGGGAAGGGTGCAGAT	12,956,070
<i>Exon1A-34</i>	GAAGGTGACCAAGTTCATGCTGCTGACCACCCAATGTTGTt GAAGGTCGGAGTCAACGGATTGCTGACCACCCAATGTTGTg	CATGAAGAGCAGGGGAAGAG	3,381,332
<i>Exon1A-35</i>	GAAGGTGACCAAGTTCATGCTGGTGCTCAACCACCTCTACc GAAGGTCGGAGTCAACGGATTGGTGCTCAACCACCTCTACa	TGGGAAGAAACCCTCAGAGA	3,442,958
<i>Exon1A-45</i>	GAAGGTGACCAAGTTCATGCTATGATAGTGATGATGACTTGTTTGa GAAGGTCGGAGTCAACGGATTATGATAGTGATGATGACTTGTTTGg	ACCTTGATTCACAGCAGGAT	11,012,144

Chapter 3 - QTL mapping of Fusarium head blight resistance in the JagMut1095 x Jagger RIL population

3.1 Introduction

Fusarium head blight (FHB), also known as scab, is a devastating disease mainly caused by *Fusarium graminearum* in the U.S. (Bai and Shaner 2004). Severe FHB epidemics have threatened wheat production in most wheat growing areas worldwide during past decades and resulted in significant losses in grain yield and quality (Bai and Shaner 2004; Wilson et al. 2017). Mycotoxins, especially deoxynivalenol (DON), produced by the pathogen can cause human sicknesses, animal feed refusal, weight losses and even death (Pestka 2010; Sobrova et al. 2010). Although application of fungicides, combined with cultural practice such as deep plowing and crop rotation, can reduce FHB damage, no efficient strategy can fully control FHB epidemics (Bai et al. 2018). In addition, application of fungicides increases production cost and environmental contamination, and no-till farming makes it impractical for deep plowing. Therefore, growing FHB resistant cultivars is the most effective strategy to minimize FHB losses (Bai et al. 2018). Many accessions of wheat and its relatives have been reported to show different levels of resistance, but most of the highly resistant materials are landraces from China and Japan, which usually show poor agronomic performance and cannot be directly used in U.S. breeding programs (Jia et al. 2018; Cai et al. 2016; Petersen et al. 2017). Thus, identifying novel sources of FHB resistance is critical to improve diversity and sustainability of FHB resistance in wheat cultivars.

Host FHB resistance can be divided into five types (Types I to V) based on the host responses to pathogen infection: resistance to initial penetration and infection

(Type I) and resistance to FHB symptom spread within a spike after initial infection (Type II) (Schroeder and Chistensen 1963); low DON accumulation (Type III) (Miller, 1985), low Fusarium damaged kernel (FDK) and FHB tolerance (Mesterhazy, 1995). Type I and Type II resistance are widely recognized resistance types. Type II has been extensively studied in wheat because it is more stable and less affected by environmental factors than Type I resistance (Bai and Shaner 2004). Since the first QTL study in 1999 (Bai et al. 1999), more than 500 QTLs have been identified for different types of resistance with about 50 QTLs located in unique chromosome locations, and seven of them have been formally named as *Fhb1* to *Fhb7* (Buerstmayr et al. 2009; Liu et al. 2009, Bai et al, 2018). Recently, *Fhb1* on chromosome 3BS has been cloned (Rawat et al. 2016; Li et al. 2019; Su et al. 2019), which shows a major and stable effect on Type II resistance in different genetic backgrounds across different environments (Bai and Shaner 2004). *Fhb2* was mapped on 6BS and conditions Type II resistance (Waldron et al. 1999; Cuthbert et al. 2007). *Fhb3* from chromosome 7Lr#1 of an alien species '*Leymus racemosus*' was transferred into wheat chromosome 7D (Qi et al. 2008). *Fhb4* and *Fhb5* were mapped on chromosome 4B and 5A, respectively, in a Chinese landrace 'Wangshuibai' (Xue et al. 2010, 2011). *Fhb6* from the perennial grass *Elymus tsukushiensis* was transferred to wheat chromosome 1A (Cainong et al. 2015). *Fhb7* on 7DS from alien species *Thinopyrum ponticum* has been recently cloned (Wang et al. 2020) and shows a major effect on Type II and III resistance.

Morphological and developmental traits such as plant height, ear compactness (Schmolke et al. 2005), flower opening (Gilsinger et al. 2005), or heading date may affect infection by creating conditions for plants to escape the pathogen infection in field (Miedaner et al. 2006; Klahr et al. 2007). Many tall or late headed genotypes

have been reported to have lower FHB disease in field experiments. QTL analysis on FHB resistance and the agronomic traits found that FHB resistance often co-located with major genes regulating plant height or heading date such as *Rht1*, *Rht2* (Draeger et al. 2007; Srinivasachary et al. 2008, 2009; Miedaner and Voss 2008; Holzapfel et al. 2008), *Rht8* and *Ppd-D1* (He et al. 2016a; McCartney et al. 2016). To clarify the effects of various *Rht* genes might have on FHB resistance, Yan et al. (2011) inoculated NILs for Type I resistance and adjusted spikes of both tall and short plants of *Rht* isolines to the same height for disease development. The results showed that FHB resistance between the isolines were similar in most pairs of NILs. Furthermore, they found that some dwarf isolines even showed significantly higher Type II resistance than their taller isolines. These results strongly suggested that, in some cases, the association between plant height and FHB resistance is due to pleiotropy or tightly linked genes and disease escape in other cases. Therefore, further research is needed to elaborate genetic relationship between FHB resistance and plant height. The objectives of this study were to identify QTLs controlling the FHB resistance in the RIL population and characterize the relationship between FHB resistance and plant height using a RIL population from ‘JagMut1095’ x ‘Jagger’.

3.2 Materials and methods

3.2.1 Plant materials

The population of 154 RILs from ‘JagMut1095’ x ‘Jagger’ was described in Chapter 2. Jagger (PI 593688) was moderately FHB susceptible and JagMut1095 was an ethyl methanesulfonate (EMS) induced mutant of Jagger (Rawat et al. 2019), which showed moderate FHB resistance and higher plant height in greenhouse experiments. The RILs and the parental lines were evaluated for Type II FHB resistance with two replications in the greenhouse at Kansas State University in

Manhattan, Kansas in fall 2018 and spring 2020, and three replications in spring and fall 2019.

3.2.2 FHB inoculation and other traits evaluation in greenhouses

A conidial spore suspension of *F. graminearum* was prepared by culturing the *F. graminearum* strain GZ3639 from Kansas in mungbean broth (Bai et al. 2000). The final inoculum concentration was adjusted to about 100,000 conidiospores ml⁻¹ by counting them in a hemocytometer under a microscope (Bai et al. 1999).

Wheat seeds were planted in plastic growing trays filled with Metro-mix 360 soil mix (Hummert International, Topeka, KS) and then were vernalized in a cold room at 4 °C for 50 days. Five seedlings per RIL or parent were transplanted into a plastic pot on greenhouse benches using randomized complete block design (RCBD) with two replications. Plants were inoculated at flowering stage by injecting 10 µl spores suspension (1000 spores/spike) into a floret of a central spikelet in a spike using a syringe (Hamilton, Reno, NV). Five spikes per pot were inoculated and the inoculated spikes were covered with sealed plastic bags to keep the moisture. After 48 h of inoculation, the plastic bags were removed. The greenhouse temperature was set at 24 °C during the day and 22 °C during the night with 14 h of light supplement. Powdery mildew was controlled by burning sulfur for 3 h during the night for 5 d in a closed greenhouse environment when mildew appeared. FHB resistance was evaluated 16 d after inoculation using the percentage of symptomatic spikelets (PSS) in a spike that were calculated by number of infected spikelets / total number of spikelets in a spike. Heading date (HD) was recorded when 50 % of spikes in each pot emerged. Before harvesting, plant height (PH) was measured from the base of the main stem to the top of the spike before harvesting, excluding awns. Spike length (SL) was measured from the base to the top of the main spike excluding awns, and

spikelets number per spike (SNS) was counted for five spikes per line and then take the average of the five as the SNS of each line.

3.2.3 DNA extraction, genotyping, linkage map construction, QTL analysis and marker development

Procedurals for DNA extraction, GBS and exome capture assay, map construction, QTL analysis and nomenclature, and marker conversion and validation were described in Chapter 2. A significant LOD score was generated for each trait (3.3-3.8) by 1000 times of permutation to claim a significant QTL. QTLs at a same or overlapping locations were considered as the same QTL. A repeatable QTL was defined as a QTL that was consistently significant in two or more experiments.

3.3 Results

3.3.1 FHB and agronomic traits in the RIL population

The mean PSS of JagMut1095 was 32% and the mean PSS of Jagger was 69% across four greenhouse environments, showing significant difference in FHB resistance between the two parents ($P < 0.001$, Table 3.1, Fig. 3.1). The mean PSS of RILs over four greenhouse environments was 43% with a wide range of segregation from 9% to 82% (Fig. 3.1). Significant transgressive segregation was observed in all four experiments suggesting that Jagger also contributed resistance QTL in this population. Continuous distribution of mean PSS of the RILs at 16 d after inoculation were observed in the greenhouse experiments (Fig. 3.1). Significant correlations ($r = 0.43 - 0.68$; $P < 0.001$) were observed among the PSS values across the four greenhouse environments (Table 3.2). The broad sense heritability for PSS was high ($H^2 = 0.83$, Table 3.1). The effects of genotype, environment, and genotype by environment interaction were highly significant in ANOVA over four greenhouse

environments ($P < 0.0001$, Table 3.4) while replications within an environment were not significant

The main HD, PH, SL, and SNS over four greenhouse environments showed continuous distributions. The two parents showed significant difference in PH, SNS, SL and HD ($P < 0.0001$, Fig. 3.1). The broad sense heritability was high for the four agronomic traits (0.75-0.89) over the four greenhouse experiments (Table 3.1). A weak, but significant, positive correlation was found between PSS and PH ($r = 0.29$, $P < 0.001$), while a weak but significant negative correlation was observed between PSS and SL ($r = -0.21$, $P < 0.05$) over the four greenhouse experiments (Table 3.3).

3.3.2 QTLs for FHB resistance and agronomic traits

Composite interval mapping identified 12 QTLs for Type II FHB resistance on chromosomes 1B, 2B, 3A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 7A and 7B (Table 3.5). Among them, four QTLs on chromosomes 3A (*QFhb.hwwg-3AS*), 4B (*QFhb.hwwg-4BS*), 5A (*QFhb.hwwg-5AL*) and 7A (*QFhb.hwwg-7AS*) were repeatable in at least two environments. *QFhb.hwwg-4BS* and *QFhb.hwwg-7AS* were both detected in three environments (2018F, 2019S and 2020S) and by the BLUE value, and Jagger contributed resistance alleles at both loci. *QFhb.hwwg-4BS* explained the largest percentage of the phenotypic variation from 7.6% to 32.8% with the LOD score from 3.50 to 16.13 (Fig. 3.2a). *QFhb.hwwg-7AS* had LOD scores from 3.75 to 6.93 and explained 7.2% to 14.0% of the phenotypic variation (Fig. 3.2 b). *QFhb.hwwg-5AL* was repeatable in all environments and BLUE, while *QFhb.hwwg-3AS* was repeatable in three of the four environments (2019S, 2019F and 2020S). These two QTLs had the resistance alleles from the mutant, and explained up to 11.3% and 14.5% of the phenotypic variation, respectively (Fig. 3.2 c and d).

Composite interval mapping also identified QTLs for PH, HD, SL and SNS in the RIL population. Five QTLs for HD were detected on chromosomes 1D, 2B, 3B, 5D and 7B. Among them, *QHd.hwwg-2BS*, *QHd.hwwg-5DL* and *QHd.hwwg-7BS* were repeatable and none of them overlapped with any repeatable FHB QTL. Seven QTLs were detected for PH on chromosomes 1A, 2B, 2D, 4B, 4D, 5A and 7B. Among them, *QPh.hwwg-4BS* was repeatable in all environments and overlapped with the FHB resistance QTL *QFhb.hwwg-4BS*. *QPh.hwwg-2BS* was significant in three environments (2018F, PH-2019S and PH-2020S) and others were detected in only one environment. Seven QTLs for SL were identified on chromosomes 2B, 3B, 4B, 5A, 5B, 6B and 7B. Among them, *QSl.hwwg-5AL* was repeatable in two environments (2019F and 2020S) and located close to *QFhb.hwwg-5AL*. Four QTLs were identified for SNS on chromosomes 2B, 3A, 5D and 7A. Among them, *QSns.hwwg-2BS* and *QSns.hwwg-5DL* were repeatable in three and four environments, respectively, but neither of them overlapped with any repeatable FHB resistance QTL.

3.3.3 Sequence analysis of *Rht1* in two parents

During the QTL analysis, *QFhb.hwwg-4BS* explained the largest phenotypic variation and overlapped with *QPh.hwwg-4BS*. *QPh.hwwg-4BS* explained 22.4 to 42.8% of the phenotypic variation for PH with the LOD score from 12.85 to 27.12, thus, was selected for further fine mapping of Type II resistance and characterization of association between FHB and PH. Because *QFhb.hwwg-4BS* overlaps with the ‘Green Revolution’ semi-dwarf gene *Rht1*, the full length of *Rht1* gene in the two parents was sequenced in an ABI 3730 sequencer using the 4B-specific-*Rht1* primers designed based on the CSv2.1 reference. Sequence comparison between the two parents identified 23 SNPs, and found the *RhtB1-b* dwarf allele in Jagger and the

RhtB1-a allele in the mutant (Peng et al. 1999). Since the contrasting *Rht-B1* alleles between two parents produced contrasting plant height, *Rht1* or a closely linked gene is a likely candidate of *QPh.hwwg-4BS*. To furtherly characterize the relationship between *Rht1* and *QFhb.hwwg-4BS*, near isogenic lines (NILs) contrasting in the alleles of *QFhb.hwwg-4BS* were isolated from residual heterogeneous inbred families (HIFs) using markers for fine mapping to determine if the FHB resistance is contributed by *Rht1*.

3.3.4 KASP markers development and delimiting candidate interval of *QFhb.hwwg-4BS*

To increase the marker density in the *QFhb.hwwg-4BS* interval, sequences flanking the SNPs discovered by an exome capture assay between the two parents were used to develop KASP markers. Eight pairs of KASP markers from exome-capture SNPs separated two parents and RILs very well and were used to delimit the candidate interval of *QFhb.hwwg-4BS* (Table 3.7). Additionally, a pair of KASP marker which was diagnostic between the *RhtB1-a* and *RhtB1-b* alleles was also added to the set of markers. Different HIFs were identified by using the set of nine KASP markers from the F5 RILs to identify new recombination in the QTL region. Six pairs of NILs with different recombination were identified and phenotyped for PSS and PH in the greenhouses. The phenotypic and genotypic data of these NILs delimited the *QFhb.hwwg-4BS* to a 1.5 Mb interval between markers *Exon4B-102* (33.5 Mb) and *Exon4B-83* (35.0 Mb) (Fig. 3.3) and 17 high confidence genes were annotated in this interval (Table 3.8).

3.4 Discussion

Wheat FHB resistance is a polygenic and quantitative trait which is greatly affected by the environment. Although large genetic variations for FHB resistance

have been found in the wheat gene pool, immune germplasm has not been found yet (Bai et al. 2018). Most of the FHB resistance in wheat accessions showed associations with undesired agronomic traits, making them hard to incorporate into breeding programs (Bai and Shaner 2004). To date, *Fhb1* from ‘Sumai 3’ and ‘Ning 7840’ is the best validated stable resistance gene with a major effect on FHB resistance and has been widely utilized in wheat breeding programs worldwide (Waldron et al. 1999; Bai et al. 1999). Thus, searching novel sources of FHB resistance and characterizing their FHB resistance mechanisms are important to enhance FHB resistance in wheat.

Type II host FHB resistance has been extensively studied in wheat, with only *Fhb1* (Rawat et al. 2016; Li et al. 2019; Su et al. 2019) and *Fhb7* (Wang et al. 2020) having been cloned. In this study, four repeatable QTLs were detected for Type II FHB resistance, which were named as *QFhb.hwwg-3AS*, *QFhb.hwwg-4BS*, *QFhb.hwwg-5AL* and *QFhb.hwwg-7AS*. The mutant contributes the resistance alleles at 3AS and 5AL QTLs. Previous studies reported the 3AS QTLs in ‘Huapei 57-2’ (Bourdoncle and Ohm 2003), ‘Wangshuibai’ (Yu et al. 2007), ‘Frontana’ (Steiner et al. 2004) and ‘AC Foremost’ (Yang et al. 2005) for different types of resistance, including Type I, Type II and FDK (Yang et al. 2011). Among these studies, most of the 3AS QTLs were located near marker *Xgwm5* (115.5 Mb) that overlaps with *QFhb.hwwg-3AS* in our study. *QFhb.hwwg-5AL* was stably detected in all the four environments among different environments, and was located between 36.1 cM and 59.2 cM using phenotypic data from all environments. Jiang et al. (2020) detected an FHB resistance QTL *QFhb-5A* from ‘Yangmai 158’, which located around 505 Mb on chromosome 5AL of IWGSC RefSeq v2.1 based on the information of their KASP marker *Excalibur_c6567_743*, which was close to *QFhb.hwwg-5AL* (407,293,640 bp to 481,245,952 bp). *QFhb.hwwg-7AS* had the resistance allele from Jagger and was

significant in three environments. A 7AS resistance QTL was previously reported in ‘Frontana’ and linked to marker *Xgwm233* (11.9 Mb) on 7AS (Mardi et al. 2006). *QFhb.hwwg-7AS* in this study was located at around 52.6 Mb and could be the same QTL as the one in ‘Frontana’.

The QTLs for the agronomic traits on 2BS, *QHd.hwwg-2BS*, *QPh.hwwg-2BS*, *QSl.hwwg-2BS* and *QSns.hwwg-2BS*, were all repeatable in at least two environments and mapped at the same region as *QFhb.hwwg-2BS* which was detected in a single environment (PSS-2019F). This 2BS region harbored heading date gene *Ppd-B1*, which located at 63.4 Mb on chromosome 2BS, thus probably had pleiotropic effects on HD, PH, SL, SNS and FHB resistance. *QSl.hwwg-5AL* was mapped closely to *QFhb.hwwg-5AL* around 450 Mb on chromosome 5AL showing the contrasting additive effects, of which the mutant contributed the alleles for longer SL and lower PSS. Meanwhile, a weak but significant negative correlation (- 0.21) was observed between SL and PSS in the greenhouse, indicating that a longer SL may slow down the FHB spread within a spike. A stable SNS QTL *QSns.hwwg-5DL* was repeatable in all the environments and overlapped with *QHd.hwwg-5DL* for HD between 444 Mb to 485 Mb where a vernalization gene *Vrn-D1* is located (470Mb). For PH, a major QTL *QPh.hwwg-4BS* overlaps with *QFhb.hwwg-4BS*, the major QTL for FHB Type II resistance that explained the largest phenotypic variation (7.6% to 32.8%) in this study. This 4BS QTL locates between markers *EXON4B-68* and *S4B_37636394* where the ‘Green Revolution’ gene *Rht1* is located.

Many QTL studies on Type I or Type II FHB resistance have found that the two types of resistance are often associated with HD and PH, such as *Rht8/Ppd-D1* (McCartney et al. 2016), *Rht-B1* (He et al. 2016), *Rht-D1* and *Vrn-A1* (Miedaner and Voss 2008; Xu et al. 2019). Among these studies, the FHB resistance on chromosome

4BS has been reported in several QTL mapping studies of common wheat. Anderson et al. (2001) identified a 4BS FHB resistance QTL in a RIL population derived from ‘Sumai 3’ / ‘Stoa’ with the resistance allele contributed by ‘Stoa’. Eckard et al. (2015) reported a 4BS QTL (*Qfhb.sdsu-4B*) in a ‘Wesley’-*Fhb1* background. In addition to hexaploid wheat, 4BS FHB resistance QTL was also reported in tetraploid wheat, for example, in three backcross populations derived from *Triticum dicoccum* × *Triticum durum*, the QTL was co-located with *Rht1* (Buerstmayr et al. 2012). Furthermore, this 4BS FHB resistance QTL was also reported from Asian sources, such as ‘Wuhan1’ and ‘Wangshuibai’ (Somers et al. 2003; Jia et al. 2005). However, the positive or negative correlation between FHB and PH, especially *Rht1*, was also observed in different studies (Saville et al. 2012; Srinivasachary et al. 2009; He et al. 2016b). Previous studies found that DELLAs encoded by *Rht* genes may differentially promote the susceptibility to biotrophs and resistance to necrotrophs (Navarro et al. 2008). Thus, further characterization of the mechanism underlying the association between FHB resistance and PH to find out whether they are tightly linked or pleiotropic gene is critical for successful use of this QTL in breeding.

The fine mapping work in this study delimited *QFhb.hwwg-4BS* into a 1.5 Mb physical interval where 17 high confidence genes including *Rht1* (*TraesCS4B03G0093100*) were annotated. Among the other 16 genes, *TraesCS4B03G0094500* encodes a Phospholipase A2 that involves in a variety of plant cellular processes including plant growth, development, and responses to stress and defense (Lee et al. 2005); *TraesCS4B03G0094900* is an F-box protein to be involved in plant defense to pathogens (van den Burg et al. 2008; Piisilä et al. 2015; Zhang et al. 2019). *TraesCS4B03G0095000* encoding 3-oxo-5- α -steroid 4-dehydrogenase was found to be down-regulated in cotton bolls and rice after insect

pest infection (Kyndt et al. 2014; Kumar et al. 2016). *TraesCS4B03G0095100* encoding a Peptidylprolyl isomerase involves in pathologic process as some pathogens can use host PPIases for their own protein modification as an essential step for the pathogen multiplication (Krominaa et al. 2008). *TraesCS4B03G0095500* encoding a Cinnamate 4-hydroxylase contributes positively to plant defense via increasing lignin content in soybean (Yan et al. 2019). *TraesCS4B03G0095900* encoding an ATP-dependent RNA helicase responds to biotic and abiotic stresses in rice and wheat (Li et al. 2008; Zhang et al. 2014). These seven genes in the 1.5 Mb of *QFhb.hwwg-4BS* interval can be candidate genes for *QFhb.hwwg-4BS*.

3.5 conclusion

In this study, 12 QTLs for Type II FHB resistance were identified on chromosomes 1B, 2B, 3A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 7A and 7B. The major QTL *QFhb.hwwg-4BS* explained up to 32.8% of the phenotypic variation and is a major QTL for FHB resistance in the population. *QFhb.hwwg-4BS* overlaps with *QPh.hwwg-4BS* for PH and was mapped in a 1.5 Mb interval harboring 17 high confidence genes including *Rht1*. Screening of recombinants in *QFhb.hwwg-4BS* interval showed high association between *Rht-B1b* (the dwarf allele) and the FHB resistance. Further fine mapping may be able to separate *QFhb.hwwg-4BS* from *Rht1* if they are linked genes. Map-based cloning and molecular characterization of the resistance mechanisms underlying *QFhb.hwwg-4BS* will facilitate determination of the gene identity and effective deployment of the gene in breeding.

Figures and tables

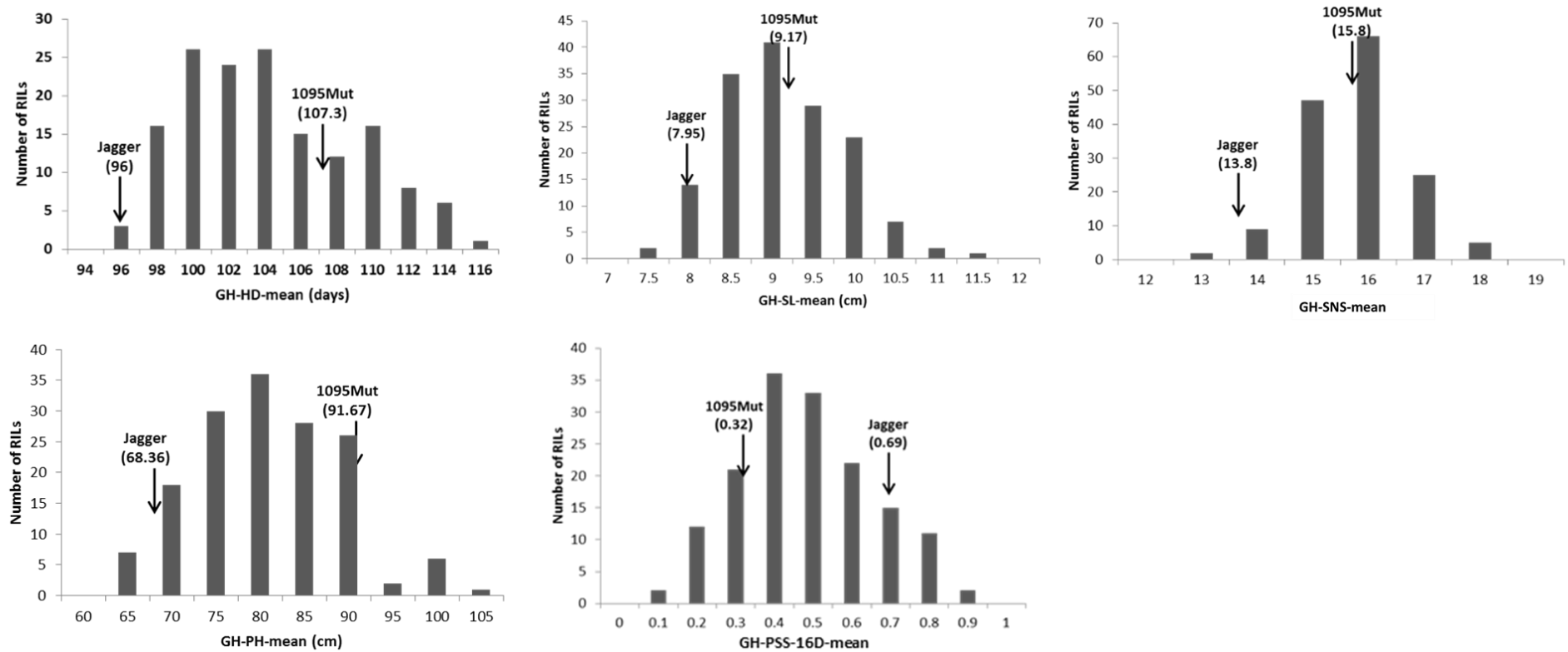
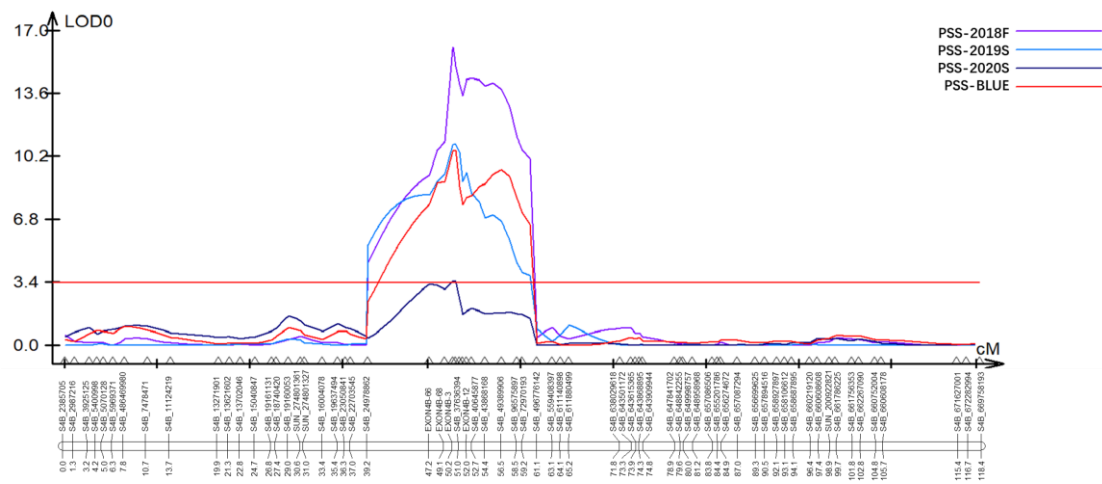
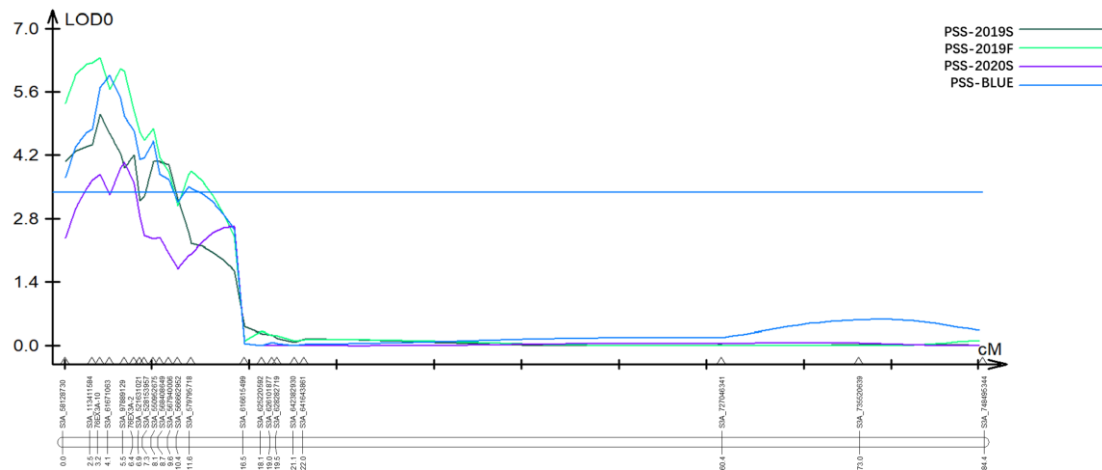


Figure 3.1 – Frequency distribution of mean plant height (PH), spike length (SL), spikelets number per spike (SNS) and heading date (HD) for the recombinant inbred lines (RILs) of JagMut1095 x Jagger evaluated in greenhouse experiments

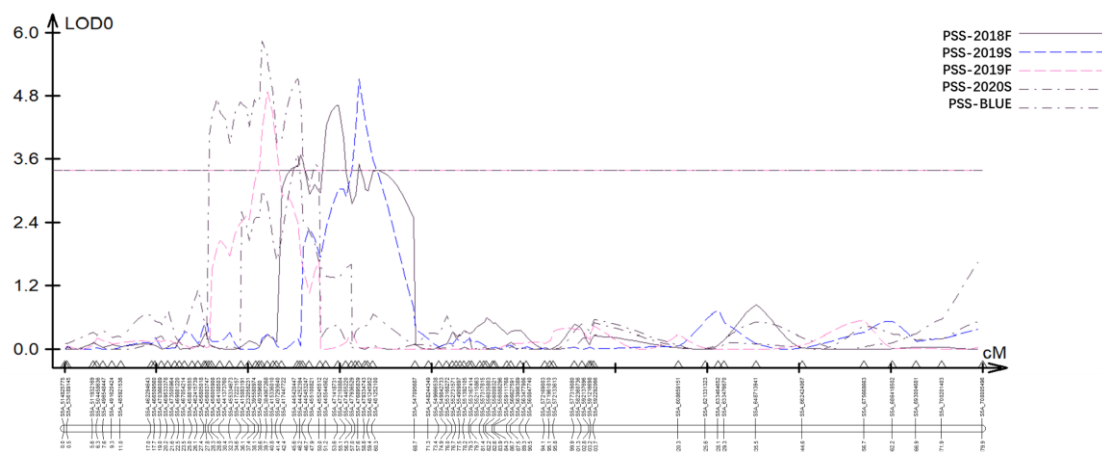
a) Chr4B



b) Chr3A



c) Chr5A



d) Chr7A

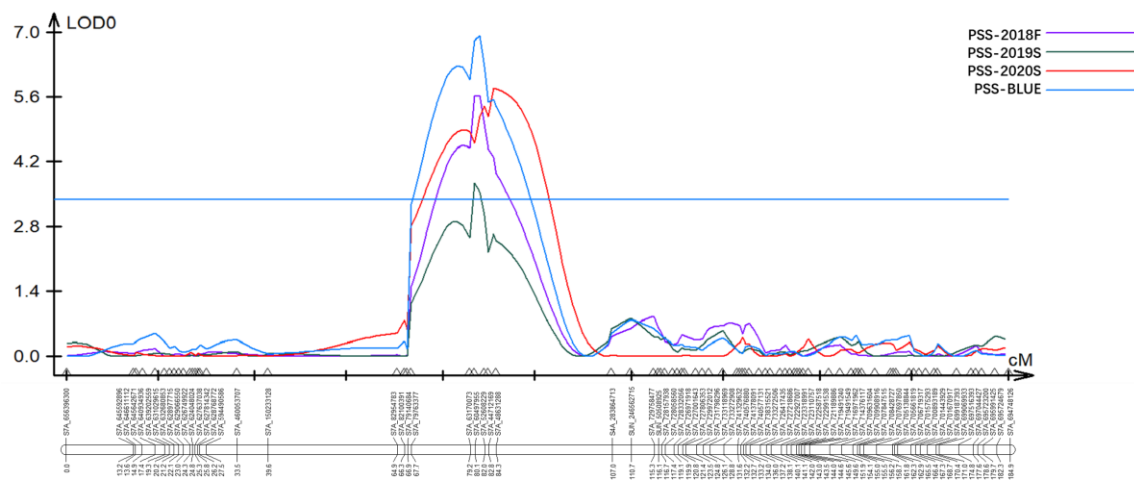


Figure 3.2 – Maps of quantitative trait loci (QTL) for FHB resistance in the recombinant inbred line (RIL) population of JagMut1095/Jagger evaluated in 2018 fall to 2020 spring greenhouse experiments.

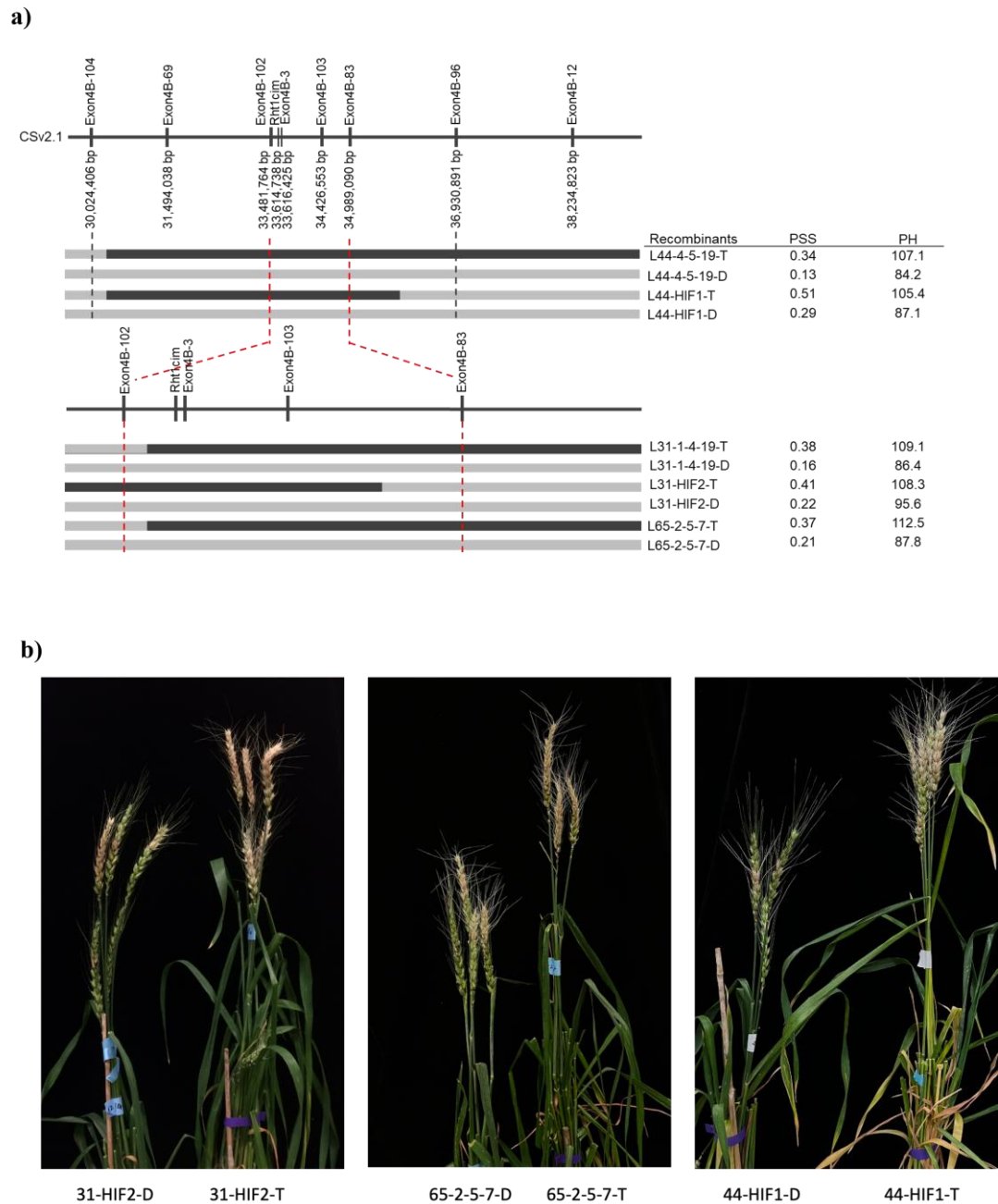


Figure 3.3 – Delimit *QFhb.hwwg-4BS* to a 1.5 Mb physical interval on chromosome arm 4BS of IWGSC RefSeq v2.1.

a) A physical map of *QFhb.hwwg-4BS* candidate interval; b) Pictures showing the phenotypes of paired near isogenic lines (NILs) derived from different heterogenous inbred families (HIFs). The black and gray bars represent susceptible and resistant genotypes, respectively. D refers to the dwarf allele of *Rht1* and T refers to the tall allele of *Rht1*.

Table 3.1 – Phenotypic variance and broad-sense heritability in JagMut1095/Jagger 154 RILs for Fusarium head blight and agronomic traits evaluated in greenhouse environments

TraitName	Mean	StdError	Minimum	Maximum	Jagger	1095Mut	Heritability
GH-PSS-2018F	0.28	0.16	0.05	0.67	0.69	0.32	0.83
GH-PSS-2019S	0.4	0.23	0.06	1.00			
GH-PSS-20219F	0.75	0.2	0.20	1.00			
GH-PSS-20220S	0.68	0.25	0.06	1.00			
GH-PH-2018F	86.2	12.7	62	118.3	68.4	91.7	0.89
GH-PH-2019S	84.6	9.7	63.5	110.0			
GH-PH-2019F	69.5	7.2	51.8	89.6			
GH-PH-2020S	75.6	7.7	54.9	96.4			
GH-SNS-2018F	16.7	1.7	12.0	20.9	13.8	15.8	0.80
GH-SNS-2019S	16.9	1.2	13.9	20.3			
GH-SNS-2019F	12.9	1.1	9.9	16.0			
GH-SNS-2020S	15.3	1.1	12.3	18.1			
GH-SL-2018F	9.19	1.48	3.20	13.55	7.95	9.17	0.75
GH-SL-2019S	9.53	0.90	7.77	12.60			
GH-SL-2019F	8.28	0.83	6.67	11.00			
GH-SL-2020S	8.40	0.92	6.00	11.00			
GH-HD-2018F	115.8	14.0	51.0	134.5	96.0	107.3	0.86
GH-HD-2019S	101.1	6.1	91.7	116.3			
GH-HD-2019F	99.8	2.9	94.7	107.7			
GH-HD-2020S	97.0	4.1	89.0	110.0			

Note: The units of PH and SL are cm; the unit of HD is days. GH refers to greenhouse; PSS refers to percentages of symptomatic spikelets in a spike; PH refers to plant height; SL refers to spike length; HD refers to heading date.

Table 3.2 – The correlation coefficients among PSS from the four greenhouse experiments

	GH-2018F	GH-2019S	GH-2019F
GH-2019S	0.695***		
GH-2019F	0.435***	0.511***	
GH-2020S	0.461***	0.550***	0.572***

Note: * Significant at $p < 0.05$ level, *** significant at $p < 0.001$ level. GH refers to greenhouse; F refers to fall and S refers to spring.

Table 3.3 – The correlation among agronomic traits and PSS over four greenhouse experiments

	HD	PH	SL	SNS
PH	0.377***			
SL	0.462***	0.426***		
SNS	0.748***	0.529***	0.540***	
PSS	-0.026	0.290***	-0.207*	-0.004

*Note: * Significant at $p < 0.05$ level, *** significant at $p < 0.001$ level. PSS refers to percentages of symptomatic spikelets in a spike; PH refers to plant height; SL refers to spike length; HD refers to heading date; SNS refers to spikelets number per spike.*

Table 3.4 – Analysis of variance of genotype, environment and replication effects for FHB over the four greenhouse environments. The degrees of freedom (df) and mean squares (MS) are shown for each analysis

Source	DF	SS	F-value	Pr>F
Replications (in environment)	6	0.36	1.48	0.18
Genotype	153	47.84	7.62	0.00
Environment	3	20.60	167.38	0.00
GxE interaction	441	25.00	1.38	0.00
Error	790	32.41		
Total	1393	126.20		

Table 3.5 – QTLs responsible for FHB resistance in the RIL population derived from 1095Mut/Jagger evaluated in the greenhouses in Manhattan, KS from fall 2018 to spring 2020.

Physical positions in CSv2.1	SNP markers	Primer sequence
30,024,406	<i>Exon4B-104_F</i>	GAAGGTGACCAAGTTCATGCTGTAGTAAATGGCCAGGTA ^T Cg
	<i>Exon4B-104_H</i>	GAAGGTCGGAGTCAACGGATTGTAGTAAATGGCCAGGTA ^T Ca
	<i>Exon4B-104_R</i>	GTGAGGCTGCTGGTGTCT
30,416,547	<i>EXON4B-66_F</i>	GAAGGTGACCAAGTTCATGCTTTGGTGTAGATCAATGATAGATAGTAATAG ^c
	<i>EXON4B-66_H</i>	GAAGGTCGGAGTCAACGGATTTTGGTGTAGATCAATGATAGATAGTAATAG ^t
	<i>EXON4B-66_R</i>	TCATCGAGAAGTGTTCCCTGA
31,175,570	<i>EXON4B-68_F</i>	GAAGGTGACCAAGTTCATGCTGACAGTATTGATGAAACTGTTGTTG ^g
	<i>EXON4B-68_H</i>	GAAGGTCGGAGTCAACGGATTGACAGTATTGATGAAACTGTTGTTG ^t
	<i>EXON4B-68_R</i>	AGTTTGCTACCTCAACCGACT
31,494,038	<i>EXON4B-69_F</i>	GAAGGTGACCAAGTTCATGCTGCTAGGGTTGCTGAAAAGG ^c
	<i>EXON4B-69_H</i>	GAAGGTCGGAGTCAACGGATTGCTAGGGTTGCTGAAAAGG ^t
	<i>EXON4B-69_R</i>	TGGTAAGGGTTCATGTGTCA
33,481,764	<i>Exon4B-102_F</i>	GAAGGTGACCAAGTTCATGCTCGTGATTGGGAGAGTGGTCTT ^g
	<i>Exon4B-102_H</i>	GAAGGTCGGAGTCAACGGATTCTGTGATTGGGAGAGTGGTCTT ^a
	<i>Exon4B-102_R</i>	CCTCTCTTGTTTCATGGTCTTCC
33,614,738	<i>Rht1_cim-KASP_F</i>	GAAGGTGACCAAGTTCATGCTCCCATGGCCATCTCSAGCT ^g
	<i>Rht1_cim-KASP_H</i>	GAAGGTCGGAGTCAACGGATTCCCATGGCCATCTCSAGCT ^a
	<i>Rht1_cim-KASP_R</i>	TCCGGTACAAGGTGCGGGCG
34,426,553	<i>Exon4B-103_F</i>	GAAGGTGACCAAGTTCATGCTATAGCCCAATTCCGGTGGT ^g
	<i>Exon4B-103_H</i>	GAAGGTCGGAGTCAACGGATTATAGCCCAATTCCGGTGGT ^a
	<i>Exon4B-103_R</i>	AGACACTTCACACGCCCTC
34,989,090	<i>Exon4B-83_F</i>	GAAGGTGACCAAGTTCATGCTGCGGAACTGCTTCCCGTT ^g
	<i>Exon4B-83_H</i>	GAAGGTCGGAGTCAACGGATTGCGGAACTGCTTCCCGTT ^t
	<i>Exon4B-83_R</i>	CCCTACTACGTCGGCGAC
36,930,891	<i>Exon4B-96_F</i>	GAAGGTGACCAAGTTCATGCTGGCACTATCGCTCCACTGT ^a

38,234,823	<i>Exon4B-96_H</i>	GAAGGTCGGAGTCAACGGATTGGCACTATCGCTCCACTGTc
	<i>Exon4B-96_R</i>	TTCCAGCGCTGTAGTCTTGT
	<i>EXON4B-12_F</i>	GAAGGTGACCAAGTTCATGCTACCGACGGCTCCCTCTCa
	<i>EXON4B-12_H</i>	GAAGGTCGGAGTCAACGGATTACCGACGGCTCCCTCTCg
	<i>EXON4B-12_R</i>	CCTTACGACAGCGTCACAG

Note: PSS refers to percentages of symptomatic spikelets in a spike. F refers to fall and S refers to spring. Contributor refers to the parent which carried the resistance allele.

Table 3.6 – QTLs for agronomic traits detected in the RIL population derived from JagMut1095/Jagger evaluated in the greenhouses in Manhattan, KS

	Trait	Chr	Position(cM)	LOD	Add	R2	Contributor	Peak interval	Flanking markers
<i>QHd.hwwg-1DL</i>	HD-2020S	1D	81.81	4.25	1.210	0.069	mutant	74.9-84	S1D_476077755-S1D_491183747
<i>QHd.hwwg-2BS</i>	HD-2018F	2B	126.31	58.05	8.377	0.791	mutant	125.8-127.6	S2B_65856187-S2B_65428602
	HD-2019F	2B	125.81	6.80	1.025	0.118	mutant	122.4-127.5	S2B_48327841-S2B_65428602
	HD-2019S	2B	126.31	30.50	4.390	0.502	mutant	126.2-127.3	S2B_65856187-S2B_64462647
	HD-2020S	2B	123.41	8.98	1.812	0.152	mutant	122.4-128.1	S2B_57826114-S2B_66203071
<i>QHd.hwwg-3B</i>	HD-2019F	3B-1	9.91	7.30	1.443	0.170	mutant	4-14.9	S3B_721782909-S3B_247268304
<i>QHd.hwwg-5DL</i>	HD-2019F	5D	180.71	11.20	-1.447	0.239	Jagger	175.8-181.9	S5D_485388092-S5D_473603213
	HD-2019S	5D	177.71	9.40	-2.155	0.124	Jagger	169.5-181.2	S5D_510666142-S5D_473603213
	HD-2020S	5D	184.91	12.83	-2.245	0.295	Jagger	178-193.6	S5D_485388092-S5D_444079953
<i>QHd.hwwg-7BS</i>	HD-2018F	7B	122.61	4.28	1.378	0.023	mutant	120.7-125.5	S7B_141171193-S7B_107012308
	HD-2019S	7B	121.71	6.96	2.294	0.083	mutant	120.2-123.1	S7B_141171193-S7B_108083737
<i>QPh.hwwg-1AL</i>	PH-2018F	1A	90.01	5.04	3.018	0.055	mutant	87.6-92	S1A_485272097-S1A_502518072
<i>QPh.hwwg-2BS</i>	PH-2018F	2B	125.81	7.82	3.728	0.081	mutant	122.4-127.5	S2B_48330832-S2B_65428602
	PH-2019S	2B	127.51	5.69	2.646	0.069	mutant	122.4-130.8	S2B_48330832-S2B_75312277
	PH-2020S	2B	127.51	5.40	4.158	0.089	mutant	125.3-128.1	S2B_57826114-S2B_66203071
	PH-BLUE	2B	125.81	5.58	2.127	0.063	mutant	122.4-128.1	S2B_48330832-S2B_66203071
<i>QPh.hwwg-2DL</i>	PH-2019S	2D	91.11	4.08	-2.933	0.074	Jagger	81.9-93.8	S2D_630906009-S2D_618996761
<i>QPh.hwwg-4BS</i>	PH-2018F	4B	50.31	27.12	8.494	0.428	mutant	49.9-50.9	EXON4B-68-S4B_37636394
	PH-2019F	4B	50.31	17.20	4.423	0.358	mutant	49.6-50.9	EXON4B-68-S4B_37636394
	PH-2019S	4B	50.31	23.88	6.271	0.377	mutant	49.9-50.9	EXON4B-68-S4B_37636394

	PH-2020S	4B	50.31	12.85	3.824	0.224	mutant	49.5-51.5	EXON4B-68-EXON4B-12
	PH-BLUE	4B	50.31	26.73	5.505	0.416	mutant	49.9-51	EXON4B-68-S4B_37636394
<i>QPh.hwwg-4DL</i>	PH-2020S	4D	8.21	5.24	-2.560	0.090	Jagger	5.6-9.2	S4D_379096550-S4D_450880247
<i>QPh.hwwg-5AL</i>	PH-2020S	5A	72.31	6.97	2.711	0.120	mutant	68.7-75.5	S5A_547095687-S5A_553168272
	PH-BLUE	5A	73.31	5.21	2.037	0.060	mutant	70.1-74.7	S5A_547095687-S5A_549842733
<i>QPh.hwwg-7BS</i>	PH-2020S	7B	121.81	3.43	1.819	0.052	mutant	115.7-130.8	S7B_245736274-S7B_7124690
<i>QSl.hwwg-2BS</i>	SL-2018F	2B	128.91	13.06	0.930	0.299	mutant	127.5-130.2	S2B_65428602-S2B_75312277
	SL-2019F	2B	122.41	6.33	0.354	0.129	mutant	119.6-125.2	S2B_46334571-S2B_65856187
	SL-2019S	2B	121.41	11.02	0.515	0.232	mutant	120.1-123.8	S2B_46972996-S2B_57826114
	SL-2020S	2B	128.11	7.88	0.353	0.144	mutant	126.3-129.9	S2B_66748856-S2B_75312277
	SL-BLUE	2B	128.11	14.71	0.388	0.271	mutant	127.2-129.4	S2B_66748856-S2B_75312277
<i>QSl.hwwg-3B</i>	SL-2018F	3B-1	49.91	5.24	-0.699	0.171	Jagger	32.2-64.2	S3B_53554720-S3B_1653526
<i>QSl.hwwg-4BS</i>	SL-2019F	4B	50.31	4.01	0.246	0.081	mutant	47.2-51.5	EXON4B-66-EXON4B-12
<i>QSl.hwwg-5AL</i>	SL-2019F	5A	38.11	5.89	0.342	0.121	mutant	36.1-40.8	S5A_415083191-S5A_415336010
	SL-2020S	5A	36.11	7.84	0.361	0.145	mutant	33.5-38.5	S5A_455334673-S5A_49359680
	SL-BLUE	5A	37.51	5.49	0.225	0.088	mutant	34.2-39.7	S5A_455334673-S5A_415336010
<i>QSl.hwwg-5BL</i>	SL-2020S	5B	148.81	4.00	-0.248	0.070	Jagger	136.8-149.9	S5B_465750834-S5B_325220571
<i>QSl.hwwg-6B</i>	SL-2019S	6B	2.01	4.43	-0.395	0.124	Jagger	0-14.2	S6B_13811641-S6B_702104604
<i>QSl.hwwg-7BS</i>	SL-2019S	7B	52.81	4.77	0.495	0.123	mutant	48.1-57.8	S7B_89455180-S5B_64593279
<i>QSns.hwwg-2BS</i>	SN-2018F	2B	121.41	20.17	1.019	0.342	mutant	120.3-123.4	S2B_46972996-S2B_57826114
	SN-2019S	2B	119.71	15.82	0.677	0.334	mutant	117.9-120.1	S2B_42435942-S2B_46972996
	SN-2020S	2B	125.81	12.00	0.577	0.252	mutant	123.8-126.3	S2B_57826114-S2B_66748856
	SN-BLUE	2B	121.41	14.73	0.479	0.240	mutant	121.3-123.4	S2B_48327841-S2B_57826114

<i>QSns.hwwg-3AS</i>	SN-2020S	3A	0.01	3.51	0.302	0.070	mutant	0-4.1	S3A_58128730-S3A_61671063
	SN-2018F	5D	186.91	6.76	-0.615	0.135	Jagger	176.2-198.6	S5D_485388092-S5D_444079953
	SN-2019F	5D	181.71	15.13	-0.757	0.333	Jagger	176.7-189.9	S5D_485388092-S5D_444079953
<i>QSns.hwwg-5DL</i>	SN-2019S	5D	180.71	6.10	-0.489	0.146	Jagger	176-195.7	S5D_485388092-S5D_444079953
	SN-2020S	5D	162.11	5.71	-0.510	0.192	Jagger	154-170.4	S5D_508721978-S5D_485388092
	SN-BLUE	5D	186.91	11.77	-0.467	0.244	Jagger	177.8-197.8	S5D_485388092-S5D_444079953
<i>QSns.hwwg-7AL</i>	SN-2019S	7A	147.71	4.32	-0.402	0.101	Jagger	144-151.7	S7A_721189886-S7A_714376117

Note: PH refers to plant height; SL refers to spike length; HD refers to heading date; SNS refers to spikelets number per spike. F refers to fall and S refers to spring. Contributor refers to the parent which carried the late allele for HD, tall allele for PH, long allele for SL, and more SNS.

Table 3.7 – A list of primers for polymorphic Kompetitive allele specific PCR-based single nucleotide polymorphisms (KASP–SNPs) that were tightly linked to *QFhb.hwwg-4BS* and their putative physical locations in Chinese Spring reference V2.1 genomes

Physical positions in CSv2.1	SNP markers	Forward primers	Reverse primers
30,024,406	<i>Exon4B-104</i>	GAAGGTGACCAAGTTCATGCTGTAGTAAATGGCCAGGTACTCg GAAGGTCGGAGTCAACGGATTGTAGTAAATGGCCAGGTACTCa GAAGGTGACCAAGTTCATGCTTTGGTGTAGATCAATGATAGATAGTAAT AGc	GTGAGGCTGCTGGTGTCT
30,416,547	<i>EXON4B-66</i>	GAAGGTCGGAGTCAACGGATTTTGGTGTAGATCAATGATAGATAGTAAT AGt	TCATCGAGAAGTGTTCTCTGA
31,175,570	<i>EXON4B-68</i>	GAAGGTGACCAAGTTCATGCTGACAGTATTGATGAAACTGTTGTTGg GAAGGTCGGAGTCAACGGATTGACAGTATTGATGAAACTGTTGTTGt	AGTTTGCTACCTCAACCGAC T
31,494,038	<i>EXON4B-69</i>	GAAGGTGACCAAGTTCATGCTGCTAGGGTTGCTGAAAAGGc GAAGGTCGGAGTCAACGGATTGCTAGGGTTGCTGAAAAGGt	TGGTAAGGGTTCATGTGTCA
33,481,764	<i>Exon4B-102</i>	GAAGGTGACCAAGTTCATGCTCGTGATTGGGAGAGTGGTCTTg GAAGGTCGGAGTCAACGGATTTCGTGATTGGGAGAGTGGTCTTa	CCTCTCTTGTTTCATGGTCTTC C
33,614,738	<i>Rht1_cim- KASP</i>	GAAGGTGACCAAGTTCATGCTCCCATGGCCATCTCSAGCTg GAAGGTCGGAGTCAACGGATTCCCATGGCCATCTCSAGCTa	TCGGGTACAAGGTGCGGGC G
34,426,553	<i>Exon4B-103</i>	GAAGGTGACCAAGTTCATGCTATAGCCCAATTCCGGTGGTg GAAGGTCGGAGTCAACGGATTATAGCCCAATTCCGGTGGTa	AGACACTTCACACGCCCTC
34,989,090	<i>Exon4B-83</i>	GAAGGTGACCAAGTTCATGCTGCGGAACTGCTTCCCGTTg GAAGGTCGGAGTCAACGGATTGCGGAACTGCTTCCCGTTt	CCCTACTACGTCGGCGAC
36,930,891	<i>Exon4B-96</i>	GAAGGTGACCAAGTTCATGCTGGCACTATCGCTCCACTGTa GAAGGTCGGAGTCAACGGATTGGCACTATCGCTCCACTGTc	TTCCAGCGCTGTAGTCTTGT

38,234,823

EXON4B-12

GAAGGTGACCAAGTTCATGCTACCGACGGCTCCCTCTCa
GAAGGTCGGAGTCAACGGATTACCGACGGCTCCCTCTCg

CCTTACGACAGCGTCACAG

Table 3.8 – A list of high confidence genes annotated in candidate interval of *QFhb.hwwg-4BS* based on Chinese Spring refseq v2.1

Gene-ID (CSv2.1)	Description	Pfam-IDs
<i>TraesCS4B03G0092800</i>	Drug/metabolite transporter	PF00892
<i>TraesCS4B03G0093100</i>	GAI-like protein 1	PF12041
<i>TraesCS4B03G0094000</i>	Glutamyl-tRNA(Gln) amidotransferase subunit A	PF01425
<i>TraesCS4B03G0094500</i>	Phospholipase A2	
<i>TraesCS4B03G0094800</i>	Phytanoyl-CoA dioxygenase	PF05721
<i>TraesCS4B03G0094900</i>	F-box family protein	PF03478
<i>TraesCS4B03G0095000</i>	3-oxo-5- α -steroid 4-dehydrogenase	PF06966
<i>TraesCS4B03G0095100</i>	Peptidylprolyl isomerase	PF00254
<i>TraesCS4B03G0095500</i>	Cinnamate 4-hydroxylase	PF14780
<i>TraesCS4B03G0095900</i>	ATP-dependent RNA helicase	PF00270
<i>TraesCS4B03G0096000</i>	Myosin-binding protein 2	PF04576
<i>TraesCS4B03G0096200</i>	DNA-binding storekeeper protein-related transcriptional regulator	PF04504
<i>TraesCS4B03G0096300</i>	Zinc finger protein VAR3, chloroplastic	PF00641
<i>TraesCS4B03G0096500</i>	Microsomal glutathione S-transferase 3	PF01124
<i>TraesCS4B03G0096600</i>	Microsomal glutathione S-transferase 3	PF01124
<i>TraesCS4B03G0096800</i>	Microsomal glutathione S-transferase 3	PF01124
<i>TraesCS4B03G0096900</i>	Basic blue protein	PF02298

Chapter 4 - QTL mapping of barley yellow dwarf virus resistance in the JagMut1095 x Jagger RIL population

4.1 Introduction

Barley yellow dwarf virus (BYDV) is not only a very serious viral disease in barley and wheat, but can also infect rye, oat and maize (Ayala et al. 2001). BYDV infected plants usually show leaf discoloration from shades of yellow to red or purple, and sometimes have stunted plants that are often mis-diagnosed as nutrient deficiency (Miller and Rasochová 1997). BYDVs are phloem-limited and transmitted by aphids in a non-propagative but persistent and circulated manner (Texcoco et al. 2002). Seven BYDV species can cause the disease, including BYDV-PAV, BYDV-MAV, BYDV-PAS, BYDV-Ker II, BYDV-Ker III, BYDV-SGV, BYDV-GAP that belong to different or unassigned genera in the family *Luteoviridae* (Hu et al. 2019). Among them, BYDV-PAV is the most prevalent worldwide and has more economic impact, and can be transmitted most efficiently by both bird cherry-oat aphid (*Rhopalosiphum padi*) and English green aphid (*Sitobion avenae*) (Parry et al. 2012; Aradottir and Crespo-Herrera 2021). In wheat, yield losses due to BYDV have been reported to be 40–50% in a severely infected fields (Larkin et al. 2002), and up to 25% even in the fields with minimal or near-invisible BYDV symptoms (Jarošová et al. 2016).

Many factors may affect BYDV symptoms. Different crops and varieties can have different symptoms, which may also be affected by aphid infestation time, host developmental stage when the infestation occurs, infestation pressure and environments (Shah et al. 2012; Choudhury et al. 2019a). BYDV can be partially controlled by agronomic practices (management of sowing date, the application of seed treatment etc.), but the response was not always consistence and did not always

result in a positive economic return (Royer et al. 2005). Also, the indiscriminated use of insecticides will increase insecticide resistance of insects (Foster et al. 2014; Bass and Jones 2018). Thus, using BYDV resistant or tolerant cultivars is currently the most effective and promising way to control this disease (Choudhury et al. 2017). To date, several BYDV tolerance genes have been reported in barley, including *Ryd1* (Suneson 1955; Rasmusson and Schaller 1959), *Ryd2* (Collins et al. 1996), *Ryd3* (Niks et al. 2004), and *Ryd4^{Hb}* (Scholz et al. 2009). However, linkage drag of this gene prevented it from wide deployment in barley breeding (Jarošová et al. 2016). In addition to the four genes, several quantitative trait loci (QTLs) for BYD-PAV resistance have also been identified on chromosomes 1H, 2H, 4H and 7H (Toojinda et al. 2000; Scheurer et al. 2001, Friedt et al. 2003). However, none of the genes were transferred to wheat.

In wheat, four BYDV resistance genes have been reported so far. *Bdv1* from a Brazilian spring wheat cultivar ‘Frontana’ was located on the short arm of chromosome 7D as *Lr34/Yr18* locus with a pleiotropic effect on the rust resistance (Singh et al. 1993). This gene showed resistance to only some BYDV species or in some environments (Zhang et al. 2009). *Bdv2*, *Bdv3* and *Bdv4* all originated from an intermediate wheatgrass (*Thinopyrum intermedium*). Wheat-*Th. intermedium* addition, substitution and translocation lines were developed and characterized to show BYDV-resistance, including *Bdv2* in 7D-7Ai#1 (Banks et al. 1995), *Bdv3* in 7D-7E (Sharma et al. 1995), and *Bdv4* in 2D-2Ai-2 (Zhang et al. 2009). Beside the four named genes, Ayala et al. (2002) identified 22 QTLs in ‘Opata’ × ‘Synthetic’ population and 7 QTLs from ‘Frontana’ × ‘INIA66’ population, including one in the similar location to *Bdv1* on 7DS. However, most of the QTLs showed minor effects explaining 6.6% to 13.0% of the phenotypic variation for visual leaf disease scores and 5.6% to 10.5% for

dwarfing and biomass reduction. Choudhury et al. (2019b) identified four QTLs for BYDV resistance on chromosomes 2A, 2B, 6A, and 7A using GWAS, explaining 4.4% - 7.4% of the phenotypic variation. Silva et al. (2022) identified significant marker-trait associations for BYDV severity on chromosomes 5AS, 7AL, and 7DL using high-throughput phenotyping and GWAS on 30 wheat cultivars and 351 advanced breeding lines. The major QTL at the 571-637 Mbp on chromosome arm 7DL reduced about 10% of disease severity and was confirmed to be *Bdv2*. Therefore, to date, most of the reported genes or QTLs for BYDV resistance show unstable minor effects in wheat, and have not been widely deployed in breeding programs. Thus, stable major QTLs/genes for BYDV resistance in common wheat is urgently needed for improvement of BYDV resistance in wheat.

Single nucleotide polymorphisms (SNPs) and insertion-deletions (InDels) are common in wheat genome (Rasheed et al. 2016). Kompetitive allele specific PCR (KASP) markers can be converted from SNPs and InDels and are more convenient for marker-assisted selection because of the low cost and medium to high throughput. For BYDV resistance in wheat, KASP markers are not available for breeding selection. In this study, we used SNP markers generated by genotyping-by-sequencing (GBS) to do QTL analysis of BYDV in 154 recombinant inbred lines (RILs) from the cross of JagMut1095 x Jagger.

4.2 Materials and methods

4.2.1 Field evaluation of BYDV of the RIL population

A total of 154 RILs were generated by single-seed-descent method from JagMut1095 x Jagger. Jagger is a U.S. hard winter wheat cultivar (PI 593688) from Kansas and shows BYDV tolerance with light BYDV symptoms in the fields; whereas JagMut1095 is an EMS-induced mutant of Jagger and shows severe BYDV

symptoms in the fields. The population and parents were natural infected and evaluated for BYDV resistance in three field experiments from 2020 to 2022 at Kansas State University (KSU) -Rocky Ford Experimental Station. Each experiment used a randomized complete block design (RCBD) with two replicates for RILs and five replicates for each parent. About 30 seeds per line were sowed in a one-meter row with 20 cm between rows. Plants were naturally infected in the fields.

The BYDV symptoms were scored for each plot by visually estimating both the percentage of infected plants and the average disease severity per plot using a 0 to 5 visual scale (Fig. 4.1), where 0 refers to all plants in a plot without obvious yellowing symptom; 1 refers to yellowing appeared only at the tip of leaves in less than 10% plants in a plot; 2 refers to ~10-30% of the plants in each plot showing yellowing symptoms on limited proportion of flag leaves and yellow or red in penultimate leaves; 3 represents 40-60% of plants showing yellowing on moderate proportion of the flag leaves and yellow or red on penultimate leaves; 4 represents ~70-90% of plants in a plot with yellow or red on a large proportion of the flag leaves and penultimate leaves; 5 represents yellowing on almost all plants in a line with some stunted plants. The BYDV symptoms were evaluated three times after spike emergency in each year with the dataset showing the largest contrast between the most susceptible and the most resistant RILs used for QTL mapping. The mean BYDV severities over the two replicates of each RIL were used for QTL mapping. Plant height (PH) in cm was measured at physiological maturity from plant base to top of spikes excluding awn. The names of the field experiments were designated by combining the testing years with trait names in each experiment, where BYDV-2020, BYDV-2021 and BYDV-2022, and PH-2020, PH-2021 and PH-2022 refer to BYDV and plant height (PH) data collected in 2020, 2021 and 2022, respectively. The best

linear unbiased estimated (BLUE) value of BYDV and PH calculated across all the environments were included in QTL analysis.

4.2.2 Genotyping, map construction and QTL analysis

DNA isolation, GBS library construction, sequencing, SNP calling and quality control, map construction, QTL analysis and how to determine stable and pleiotropic QTL were described in Chapter 2. The threshold of logarithm of the odds (LOD) value to claim significant QTLs was settled at 3.3 for BYDV resistance and at 3.5 for PH based on 1000-times permutation using QTL Cartographer version 2.5.

4.2.4 Development of KASP markers

Once a major QTL was found, Kompetitive allele specific PCR (KASP) primers were designed using the SNP flanking sequences in the major BYDV QTL interval generated from exome capture using Primer3web version 4.1.0 (<http://primer3.wi.mit.edu/>) (Untergasser et al. 2012). The protocol for KASP assays was described in Chapter 2.

4.3 Results

4.3.1 BYDV severity in parents and RILs

The mean BYDV symptom scores were significantly different between the two parents in all three experiments ($P < 0.001$, Fig 4.2), with the BLUE value of 3.1 for JagMut1095 and 1.4 for Jagger across the three field environments. The BYDV severities also varied among the three field experiments due to the natural infection. A normal distribution was observed for the BLUE values of RIL population across the three environments (Shapiro-Wilk test $P > 0.1$), but not for BYDV scores in an individual environment (Fig. 4.2, Shapiro-Wilk test $P < 0.0001$). Significantly positive correlations in BYDV severity were observed among the three field environments (0.25 - 0.50, $P < 0.001$, Table 4.1). The broad sense heritability for

BYDV was medium high (0.66). Correlation between BYDV severity and PH was not significant in any of the tested environments, suggesting that virus did not cause obvious stunting plants (Table 4.2). The effects of genotype, environment, and genotype by environment interaction were highly significant on BYDV severity (ANOVA, $P < 0.0001$, Table 4.3) across three environments.

4.3.2 SNP genotyping and linkage map construction

As described in chapter 2, 3,659 high quality GBS-SNPs were used to build the linkage map and 1,003 unique SNPs were mapped in 22 linkage groups corresponding to the 21 chromosomes. The final map is 3,106 cM with a marker density of 3.1 cM per marker.

4.3.3 QTL for BYD resistance and PH

Composite interval mapping using the SNP map identified five QTLs for BYDV resistance on chromosomes 1D, 2A, 2D, 5A and 7A using the data from the three field experiments (Table 4.4). Among them, *QByd.hwwg-2DL* has been consistently detected in all three experiments and the BLUE values. *QByd.hwwg-2DL* was located between markers *S2D_642415923* and *Exon2D-3* in the BYDV-2020 and BYDV-2021 experiments and with BLUE, and between *2D-642166246* and *Exon2D-19* in the BYDV-2021 experiment. This 2DL QTL explained 11.6 to 44.6% of the phenotypic variance (Table 4.4, Fig 4.3) with LOD score from 5.04 to 21.56. *QByd.hwwg-1DL* was detected only in the BYDV-2021 experiment, explaining 12.4% of the phenotypic variation with the LOD score at 4.16. *QByd.hwwg-5AL* was detected only in the BYDV-2020 experiment, explaining 10.8% of the phenotypic variation with the LOD score at 4.49. *QByd.hwwg-7A* was detected only in the BYDV-2022 experiment, explaining 7.1% of the phenotypic variation with the LOD score at 3.32. Jagger contributed the resistance alleles at all the four QTLs. The

mutant only contributes the resistance allele at *QByd.hwwg-2AL* that was detected in the BYDV-2020 experiment explaining 11.5% of the phenotypic variation.

4.3.4 Convert GBS SNPs in the major QTL region to KASP markers

In this study, flanking sequences of four GBS-SNPs in the *QByd.hwwg-2DL* region were used to design KASP primers for KASP markers. Genotyping of RILs using these four KASP markers showed the same segregation pattern as the original GBS-SNPs. The new markers filled in most of the missing datapoints. Additionally, six co-dominant KASP markers were designed using SNPs discovered from an exome capture assay of the two parents to increase SNP density in the *QByd.hwwg-2DL* region. These exome capture KASPs were distributed from 642,419,369 bp to 643,117,096 bp and the marker clusters separated two parents and the RIL population well, thus, were used to update the linkage map of chromosome 2DL (Table 4.5).

4.3.5 Candidate gene(s) underlying *QByd.hwwg-2DL*

The candidate interval of QTL *QByd.hwwg-2DL* was delimited into the region between markers *2D-642415923* and *Exon2D-19* (642,415,923 bp and 642,998,925 bp) on chromosome arm 2DS. In this candidate interval, 14 high confidence genes were annotated, and seven of them were predicted to be involved in disease resistance, including genes encoding wall-associated receptor kinase and lectin-receptor kinase, glutathione peroxidase, defensins, and cytochrome P450 (Table 4.6).

4.4 Discussion

The BYDV severity of the RIL population showed significant variation in each environment and the heritability of the BYDV severity was medium high (0.66) among the three environments, suggesting relatively high consistency of BYDV phenotypic data across the three experiments even under natural infection in the field. Usually, plant resistance to insect transmitted diseases has a low heritability since the

transmission is highly influenced by the environments (Price and Schluter 1991). Non-significant correlation between BYDV and PH suggested that BYDV did not cause significant plant stunting in this study.

To date, only a few BYDV resistance genes have been reported in wheat including *Bdv1*, *Bdv2*, *Bdv3* and *Bdv4*. Among them, *Bdv4* was mapped on chromosome 2D, which originated from an intermediate wheatgrass *Thinopyrum intermedium* (Zhang et al. 2009). Two wheat cultivars, ‘Zhong 4’ and ‘Zhong 5’, were reported to carry the *Bdv4* with the donor from a partial wheat-Th. *intermedium* amphiploid (Zhang et al. 2009). In this study, the major QTL *QByd.hwwg-2DL* for the BYDV resistance is from Jagger, and does not carry any translocation from *Thinopyrum intermedium*. Also, the physical position of *Bdv4* is reported as a 2D-2Ai-2 centric fusion (Choudhury et al. 2019), but *QByd.hwwg-2DL* was located at the distal end of the 2DL. Therefore, *QByd.hwwg-2DL* is most likely a new resistance locus different from *Bdv4*.

As an aphid transmitted disease, it is possible that BYDV resistance in our study may be due to aphid resistance in wheat. Currently, at least 11 species of BYDV can be transmitted by cereal aphids, which established complex virus-vector interactions with variations in specificity and transmission efficiency (Aradottir and Crespo-Herrera 2021). Among the aphid resistance loci identified in wheat, *QGb.slu-2DL* was reported as a novel resistance QTL to *Schizaphis graminum*, which comes from the synthetic hexaploid wheat ‘CWI76364’ in 2014 (Crespo-Herrera et al. 2014). This QTL was located from 612.2 to 615.7 Mb on wheat chromosome 2D, physically about 27 Mb away from *QByd.hwwg-2DL* identified in this study. Previously, Jagger has been reported to carry the introgression 6 on chromosome 2DL from a wheat relative *Aegilops markgrafii* or *Aegilops umbellulata* ($2n = 2x = 14$, U) (Keilwagen et

al. 2022). Whereas *QGb.slu-2DL* was from ‘CWI76364’ selected from the cross between *Triticum dicoccum* (PI 94623) and *Aegilops tauschii* (WX1027), therefore *QByd.hwwg-2DL* in our study is different from *QGb.slu-2DL*. Further investigation is needed to determine the BYDV resistance mechanisms of *QByd.hwwg-2DL*.

Based on IWGSC refseq 2.1 (IWGSC, 2018), the *QByd.hwwg-2DL* candidate region (642,415,923 bp to 642,998,925 bp) has 14 annotated high confidence genes and 7 of them were predicted to be pathogen defense related genes in different plant species (Table 4.6). *TraesCS2D03G1277500* encodes wall-associated receptor kinase-like protein to be involved in the response to pathogen attacks (He et al. 1998; Morris and Walker 2003). *TraesCS2D03G1277700* encoding glutathione peroxidase was critical to reduce the H₂O₂ accumulation by oxidizing reduced glutathione (GSH) to the disulfide form (GSSG) (Zhang et al. 2019). Plant glutathione S-transferases (GSTs) have glutathione peroxidase activities and can detoxify toxic lipid hydroperoxides that were accumulated during pathogen infections (Gullner et al. 2018). *TraesCS2D03G1278700* and *TraesCS2D03G1278900* encoding defensins may involve in diverse defense mechanisms against different pathogens including viruses, bacteria and fungi (Sher Khan et al. 2019). *TraesCS2D03G1279000* encoding a lectin-receptor kinase might play an important role in plant–virus interactions and plant innate immunity (Singh and Zimmerli 2013; Macho and Lozano-Duran 2019). *TraesCS2D03G1279300* and *TraesCS2D03G1279400* encode Cytochrome P450. Cytochrome P450 is a heme-containing membrane-bound enzyme and plays important roles in plant defense through several types of oxidation-reduction reactions in secondary metabolite biosynthesis in the classical xenobiotic detoxification pathway (Schuler and Werck-Reichhart 2003; Xu et al. 2015). These genes can be selected as candidate genes for further functional analysis. Among them,

TraesCS2D03G1277500, *TraesCS2D03G1277700* and *TraesCS2D03G1279400* have SNPs between two parents from the exome-capture assays, these SNPs have been successfully converted to KASP markers as *Exon2D-3*, *Exon2D-13* and *Exon2D-19*, respectively. These markers could be used for selection of *QByd.hwwg-2DL* in breeding.

4.5 Conclusion

In this study, we analyzed the BYDV resistance in wheat RILs derived from JagMut1095 x Jagger, and found *QByd.hwwg-2DL* as the consistent, major resistance QTL for the BYDV resistance. This resistance QTL is from Jagger, and physically delimited in a 583 Mb interval between 642,415,923 bp and 642,998,925 bp harboring 14 high confidence genes. Further fine mapping of *QByd.hwwg-2DL* will facilitate map-based cloning and further characterization of the mechanisms of wheat BYDV resistance.

Figures and tables

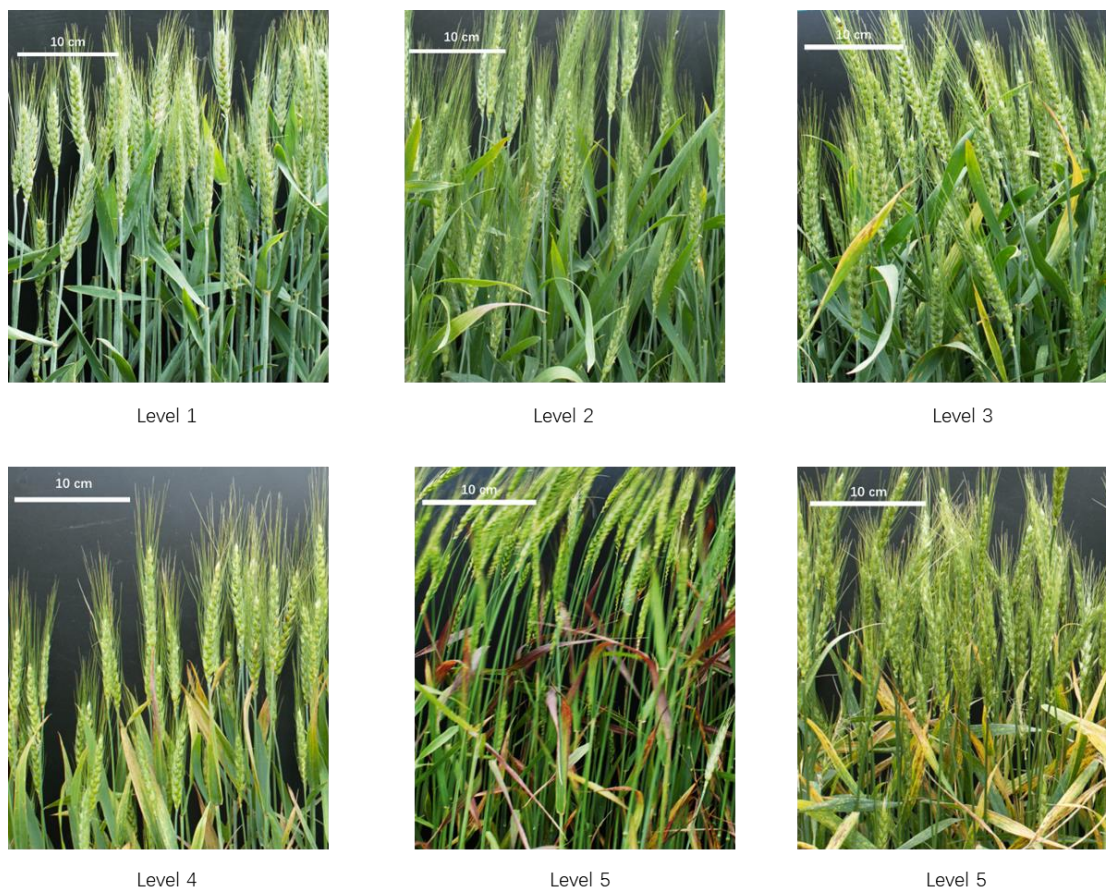


Figure 4.1 – Sample pictures of wheat plants with different BYDV scores in the population of JagMut1095 x Jagger recombinant inbred lines (RILs).

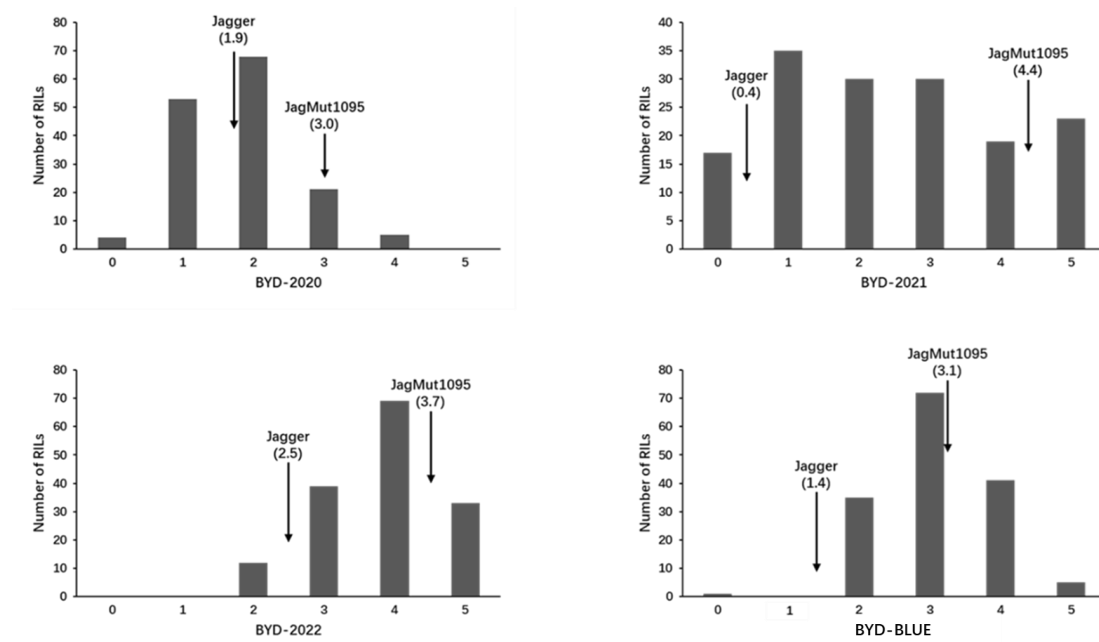


Figure 4.2 – Frequency distribution of mean BYDV of JagMut1095 x Jagger recombinant inbred lines (RILs) evaluated in three field experiments and the BLUE.

Table 4.1 – The correlation coefficients of BYDV scores among the three environments

Environment	2020	2021
2021	0.3506***	
2022	0.2489**	0.4983***

*Note: ** Significant at $p < 0.01$ level, *** significant at $p < 0.001$ level.*

Table 4.2 – Correlations between BYDV severity and plant height in field in Manhattan, KS

Environment	Correlations
2020-Rockyford	-0.034
2021-RockyFord	-0.092
2022-RockyFord	-0.073
Across Environments	-0.030

Table 4.3 – Analysis of variance of genotype, environment and replication effects for BYDV severity over the three environments. The degrees of freedom (df) and Sums of Squares (SS) are shown for each analysis

Source	DF	SS	F-value	Pr>F
Block(Environment)	3	3.61	1.19	0.31
Genotype	153	651.70	4.22	0.00
Environment	2	653.52	323.53	0.00
GE-interaction	302	510.91	1.68	0.00
Error	451	455.51		
Total	911	2275.25		

Table 4.4 – QTLs identified for BYDV resistance in the RIL population derived from JagMut1095/Jagger evaluated in 2020, 2021 and 2022 field in Manhattan, KS

QTL name	Trait	Chr	Peak position(cM)	LOD	Add	R ²	contributed by	Peak interval	Flanking of the peak
<i>QByd.hwwg-1DL</i>	BYDV-2021	1D	67.81	4.16	0.65	0.124	Jagger	57.8-79.2	S1D_466621318-S1D_484144587
<i>QByd.hwwg-2AL</i>	BYDV-2020	2A	66.41	4.51	-0.35	0.115	mutant	61.4-66.8	S2A_693338820-S2A_722798652
<i>QByd.hwwg-2DL</i>	BYDV-2020	2D	96.81	5.04	0.26	0.116	Jagger	95.9-98.2	2D-642415923-Exon2D-3
	BYDV-2021	2D	96.81	19.47	1.01	0.383	Jagger	95.9-98.3	2D-642415923-Exon2D-3
	BYDV-2022	2D	98.21	11.59	0.44	0.254	Jagger	97.2-98.8	2D-642166246-Exon2D-19
	BYD-BLUE	2D	97.21	21.56	0.51	0.446	Jagger	96.2-98.3	2D-642415923-Exon2D-3
<i>QByd.hwwg-5AL</i>	BYDV-2020	5A	57.01	4.49	0.39	0.108	Jagger	55.3-61.3	S5A_415083191-S5A_445420447
<i>QByd.hwwg-7A</i>	BYDV-2022	7A	154.11	3.32	0.28	0.071	Jagger	149.6-158.2	S7A_150233128-S7A_628768772

a *LOD* log likelihood ratios, which is significant when comparing hypotheses H_1 (there is QTL linked) vs. H_0 (there is no QTL linked)

(Churchill and Doerge 1994)

b *PVE* phenotypic variation explained

c *ADD* additive effect in which a positive value indicates increased BYDV level contributed by the JagMut1095

Table 4.5 – A list of primers for polymorphic Kompetitive allele specific PCR-based single nucleotide polymorphisms (KASP–SNPs) that were tightly linked to *QByd.hwwg-2DL* and their putative physical locations in Chinese Spring reference V2.1 genomes

CSv2.1	Marker	Sequence
642,419,369	Exon2D-3_F	GAAGGTGACCAAGTTCATGCTCTCCCGAATTTATTGTGGCAAAT
	Exon2D-3_H	GAAGGTCGGAGTCAACGGATTCTCCCGAATTTATTGTGGCAAAC
	Exon2D-3_R	GTTAGCATAGCGGCTCTGGA
642,664,383	Exon2D-13_F	GAAGGTGACCAAGTTCATGCTTTGTGAATGTCGCATCTCGG
	Exon2D-13_H	GAAGGTCGGAGTCAACGGATTTTGTGAATGTCGCATCTCGA
	Exon2D-13_R	GCCAGACCACTGCAAGGATA
642,998,925	Exon2D-19_F	GAAGGTGACCAAGTTCATGCTTGGATGCAATCTAAAAGTTTCCTTC
	Exon2D-19_H	GAAGGTCGGAGTCAACGGATTTGGATGCAATCTAAAAGTTTCCTTG
	Exon2D-19_R	GCAGAACTCCACTACATGCA
643,117,096	Exon2D-29_F	GAAGGTGACCAAGTTCATGCTTGGAAAACAAACCTTGAATCCCA
	Exon2D-29_H	GAAGGTCGGAGTCAACGGATTTGGAAAACAAACCTTGAATCCCG
	Exon2D-29_R	CAGGGCGTGGTGATACAACA

Table 4.6 – A list of genes in candidate interval of *QByd.hwwg-2DL* in Chinese Spring reference V2.1 genomes

Gene-ID (CSv2.1)	Description	Pfam-IDs
<i>TraesCS2D03G1277500</i>	Wall-associated receptor kinase-like protein	PF13947
<i>TraesCS2D03G1277600</i>	AT hook motif DNA-binding family protein, putative	PF03479
<i>TraesCS2D03G1277700</i>	Glutathione peroxidase	PF00255
<i>TraesCS2D03G1277800</i>	DNA-directed RNA polymerase subunit beta	
<i>TraesCS2D03G1277900</i>	C2 calcium/lipid-binding and GRAM domain protein	PF00168
<i>TraesCS2D03G1278100</i>	Oxidoreductase/ transition metal ion binding protein	
<i>TraesCS2D03G1278200</i>	Envelope glycoprotein B	
<i>TraesCS2D03G1278500</i>	Acetyl-coenzyme A synthetase	PF00501
<i>TraesCS2D03G1278700</i>	Defensin	PF00304
<i>TraesCS2D03G1278900</i>	Defensin	
<i>TraesCS2D03G1279000</i>	lectin-receptor kinase	PF00069
<i>TraesCS2D03G1279200</i>	Dihydroflavonol 4-reductase	PF01370
<i>TraesCS2D03G1279300</i>	Cytochrome P450	PF00067
<i>TraesCS2D03G1279400</i>	Cytochrome P450	PF00067

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