COMPARATIVE QTL MAPPING OF HAGBERG FALLING NUMBER, PRE-HARVEST SPROUTING, AND LATE MATURITY ALPHA-AMYLASE IN UI PLATINUM BY SY CAPSTONE DERIVED SPRING WHEAT POPULATION

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Authorization to Submit Thesis

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Abstract

The Hagberg falling number (FN) is an important quality trait in common wheat. Assessment of FN being used on flour samples from harvested grain is costly and affected by environments where the grains were produced. Pre-harvest sprouting (PHS) and late maturity α-Amylase (LMA) are the two main sources of low FN in wheat. Use of quantitative trait loci (QTL) and tightly linked molecular markers for FN, PHS, and LMA are good alternatives to use in early generation selection and for improvement of selection efficiency. The present study uses a comparative QTL mapping for the three related traits in a doubled haploid population derived from two hard white spring wheat cultivars: UI Platinum (UIP) and SY Capstone (SYC). FN data from nine trials, PHS data from eight datasets in two experiments, and LMA data from one trial with two treatments were used in the QTL analysis. Out of sixteen QTLs for FN detected, five (*QFN.UIA-1B-1*, *QFN.UIA-2B*, *QFN.UIA-3B-1*, *QFN.UIA-5A-1*, *QFN.UIA-7A-1*) were detected in three or more data sets, explaining 10 to 34% of phenotypic variation. Nine QTLs for PHS were identified in three or more data sets on six chromosome regions, explaining 10 to 25% of phenotypic variation. Three QTL for LMA were identified on chromosomes 4A, 5A, and 7A-1. The QTL on 5A was associated with all three traits, and UIP contributed to higher FN, resistance to PHS and LMA. The QTL on 7A-1 was associated with FN and LMA and UIP contributed to lower FNs and higher PHS scores. QTL on 1B-1 was associated with FN and PHS and UIP contributed to lower FNs and higher PHS scores. This study suggests that the three traits may share some common genetic information; therefore, selecting one trait may permit the indirect selection of other related traits. This study also suggests that lines with improved resistance to FN, PHS, and LMA may be achieved through genetic recombination for QTL from the two parents. Compared to the physical map of Chinese Spring, the 5A QTL is in the flanking region of the *Amy 3* gene. This suggests that it is necessary to study the *Amy 3* gene in order to understand the genetic control of the three traits assessed in the present study.

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Dedication

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Chapter 1. LITERATURE REVIEW

1.1 Common Wheat and its Quality

Common wheat, also known as "bread wheat" (*Triticum aestivum* L. em Thell; AABBDD; 2*n* = 6*x* = 42), is considered an essential food crop as it provides a large portion of protein and calories for the human diet. Nearly 20% of the total dietary protein worldwide is known to be supplied by common wheat (Braun et al. 2010). According to the FAOSTAT (2016), world wheat production was about 730 million tons and harvested from an area of over 220 million ha during the year 2014 (FAO-Stat 2016). Based on worldwide production, wheat (751.1 million tons) stood second after maize (1,341.7 million tons), but the production was more than rice (480 million tons) in 2016 (USDA-ESMIS, 2016). Wheat has a wide adaptation and is capable of growing in temperate climates, irrigated to dry and high-rain-fall areas, as well as in warm, humid to dry, and cold environments. More specifically, wheat can grow from 67^{o}N to 45^{o}S latitude (Trethowan, et al. 2005).

Wheat is a C3 type annual plant belonging to the family *Poaceae* (Gramineae) and genus *Triticum*. Common wheat evolved roughly 8,000 years ago from the natural hybridization of three wild diploid grasses known as *Triticum urartu, Aegilops speltoides,* and *Aegilops tauschii* (Trethowan, et al. 2005). After hybridization, allohexaploid wheat was formed, which contains 21 pairs of chromosomes originating from the A, B, and D genomes (Mangelsdorf, 1953). The wheat genome size is 17 Gb and the genome include 94,000 to 96,000 genes (Brenchley, et al. 2012).

Wheat is classified as winter, spring and facultative based on whether vernalization is required. Winter wheat must go through a period of cold temperature to be able to produce seeds, while spring and facultative wheat do not. Winter wheat is planted in fall and harvested in summer of the following year, while spring wheat is planted in spring and harvested in summer of the same year. Facultative wheat has a flexible planting period. By also considering grain hardness and seed color of winter and spring wheat, wheat is classified as

soft white winter, soft red winter, hard red winter, hard white winter, soft white spring, soft red spring, hard red spring, and hard white spring. These classes of wheat have different adaptation areas in the world and produce different end-use products.

Wheat contains about 13% protein (weight basis), including many essential amino acids, important minerals and vitamins, beneficial phytochemicals, and other fiber components for the human diet (Shewry, 2009). The majority of plant-based protein in our daily diet comes from wheat as it has relatively higher protein content compared to other cereal crops. Therefore, more than 30% of the human population considers wheat a staple food (International Wheat Genome Sequencing 2014). A wide range of foods such as bread, noodles, and tortillas use wheat flour as its main ingredient. Whole wheat products have the potential to reduce the risk of chronic diseases like diabetes and cancer; it can also reduce the risk of obesity and assist in improved weight control ("Wheat facts," 2017). In addition to its high nutritional value, wheat is an important food crop due to its long shelf life and transporting qualities.

1.2. Hagberg Falling Number and Its Relation to Wheat Quality

Hagberg falling number (FN) is a critical quality trait used in wheat grading in grain elevators, export markets and baking industries. The FN method, also referred to as the Hagberg number or Hagberg-Perten number, measures the degradation in wheat flour or meal of starch that results from α-Amylase activity (Perten, 1964). In the FN test, 6 to 7 grams of flour are placed in a test tube, and 25 mL of distilled water is added. The tubes are shaken to mix the water and flour. The wheat flour- water mixture is gelatinized upon boiling. Finally, the flour-water mixture is stirred for one minute, with stir rods, that are then dropped from the top to the bottom of the tube. The FN instrument measures the number of seconds it takes for the metal stir rod to fall in the gelatinized paste (Kweon, 2010). The excess enzyme, α-Amylase, in the wheat flour and water mixture will break down the starch and reduce the viscosity of the slurry, making the stir rod fall faster, resulting in a lower FN of the sample (Kweon, 2010). A wheat line with an FN less than 300 seconds indicates the starch is damaged, or hydrolyzed, mostly by over-expressed α-Amylase in the wheat grain sample (Instruments, 2019).

The falling number test was initially developed in 1960 by Sven Hagberg as a rapid and original method to determine the starch damage of pre-harvest sprout (PHS) damaged wheat grains and the name was later changed from the Hagberg Falling Number to merely the "falling number" (Kweon, 2010). At the time that Sven Hagberg established the FN test, there were some well-known tests for measuring starch damage. To name a couple of these methods there is the Wohlegmuth method that involved using the dextrinizing properties of α-Amylase (Bendelow, 1963, Hagberg, 1960), and the Sandstedt, Kneen, and Blish method which involved measuring enzyme activity for a standard decrease to occur in the starch-iodine (Briggs, 1961, Hagberg, 1960). However, these and other tests were too time-consuming and could only measure a few samples at a time. Often, they also required an expensive apparatus to perform the tests, or the results collected from those tests did not accurately reflect the amount of damaged starch (Hagberg, 1960). These tests also often had a common defect in that they measured the time required to cleave starch to a much higher degree than what is necessary during bread-baking (Hagberg, 1960). Hagberg's FN method proved to be reliable over a wide range of tests and quickly became one of the most common methods used to test starch damage (Hagberg, 1960).

Starch can be damaged due to a number of reasons. Starch damage can occur due to technological interferences such as milling. Such damage can be improved by milling methods and the mechanics used. Excessive moisture prior to and during harvest, causes PHS, and extreme temperature changes (cold or hot), which cause Late Maturity Alpha Amylase (LMA), during grain fill also induce elevated α-Amylase activities in mature grain subsequently causing starch damage and reduce quality of baked products. In baking industries, a certain amount of α-Amylase activity is needed to break the starch into sugars that aid as fuel for the fermentation process. Too much α-Amylase activity in low falling number (below 300 seconds) flour results in high sugar content that causes cakes to fall, make noodles sticky and results in bread that does not rise well (Steber, 2017). However, too low of α-Amylase activity and an FN over 350 seconds, may result in dry breadcrumbs and a diminished volume (Instruments, 2019). Optimal α-Amylase activity with an FN around 300 seconds is desirable for a high-volume bread with a firm and soft texture (Instruments, 2019).

FN is used in grain elevators to differentiate between a successful growing season and a devastating one. If the FN falls below the optimal level (300 seconds), then the value of the wheat can be discounted by $0.25/bu for every 25 seconds below 300 (Steber, 2017). In 2016, wheat farmers in the Pacific Northwest (PNW) lost several million dollars in profit due to low falling number (LFN). Since the loss in 2016 and due to other incidents, e.g. in 2013 and 2014 growers reported to have a higher than average number of cases of PHS across the PNW region, the PNW region has committed substantial resources to determine the leading causes of LFN and how to counter them.

To breed wheat cultivars with desirable FNs in diverse environments, it is essential to understand the genetic control of FN. QTL mapping can help to determine the genes and chromosome regions underlying FN. Molecular markers tightly linked to FN can be used in the selection of resistant plants in early generations for cultivar improvement. Researchers have determined that significant QTLs related to FN are located on 14 chromosomes: 1B, 2D, 3A, 6B (Zhang, et al., 2014), 3B, 3D, 4A, 4D, 6A, 6D (Tang et al., 2017), 5A, 5D, (Bőrner,et al.,2017) and 4B, 7B (Zhang, et al., 2014Tang et al., 2017).

Chromosome 4A has been shown to be associated with PHS, specifically seed dormancy and several other smaller traits connected to controlling PHS (Lin, et al., 2016). PHS is one of the primary causes of LFN and is also better understood compared to LMA, a defect just as devastating and a more recently discovered cause of LFN. Chromosomes 6A and 6D are connected to the synthesize of α-Amylase and is often associated with LMA (Barrero, et al., 2013). Both PHS and LMA will be discussed further in the following sections.

1.3. Pre-harvest Sprouting (PHS) and Its Effects on Wheat

PHS results when wheat germinates in the grain head due to excess rainfall prior to harvest (Thomason et al., 2019). Germination is activated by GA3-induced alpha amylase (Mrva & Mares, 1999). Genetic variations for PHS were observed and associated with known genes controlling seed color, dormancy, and spike morphology (Kocheshkova, et al., 2017).

Various features influence PHS resistance in the spike morphology; spike shape, presence of awns, the openness of florets, glume rigidity, and germination inhibitors in husks (Kocheshkova, et al., 2017). The seed coat determines seed dormancy and its ability to resist

PHS. The seed coat can contain germination inhibitors, and it determines the rate moisture penetrates the seed (Kocheshkova, et al., 2017). In addition to the aforementioned features, the seed's red color is also connected to seed dormancy (Kocheshkova, et al., 2017).

Red color wheat is associated with the triplicate *R* homoeoloci gene, which is a significant gene in controlling PHS (Andreoli, Bassoi, & Brunetta, 2006). However, the red wheat cultivar will not guarantee a complete or adequate PHS resistance. For optimal resistance, the *Phs1* gene, which affects seed dormancy, is needed (Andreoli, Bassoi, & Brunetta, 2006). In addition to triplicate *R* homoeoloci and *Phs1,* two causal genes for non-grain color have been cloned and designated as *TaPHS1* and *TaMKK3-A,* respectfully (Lin et al., 2018). Both of these cloned genes have additive effects that can significantly reduce PHS and its impact in a wheat cultivar. To fully utilize these seed dormancy and seed color genes, wheat breeders have been studying the QTLs associated with these genes (Mares & Mrva, 2014).

The *Phs1* QTL is located on chromosome 4A, and the triplicate *R* homoeloci's QTL located on chromosomes 3A, 3B, and 3D (Torada, et al., 2016). The *TaPHS1* and *TaMKK3-A* QTLs are located on chromosomes 3AS and 4AL (Lin et al., 2018), respectively. However, the *TaPHS1* QTL effects vary based upon the environment the cultivars are grown in, such as temperature, humidity, and rainfall. The gene sources of the cultivar also influence the effects of *TaPHS1*, while in contrast, *TaMKK3-A* effectiveness was only influenced by the environment (Lin et al., 2018). As all of the mentioned genes are affected by the environment, it is essential to determine how resistant a cultivar is to PHS. There are several methods to test a wheat cultivar's resistance to PHS, including artificial sprouting of intact spikes, germination tests, natural weathering in field trials, artificial weathering trials and indirect assessment of sprouting by measuring the falling number (DePauw, et al., 2012).

Three genes synthesize α-Amylase, *Amy1*, *Amy2*, and *Amy3*. *Amy1* is located on chromosomes 6A, 6B, and 6D (Cheng, et al., 2013). *Amy2* is located on group 7 chromosomes- 7A, 7B and 7D in wheat (Mares & Mrva, 2014). *Amy3* is located on chromosome 5A. *Amy1* was reported as having an association with PHS (Laethauwer, et al., 2013), while the relationship between PHS and *Amy2* and *Amy3* is not clear. The most significant way to resist PHS resistance would be through a cultivar without genes relating to

seed color, dormancy and morphology. Such QTLs have been reported on 2D (Munkvold et al., 2009), but the effects of these QTLs were much smaller than those known genes. There is still a need to identify additional such QTLs for PHS.

The artificial sprouting of intact spikes, which is the most common method of assessing PHS, exposes the wheat seeds to conditions that will induce PHS while still in the spikes. This method was utilized to test the cultivars BRS Pardela and BRS 220, which are two varieties that are considered PHS susceptible after threshing. When these cultivars were tested using intact spikes, the seeds proved to be resistant while still within the spike (Gavazza, et al., 2012). This research supports what has been stated earlier in this section that spike morphology plays a vital role in a cultivar's ability to resist PHS.

The third and final factor is the genetics of the cultivar. *Amy 1*, as mentioned earlier in this section, is the gene responsible for the majority of the production of α-Amylase after a wheat plant reaches physiological maturity (PM). The higher the levels of *Amy1* that are expressed in a cultivar, the more PHS will be able to damage the wheat starch (Laethauwer, et al., 2013). Thus, it is imperative to choose a cultivar that expresses lower levels of *Amy1* (Laethauwer, et al., 2013).

1.4. Late Maturity α-Amylase (LMA) and Its Effects on Wheat

LMA is a newer field of research in wheat breeding that deals with the early production of α-Amylase during the middle stages of seed development when exposed to extreme temperatures (e.g. excessive low temperatures) (Mares & Mrva, 2014). Unlike PHS, LMA is a genetic defect that is widespread in bread wheat germplasm and causes high levels of α-Amylase in seeds when the environment lacks excessive moisture levels. (Barrero, et al., 2013). LMA however, is not induced simply by extreme temperature. Rather, this is a complex mechanism that is not fully understood but involves extreme temperature shock (large shifts in daily temperatures, either up or down) (Barrero, et al., 2013). It also occurs at a specific window during kernel development (25-30 days post-anthesis) (Emebiri, et al., 2010). This defect is a recessive trait with a significant QTL found on the long arm of chromosome 7B (Mrva & Mares, 2001). Other QTLs, though less prominent than 7B, have been found on 3B and 4B (Mrva & Mares, 2001). As this is a genetic issue, the best way to reveal what

genotypes are more susceptible to LMA's effects. is to study the genes that form α-Amylase (Barrero, et al., 2013).

As mentioned in Section 1.2, α-*Amy1* and *α-Amy2* are the genes that produce α-Amylase; more specifically, they create pI isozymes. There are two primary forms of pI isozymes; a high pI α-Amylase (associated with α-*Amy1* or the germination isozyme) and a low pI α-Amylase (associated with *α-Amy2* or the development isozyme) (Cheng, et al., 2013). The ability to distinguish between these two types of pI α-Amylase is crucial, as it is the high pI α-Amylase that is active in cultivars with the LMA quality defect during later stages of grain development (Cheng, et al., 2013). The best method to predict the LMA defect is to analyze the QTL using the *α-Amy1* sequences of established expressed sequence tags (ESTs) (Cheng, et al., 2013). Since the QTL for LMA is located on the long arm of chromosome 7B, which is associated with *α-Amy2*, many would expect this QTL to produce only low pI isozymes α-Amylase. However, this particular QTL is distant and genetically independent from the *α-Amy2* gene allowing it to create high pI isozymes (Mrva & Mares 2002).

Typically, LMA occurs when the plant is exposed to extreme temperature changes while the seed is still developing, roughly between 24-32 days after anthesis or flowering (DF). However, it is highly unpredictable, and several factors can affect the influence of LMA. Temperature, field location, individual plants, the spikes of different plants, even the grains themselves can influence how LMA will affect the wheat (Mares & Mrva, 2008). These factors and their unpredictability have made it difficult for wheat breeders to develop an effective screening method for LMA. One reliable method was initially designed in an Australian breeding program.

This method involves a two-phase process where in the first phase, the wheat plants are raised in a controlled environment and subjected to a cold temperature treatment during the grain development stage (26-32 DF) (Mrva & Mares 2002). The second phase tests the α-Amylase that is synthesized in the grains due to the induction of LMA (Mrva & Mares 2002). This method has proved to be effective because it can be used on wheat grown in controled conditions or wheat grown in the field under natural conditions. It has been implemented in

numerous breeding programs around the world (Mrva & Mares 2002). A significant advantage of this screening method is that allows breeders to determine the influence a particular field and environment has on LMA. The implementation of this two-phase method is assisting wheat breeding programs as they make advancements to eliminate LMA (Mrva & Mares 2002). With knowledge of the QTL that affects LMA and by developing an effective screening method, breeders have been working on selecting cultivars resistant to LMA. Using the numerous tests to determine what causes LMA, breeders discovered a new connection between LMA and wheat cultivars that possess the semi-dwarfing gene *Rht-Dtb* (*Rht-Dtb*) (Farrell, et al., 2013).

When comparing the tall cultivars to those with the semi-dwarfing gene, breeders discovered that semi-dwarf varieties show greater resistance to LMA (Farrell, et al., 2013). As a result, breeders began to look more closely at the genes found in the semi-dwarfing types. They found that cultivars possessing the semi-dwarfing gene *Rht-D1b* and carrying a GA-insensitive semi-dwarfing alleles had greater LMA resistance (Farrell, et al., 2013). With this knowledge, breeders began using semi-dwarfing genes; however, not every dwarfing gene helps with LMA resistance. In fact, in some of the very same semi-dwarfing cultivars, there was another set of genes known as B1/R1 that were prone to express LMA (Farrell, et al., 2013). In spite of this, *Rht-D1b* continues to be utilized since *Rht-D1b* and B1/R1 work independently from each other. A cultivars resistance to LMA is determined by whether *Rht-D1b* is absent from the cultivar and the strength of the presence of B1/R1 (Farrell, et al., 2013).

However, despite the knowledge we have gained on the genetics behind LMA; the mechanisims are still largely unknown, and its effects are not fully understood (Ral, et al., 2015). The Australian National University, the International Rice Research Institute in Manilla, Philippines, and the Commonwealth Scientific Industrial Research Organisation (CSIRO) are working to increase the world's understanding of LMA (Ral, et al., 2015). The research performed by this collaboration found that wheat overexpressing an isoform of α-Amylase known as Ta*Amy*3, in the endosperm, resulted in low FN. These FN results were similar to those seen in LMA- or PHS- affected grains (Ral, et al., 2015). However, inspite of the increase of α-amylase levels from Ta*Amy*3, there was no detrimental effect on the starch

structure or flour composition, instead Ta*Amy*3 enhanced the baking quality in small-scale (10g) baking tests (Ral, et al., 2015). From these small-scale tests, they discovered that overexpression of Ta*Amy*3 led to increased loaf volume and Maillard-related browning at levels higher than those in control flours when baking improver was added. These findings, challenge the assumption that all LMA is detrimental to the end product quality and that a low FN is always an indication of a reduction in quality (Ral, et al., 2015).

1.5. Starch Genetics

Since wheat is one of the top and most vital cash crops around the world, it is crucial that production of high-quality end products continues. With the FN crisis that struck the Pacific Northwest of America in 2016, the United States Department of Agriculture (USDA) determined that it was necessary to investigate the cause of this crisis to save one of the country's major economic commodities (USDA, 2019). As mentioned in Section 1.1, the FN test is a means of measuring starch damage in wheat from PHS and LMA. However, to understand how PHS and LMA can damage the starch, it is best to have a basic understanding of starch genetics and how the environment can influence it. Starch is divided into two types based on the mode of synthesis and utilization; the first is transitory starch which is synthesized in leaves during the day and consumed at night (Rahman, et al., 2019). The second type, reserve starch, is deposited in storage organs for later use by the plant; this is the starch found in wheat grains (Rahman, et al., 2005). Reserve starch is typically divided into two main types of granules, the large, disk-shaped A-type granules and the small, spherical B-type granules. A-type granules are distinct in that they have more amylose and long-B2 amylopectin than B-type granules (He et al, 2019). There is a third type of granule known as C-type granules; however, it is uncertain whether these are a type of granule or just a step in the A or B starch granule development (He et al, 2019).

The higher amylose levels and long B2 amylopectin give A-type granules a higher viscosity than B-type granules (He et al, 2019). In addition to size and shape, A-type and B-type granules are also different in their composition. All starch is constructed from two linear α-glucans known as amylose and amylopectin. Amylose forms about 20-30% of the starch structure; it has α-1-4 glycosidic linkages and forms a straight-chain structure (Zhang, et al.,

2017). Amylose is less soluble in water, so it does not create a gel when hot water is added, and it is a great storage system for energy (Zhang, et al., 2017).

Amylopectin makes up the other 70-80% of the starch structure; it has α-1-4 glycosidic linkages, but it also has α-1-6 glycosidic linkages, and instead of forming a straight-chain it forms a branched-chain polymer (Zhang, et al., 2017). With this branched structure, it also has more complicated molecular chains, which are classified into A, B1, B2, B3 and B4 chains (He et al, 2019). It is more soluble in water than amylose, and unlike amylose, it is only able to store a small amount of energy (Zhang, et al., 2017). The overall fraction ratio of these two glucose polymers affects the processing quality and digestibility of starch-based food products; digestibility, in turn, determines nutritional quality (Mishra, et al, 2016). For instance, a starch that has a higher amylose content is considered a resistant or healthy starch and is very desirable for preventive measures against obesity and related health conditions (Mishra, et al, 2016). The α-Amylase produced from PHS causes degradation of the A-type granules, which contributes to the end-product quality and stability (Simsek, et al., 2014). However, it has been hypothesized that part of what causes low FNs is not only a degradation of the A-type granules but also a surplus of B-type granules. This hypothesis was tested in 2019 through a collaboration between the Idaho Wheat Commission and the University of Idaho (Shao, et al., 2019).

The experiment was conducted on two soft white spring (SWS) wheat cultivars, UI Stone and Alturas, along with an SWS wheat elite line, SA043 (Shao, et al., 2019). The samples of the three cultivars used were grown in 2013 and 2014. The wheat seeds were ground into a mill using a lab grinder with a 0.8 mm screen and tested for FN as well as several other starch rating tests (Shao, et al., 2019). The FNs for all three of the 2013 cultivars were above 300 seconds (s); however, the FNs of the samples from 2014 all fell below 200 s with SA043's FN decreasing from 337 to only 62 s. The starch tests were used to help explain these drastic decreases of the FNs for these cultivars. Researchers looked at the starch granule morphology, size and the susceptibility of A-type and B-type starch to α-Amylase.

When observing the morphology of isolated starch, the researchers found that both the A-type and B-type granules showed smooth surfaces, and most of the starch granules were intact (Shao, et al., 2019). However, in both 2013 and 2014 there were a small number of

dents observed on the surface of the A-type wheat granules in all three cultivars (Shao, et al., 2019). For granule size, the researchers found that the proportion of B-type granules showed a statistically significant increase ($p < 0.05$) for all three cultivars in 2014; for example, the percentage of B-type granules found in UI Stone increased from 88.6% to 93.2% (Shao, et al., 2019). Finally, in determining the susceptibility of the A-type and B-type starch granules to α-Amylase, the researchers found that the B-type granules proved to be more susceptible α-Amylase than the A-type granules under normal hydrolysis conditions (Shao, et al., 2019). Interestingly enough, when the two granules were gelatinized, the A-type granules was more vulnerable to α-Amylase when compared to the B-type granules (Shao, et al., 2019).

Like FN, PHS and LMA, one of the best ways to understand and control starch issues is to study the genetic process by which starch is formed (Rahman, et al., 2005). There are several genes connected to starch synthesis: ADP-glucose pyrophosphorylase (large subunits and small subunits), granule bound starch synthase I (GBSS I), starch synthase (SS I, SS II, SS III, SS IV), starch branching enzyme I (SBE I, SBE IIa, SBE IIb) isoamylase I (ISA 3) and pullulanase (Rahman, et al., 2005). These genes are located on 1AS, 1B, 1D, 2A, 2AL, 2B, 2BL, 2D, 2DL, 4AL, 7AS, 7B, 7BS, 7D, and 7DS (Rahman, et al., 2005). The removal or alteration of any one of these genes can result in starch alterations; for example, a loss of ADP-glucose pyrophosphorylase will lead to a loss of the total starch synthesized and result in slight alterations to grain size (Rahman, et al., 2005).

1.6. Perspectives and Challenges of Solving Low Falling Numbers Issues in Wheat

As has been stated in the previous sections, LFN wheat has been a significant issue since 2014 and was a noticeably more critical issue between the years of 2014 and 2016. Since 2016, wheat breeders and other plant scientists have been working to help prevent LFN. This is not a simple task given the environment, more specifically the weather, is the biggest challenge to preventing LFN and is something growers have limited control over (Steber, 2017). PHS is the initiation of grain germination on the mother plant in response to rain before harvest. LMA is the induction of α-Amylase in response to large temperature changes during late-grain maturation (Steber, 2017). In 2016, rain before harvest and great changes in temperature induced low FN. The most effective method to combat LFN is to develop and plant wheat cultivars that are genetically resistant to PHS and/or LMA and to harvest wheat

quickly, after it reaches full maturity, to help reduce the chances of rain damage (Steber, 2017).

However, in addition to countering the effects of the weather, LFN prevention also involves considering crop storage. (Steber, 2017). Since α-Amylase is an enzyme catalyst, even a small amount can cause severe FN problems. Researchers at the University of Minnesota and Northern Crops Institue, reported placing just two highly sprouted seed into 200 grams [~5500 to 6500 grains] of sound seeds was sufficient to drop the FN of the sound seeds by 100 seconds (Ross, 2011). Improving storage methods and avoiding the blending of grain with different FNs is another big step in preventing LFN. Using these methods can help farmers reduce the damaging effects of LFNs.

Even with these advancements, there are still many other factors and issues related to LFN, which we do not fully understand or are unable to control. In addition to starch damage caused by α-Amylase due to PHS and LMA, there are a number of other challenges, including the need to better understand how the composition of the starch itself can affect the wheat's FN. Thus, continued research is essential to better understand the complexities of LFN. In an attempt to expand the world's understanding of LFN, our research looked into the idea that there could possibly be a genetic link between PHS and LMA that is also connected to FN. If such a connection does exist, it could potentially lead to the development of wheat germplasms that could eventually be used to breed resistance to PHS and LMA with naturally higher FN. In order to do this, we used a mapping population with 111 double haploid lines (DHL) that were developed from a cross of two photo-insensitive and semi-dwarfing cultivars known as UI Platinum and SY Capstone. Our first objective with these DHLs was to characterize the cause and effects of LFN. Second, we wanted to assess PHS and LMA under controlled conditions. Finally, our ultimate objective, as mentioned earlier, was to identify candidate QTLs controlling LMA and PHS once objectives 1 and 2 were completed.

Chapter 2. COMPARATIVE QTL MAPPING OF HAGBERG FALLING NUMBER, PRE-HARVEST SPROUTING, AND LATE MATURITY ALPHA AMYLASE IN HARD WHITE SPRING WHEAT

INTRODUCTION

The Hagberg falling number (FN) is a critical grain quality trait used for grading in grain elevators, the baking industry, and export markets. A FN less than 300 seconds is used to discount grain samples in grain elevators (Steber, 2017). In 2016, wheat farmers in the Pacific Northwest (PNW) lost several million dollars in profit due to low falling number (LFN). With 2016 and as a result of other incidents over the years, e.g. in 2013 and 2014 growers reported to have a higher than average number of cases of PHS across the PNW region, the PNW region has committed substantial resources to determine the leading causes of LFN and how to counter them.

The cause of low FN (LFN) is starch damage due to mechanical damage during milling enzyme hydrolysis, elevated alpha amylase activities, and other unknown factors. Excessive moisture at the plant physiological maturity can cause elevated α-Amylase activity and germination on wheat spikes, or pre-harvest sprouting (PHS). Cold shock during the grain-filling stage can also cause elevated α-Amylase activity and low FN in sound grain after harvesting. This phenomenon is called late maturity alpha amylase (LMA). FN data cannot differentiate if a low FN is caused by PHS or LMA. Phenotyping FN, PHS, and LMA is very difficult and costly. The QTL mapping of LFN, PHS and LMA may help us understand the similarities or differences in chromosomal locations underlying their genetic controls. QTL mapping and associated markers can be used in early generation selections and progeny predictions.

QTLs associated with FN has been reported in several publications, as reviewed in greater detail in Chapter 1 above. Approximately 21 QTL in twelve chromosome regions (1B, 2A, 2B, 3D, 4A, 4B, 4D, 5A, 5D, 7A, 7B and 7D) are associated with FN according to prior

publications (Zhang et al., 2014; Tang et al., 2017; Bòrner, et al., 2017; Martinez et al., 2018). Chromosomes 2B, 4A, 5A, 5D, 6B, 7B were reported to have significant QTLs in more than two papers; however, the QTL positions cannot be compared as different marker platforms were used. All of the studies acknowledged further study was needed to validate these QTLs due to either small population size, few field trials, older marker platforms, or environmental interference.

The phenotyping of PHS by using the spike germination method is easier than measurements for FN and LMA; therefore, more QTL have been identified for PHS and two QTLs controlling PHS have been cloned, including the QTL on chromosome 3AS (Liu et al. 2013 and 2018; Nakamura et al., 2011) and chromosome 4AL (*Phs1*) (Barrero et al., 2015; Torada et al., 2016). The cloned genes on the two chromosome regions are associated with seed color or seed dormancy. There were some technical limitations in using these QTL in breeding when pyramiding with other traits. Therefore, additional QTLs are needed in universal genetic backgrounds.

Limited QTL mapping studies have been conducted for LMA because accurate phenotyping of LMA is very difficult compared to testing FN and PHS. This is due to LMA being affected by multiple factors such as, the temperature, field locations, wheat spikes or even the wheat grains themselves. One major QTL on chromosome 7B and two minor QTL on 3B and 4B were reported for LMA in 158 doubled haploid lines derived from Australian germplasm (Mrva & Mares, 2001). This paper used a patented method that is has not been repeatable in US germplasm. Therefore, it is necessary to do an additional QTL mapping the locally adapted cultivars using a more developed genotyping platform.

Previous QTL studies were primarily conducted on one of the three traits at a time, and in different genetic materials, such as using an association mapping population instead of linkage mapping populations. Few studies have investigated FN and PHS in the same population, and no studies worked on the three traits in the same population. The primary objective of the present study was to map QTLs associated with FN, PHS, and LMA in the same set of doubled haploid lines, to identify candidate QTLs controlling LFN,PHS and LMA.

MATERIALS AND METHODS

Mapping Population and Field Experiments

A mapping population of 111 double haploid (DH) lines was developed from the F_1 generation of UI Platinum x SY Capstone using wheat by maize hybridization (Lui et al., 2018) under service from Heartland Plant Innovation (Manhattan, Kansas). UI Platinum (UIP) and SY Capstone (SYC) are two high-yielding hard white spring wheat cultivars. Both are photo-insensitive and semi-dwarfing. SYC was developed by Syngenta Cereals and released in 2011 (Marshall, et al, 2013). UIP was developed by the Idaho Agricultural Experiment Station and released in 2014 (Chen, et al., 2015). It should be noted that this DH population was not originally designed to be used for this study. It was originally intended to be used for physiological trait analysis. Due to this the difference FN, PHS and LMA between the UIP and SYC was not as large as we expected but was still good and could be used in QTL analysis. However, the transgressive segregation of this population was good and the use of elite cultivars as parents meant that we could easily translate the QTL markers from this study to other populations in our breeding program. This would allow the marks to be immediately used in other studies.

Field experiments for the DH population were performed in nine separate trials from late March to August in Aberdeen (AB) (42.96^o N, 112.8^o W, elevation 4403') in 2015 to 2019 (15-19) and one year at Soda Springs (SD), Idaho (42.66^o N, 111.6^o W, elevation 5774') in 2016 (16). Trials in Aberdeen, Idaho (15F206, 15F210, 15F309, 16AB, 17AB1, 17AB2, 18AB, 19AB) were irrigated, while the trial in Soda Springs (16SD) was under dryland. The temperature data for those years are summarized in Figure 2.1 and table 2.1. The irrigation management was the same in all Aberdeen trials except for the difference in rainfall received (see Table 2.1.). Based on the rainfall data in, five trials in 2015 and 2016 (15F206, 15F210, 15F309, 16AB, 16SD) received more rainfall in July and August than the four trials (17AB1, 17AB2, 18AB, 19AB) in 2017 and 2019.

For each trial, the population and the parental lines were planted in a randomized complete block design with one to two replications. All trials consisted of seven row plots measuring 3.0 m in length, 1.5 m in width, and 0.25 m between rows. For optimal trial management, standard fertilizer and weed control were applied to the plots when necessary

this was applied to all of the trials; the wheat borders were planted to minimize edge effect. Heading and anthesis dates were collected for each year along with height and yield data.

Falling Numbers Evaluation

Falling Number (FN) data was collected in all nine trials. Considering the workload, only one replicate of each DH line was tested for FN in each trial. After harvesting and cleaning the glume and awns from the grains, a 200 g sample from each line was ground into a flour meal using a UDY Cyclone Sample Mill grinder (UDY Corporation, USA) with a 0.05 mm screen. A Kern MLB_N moisture analyzer (Kern & Sohn GmbH, Germany) was used to help determine the correct sample size for preforming the FN tests. After acquiring the moisture percentage (%) for the samples, we used a moisture percent to weight (g) conversion chart to find the correct sample size of the meal to utilize in the FN rating tests. Two test tubes were filled with the meal at the correct sample size.

25ml of water was added to the tubes that were then capped and put in a Perten 1095 SM shaker (Perten Instruments, USA) to mix the water and flour. Stirring rods were then placed into the tubes. The tubes were placed into a water bath, set to 100^{o}C, in a Perten FN 1000 Falling Numbers machine (Perten Instruments, USA). Five seconds after the test tubes were placed in the water bath, the FN machine stirred the water-flour meal mix for 55 s with the stir rods. As the water-flour meal mix was stirred after 60 s a doughy gelatin substance formed and the machine released the stir rods measuring the time, in seconds, it took for the rods to reach the bottom of the tubes to determine the raw FN scoring. For each line in each trial, two duplicate samples were measured at once, and the average scores of the two FNs were considered for each of the DH lines. If the two ratings were not within 20 s of each other, the rating test would be redone to ensure accuracy.

Pre-harvest Sprouting Test

For rating pre-harvest sprouting (PHS) resistance of this population, we used an established protocol of artificial sprouting of intact spikes (DePauw, et al., 2012). With an

established rating system developed at (Cornell University) (Paterson et al., 1989) (See Figure 2.2). The samples were grown in the field where we also collected physiological maturity dates (PMD). Once the samples reached PMD, we gathered ten heads from each plot, and tagged them with the plot number and double haploid (DH) line IDs. The spikes were taken to the greenhouse, where we placed them in 13 $^{3/4}$ 26 ^{¾} inches or 10x20 cell seedling foam trays.

The trays were placed in a misting tent that had been prepared beforehand. The tent was made of clear plastic tarps and surrounded one of the tables in the greenhouse; it was designed to maintain a temperature of 66 to 75^{o} F and a humidity of 74% (Paterson et al., 1989). The table the tent was built on was selected as it also had a misting system built over the table. The misting system was also within the tent and was set to mist the samples for thirty seconds every five minutes, which ran for twenty-four hours for one week. We used an established artificial sprouting of intact spikes method, but we needed to determine what day would show the most variation of PHS injury between the samples. The samples were rated on specific days throughout the week to find which day would show a significant variation. In a preliminary experiment in 2017, the PHS was rated on the third day, the fifth day, and the seventh day.

However, the variation was limited on the 3rd and 7th days; therefore, we optimized our protocol and implemented PHS rating on the 4th, 5th, and 6th day in the 2018 and 2019 experiments. The rating system was based on a 0-9 assessment scale. With 0-2 showing high resistance to PHS, 3-6 showing moderate resistance, and 7-9 showing low resistance to PHS (Paterson et al., 1989).

Late Maturity Alpha Amylase Test

We used a detached spike method developed by Mrva and Mares (Mrva & Mares 2002) with modifications. Prior to this study, a preliminary study was conducted on fifteen selected lines from the DH population. Based on the flowering date, wheat spikes with stem were cut at 20, 22, 24, 26, 28, and 30 days after flowering (DAF). We tested these 15 lines to determine which day would provide the greatest variation. On each testing day, stalks from the field plots were collected by cutting three bundles of fifty stalks each. The bundles were

tagged with the plot numbers and the doubled haploid ID. The bundles were then placed in 1.5-gallon buckets filled roughly three-quarters of the way with water to keep the stalks alive until they reach maturity. Two of the three bundles were placed in a growth chamber for three days, with the third set in the greenhouse as the control sample. In the growth chamber, the bundles were exposed to twelve hours of light and a temperature of 50^{o}F after being exposed to a temperature range of 65 to 75^{o}F to induce cold shock in the samples. After three days in the growth chamber, the detached spike samples were placed in the greenhouse on station where they remained until they reached full maturity, and then the spikes were harvested from the stalks. The seeds were then cleaned and ground using a UDY Cyclone Sample Mill grinder (UDY Corporation, USA) with a 0.05 mm screen. We used the flour meal to perform the Hagberg FN test to rate LMA results. The results of these preliminary tests showed that 28 DAF was the optimal day to assess the LMA resistance and thus, was used in the present study.

Phenotypic Data Analysis

FN data in parents and the derived lines was used to calculate population mean and standard deviation in each trial and in the correlation analysis among the nine trials. Based on the FN distribution, population mean, and weather data, the best linear unbiased predictions (BLUPs) and broad-sense heritability were estimated for the 2015-2016 trials and the 2017-2019 trials. The BLUPs were calculated using the grand mean for the entire population subtract the mean for each the 111 induvial lines. The BLUPs considered the special variation of the trait. The distribution of the BLUP data in general has a normal distribution.

This is more effective in data analysis than simply using the means. The correlation rating used for all three traits were measured using the R values ranges, 0.2 or below showed low correlations, 0.3 to 0.5 showed moderate correlations. R values that ranged from 0.6 and up showed high correlations. We used the correlation tests to determine how the results were affected by the environment.

Based on daily ratings of PHS, six datasets for PHS (PHS18-D4, PHS18-D5, PHS18-D6, PHS19-D4, PHS19-D5, PHS19-D6) were created for the parents and DH population in

two experiments. PHS data in the parents and the derived lines were used to calculate the population mean and standard deviation in each dataset as well as the correlation analysis among the six datasets from the two experiments. The BLUPs were created to encompass days four through six for 2018 and 2019 (PHSD4_BLUP, PHSD5_BLUP, PHSD6_BLUP).

LMA data in the parents and the derived lines was used to calculate the population mean and standard deviation in each trial as well as in the correlation analysis among the four trials (DSFNC18, DSFNT18, DSFNC19, DSFNT19). BLUPs were created to encompass the two years of control and treated trials (DSFNC18, DSFNT18).

Broad-sense heritability (H^2) was estimated based on the following equation $H^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_E/er)$ (Fehr, 1987). Where σ^2_G is the variance of genotype, σ^2_E is the error in variance, e is the number of environments and r is the number of replications (Fehr, 1987). Broad-sense heritability is the ratio of total genetic variance to total phenotypic variance, while narrow-sense heritability is the ratio of additive genetic variance, focusing on one specific allele, to the total phenotypic variance. The reason we chose the broad-sense heritability is because we needed to see the effects both parental alleles had on the phenotypic data. All data analyses were conducted using the JMP Genomics v8.0 (JMP) statistics program (JMP®, Version 8 SAS Institute Inc., Cary, NC, 1989-2019).

Genotypic Data Analysis and Linkage Map Construction

This study used previously published linkage maps in the mapping population (Liu et al., 2018; Wang et al., 2018). Briefly, the DH population was genotyped using the 90K SNP iSelect platform (Wang, et al., 2014), and 11,632 polymorphic SNP markers were selected and obtained. Among them, 4,213 remained after filtering for monomorphism, high frequency of missing values, or segregation distortion. Forty-two linkage groups (LG), representing all 21 chromosomes of wheat, were identified from the linkage analysis (Liu et al., 2018).

QTL Analysis

For the QTL analysis, the major and minor QTLs were identified using the composite interval mapping model (CIM) in JMP (JMP®, Version 8 SAS Institute Inc., Cary, NC, 1989-2019). The expectation-maximization (EM) (Dellaert, 2002) threshold of 2.5 (LOD>2.5) was used to identify all significant QTLs. The phenotypic variance (R^2) and the additive effects of the QTLs were also obtained from the JMP software. The effect contributions from SYC and UIP were indicated with negative or positive numbers of the additive effect, respectively.

RESULTS

Falling Number Performance in Nine Trials Over 2015 to 2019

FN data of parents and the derived lines were summarized for all nine trials in Table 2.2. Four trials conducted in 2017-2019 had a very limited number of lines (max of five) with FNs less than 300 seconds, while five trials in 2015-2016 had 29 to 85 lines with FNs less than 300 seconds. Correlation values among the 2015-2016 trials were much higher than correlation values among the 2017-2019 trials (Table 2.3). This performance reflects weather data between the flowering and physiological maturity dates of each year. Trials in 2015-2016 matured about two weeks earlier (around July 11) than the trials in 2017-2019 (matured around July 29). Relatively lower temperature and higher moisture occurred during the physiological maturity days (PMDs) (Table 2.1). Therefore, two BLUPs were estimated, BLUP1516FN from the 2015 to 2016 trials, and BLUP1719FN from the 2017 to 2019 trials. The two BLUPs showed a normal distribution, but BLUP1516FN had a much higher broad sense heritability than BLUP1719FN (0.82 vs. 0.45) (Figure 2.3). The FN data in UIP was much higher than that in SYC in trials in 2015-2016, but the difference of the two parents was minimal in trials in 2017-2019. For the correlation ratings, high correlations were set at 0.6 and up, moderate correlations were set at 0.3 to 0.5, and low correlations were 0.2 and below. The FN values from 2015, 2016 and 2017 showed low to high correlations (r^2 ranged from 0.20 to 0.82) between the various environments. The 2018 and 2019 FN values showed little to no relationship with any of the environments confirming that there were stark differences between these two years and the other trials.

PHS Resistance in Two Greenhouse Experiments

PHS data in parents and the derived lines was summarized for all six trials in Table 2.4. PHS scores from Day 4 to Day 6 changed from 3.47 to 7.24 based on the BLUP data (Table 2.4). The broad sense heritability at Day 6 was the highest, while the lowest was at Day 4. The parental line, SY Capstone (SYC), showed a lower PHS rating than UI Platinum (UIP) although, the differences between the two parents were small (e.g. day four's rating for both years were exactly the same) Both parents were moderately susceptible to PHS, but transgressive segregation was commonly observed (Table 2.4). Correlation values among the three rating days (day 4, 5 and 6), within the same experiment year (2018 or 2019), were much higher, ranging from 0.60 to 0.92, than those between the two experiments (Table 2.5), or two years (2018 and 2019), ranging from 0.26 to 0.37. Correlation values between individual ratings with the PHS BLUP data were all high; ranging from 0.60 to 0.92 (Table 2.5).

LMA Resistance in a Detached Spike Greenhouse Experiment

We used FN data to measure LMA that was induced by cold temperature, which is a commonly accepted approach to assess resistance to LMA. Both parents responded to the cold treatment, but their FN stayed above 300 seconds. The FN of UIP changed from 397 to 362 seconds, while the FN of SYC changed from 388 to 372 seconds (Table 2.6). When the FN of a line decreased to less than 300 seconds in the cold-treated sample, the line was called susceptible. When a line remained unchanged or the FN increased, the line was called resistant. In the 2018 detached spike test, in the cold-treated lines, 8 lines were susceptible, 24 lines were resistant, and 76 lines had a decrease in FN but stayed above 300 seconds. The FN correlation value was 0.66 between the FN of untreated and cold treated among lines in the population. The histogram of the FN distribution of untreated and cold treated samples showed a broad but normal variation of the results, suggesting the mode of multiple gene control (Figure 2.5). In this one-year experiment, we were not able to estimate broad sense heritability.

QTL Detection

QTL Associated With FN

A total of sixteen QTL were detected on seven chromosome regions (Table 2.7), explaining 10 to 34% of phenotypic variation. Most of the QTL were detected in trials in 2015-2016. Five of 16 QTL were detected in three or more data sets. These major QTL include *QFN.UIA-1B-1, QFN.UIA-2B, QFN.UIA-3B-1, QFN.UIA-5A-1,* and *QFN.UIA-7A-1*. *QFN.UIA-2B* was detected in four of the five trials in 2015-2016 and one of the four trials in 2017-2019. *QFN.UIA-1B-1, QFN.UIA-5A-1, QFN.UIA-7A-1* were only detected in trials in 2015-2016, while *QFN.UIA-3B-1* was only detected in trials in 2017-2019. UIP contributed to the high FN for *QFN.UIA-5A-1,* effect shown as a positive number, while SYC contributed to the high FN for the other four QTL and the effect shown was displayed as a negative number. This was done to help distinguish between the two parental alleles and to show which had the greater positive influence in the QTLs for all three traits.

QTL Associated With PHS

A total of nine QTLs were located on six chromosome regions (Table 2.8), that explain 10 to 34% of the phenotypic variation for PHS. Three of the nine QTLs were detected in three or more data sets. These major QTL include *QPHS.UIA-1A, QPHS.UIA-1B* and *QPHS.UIA-5A. QPHS.UIA-5A* was detected in two data sets in 2018, one in 2019, and two BLUPs. *QPHS.UIA-1A* was only detected in two data sets in the 2018 experiment and two BLUPS while *QPHS.UIA-1B* was only detected in three data sets in 2019. SYC contributed higher PHS scores for *QPHS.UIA-1A* and *QPHS.UIA-5A,* while UIP contributed higher PHS scores for *QPHS.UIA-1B*.

QTL Associated With LMA

FN data from non-treated and cold-treated lines was used separately in QTL detection. A total of seven QTL on seven chromosome regions were detected (Table 2.9), explaining 11 to 29% of the phenotypic variation for LMA. Three QTL, *QLMA.UIA-4A, QLMA.UIA-5A,*

and *QLMA.UIA-7A* were associated with FN data from both treated and non-treated lines; however, the QTL effect (29.76 sec) of the *QLMA.UIA-7A* treated lines was much higher than that on the non-treated lines (20.78 sec.). UIP contributed to the higher FN for *QLMA.UIA-4A* and *QLMA.UIA-5A*, while SYC contributed to higher FN for *QLMA.UIA-7A*. Two QTL, *QLMA.UIA-2B* and *QLMA.UIA-5B* were associated with FN only from non-treated lines, while *QLMA.uiA-2D* and *QLMA.UIA-3B* were only associated with FN from the treated lines.

DISCUSSION

The present study used a unique mapping population consisting of 110 double haploid lines of hard white spring wheat derived from two high yielding cultivars, UI Platinum (UIP) and SY Capstone (SYC). The population and parental lines were assessed simultaneously for FN, PHS and LMA and genotyped with the advanced genotyping platform, Wheat 90K SNP Illumina iSelect. QTL information for the three traits will help us to understand the similarities and differences of the three traits, facilitate the selection method development, and improve breeding efficiency.

Phenotypic Performance Strongly Affected by Environment

FN assessment in the parents and the derived DH lines were conducted in nine field trials. Five trials in 2015-2016 had a number of lines with an FN less than 300 sec. and were considered as low FN (LFN) years, while the four trials in 2017-2019 had very few lines with low FNs and were considered as normal crop years. This can be seen in the BLUPs for the two sets of trials. Weather conditions (rainfall and temperature) during grain filling (from flowering to physiological maturity) and maturation (from physiological maturity to harvesting) are possible reasons for these differences (Table 2.1 and Figure2.1). There was a cold temperature (below 60^{o}F) event around July 10th, about twenty-five days after flowering (DAF) occurred for the trials in AB in 2015 and 2016. There was another cold event around July 28th, seventeen days after PMD coupled with 0.07 inches of rain occurred in AB in 2015. The two AB trials received 4.32 and 4.65 inches of rainfall and irrigation waters for 2015AB and 2016AB trials, respectively. In contrast, there was no cold temperature events occurred in

trials in AB over 2017 to 2-18 (Figure 2.1), the irrigation water received in the three trials (2.38” in 2017AB, 1.47” in 2018AB, 0.91” in 2019AB) was much smaller than that in 2015AB and 2016AB (Table 2.1). 2016SD is a dryland location, it had a cold event around July 10th, fourteen DAF, coupled with 0.09 inches of rainfall, but this trial only received 0.2 inches rainfall after PMD before harvesting. It is hard to understand why so many LFN lines showed in that trial/year. To fully understand the weather effects on the FN performance, it is necessary to conduct additional experiments with more accurate weather data and more accurate plant growth stage record data in the future.

Although PHS experiments were conducted in the greenhouse, the correlation was much smaller (<0.41) between the two greenhouse experiments than among the three PHS ratings within the same experiment (>0.78). The two field trials in 2018 and 2019 had close physiological maturity dates (PMD) (July 23 to July 31), but flowering dates (FD) in 2018 occurred from June 17 to June 23, approximately one week earlier than in 2019 (June 25 to July 1). FD difference may have caused the poor correlation between the two years, although PHS sampling was based on PMD.

Accurate screening of LMA resistance was also affected by environments in the detached spike test. We observed sprouting in some non-treated lines during cold treatment due to limited growth chamber space, and there were too many samples that were treated simultaneously; therefore, we were unable to use the 2019 data in the final analysis.

QTL for FN, PHS, and LMA

QTL for FN

Of the sixteen QTL identified, five (*QFN.UIA-1B-1, QFN.UIA-2B, QFN.UIA-3B-1, QFN.UIA-5A-1, QFN.UIA-7A-1*) were detected in three or more data sets. QTL for FN on 1B was reported by Zhang, and others (2014), but its position was different from that of *QFN.UIA-1B-1*, which was identified in the present study. The *QFN.UIA-1B-1* was found in three environments and showed strong LOD scores ranging from 4.18 to 5.60, explaining 16 to 21% of the genetic variation. SYC contributed to the high FN

A QTL on *QFN.UIA-2B* was detected in six environments with strong LOD scores ranging from 3.77 to 9.61, explaining 10 to 34% of the genetic variation. QTL on 2B was reported by Tang, and others (2017), but its position cannot be compared because they used the SSR marker platform. QTL on 2B was also reported by Zhang and others (2014), but its position they found was different from that of *QFN.UIA-2B* identified in the present study. SYC contributed to the high FN score.

QFN.UIA-3B-1 was found in three environments with strong LOD scores ranging from 3.43 to 5.47 and explains 13 to 18% of the genetic variation. QTL for FN on 3B has been reported in previous research but was a minor QTL in the study by Tang and others (2017). In this study it was detected as a major QTL for FN. SYC contributed to the high FN score.

QFN.UIA-5A-1 was found in three environments with strong LOD scores that ranged from 3.64 to 6.37. *QFN.UIA-5A-1* explaining 10 to 30% of the genetic variation. QTL for FN on 5A was reported by Bȍner and others (2017)and Martinez and others (2018), but its position in Martienz and others (2018), is different from that of *QFN.UIA-5A-1* in the present study, and it cannot be compared with Bȍner and others (2017) because of the different marker platform Bȍner used. UIP contributed to the high FN score.

QFN.UIA-7A-1 was detected in three environments with one LOD score that was weaker than most but still good, and the other two were very good; they ranged from 2.87 to 6.25; explaining 11 to 24% of the genetic variation. QTL for FN on 7A was reported Martinez and others (2018), but its position is different from that of *QFN.UIA-7A-1* in this study. SYC contributed to the high FN score.

QTL for PHS

Of nine QTLs identified, three (*QPHS.UIA-1A*, *QPHS.UIA-1B* and *QPHS.UIA-5A*) were detected in two or more data sets. QTLs for PHS on 1A and 1B were reported by Martinez and others (2018), but their positions were different from those identified in the present study. *QPHS.UIA-1A* was only detected in two data sets in the 2018 experiment and two BLUPs, explaining 11 to 14% of the genetic variation, while *QPHS.UIA-1B* was only

detected in three data sets in the 2019 experiment, explaining 19 to 34% of phenotypic variation. UIP contributed to the low PHS score for *QPHS.UIA-1B*. SYC contributed to the low PHS score for *QPHS.UIA-1A*.

QPHS.UIA-5A was detected in three data sets explaining 11 to 25% of the genetic variation. A QTL on 5A for PHS was reported by Martinez and others (2018); the position, however, was different from that of *QPHS.UIA-5A*. SYC contributed to the low PHS score.

QTL for LMA

A total of seven QTL on seven chromosome regions were detected (Table 2.9), explaining 11 to 29% of the phenotypic variation for LMA. Three QTL, *QLMA.UIA-4A*, *QLMA.UIA-5A*, and *QLMA.UIA-7A*, were associated with FN data from both treated and non-treated lines; however, the QTL effect (29.76 sec.) of the *QLMA.UIA-7A* on treated lines was much higher than that on the non-treated lines (20.78 sec.). Two QTL, *QLMA.UIA-2B* and *QLMA.UIA-5B*, were associated with FNs only from non-treated lines, while *QLMA.UIA-2D* and *QLMA.UIA-3B* were associated with FNs only from treated lines. QTL for LMA on 4A was reported in Emebiri et al., 2010 but its position cannot be compared because of the different marker platform used. UIP contributed to the low LMA score. *QLMA.UIA-2B*, *QLMA.UIA-5B* and *QLMA.UIA-3B* were also present in the FN QTL analysis of this study. *QLMA.UIA-5A*and *QLMA.UIA-7A*are novel QTLs since they have not been reported in previous LMA studies. UIP contributed to the high FN (LMA resistance) for *QLMA.UIA-5A* and SYC contributed to the low FN (LMA susceptibility) for *QLMA.UIA-7A*.

Comparisons of QTL for FN, FN, PHS, and LMA and Possible Breeding Strategies

The present study conducted a comparative QTL mapping for the three related traits in a doubled haploid population derived from two hard white spring wheat cultivars, UIP and SYC. Four chromosomal regions each has multiple QTL associated with two to three traits assessed in the present study (Figure 2.6). QTL on 5A was associated with all three traits, and UIP contributed to higher FN, resistance to PHS, and LMA. Compared to the physical map of

Chinese Spring, the 5A QTL is in the flanking region of the *Amy 3* gene (Figure 2.7). This is the first report that describes the *Amy 3* gene in relation to FN, PHS, and LMA. This finding will help us to understand the relationships among FN, PHS, and LMA, as well as to develop selection strategies of the three traits after they are confirmed.

QTL on 7A-1 was associated with FN and LMA, SYC contributed to higher FN and higher FN induced by cold treatment (LMA resistance). QTL on 1B-1 was associated with FN and PHS, UIP contributed to the lower FN and higher PHS score. QTL on 2B was also associated with FN and LMA, SYC contributed to the higher FN and lower FN-LMA score.

Based on above analysis both parents have desirable and complementary alleles for different QTL in different chromosome regions, suggesting that resistant lines to FN, PHS and LMA can be selected through genetic recombination. The present study suggests that the best strategy is to select UIP alleles at QTL on chromosome 5A, but SYC alleles at QTL on chromosome on 1B-1, 2B, and 7A-1. Using this strategy, few lines were selected in the present population that have improved PHS and LMA resistance over both parents.

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Table 2.1 Dates of heading, anthesis, physiological maturity (PMD), harvesting, rainfall and irrigation (“) during the four wheat-growing seasons from the Aberdeen and Soda Spring of the eight trials.

Trial*	Heading	Anthesis	PMD	Harvesting	Rainfall after PMD Dates	Irrigation after PMD
2015AB	6/8/2015	6/15/2015	7/11/2015	8/18/2015	0.25, 7/15; 0.25, 7/22-24; 0.07, 7/27; 0.35, 8/07-08; 0.01, 8/10; 0.03, 8/14	1.89, 7/13-14; 1.47, 7/15
2016AB	6/8/2016	6/15/2016	7/15/2016	8/15/2016	None	1.89, 7/13; 1.26, 7/20; 1.5, 7/27
2016SD	6/19/2016	6/26/2016	7/26/2016	8/26/2016	0.03, 7/31; 0.01, 8/04; 0.11, 8/07; 0.04, 8/17; 0.01, 8/20	Dryland trial
2017AB	6/19/2017	6/26/2017	7/27/2017	8/23/17	0.16, 7/26-27; 0.26, 8/08; 0.02, 8/11; 0.05, 8/14	1.89, 7/25
2018AB	6/11/2018	6/18/2018	7/25/2018	8/14/2018	None	1.47, 7/24
2019AB	6/21/2019	6/28/2019	7/29/2019	8/23/2019	0.07, 7/29	0.84, 8/01

*Trial names were defined in materials and methods.

Table 2.2 Summary of FN performance in parents and the derived lines in nine trials over 2015 to 2019

Trial*	----------Parents----------		----------DHLs----------				
	UIP	SYC	MAX	MIN	Mean	SD	h^2_b
15ABF206	344	265	396	190	313.94	36	
15ABF210	338	273	406	205	313.21	38	
15ABF309	351	297	408	199	318.95	37	
16AB	344	334	366	200	311.50	25	
16SD	284	174	360	173	272.56	38	
15-16BLUP	330	271	363	233	306.40	21	0.82
17AB1	377	366	464	287	362.19	34	
17AB2	355	339	421	294	351.65	25	
18AB	331	352	470	253	364.87	37	
19AB	421	425	527	351	430.34	36	
17-19BLUP	375	372	399	353	377.45	9	0.45

*Trial names were defined in materials and methods.

Table 2.3 Correlations among FN data from different trials

Trial*	15ABF206	15ABF210	15ABF309	16AB	16SD	17AB1	17AB2	18AB	19AB	15-16BLUP
15ABF210	0.82***									
15ABF309	0.73***	0.72***								
16AB	0.42***	0.49***	0.41***							
16SD	0.33**	0.33**	0.32**	0.23*						
17AB1	0.43***	0.38***	0.36**	0.26**	0.22*					
17AB2	0.45***	0.53***	0.50***	0.47***	0.20*	0.22*				
18AB	0.04	0.14	0.09	0.30**	0.06	0.19	0.34**			
19AB	0.15	0.20*	0.21*	0.22*	0.02	0.10	0.16	0.10		
15-16BLUP	0.87***	0.89***	0.84***	0.62***	0.60***	0.43***	0.55***	0.16	0.19	
17-19BLUP	0.40***	0.48***	0.43***	0.48***	0.17	0.61***	0.62***	0.67***	0.58***	0.50***

*Trial names were defined in materials and methods.

Table 2.4 Summary of pre-harvest sprouting data in parents and derived doubled haploid lines in two spike germination experiment from 2018 to 2019

PHS*	----------Parents----------		----------DHLs----------				
	UIP[a]	SYC[a]	MAX	MIN	Mean	SD	h^2_b
PHS_D4-18	4	4	6	1	3.29	1.06	
PHS_D4-19	4	4	6	2	3.70	0.98	
BLUP_PHSD4	4	4	4	3	3.47	0.32	0.42
PHS_D5-18	6	5	8	2	5.25	1.09	
PHS_D5-19	5	5	8	2	5.21	1.10	
BLUP_PHSD5	5	5	6	4	5.18	0.44	0.53
PHS_D6-18	8	8	9	4	7.42	1.18	
PHS_D6-19	8	7	9	3	7.15	1.12	
BLUP_PHSD6	8	8	8	6	7.24	0.55	0.58

*PHS scores assessed on day 4, 5, 6 and the BLUPs of each day over two years in 2018 and 2019, respectively.

Table 2.5 Correlations among different PHS data sets derived from 2018 and 2019

PHS*	PHS_D4-18	PHS_D5-18	PHS_D6-18	PHS_D4-19	PHS_D5-19	PHS_D6-19	BLUP_PHSD4	BLUP_PHSD5
PHS_D5-18	0.77***							
PHS_D6-18	0.67***	0.86***						
PHS_D4-19	0.26**	0.35**	0.32**					
PHS_D5-19	0.30**	0.36***	0.37***	0.88***				
PHS_D6-19	0.33**	0.40***	0.41***	0.78***	0.91***			
BLUP_PHSD4	0.81***	0.71***	0.63***	0.78***	0.73***	0.69***		
BLUP_PHSD5	0.65***	0.82***	0.74***	0.74***	0.83***	0.80***	0.87***	
BLUP_PHSD6	0.60***	0.75***	0.85***	0.64***	0.76***	0.83***	0.78***	0.92***

*PHS scores assessed on day 4, 5, 6 and the BLUPs of each day over two years in 2018 and 2019, respectively.

Table 2.6 Summary FN derived from detached spike method for late maturity alpha amylase in parents and derived doubled haploid lines in 2018

Treatment	----------Parents----------		----------DHLs----------				
	UIP	SYC	Max	Min.	Aver.	SD	h^2_b
DSFNC18	397	388	489	288	394.05	38	
DSFNT18	362	372	458	261	363.67	42	

Table 2.7 Significant QTL for falling number traits identified

QTL	Trial*	Peak position (cM)	LOD	Effect[a]	R2 (%)
QFN.UIA-1B-1	15ABF210	86.47	5.01	-28.08	19
	15ABF309	86.47	5.6	-30.04	21
	15-16BLUP	86.47	4.18	-14.52	16
QFN.UIA-1B-2	15ABF206	156.4	4.86	-26.19	18
QFN.UIA-2A-1	16SD	38.51	2.97	-20.37	12
	15-16BLUP	43.06	4.47	-13.56	17
QFN.UIA-2A-2	17AB2	100.96	5.42	19.546	20
	17-19BLUP	102.77	6.68	7.6742	24
QFN.UIA-2A-3	16AB	171.4	5.25	20.318	20
QFN.UIA-2B	15ABF206	20.95	6.97	-29	25
	15ABF210	21.86	9.61	-34.82	34
	15ABF309	20.95	2.6	-17.29	10
	16AB	25.5	3.77	-15.53	15
	15-16BLUP	20.95	5.25	-14.37	20
	17AB1	25.5	6.81	-30.65	25
	17-19BLUP	25.5	5.11	-6.124	19
QFN.UIA-3B-1	17AB1	87.66	4.63	-24.93	18
	18AB	94.94	3.43	-23.75	13
	17-19BLUP	85.84	5.47	-6.446	20
QFN.UIA-3B-2	16AB	126.38	4.85	-18.39	18
QFN.UIA-5A-1	15ABF206	6.37	2.53	19.755	10
	15ABF210	6.37	8.33	30.87	30
	16SD	3.64	3.17	21.16	12
	15-16BLUP	6.37	3.76	12.995	15
QFN.UIA-5A-2	15ABF309	57.21	6.01	29.134	22
	15-16BLUP	59.21	7.84	19.248	28
QFN.UIA-5A-3	18AB	166.89	2.67	-20.84	11
QFN.UIA-5A-4	19AB	147.77	2.8	19.891	11
QFN.UIA-5B	17AB1	96.72	3.89	25.359	15
QFN.UIA-7A-1	15ABF206	37.38	2.87	-20.85	11
	15ABF210	37.38	6.25	-28.81	24
	15ABF309	31.92	5.35	-26.47	20
	15-16BLUP	24.61	6.3	-16.3	23
QFN.UIA-7A-2	16SD	156.35	4.13	24.628	16
QFN.UIA-7A-3	19AB	177.33	4.59	-26.43	17

*Trial names were defined in materials and methods.

[a] The effect contribution from SYC is indicated by a negative and UIP is indicated by a positive number.

Table 2.8 Significant QTL identified in different PHS data sets

QTL	Data Set	Peak position (cM)	LOD	Effect[a]	R2 (%
QPHS.UIA-1A	PHS_D5-18	57.29	2.65	-0.68	11
	PHS_D6-18	57.29	3.58	-0.86	14
	BLUP_PHSD5	57.29	4.28	-0.35	16
	BLUP_PHSD6	57.29	6.12	-0.51	23
QPHS.UIA-1B	PHS_D4-19	56.09	5.03	0.817	19
	PHS_D5-19	56.09	9.89	1.243	34
	PHS_D6-19	54.28	5.03	0.856	19
QPHS.UIA-2A-1	PHS_D4-18	29.37	2.54	-0.59	10
QPHS.UIA-2A-2	PHS_D6-19	86.36	6.02	-0.95	22
QPHS.UIA-2A-3	BLUP_PHSD5	62.29	2.7	-0.24	11
QPHS.UIA-3D	PHS_D6-19	177.03	3.22	0.656	13
QPHS.UIA-5A	PHS_D5-18	3.64	5.93	-0.89	22
	PHS_D6-18	3.64	6.85	-1.04	25
	PHS_D4-19	6.37	2.86	-0.55	11
	BLUP_PHSD5	3.64	4.97	-0.32	19
	BLUP_PHSD6	3.64	5.1	-0.4	19
QPHS.UIA-7A-1	BLUP_PHSD5	37.38	3.09	0.251	12
QPHS.UIA-7A-2	BLUP_PHSD6	170.94	2.97	0.302	12

[a] The effect contribution from SYC is indicated by a negative and UIP is indicated by a positive number.

Table 2.9 Significant QTL for LMA resistance identified from 2018 detached spike test

QTL	Environment	Peak position (cM)	LOD	Effect[a]	R2 (%)
QTLMA.UIA-2B	DSFNC18	83.78	3.76	-22.91	0.15
QTLMA.UIA-2D	DSFNT18	119.48	2.81	21.54	0.11
QTLMA.UIA-3B	DSFNT18	6.39	2.88	-22.44	0.12
QTLMA.UIA-4A	DSFNT18	1.82	7.94	37.26	0.29
	DSFNC18	22.77	7.01	32.13	0.26
QTLMA.UIA-5A	DSFNT18	0	3.93	24.61	0.16
	DSFNC18	3.64	3.18	21.00	0.13
QTLMA.UIA-5B	DSFNC18	163.04	3.52	22.10	0.14
QTLMA.UIA-7A	DSFNC18	31.92	2.94	-20.78	0.12
	DSFNT18	31.92	5.07	-29.76	0.20

[a] The effect contribution from SYC is indicated by a negative and UIP is indicated by a positive number.

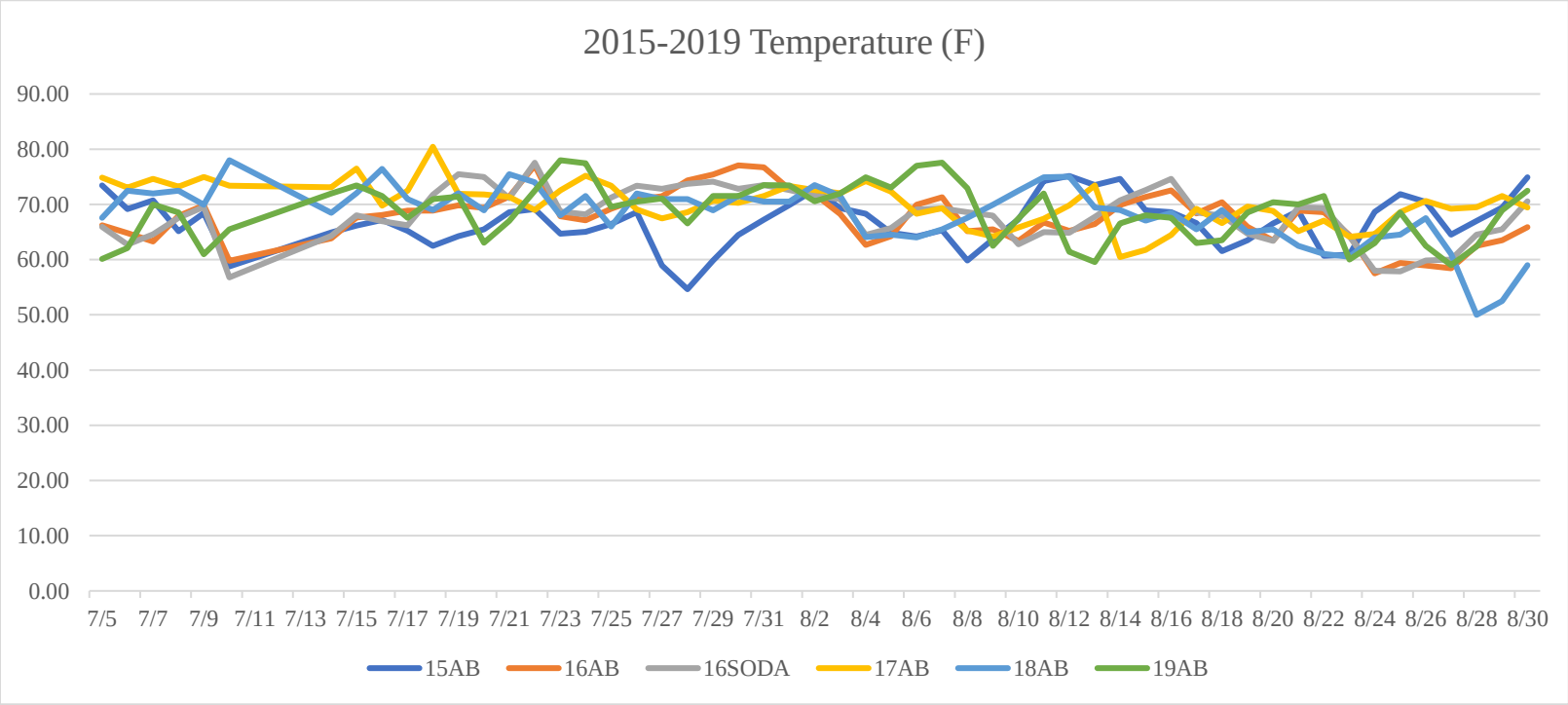
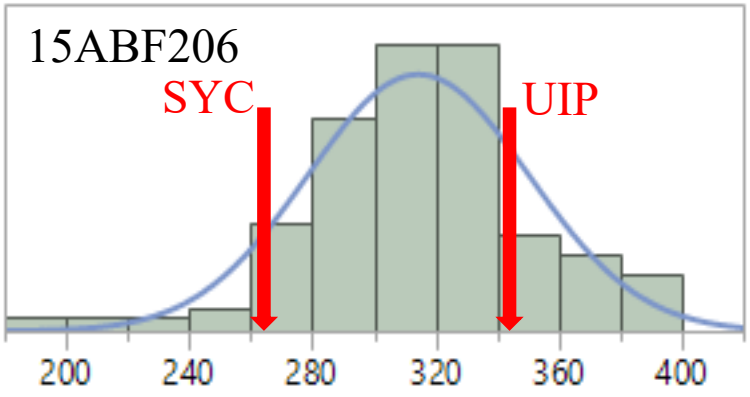
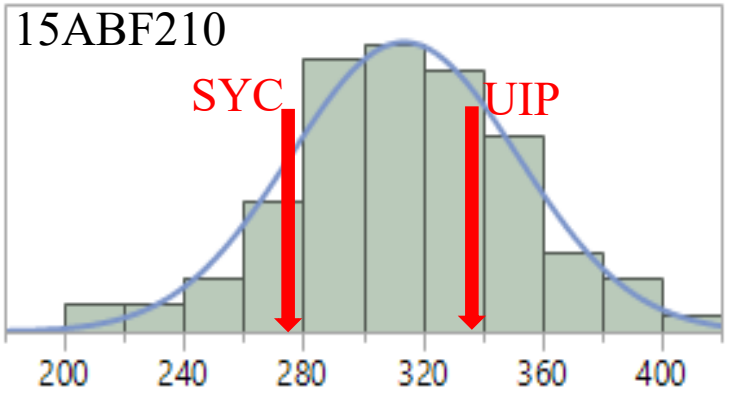


Figure 2.1 Temperature (ºF) during the four wheat-growing seasons from the Aberdeen and Soda Spring of the eight trials.

Figure 2.2 Pre-harvest sprouting (PHS) rating system developed at (Cornell University) (Paterson et al., 1989) 0-2 showing high resistance to PHS, 3-6 showing moderate resistance, and 7-9 showing low resistance to PHS


15ABF206
SYC
UIP
200
240
280
320
360
400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UIP
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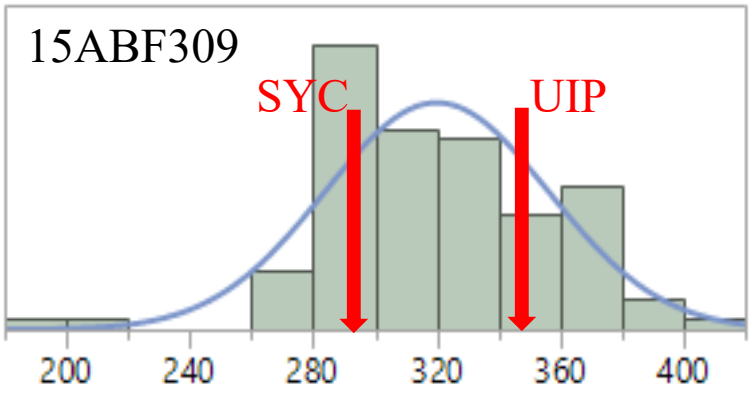
400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SYC
UIP
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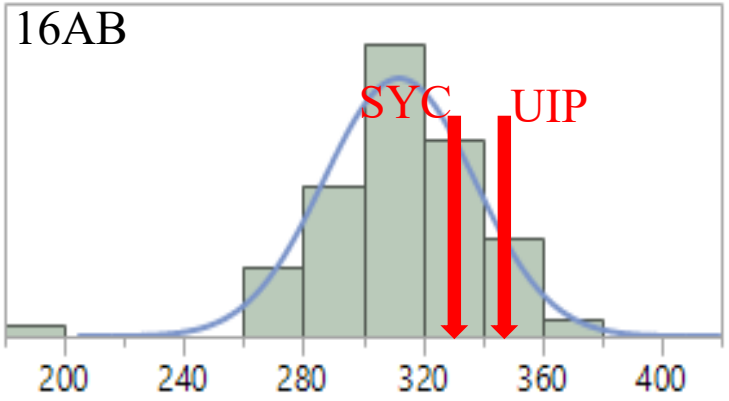
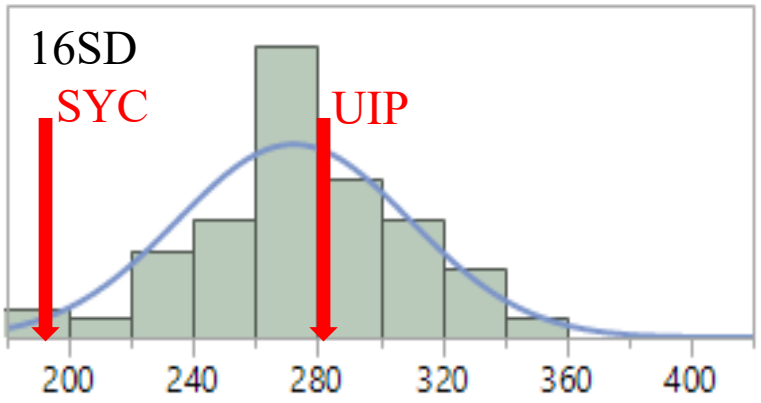

16AB
SYC
UIP
200
240
280
320
360
400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SYC
UIP
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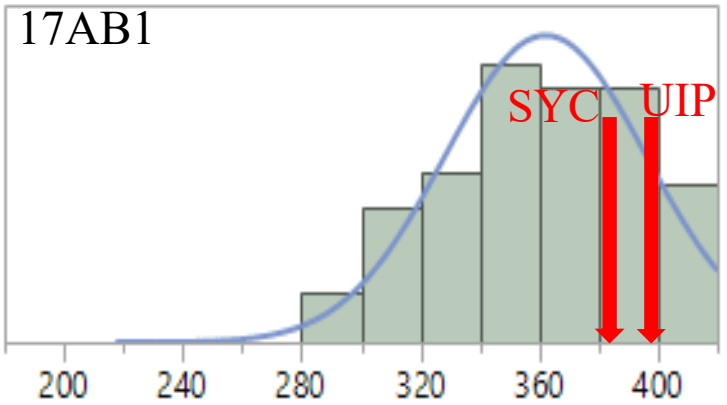
280
320
360
400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UIP
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280
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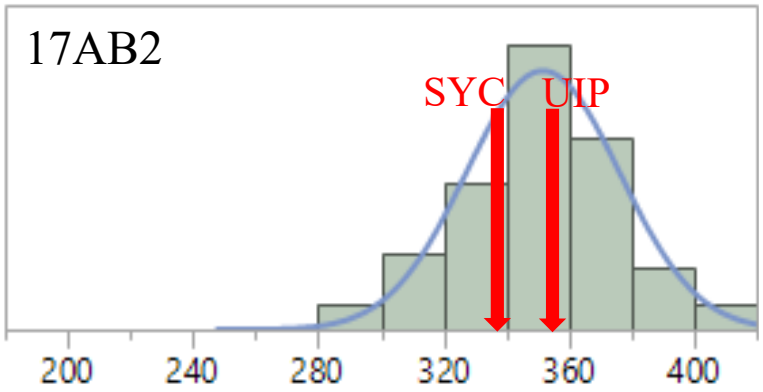
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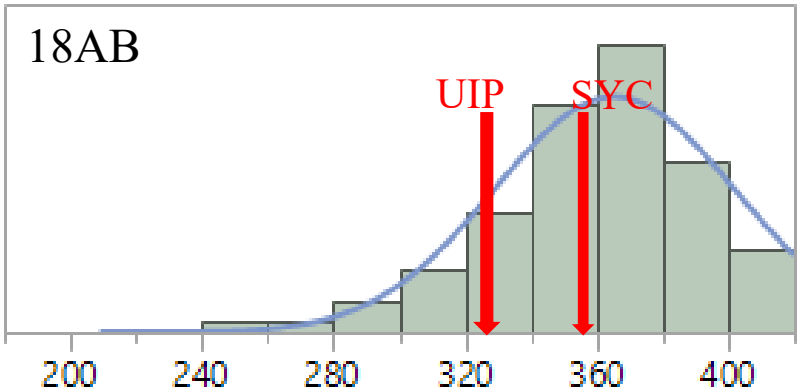
18AB
UIP
SYC
200
240
280
320
360
400

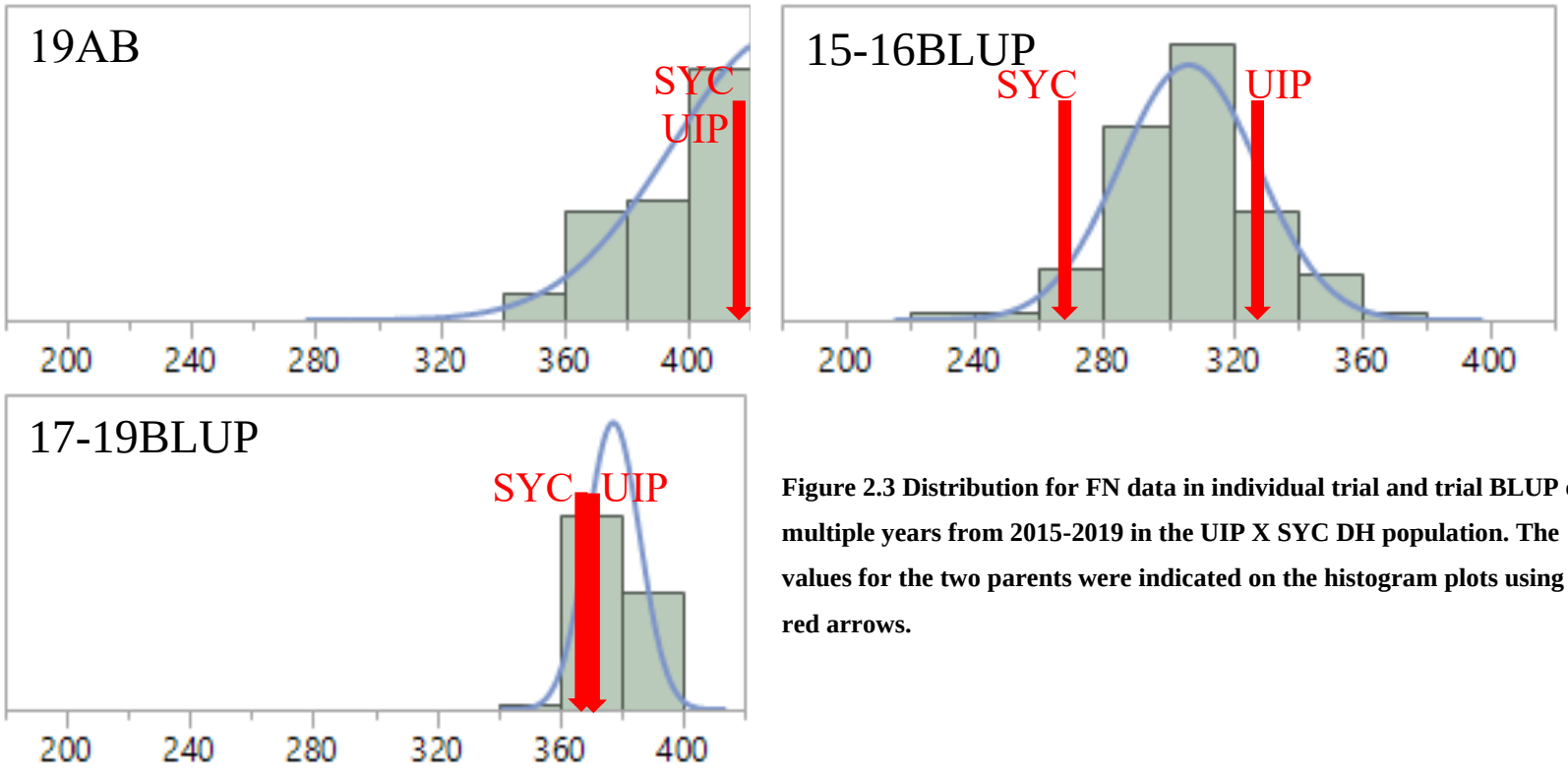

Figure 2.3 Distribution for FN data in individual trial and trial BLUP of multiple years from 2015-2019 in the UIP X SYC DH population. The values for the two parents were indicated on the histogram plots using red arrows.

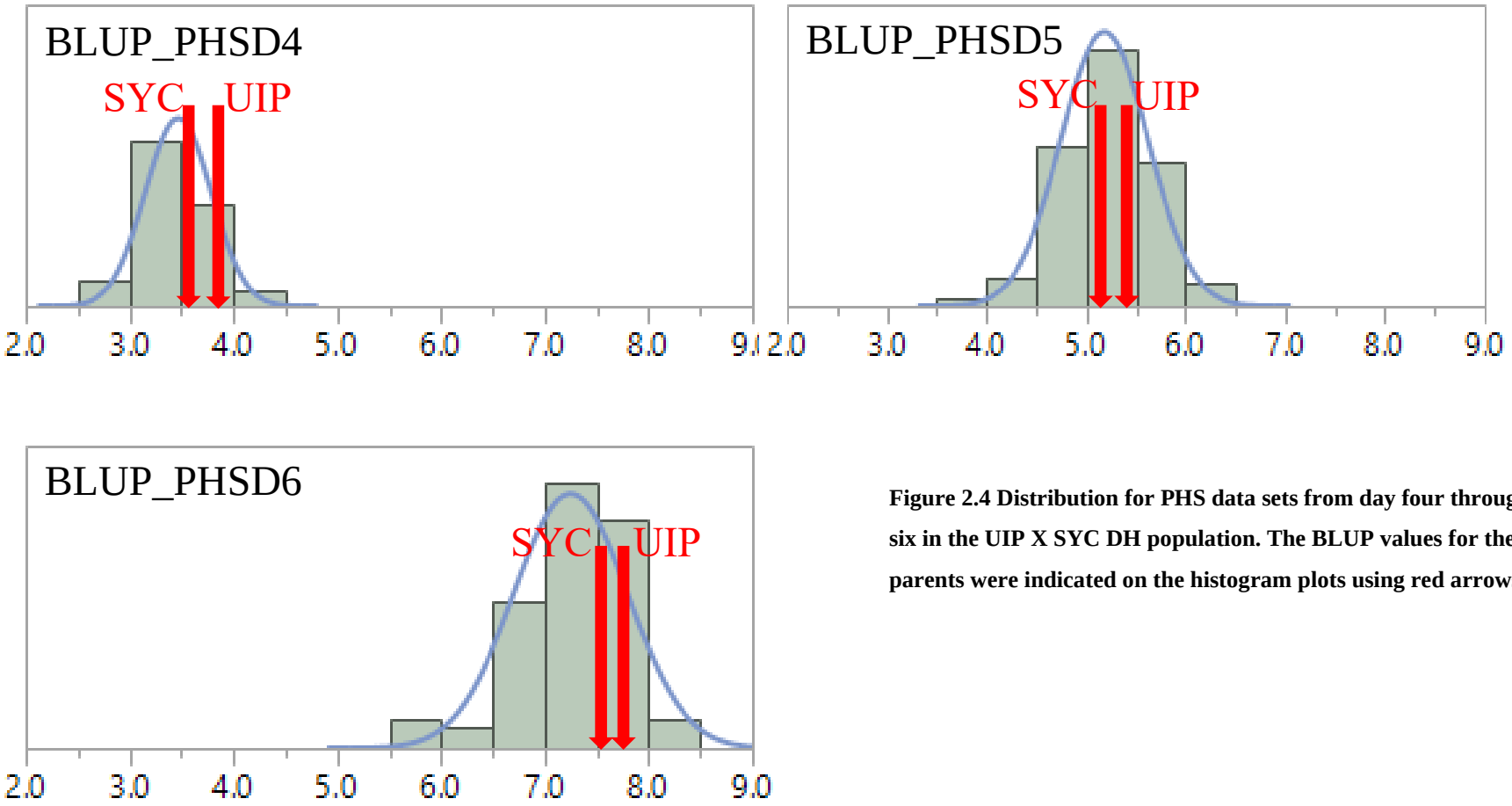

Figure 2.4 Distribution for PHS data sets from day four through day six in the UIP X SYC DH population. The BLUP values for the two parents were indicated on the histogram plots using red arrows.

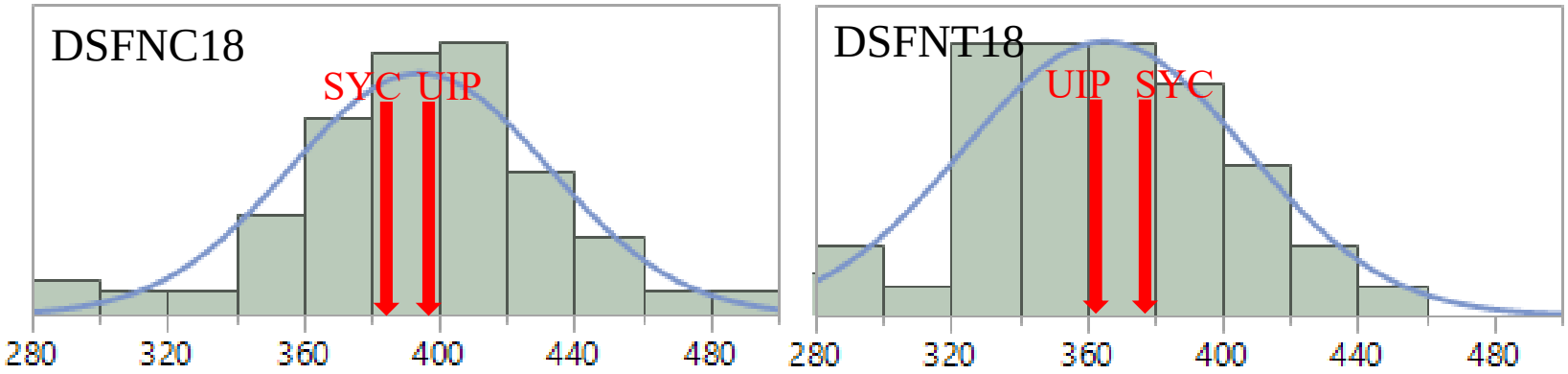

Figure 2.5 Distribution for the detached spike LMA trials from 2018 in the UIP X SYC DH population. The values for the two parents were indicated on the histogram plots using red arrows.

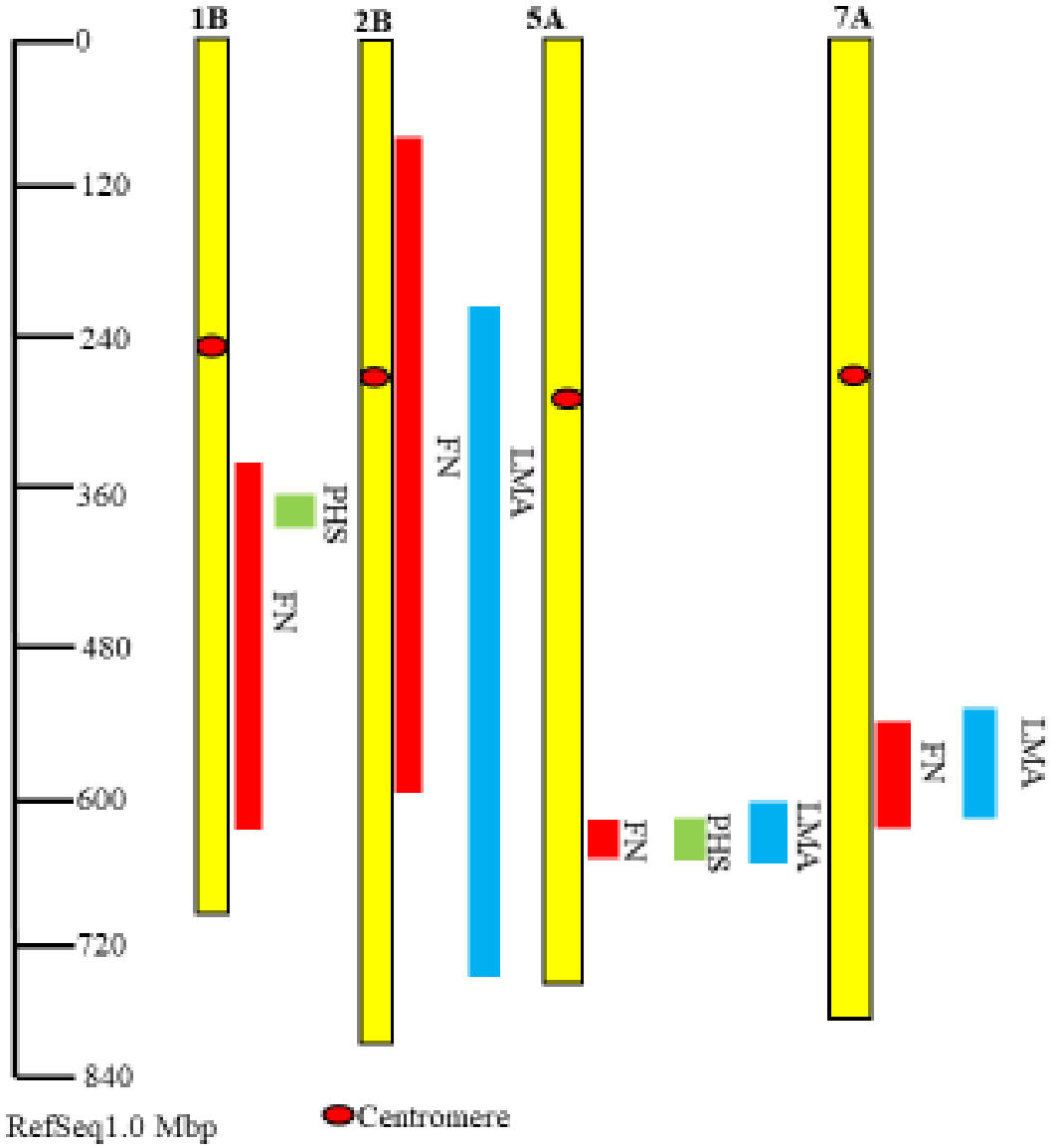

Figure 2.6 Major QTL and map co-locations of QTLs for falling numbers (FN), pre-harvest sprouting (PHS) and late maturity alpha amylase (LMA).

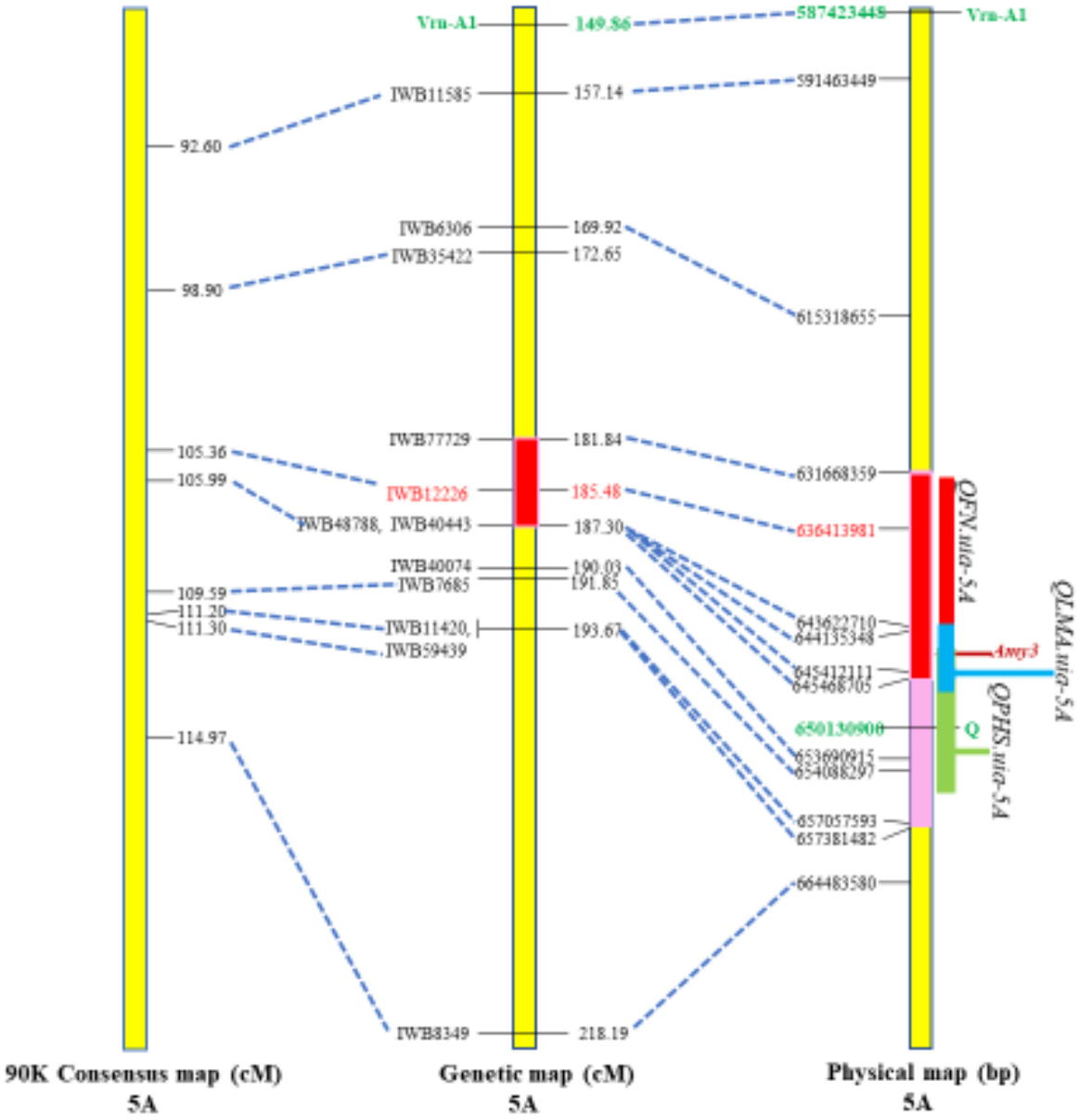

Figure 2.7 QTL 5A and map co-locations for FN, PHS and LMA. *Amy3*'s location is also shown on this map.