AN ABSTRACT OF THE DISSERTATION OF

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Title: Identification of Quantitative Trait Loci Underlying Kernel Extra-softness and Related Traits by Linkage and Association Mapping in Wheat (*Triticum aestivum* L.)

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Kernel hardness (KHA) is a major factor determining break flour yield (BFY) and end-use quality of common wheat (*Triticum aestivum* L.). Within the soft wheat class, genotypes with consistently softer grains than common soft wheat are considered to be 'extra-soft'. In addition, 'extra-soft' wheats have greater BFY than common soft wheat lines. In order to better understand this interrelationship, a set of 164 F_6-recombinant inbred lines (RILs) developed from a soft × 'extra-soft' wheat cross was evaluated for KHA, BFY, and other related traits in six field environments. The estimates of broad-sense heritability for KHA and BFY ranged from 0.84 to 0.96 and 0.56 to 0.76, respectively. Significant environmental effects and genotype by environment interactions were detected for all traits evaluated.

A comprehensive genetic linkage map was created with 650 molecular markers based on this mapping population. Three chromosome translocations, 1BL.1RS, 2NˇS-2AS.2AL and 5B:7B, were identified during linkage analysis. A total of 47 quantitative trait loci (QTL) were identified for nine traits including KHA, BFY, bran yield (BRN), unground middling yield (MID), plant height (PHT), days to heading (HDD), thousand-kernel weight (TKW), grain protein content (GPC), and test weight (TWT). The number of QTL per trait ranged from three for MID to nine for GPC. The phenotypic variance explained by individual QTL ranged from 5.8 to 47.6%. Among five QTL identified for

KHA, the two most important QTL were located on chromosomes 4DS (*Xbarc1118-Rht-D1 interval*) and 4BS (*Xwmc617-Rht-B1 interval*), indicating that the 'extra-soft' characteristic was not controlled by the 5DS Hardness (*Ha*) locus which encodes the two puroindoline genes *pinA* and *pinB*. The co-location of QTL for KHA, BFY, BRN, and MID on 4DS suggested that genetic factors affecting KHA may have a pleiotropic effect on BFY. Two co-located QTL for TWT, TKW and PHT were detected on 4DS and 4BS, and a QTL for HDD was detected on 4DS, indicating that these QTL may represent the consequence of the semi-dwarfing green-revolution genes *Rht-D1* and *Rht-B1* located on 4DS and 4BS, respectively. Additional analysis suggested that the QTL for KHA on 4DS and 4BS are the effects of genes linked to *Rht-D1* and *Rht-B1*, rather than pleiotropic effects of these genes. Some coincident QTL for the traits that were evaluated represent the interrelationships of phenotypic traits, where both KHA and BFY were associated with HDD and TWT based on path coefficient analysis.

Association mapping can be an effective means for identifying, validating, and fine mapping genes and QTL in crop plants. To test this approach, a set of 94 diverse elite wheat lines was phenotyped for five important traits and genotyped with 487 molecular markers. In this study, the marker-trait association analysis showed that the gene *pinB* (*Ha* locus) was significantly associated with KHA as it is known to define the difference between soft and hard wheat classes. Additionally, the significant associations of marker *XwPt-7187* with KHA, *XwPt-1250* and X*wPt-4628* with TWT, and *Xgwm512* with PHT mark the first report of such associations in these genomic regions.

This study, aiming at the genetic dissection of wheat kernel extra-softness and related traits, enhanced our understanding of both genetic control of and environmental effects on these important traits. Path coefficient analysis showed the promise of an alternative phenotypic selection approach that is more cost effective than direct measurement of kernel quality. Three chromosome translocations were discovered and their approximate chromosome break points were located. Numerous QTL were identified for these important traits. The major QTL can serve as a start point for fine mapping that eventually lead to the cloning of the QTL through map-based or candidate gene approach. Association mapping, as an alternate approach and

complementary tool to QTL mapping, was demonstrated feasible in wheat for identification of marker-trait associations and cross validation of QTL or genes identified from bi-parent mapping populations.

Identification of Quantitative Trait Loci Underlying Kernel Extra-softness and Related Traits by Linkage and Association Mapping in Wheat (*Triticum aestivum* L.)

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Guomei Wang, Author

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CHAPTER 1

Introduction

Guomei Wang

Wheat (*Triticum* spp.), which originated in the Fertile Crescent region of the Near East, belongs to the Gramineae (Poaceae) family. As one of the most important cereal crops in the world, wheat contributes more than 20% of the calories consumed by humans. Most wheat cultivated today can be classified into two types, durum and bread wheat. Durum wheat (*Triticum turgidum* L. var. *durum*, 2*n* = 4*x* = 28, AABB genomes) is a tetraploid hard wheat with an amber color, which is coarsely ground into semolina and used to make pasta and other semolina products. Common, or bread wheat (*Triticum aestivum* L., 2*n* = 6*x* = 42, AABBDD genomes) is a hexaploid that evolved from the hybridization of diploid progenitors *Triticum urartu* Tum. ex Gand. (2*n* = 2*x* = 14, A genome), a specie related to *Aegilops speltoides* Tausch (2*n* = 2*x* = 14, S genome) and *Aegilops tauschii* Coss. (2*n* = 2*x* = 14, D genome) (Feldman et al., 1995; Kihara, 1944; McFadden and Sears, 1946). Compared to durum wheat, common wheat is softer and varies in color from light cream to a deep reddish-brown. As the major source of gluten-containing flour, common wheat accounts for about 95% of all the consumed wheat in the world. It is used primarily in making bread, but also used in cakes, cookies, crackers, and noodles.

Common wheat is cultivated worldwide and provides up to 60% of daily calorie intake for humans in some developing countries (Cakmak, 2008). The marketing classification of common wheat types is based on whether the plant requires a vernalization period prior to flowering, therefore dictating the time of planting and the physiochemical characteristics of kernel color and texture. Common wheat can be classified as soft or hard based on kernel hardness, and further divided into five major classes: 1) hard red winter; 2) hard red spring; 3) soft red winter; 4) soft white; and 5) hard white. Among the five major wheat classes grown in the United States, soft wheat is usually used to make pastry-type products, such as cookies and cakes (Faridi et al., 1994; Giroux and Morris, 1998; Hoseney, 1994). In the United States, soft white wheat is

grown in the Pacific Northwest states including Washington, Oregon and Idaho, and also in Michigan and New York (Tanhehco and Ng, 2008).

Wheat endosperm

Kernel anatomy and quality

Wheat kernels consist of approximately 80-85% endosperm, 13-17% bran and 2-3% germ on a dry matter basis (Belderok et al., 2000). Since endosperm is the largest component of wheat kernel and is the source of starch and protein that form the various types of bread made worldwide, wheat endosperm has been studied intensely. When white flour is the desired end product, the embryo (germ) and seed coat (bran) are removed in the milling process (Olsen, 2004).

Endosperm is triploid ($3n = 6x = 63$) in nature and originates from the fertilization of two haploid polar nuclei in the embryo sac and a haploid sperm nucleus from the germinating pollen grain. The other sperm nucleus fertilizes the egg cell to form the diploid embryo ($2n = 6x = 42$). The development of the endosperm nucleus is completed through subsequent cell divisions, which includes three major phases: early development, differentiation, and maturation (Sabelli and Larkins, 2009). Cell divisions in the wheat endosperm continue until 12-14 days post-fertilization, resulting in fully developed endosperm. The fully developed tissue consists of four main parts: starchy endosperm, aleurone layer, transfer cells, and cells of the embryo-surrounding region (Huber and Grabe, 1987; Olsen, 2004). The main function of the endosperm is to provide nutrients to the developing and germinating embryo. Although many studies provide insight into the genetic factors that regulate the development and structure of wheat endosperm, the molecular and genetic factors controlling cell division, cell shape, and other development of endosperm remain unknown.

One quantitative measure of endosperm texture is the relative hardness or softness of the kernel. The single kernel characterization system (SKCS) is used to assess

wheat kernel texture as a hardness index. The hardness value is estimated based on an algorithm related to the raw crush-force-time profiles of each grain sample by accounting for the weight, electrical current, and force needed to crush each individual kernel in the sample. The SKCS is used to classify wheat into two categories based on kernel texture; hard wheat (hardness index ≥ 46) and soft wheat (hardness index <46) (Pearson et al., 2009; Turnbull and Rahman, 2002). Within the soft wheat class, a further distinction can be made for wheat kernels which have an extremely low hardness index: 'super-soft' or 'extra-soft.'

Kernel hardness and milling properties

Kernel hardness or softness impacts the milling process by affecting flour particle size, starch damage and milling yield, which in turn affect the end-use quality of the flour (Delwiche, 1993). Flour milling involves removal of the multilayer pericarp and the germ from the endosperm. Wheat endosperm is gradually reduced in size until it is fine enough to be known as 'flour.' Flour contains 68-75% starch, 6-18% protein, 11-14% moisture, 2-3% gums, 1-1.5% lipids and ash (Figoni, 2007). In general, hard wheat requires more energy to mill and yields flour with a coarser particle size, with higher gluten content, and consequently higher protein content than soft wheat (Campbell et al., 2001). In contrast, flour milled from soft wheat has a smaller particle size with less starch damage (Gaines et al., 2000).

During the breeding cycle, elite wheat lines are evaluated for a variety of phenotypes including the predicted flour yield. Several quantitative measures of flour quality can be obtained from the micro-milling process including break flour yield, bran recovered yield, the percentage of bran from break rolls by weight of the total products, and unground middling stock, the percentage of middling from break rolls by weight of the total products. Break flour yield, the portion of flour from break rolls relative to the weight of the total products, is an excellent indicator of flour yield (Yamazaki and Donelson, 1983). In addition, break flour milled from soft wheat has a higher flour yield

with less starch damage and smaller particles than that from hard wheat. This difference is fundamental to the profound divergence of the end-use quality of flour, which encompasses different appearances, textures, flavors, and nutritional values (Bettge and Morris, 2000).

Milling samples to produce meaningful measurements of flour yield often require more seeds than available from lines in early breeding generations. Micro-milling quality testing developed to accommodate samples of a small amount of seeds is an efficient and rapid method used by wheat breeding programs to predict flour yield in early generation tests (Seeborg and Barmore, 1957). The micro-milling protocol requires wheat kernels to be softened enough for easy grinding (tempered) so that the pericarp does not shatter while being crushed. Hard wheat is tempered to higher moisture content than soft wheat. The purpose of different moisture adjustment in the hard and soft wheat grain is to maximize the separation of bran from endosperm in each. After tempered, the grains are ready for the micro-milling process. Because even micro-milling is a fast process, hardness is often used as a rapid proxy to predict break flour yield and assess the overall wheat quality and end-use potential that are important to the miller (Bettge and Morris, 2000; Morris et al., 2001; Pomeranz and Williams, 1990).

Other related traits

Kernel hardness is associated with other traits, including grain protein content, grain yield, and break flour yield (Gwirtz, 1998). Grain protein content in wheat is affected by complex genetic system, genotype, and environment, and is slightly positively correlated with kernel hardness (Bettge and Morris, 2000; Payne et al., 1984). Conversely, protein content is negatively correlated with grain yield (Costa and Kronstad, 1994; Turnbull and Rahman, 2002). Soft white wheat flours with 7-10% protein are reported as the best for cake and cookie making. As a result, reducing protein synthesis or increasing starch deposition in the endosperm may improve break flour yield of soft wheat and the quality of cakes and cookies. In addition, kernel

hardness is correlated with test weight and thousand-kernel weight in hard wheat (Ohm et al., 1998; Pomeranz et al., 1985). Break flour yield is correlated with plant height, thousand-kernel weight, days to heading, and number of spikes per head (Kashif and Khaliq, 2004; Okuyama et al., 2004).

Mapping quantitative traits

Qualitative and quantitative traits

In general, the inheritance of traits can be classified into two types: qualitative and quantitative. Qualitative (or Mendelian) inheritance is observed when a trait or observable characteristic (such as color, shape, and height) is largely determined by only one or a few major loci or genes. Thus, phenotypes show discrete variation and provide information about the underlying genotypes. In contrast, quantitative (or complex) inheritance is determined by several to many factors or genes of varying effects. Thus, phenotypes show continuous variation, yield, some disease and insect resistance, abiotic stress tolerance, quality traits, and other traits of agronomic importance for example. Due to their importance, quantitative traits have been the subject of genetic studies for over a century (Fisher, 1918). Two common approaches are used to genetically dissect traits that show quantitative inheritance. One is quantitative trait loci (QTL) mapping using mapping populations segregating for the traits of interest. Another is association mapping or linkage disequilibrium mapping using a population of well chosen lines, accessions, or cultivars showing variation for the traits of interest.

QTL mapping

QTL mapping can be defined as the molecular marker-facilitated genetic dissection of the variation of complex phenotypes. The aim of QTL mapping is to identify chromosomal regions that affect the quantitative trait of interest, and to estimate the effect of QTL on the trait. In general, QTL analysis is performed with a well-developed

genetic linkage map and reliable phenotypic data from a mapping population. The power of QTL mapping and the accuracy of QTL estimation mostly depend on the selection of two parents, type and size of the mapping population, marker density, genome coverage of the genetic linkage map, quality of phenotypic trait data, and statistical method used.

Mapping population

QTL mapping in plants is usually conducted in a segregating experimental population derived from a cross between phenotypically different parental lines, such as F_2, F_3, F_4, backcross (BC), recombinant inbred line (RIL), recombinant substitution line (RSL), doubled-haploid (DH) or near-isogenic line (NIL) populations (Kearsey and Farquhar, 1998). F_2, F_3 and F_4 populations are frequently used for linkage map construction because they are easier to produce in a short time. RIL, RSL, and DH populations are immortal mapping populations and useful for QTL analysis because a large number of plants of each homozygous line allow replication (Varshney et al., 2006). Of these, RILs produce robust genetic mapping information.

A suitable mapping population having a reasonable sample size and segregating for phenotypic traits is essential for the construction of a saturated genetic map and QTL analysis. It is critical to select parental lines with genetic diversity for the trait(s) of interest. This will enhance the possibility of identifying a large set of polymorphic markers covering the whole genome. In general, the larger the population size, the higher the mapping resolution and the more accurate and reliable the QTL detection (Liu, 1998; Melchinger et al., 1998; Mohan et al., 1997). A study of the effects of population size on QTL mapping in barley reported that the number of QTL detected increased with increasing population size (Vales et al., 2005). Even though major QTL can be identified in a small population, minor QTL are under detection. Thus, one advantage of a large population is the ability to detect minor QTL. However, population

size is often governed by practical concerns as well as the cost of genotyping and phenotyping.

Genetic linkage map

Genetic linkage maps are constructed based on the recombination rates between marker loci that are sampled in an experimental cross. This information is then used to reconstruct the order, position, and relative distance of markers along a chromosome. In turn, linkage maps can be used to identify chromosomal regions containing genes and QTL for traits of interest. Construction of a genetic map is begun by ascertaining the allelic status of each marker locus for each individual of the mapping population. The combination of the polyploidy level, chromosome number, and genome size increase the difficulty of linkage map construction in wheat. Nevertheless, the use of genetic linkage maps has steadily increased with the improvements in molecular marker technology since the 1980s. The total genetic distance of wheat linkage maps has been reported with ranges from 1500 cM to 3800 cM. One cM corresponds to approximately 5.3 Mb assuming the total genetic distance of wheat is 3,000 cM (Akbari et al., 2006; Somers et al., 2004).

Statistical methods of QTL analysis

QTL analysis is used to genetically dissect complex traits. To improve QTL detection power and to estimate QTL location and effect for quantitative traits, several statistical methods are used, including single marker analysis, simple interval mapping (SIM), composite interval mapping (CIM), and multiple QTL mapping (MQM). The latter two methods are commonly used for QTL mapping because they are considered to be more powerful and precise. Single marker analysis is the simplest method to detect significant difference between phenotypic means of lines grouped by marker class based on *t*-test, analysis of variance (ANOVA) and linear regression. SIM is an extension of single marker analysis, which is used to estimate the genetic effects and genome

locations of QTLs controlling quantitative traits using the genotype of two adjacent markers to group lines (Lander and Botstein, 1989). CIM combines SIM with linear regression and uses phenotypic means while including additional markers as covariates to account for effects of other QTLs and to reduce residual variance when testing putative QTLs in an interval (Zeng, 1993; Zeng, 1994). MQM, an extension of interval mapping, allows one to better infer the location of QTL as well as interactions between QTL (Van Ooijen, 2004). Besides the above described QTL mapping methods, another recently proposed approach is Bayesian method. Available computer programs for QTL analysis include MapQTL, QTL Cartographer, MapManager QTX and QTLNetwork (Manly et al., 2001; Van Ooijen, 2004; Wang et al., 2007; Yang et al., 2008).

Application of QTL mapping

Genetic or QTL mapping has become an important tool for wheat breeding by allowing geneticists and breeders to identify, tag, and clone genes, and to manipulate plants at the DNA level . For example, a stripe rust resistance gene, *Yr36*, was recently cloned from wheat through map-based cloning approaches (Fu et al., 2009). In the past decade, considerable progress has been made in terms of positional cloning in wheat, with examples including the vernalization gene *VRN1* and the leaf rust resistance gene *Lr21* (Huang et al., 2003; Krattinger et al., 2009; Yan et al., 2003). Therefore, identification of QTL for traits of interest has provided a window of opportunities for the molecular characterization of QTL via map-based cloning.

Association mapping

Principle of association mapping

As an alternative method to linkage and QTL mapping, association mapping allows the identification of associations of marker loci with quantitative phenotypic traits based on a diverse population of individuals rather than a mapping population

derived from two parental lines. Association mapping was first developed and applied in human genetics to identify causal mutations for common complex human genetic traits (Kerem et al., 1989). The primary objective of association mapping is to identify the associations between marker loci and the trait of interest by calculating the differences in observed and expected haplotype frequencies on the basis of linkage disequilibrium (LD). Linkage disequilibrium or gametic phase disequilibrium measures the degree of non-random association between alleles at different marker loci (Zhu et al., 2008).

Several statistical methods have been used in association studies, including linear regression, analysis of variance (ANOVA), and chi-square test. A potential problem with association mapping is that marker-trait associations are of high chance of being false positive because of underlying population structure and relatedness among members of the panel. Both population structure and relative kinship can be taken into consideration in a newer model, mixed linear model (MLM), which is used to control confounding factors including population structure (Q-matrix) and relative kinship (K-matrix) (Yu and Buckler, 2006).

Factors affecting association mapping

A number of factors that potentially affect the association analysis via the LD approach have been reported, including population structure, pleiotropic effect, epistatic interaction, genotype by environment interaction, experimental design, statistical test or inference, population size, complexity of the trait under study, and quality of the phenotypic trait data. Many factors, individually or in combination, may lead to spurious associations. Selection of germplasm in the population is critical to the success of association analysis, because the mapping resolution, marker density, statistical methods and mapping power are determined by genetic diversity, extent of genome-wide LD, and relatedness within the population (Flint-Garcia et al., 2003). Generally, population stratification exists when the total population has been formed by admixture among subpopulations and when admixture proportions vary among

individuals, where admixture proportion is defined as the proportion of the genome that has ancestry from each subpopulation (Hoggart et al., 2003). Despite these disadvantages, association mapping can be considered as an alternative and complementary approach to traditional QTL mapping.

Application of association mapping in wheat

In the past few years, association mapping has been applied to the studies of plant genetics because of the potential for identifying polymorphisms underlying complex traits (Flint-Garcia et al., 2003). Examples of plant species used for association studies include *Arabidopsis*, maize, rice, barley, wheat and potato (Breseghello and Sorrells, 2006; Kruglyak, 1999; Nordborg et al., 2002; Remington et al., 2001; Simko et al., 2004). In wheat, markers associated with kernel size and milling quality traits were identified using association mapping in a diverse population of 95 soft winter wheat cultivars (Breseghello and Sorrells, 2006).

Although QTL analysis has been used widely in dissecting complex traits in bi-parental mapping populations, QTL are often inconsistently across different mapping populations. Association analysis using diverse germplasm collections or natural populations offers the advantage of significant time and resource savings relative to the development of a bi-parental mapping population. Thus, association mapping is considered as an efficient and effective tool that may be used to validate and confirm QTLs detected through traditional genetic mapping approaches (van Berloo, 2008).

Recent studies of kernel hardness

Kernel hardness is a quantitative trait controlled by multiple genes as well as environmental conditions (Bassett et al., 1989). Because of its importance to the baking and milling industries, wheat kernel hardness has been studied intensely. The following is a brief summary of different factors that affect kernel hardness.

Biochemical factors

Greenwell and Schofield (1986) identified a 15 kDa protein complex in starch granules from soft wheat but absent or in limited amounts in hard wheat. This protein complex was termed friabilin that is encoded by a hardness (*Ha*) locus located on the short arm of chromosome 5D (Jolly et al., 1996). Another study of variation of friabilin content among hard, soft, and 'extra-soft' wheat reported that 'extra-soft' wheat has more friabilin than soft wheat, probably indicating that a contribution of friabilin to the 'extra-soft' phenotype (Rybalka, 2008).

Further work has shown that two separate proteins, termed puroindolines, are the constituents of the friabilin protein complex. The two puroindoline genes, *pinA* and *pinB*, are closely linked to the *Ha* locus on chromosome 5DS (Jolly et al., 1996). Consequently, it has been suggested that the lack of *pinA* and *pinB* are the cause of grain hardness in wheat (Giroux and Morris, 1998). Subsequent work has confirmed that the puroindoline genes are the primary genetic factors in the determination of wheat grain hardness (Hogg et al., 2004). Transgenic wheat genotypes were used to test the effect of expression levels of puroindoline genes *pinA* and *pinB* on grain hardness (Hogg et al., 2004; Turnbull and Rahman, 2002). The results showed that increasing puroindoline transcript levels significantly increased puroindoline content and decreased grain hardness resulting in wheat lines with 'extra-soft' grain texture.

Pentosans, polymers of five carbon sugars, are another cellular component shown to affect kernel hardness. Pentosans are the primary non-starch polysaccharide of the endosperm cell-wall comprising about 2.2% of flour weight (Saulnier et al., 2007). As one of the major polysaccharide components in endosperm cell walls, together with cellulose and beta-glucan, arabinoxylan (AX) is composed of approximately 75% water-soluble AX and 25% water-insoluble AX (Saulnier et al., 2007). A previous study showed that pentosan content was negatively correlated with kernel softness and cookie diameter among soft wheat samples, indicating that pentosans affect kernel texture and

the end-use quality of flour (Bettge and Morris, 2000). Similarly, a significant association between AX concentration and kernel hardness was also observed in barley (*Hordeum vulgare* L.) and maize (Gamlath et al., 2008) because AX is the major component of pentosans. Although correlations have been described, the mechanisms of the effect of pentosan concentration on hardness are unclear.

Genetic factors

The genetic basis of wheat kernel hardness has been extensively studied through QTL mapping methods. Besides the *Ha* locus which encodes both puroindolines, numerous QTL that affect wheat kernel hardness have been revealed. Sourdille et al. (1996) identified four QTLs for kernel hardness, one each on chromosome 2A, 2D, 5B and 6D. In an $F_{2:5}$ -derived RIL population of a soft X hard wheat cross, a QTL at the *pinB* locus on 5DS explained 60% of phenotypic variance for kernel texture. Three other QTLs for kernel texture were mapped on chromosomes 2A, 2D, 6B (Campbell et al., 1999). At least 10 other QTL were mapped on chromosome groups 3 to 7 for grain hardness based on a hard X hard cross population (Arbelbide and Bernardo, 2006; Breseghello et al., 2005; Campbell et al., 2001; Nelson et al., 2006; Perretant et al., 2000; Pshenichnikova et al., 2008; Zanetti et al., 2001). Of these, another major QTL for grain hardness was mapped on chromosome 1BL, accounting for 28% of phenotypic variance of kernel hardness, while only about 8% of phenotypic variance of kernel hardness was explained by the QTLs mapped closely to the *Ha* locus and the puroindoline genes on chromosome 5DS (Li et al., 2009). In summary, the above studies were focused on the difference between hard and soft wheat. However, the genetic factors underlying the variation from soft to 'extra-soft' wheat are still unclear.

Environmental factors

In addition to biochemical factors, kernel hardness is influenced by environmental conditions, such as temperature and rainfall (Bushuk, 1998; Marshall et

al., 1986). The degree of the environmental effect varies and the effect is generally less significant than that of genetic effects. A study, which investigated winter wheat cultivars grown in 11 locations, showed that environment had less effect than genotype on the variation of kernel hardness, where the ratio of genotypic variance to environmental variance is around six-fold (Pomeranz et al., 1985). A similar slight influence of environmental conditions on kernel hardness was observed in a study of 39 spring wheat cultivars and advanced lines (Yong et al., 2004). In a study of French bread wheat, no significant environmental effects were observed on the relative concentrations of puroindoline A and B, the proximate modifiers of kernel hardness (Igrejas et al., 2001). Although previous studies indicated both genotype and environment affect the development and growth of kernel endosperm, variation in kernel hardness is primarily explained by genotypic variance (Finlay et al., 2007).

Research background and objectives

Most previous QTL mapping studies were focused on genetic effects of hardness using mapping populations developed from a hard X soft wheat or hard × hard wheat cross. Despite studies on the genetic basis of kernel hardness, little is known about the genetic control of the 'extra-soft' characteristic in wheat. The 'extra-soft' grain characteristic, which positively affects break flour yield and end-use quality (e.g.; greater cookie diameter and higher sponge cake volume), has fostered the development of a novel class of soft white wheat with superior end-use quality compared to common soft wheat. The potential of this new class of wheat to widen export markets has created an interest in understanding the genetic basis of the trait. This study is an effort to address some of these issues.

The aim of this research was to increase our understanding and knowledge with respect to QTL related to the 'extra-soft' characteristic of wheat, and ultimately to determine the genetic basis of the difference between soft and 'extra-soft' wheat at the

molecular level. The primary objectives were to detect QTL on genetic linkage maps by QTL mapping, identify molecular markers associated with kernel hardness and related traits by association analysis, and dissect the underlying genetic factors controlling these traits. The specific objectives of this study were:

1) To build a comprehensive genetic linkage map with SSR and diversity arrays technology (DArT) markers using a soft winter wheat mapping population derived from a cross between two elite soft white genotypes OS9A (Stephens) and QCB36 (OR9900553);
2) To estimate the impacts of environmental conditions on traits of interest and to assess the interrelationships of evaluated traits using path coefficient analysis;
3) To identify the QTL controlling kernel hardness, break flour yield, and other related traits using QTL mapping and to elucidate the QTL position controlling kernel extra-softness;
4) To identify and validate QTL of investigated traits using association mapping on a collection of 94 diverse wheat lines.

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CHAPTER 2

Genotype-by-environment Interaction and Interrelationships of Kernel Hardness and Grain Quality Related Traits in a Soft White Wheat Population

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Abstract

Kernel hardness is a complex trait that affects milling yield and end-use quality of wheat. Understanding the interrelationships among phenotypic traits and the effects of environmental conditions on wheat kernel hardness and milling yield will facilitate phenotypic selection in wheat breeding programs. This study was conducted to evaluate genotype-by-environment (GE) interactions and investigate the interrelationships of milling yield-related traits. A set of 164-F_6 recombinant inbred lines, derived from a cross between hexaploid wheat varieties OS9A and QCB36, was used in this study. Significant environmental effects and GE interaction were detected for seven agronomic traits and milling yield. Break flour yield and kernel hardness had broad-sense heritability (h^2) ranging from 0.56 to 0.84 and 0.84 to 0.96, respectively. Path coefficient analysis revealed that both hardness and break flour yield were directly associated with days to heading, test weight and grain protein content. Increases in grain hardness were associated with reduced break flour yield in all environments; similarly, high grain protein content was coupled with reduced break flour yield. Conversely, higher test weight was associated with higher break flour yield. Test weight also had a significant positive indirect effect through hardness on break flour yield. In addition, thousand-kernel weight had a significant negative direct effect on break flour yield. Plant height had a positive indirect effect through test weight on break flour yield; however, days to heading had a negative direct effect and a negative indirect effect through test weight on break flour yield. The air temperature in June substantially affected BFY, and the daily air temperature from April to June and the cumulative rainfall in March and June largely affected KHA; indicating that the weather condition during the period of grain filling and ripening was important to KHA and BFY. Thus, the genetic improvement of BFY can be effectively accomplished by concentrating on the selection of genotypes with low kernel hardness, high test weight, and early heading date.

Introduction

Kernel or grain hardness (KHA), a major determinant index of grain texture, is an important characteristics that affects milling, baking and end-use qualities of wheat (Bettge and Morris, 2000). Common wheat (*Triticum aestivum* L.) is classified as hard or soft based on KHA measurements. Hard wheat usually is used to make bread, while soft wheat generally is used to make cakes, pastries, and cookies (Faridi et al., 1994; Giroux and Morris, 1998). Compared to hard wheat, soft wheat has smaller particles and higher break flour yield (BFY, portion of flour from break rolls relative to the weight of total products) during milling, suggesting that BFY is negatively correlated to KHA (Bettge and Morris, 2000; Campbell et al., 2007; Gaines, 1985). As an essential criterion of end-use quality, increased BFY is one of the primary objectives in soft wheat breeding programs. However, it is challenging to significantly improve BFY of soft wheat because of the limited understanding of genetic control of KHA and BFY and inadequate knowledge of how yield-related traits interact with the environment to affect KHA and BFY.

Both KHA and BFY are affected by genetic components, yield-related traits and environmental factors, including temperature and rainfall (Bushuk, 1998; Marshall et al., 1986). In addition, BFY is known to be affected by other yield-related traits such as kernel size, test weight, grain hardness and grain protein (Marshall et al., 1986; Ortiz et al., 2007). Although test weight has been used to estimate milling yield, the relationship between test weight and flour yield appears to depend on both cultivar and environmental conditions (Marshall et al., 1986). Thus, test weight cannot be reliably used as the only selection criterion of wheat grain yield or milling yield (Gimelfarb and Lande, 1995). As a consequence, a combined selection for genetic factors and early-generation phenotypic performance would probably be more efficient (Zhang et al., 2005).

Path coefficient analysis or path analysis can be used to test theoretical models that explain the interrelationships among a set of observed variables, and provide information on the causal systems among complex traits (Rao and Province, 2000). Due to the limited information supplied by simple correlation analysis, path analysis has been used in many studies to determine the causal relationships among investigated traits and to dissect the correlation coefficients into direct and indirect effects. In agriculture, path analysis was first used to analyze crested wheatgrass seed production by partitioning simple correlation coefficients within traits into direct and indirect effects (Dewey and Lu, 1959). Path analysis was also successfully used to investigate the complex interrelationships between final yield and yield-related traits in wheat (Aycicek and Yildirim, 2006; Dencic et al., 2000; Ehdaie and Waines, 1989; Kashif and Khaliq, 2004; Okuyama et al., 2004). Therefore, path analysis can provide insight into the causal relationships among traits and help to define how genetic mechanisms of target traits are influenced by various physiological traits.

The objective of this study was to understand the impacts of genetic and environmental factors on wheat KHA and BFY as well as genotype by environment interaction. To determine which traits contribute most to the variation of BFY under various environmental conditions, we investigated the interrelationships of KHA and BFY with yield-related and agronomic traits using path analysis. A total of four weather parameters, including temperature and rainfall from January to June, were used to determine which climatic factors impacted the investigated traits.

Materials and methods

Plant materials and experimental design

A set of 164 F_6-derived recombinant inbred lines (RILs) were generated from the cross between soft wheat OS9A and QCB36. OS9A is a single plant selection from the cultivar ‘Stephens’, a widely adapted and high-yielding semi-dwarf variety with durable

high-temperature adult-plant (HTAP) resistance to stripe rust (*Puccinia striiformis* Westend f. sp. *tritici* Ericks) (Chen and Line, 1995). QCB36 is a single plant selection from the elite breeding line OR9900553, a high yielding and facultative semi-dwarf white wheat breeding line with very soft ('extra-soft') grain kernel texture and superior end-use quality (Riera-Lizarazu et al., 2010).

The 164 RILs and the two parents were first planted in Hyslop Field (Corvallis, OR) in 2007, and then were grown in a wide range of environments in the U.S. Pacific Northwest (Corvallis, Pendleton, and Moro, OR; Pullman, WA; and Moscow, ID) in 2008. The plants were grown in each field in a randomized complete block design (RCBD) with two replications. These five environments are diverse and representative of wheat-producing regions in the U.S. Pacific Northwest.

Measurements of agronomic and quality traits

A total of 10 agronomic traits were measured in the OS9XQ36 population and the two parents. After physiological maturity, plant height (PHT, cm) was determined by measuring the height of the stem from the soil surface to the tip of the spike excluding the awns. Days to heading (HDD, days) was recorded as the number of days from January 1st until 50% of the spikes in a plot were completely emerged. After harvest, test weight (TWT, kg m^{-3}) was measured using a GAC2100 GI analyzer (DICKEY-john Corporation, Auburn, IL), and grain protein content (GPC, %) was determined using the Infratec1241 grain analyzer (FOSS, Eden Prairie, MN). Kernel hardness (KHA, hardness index), kernel weight (KWT, mg) and kernel diameter (KDM, mm) were determined on 300-kernel samples with a single kernel characterization system (SKCS) (model 4100, Perten Instruments AB, Huddinge, Sweden) (Martin et al., 1993; Osborne and Anderssen, 2003).

Micro-milling, an efficient and rapid method to predict flour yield when seed quantities are limited (Gaines et al., 2000; Seeborg and Barmore, 1957), was also used to evaluate milling-yield related traits of grain samples from each environment. Grain

samples (~15 g) were equilibrated to 13% moisture before milling using a short flow micro-mill (Kitterman et al., 1959). Break flour yield (BFY), bran recovered (BRN, a percentage bran from break rolls by weight of the total products) and unground middling stock (MID, middling stock from break rolls as a percentage of the total products) were measured. Percentages (%) were converted to grams per kilogram (g kg^{-1}). Only grain samples from Corvallis, Moro, Pendleton (OR) and Pullman (WA) produced in 2008 were tested for milling yield.

Weather data collection

Weather data from January to June during 2007 and 2008 was obtained from weather stations nearest the experimental plots. The parameters included cumulative rainfall (mm), average daily air temperature (˚C), average maximum air temperature (˚C), and average minimum air temperature (˚C).The total amount of liquid equivalent precipitation recorded in 24 hours is referred to as cumulative rainfall. Average daily temperature is the mean temperature recorded in 24 hours. The weather data in Corvallis (OR, Hyslop farm) in 2007 and 2008 were obtained from the Hyslop weather station. The weather data from Moro and Pendleton (OR) were obtained from the closest weather stations in Moro and Pendleton, respectively. In Pullman (WA), the weather data were from the weather station at the Pullman airport, about 1 mile from the field. In addition, the weather data of Moscow (ID) were extracted from the monthly weather data sheets of the Moscow Plant Science Farm (MPSF), which is the weather station closest to the field at this location.

Statistical analysis

The phenotypic data for each trait from each environment and across environments were analyzed for normality by the PROC UNIVARIATE procedure of SAS 9.1 (SAS Institute, Cary, NC). On the basis of these normality tests, all trait data showed normal distributions or nearly-normal distributions. Analysis of variance (ANOVA) was

performed to partition the different sources of variation for the traits in each environment and multi-environments using PROC GLM and PROC MIXED procedures, respectively, where all effects were assumed random. Broad-sense heritability or repeatability (h^2) estimates on RIL mean basis were calculated using variance components (σ_G^2 , σ_{Gr}^2 , σ_{GE}^2 and σ_e^2). The heritability of each trait was estimated for each environment using $h^2 = \sigma_G^2 /(\sigma_G^2 + \sigma_{Gr}^2 / r)$ based on one-way ANOVA and using $h^2 = \sigma_G^2 /(\sigma_G^2 + \sigma_{GE}^2 / E + \sigma_e^2 / rE)$ across environments according to a two-way ANOVA, where σ_G^2 is the genotypic variance for the RIL population, σ_{Gr}^2 is the variance of interaction between genotype and replication, σ_{GE}^2 is the variance of genotype-by-environment interaction, σ_e^2 is the error variance among RILs, *r* is the number of replications in a single environment (*r*=2) and *E* is the total number of environments (Hallauer and Miranda, 1981).

Phenotypic correlation coefficients among traits were estimated using PROC CORR in SAS for each of the four environments and the average across four environments (Corvallis, Moro, and Pendleton in Oregon (OR) and Pullman, WA) in 2008). Path analysis was performed using PROC CALIS in SAS (Dewey and Lu, 1959; García del Moral et al., 1991; Gebeyehou et al., 1982). The interrelationships of traits in the path causal model were hypothesized based on the ontogeny of the wheat plant. BFY was the dependent variable and other traits were considered independent variables (Kozak et al., 2007). Kernel diameter was excluded from path analysis because it is highly correlated with thousand-kernel weight (TKW, g) with a correlation coefficient of 0.96. Both PHT and HDD, early growth traits in the ontogeny of wheat, were chosen to test the direct effects on TWT, GPC and TKW. Further, the direct and indirect effects of TWT, GPC and TKW on KHA or BFY also were investigated in path models. The path was kept in the model if the coefficient of the added path was significant at $p<0.05$ level and Chi-square tests did not reject the model. Otherwise the hypothetical path was

discarded and a new path was tried. The final best-fitting path structure model was considered as the best interrelationships for these traits. For weather data, stepwise regression selection was used to determine the most important climatic characteristics affecting a given quality and agronomic trait, and the p-value corresponding to each parameter in the stepwise selection was recorded.

Results

Phenotypic data and broad-sense heritability

The least square (LS) means and the range of phenotypic traits for the two parents and RILs across environments are presented in Table 2.1. OS9A and QCB36 differed significantly ($p<0.05$) for all traits except TWT and TKW. The two parents had similar grain yield, where the TWT for OS9A and QCB36 were 755 and 750 kg m^{-3}, and TKW were 44.3 and 39.5g, respectively. As expected, the 'extra-soft' wheat QCB36 had a lower hardness index than soft-wheat OS9A; the KHA index of OS9A was twice the value of QCB36. Also, KHA in the RIL population varied continuously from 5.4 to 40.8. Significant differences in BFY were observed between the parents, and BFY of the RIL population ranged from 112 to 178 g/kg. In addition, QCB36 had a significantly higher BRN and a lower MID than OS9A. QCB36 had a later HDD and a higher GPC than OS9, but a lower PHT, and KDM. These traits segregated within the RIL population with a wide range of values.

Most broad-sense heritability estimates were relatively high in single environments and across environments (Table 2.1). The heritability calculated across environments for KHA, MID, PHT, HDD, TKW and KDM were 0.96, 0.90, 0.96, 0.93, 0.91 and 0.91, respectively. The heritability of KHA ranged from 0.84 to 0.97 in the six environments. The estimates of heritability for three flour yield measurements, BFY, BRN, and MID, were similar, ranging from 0.56-0.84, 0.68-0.85, and 0.71-0.90,

respectively. Plant height exhibited consistent high heritability in single environments and across environments with heritability estimates ranging from 0.82 to 0.96.

Estimation of GE interaction and variance components

The F-value and variance components for genotypes, environments and GE interaction were calculated for all 10 investigated traits (Tables 2.2, 2.3). The genotype and environment main effects were highly significant ($p<0.001$) for all 10 measured traits except the environmental main effect of MID ($p<0.01$). For seven agronomic traits, the environmental variance components were larger than corresponding genotypic variance components, and GE variance components were smaller. Significant differences among RILs and RIL-by-environment interactions were observed for all traits ($p<0.01$). The parent-by-environment interaction was significant for KHA, HDD, TKW, and KDM ($p<0.01$). Compared to genotypic variance components, large environmental variance components were found for all traits except BFY, BRN, and MID which had larger genotypic variance components than environmental variance components.

Phenotypic correlation and path coefficient analysis

The phenotypic correlations for the 10 investigated traits in four single environments and across environments for the 164-RIL population are reported in Table 2.4. Kernel hardness had a negative correlation to BFY and to BRN ranging from -0.464 to -0.613 and -0.215 to -0.391 in all four environments, respectively (Table 2.4). Conversely, KHA had positive correlations with MID. Both KHA and BFY were associated positively with TWT, and significant positive correlations were observed between KHA and GPC. Break flour yield was negatively correlated with GPC. Negative correlations were also observed between PHT and HDD, and PHT and GPC in all environments. Positive correlations were observed between PHT and TWT, PHT and TKW for most of the environments. The correlation coefficients between KHA and PHT, KHA and TKW, BFY and PHT were inconsistent in four environments. Thousand-kernel weight had a

positive correlation to kernel diameter (0.837 to 0.955). Thus only TKW was included in the path coefficient analysis.

A cause-effect diagram of traits was derived from the described ontogeny of wheat (García del Moral et al., 1991). Direct effects of yield-related traits on BFY and the complex interrelationships within these traits in each environment and across environments were added to this model (Figure 2.1-2.5). A significant direct effect of KHA on BFY was found in the range from -0.486 to -0.608 in all environments, indicating milling yield of soft wheat was greatly affected by kernel texture. There was a positive direct effect between TWT and BFY, varying from 0.081 to 0.286, in all environments. In contrast to TWT, GPC had a negative direct effect on BFY ranging from 0.085 to 0.155 in all environments except Pullman (WA). Also, TKW presented negative direct effects on BFY except in Corvallis and Pendleton (OR). A direct effect of HDD and an indirect effect through GPC or TWT were observed in several environments. A significant direct effect of PHT on BFY was only observed across environments, however, indirect effects through TWT, TKW and GPC on BFY were found in several environments.

With regard to kernel hardness, HDD, PHT, TWT and GPC had direct effects on KHA over two environments (Figure 2.1-2.5). Negative direct effects of HDD on KHA were observed in three environments, while the various direct effect of PHT on KHA were found in Corvallis and across environments. There was a positive direct effect of TWT on KHA with correlation coefficients ranging from 0.127 to 0.202 in four environments. Similarly, a positive direct effect of GPC on KHA was observed in Moro and across environments. For the effect of TKW on KHA, a negative direct effect was only observed in Pendleton. Thus, various direct effects of other traits on KHA revealed that KHA is likely influenced by events at various ontogenetic phases of development and environmental conditions in the field.

Impacts of weather parameters on traits

In this study, a total of four weather parameters from January to June showed a wide range of variation across six environments (data not shown), and the effects of weather parameters on BFY, KHA, PHT, HDD, TWT, GPC and TKW were estimated using stepwise regression selection (Figure 2.6). The air temperature in June was the most important environmental factor affecting BFY. KHA was significantly affected by daily air temperature from April to June and the cumulative rainfall in March and June, indicating that weather during the period of grain filling and ripening was critical. PHT was highly affected by cumulative rainfall and air temperature parameters during March (Figure 2.6). Most of the weather parameters caused significant effects on HDD, suggesting that weather variation was an important environmental determinant for flowering. The effects of weather on TWT, TKW and GPC were complex but these traits appeared to be affected by most of the weather parameters from January to June in which we had the weather data in the analysis.

Discussion

Except for HDD, relatively high heritability estimates suggest the experimental conditions and evaluation methods used in this study were consistent and reproducible. The heritability estimates of PHT, HDD, TWT, GPC, and TKW are similar to previous reports (Huang et al., 2006). Also, the estimates of heritability for KHA and BFY were similar to other studies, indicating the possibility of improving milling yield through direct selection of milling yield-related traits (Bergman et al., 1998; Yamazaki and Donelson, 1983).

Environmental effects and unexplained GE interactions are important factors in the selection and evaluation of grain yield-related traits (Gómez-Becerra et al., 2010). Environmental factors, such as temperature and rainfall, affect the growth and development, and in turn affect the grain formation and final flour yield (Zhao et al.,

2007). For example, rainfall, temperature, and soil fertility show greater impacts on GPC than genetic factors (Groos et al., 2003; Triboi et al., 2000; Yong et al., 2004). In the present study, the significant environmental main effects and GE interactions were responsible for the phenotypic variation of traits under diverse environmental conditions. In the case of KHA, we found that the environmental variance component was greater than the genotypic variance component. Similar observations have been reported by others (Law et al., 1978; Pomeranz et al., 1985). BFY was likely affected by the temperature during June, in which heading and the critical period of wheat grain filling in the Pacific Northwest fall into. Thus, BFY was apparently affected by conditions around heading time and from heading to maturity, which in turn directly affected the grain filling period. We believe the delays in heading date in Pendleton (OR) and Moscow (ID) were affected by the cooler conditions in May and June of 2008.

Relationships among complex traits are difficult to unravel and interpret because of limited information provided by simple correlation analysis. Path analysis has been used in many studies of complex agronomic and quality traits of wheat, such as grain yield, kernel weight, plant height, and days to heading to investigate the causal relationships among traits by dissecting the simple correlations into direct and indirect effects (Akanda and Mundt, 1996; Kashif and Khaliq, 2004; Li et al., 2006). In the present path analysis, the most notable relationship between traits was the negative direct effect of KHA on BFY, where a softer kernel (low hardness index) has a higher BFY (Bettge and Morris, 2000; Ohm et al., 1998). The significant negative association of GPC with BFY is consistent with previous reports that high protein wheat has a relatively low milling yield (Gaines, 1985; Gómez-Becerra et al., 2010; Gonzalez-Hernandez et al., 2004; Lehmensiek et al., 2006; Nelson et al., 2006; Yamazaki and Donelson, 1983). Thus, achieving a right balance between GPC and milling yield is one of the objectives in wheat breeding programs (Oury et al., 2010). Although simple correlation between HDD and BFY was weak, significant negative direct effects of HDD on BFY were detected using

path coefficient analysis. This relationship could be explained by the indirect effect of HDD on BFY through GPC. Grain protein content was apparently increased in early heading genotypes, which in turn decreased BFY because of the negative correlation between GPC and BFY. In addition to trait interrelationships, GPC was probably enhanced by high temperature which reduced starch storage in grains during the wheat grain filling period (Zhao et al., 2007). In our study, all the air temperature parameters were important for GPC except the average daily air temperature in April and the average maximum air temperature in May. This is probably due to the presence of earliness in some RIL lines that allowed them to avoid high temperature conditions during the most sensitive phases of kernel filling and development (Royo et al., 2006).

In addition to phenotypic correlation, underlying genetic factors for wheat KHA and BFY probably affect milling yield and other end-use quality traits. The alleles of loci *pinA* and *pinB*, encoded by the wheat kernel hardness (*Ha*) locus on chromosome 5DS, correlate with wheat end-use quality (Giroux and Morris, 1998). Some soft-grain wheat lines with the *Pinb-D1b* allele had a higher milling yield compared to wild type lines (Chen et al., 2007). The path coefficient analysis implied that KHA was the best predictor of BFY and revealed the potential underlying genetic correlation between KHA and BFY. In addition, positive direct effects of PHT on TWT and TKW were detected, indicating that genetic factors of plant height probably have some impacts on KHA. We expected that plants with height far beyond the normal range for Pacific Northwest elite wheat cultivars would exhibit different BFY performance. Although this was not the case, we did note that PHT showed some direct effects on KHA in Corvallis and TWT, indicating that BFY was probably indirectly affected by PHT through KHA or TWT. Previous studies suggested that PHT is directly controlled by the dwarfing genes *Rht-B1*and *Rht-D1* and account for a large part of yield performance (Aycicek and Yildirim, 2006; Flintham et al., 1997). However, a significant direct effect of PHT on BFY was observed only in the analysis across environments. Our inability to detect this association in datasets from

individual environments may be due to the exclusion of extreme tall or short plants in the RIL population, which results in a narrow range of PHT phenotypes in our current RIL population.

The present results suggest that BFY is not only affected by environmental conditions, but also by other yield-related traits, such as KHA, HDD, TWT, and GPC. The genetic improvement of BFY can be effectively accomplished by concentrating on the selection of genotypes with low kernel hardness, high test weight, and early heading date. Understanding the environmental effects on genotypes and the clarification of the interrelationships among these investigated yield-related traits are of great significance for the improvement of quantitatively inherited yield traits. More information about the underlying genetic mechanism of kernel hardness and flour yield-related traits will be provided by QTL analysis.

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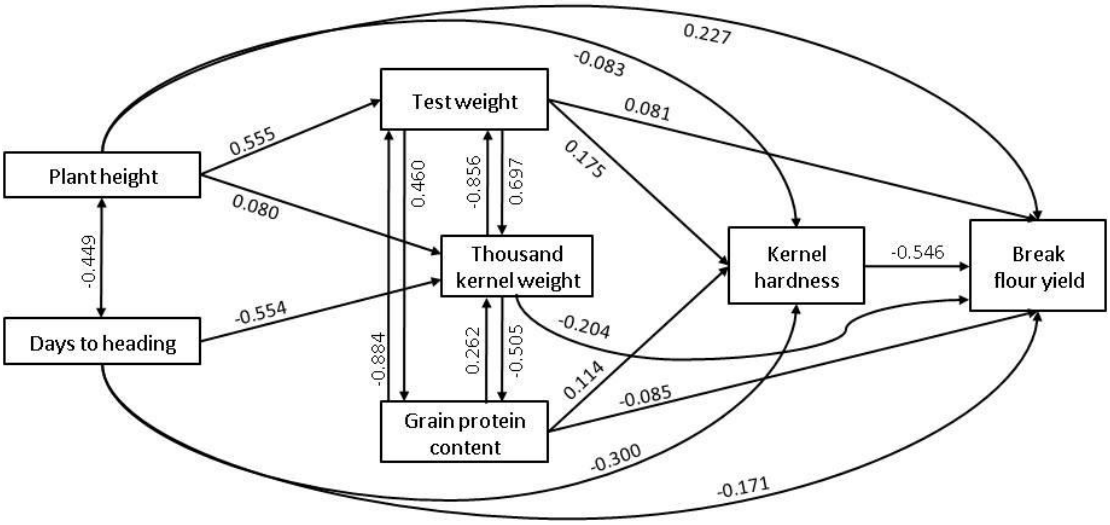

Figure 2.1 Path coefficient diagram presenting the causal interrelationship among traits across four field environments (Corvallis, Moro, Pendleton in OR and Pullman in WA) in 2008. The double-headed arrow indicates the simple negative correlation between plant height and days to heading , while the single-headed arrow shows the significant direct effect of one trait on another ($p<0.05$). The path coefficient next arrow represents the positive or negative direct effect.

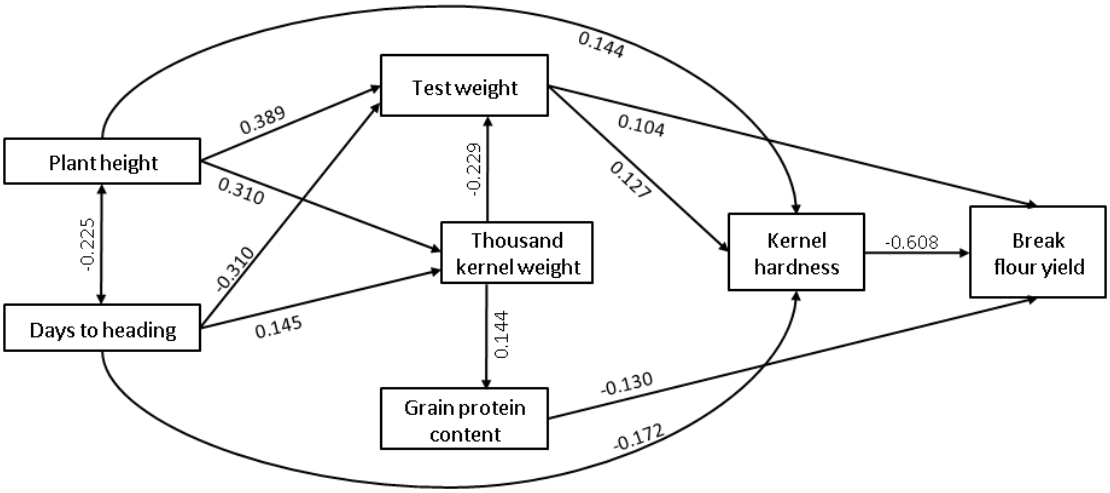

Figure 2.2 Path coefficient diagram presenting the causal interrelationship among traits of wheat grown at Corvallis, OR in 2008.

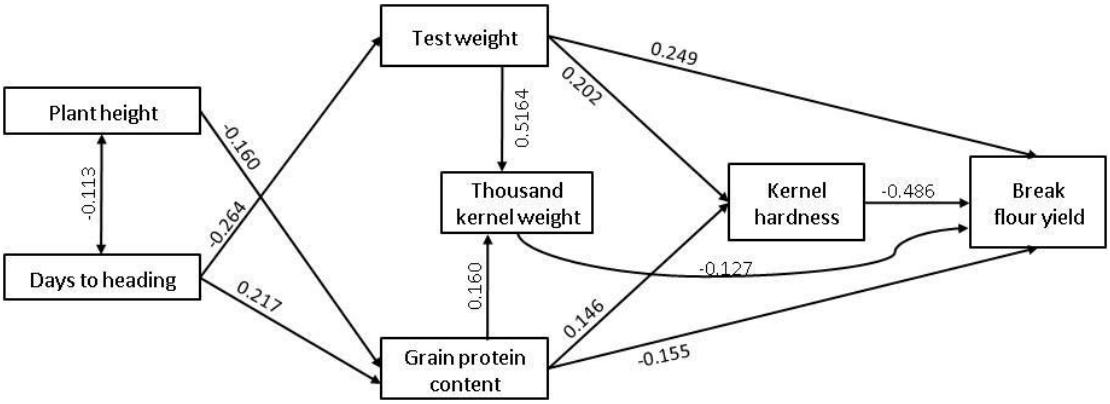

Figure 2.3 Path coefficient diagram presenting the causal interrelationship among traits of wheat grown at Moro, OR in 2008.

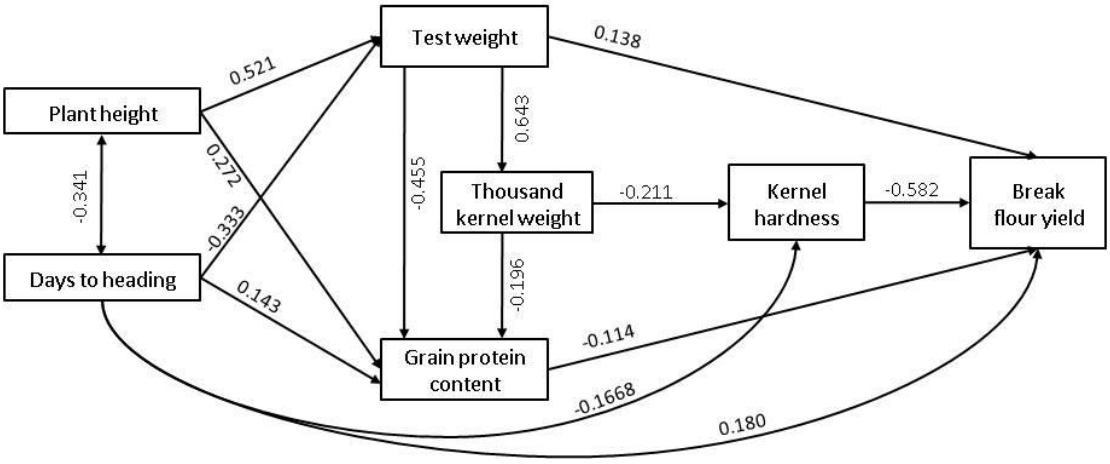

Figure 2.4 Path coefficient diagram presenting the causal interrelationship among traits of wheat grown at Pendleton, OR in 2008.

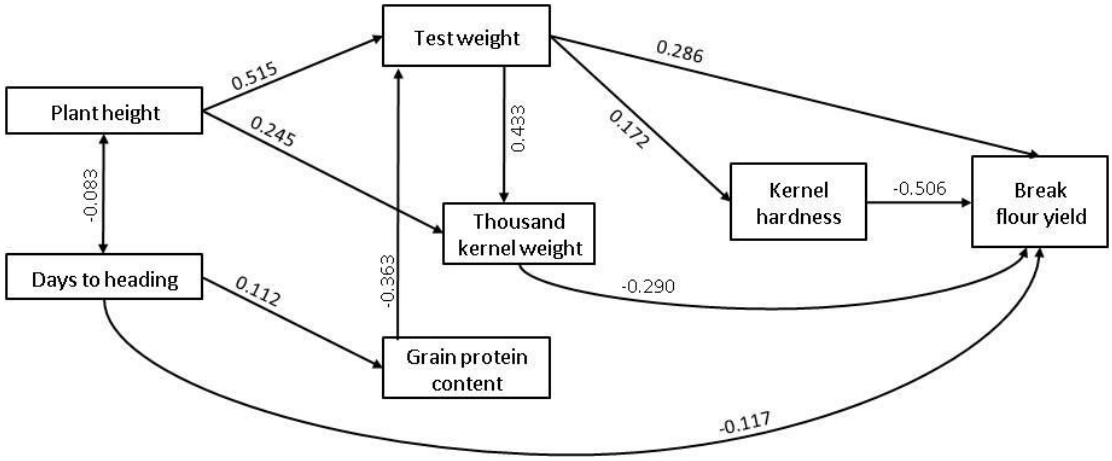

Figure 2.5 Path coefficient diagram presenting the causal interrelationship among traits of wheat grown at Pullman, WA in 2008.

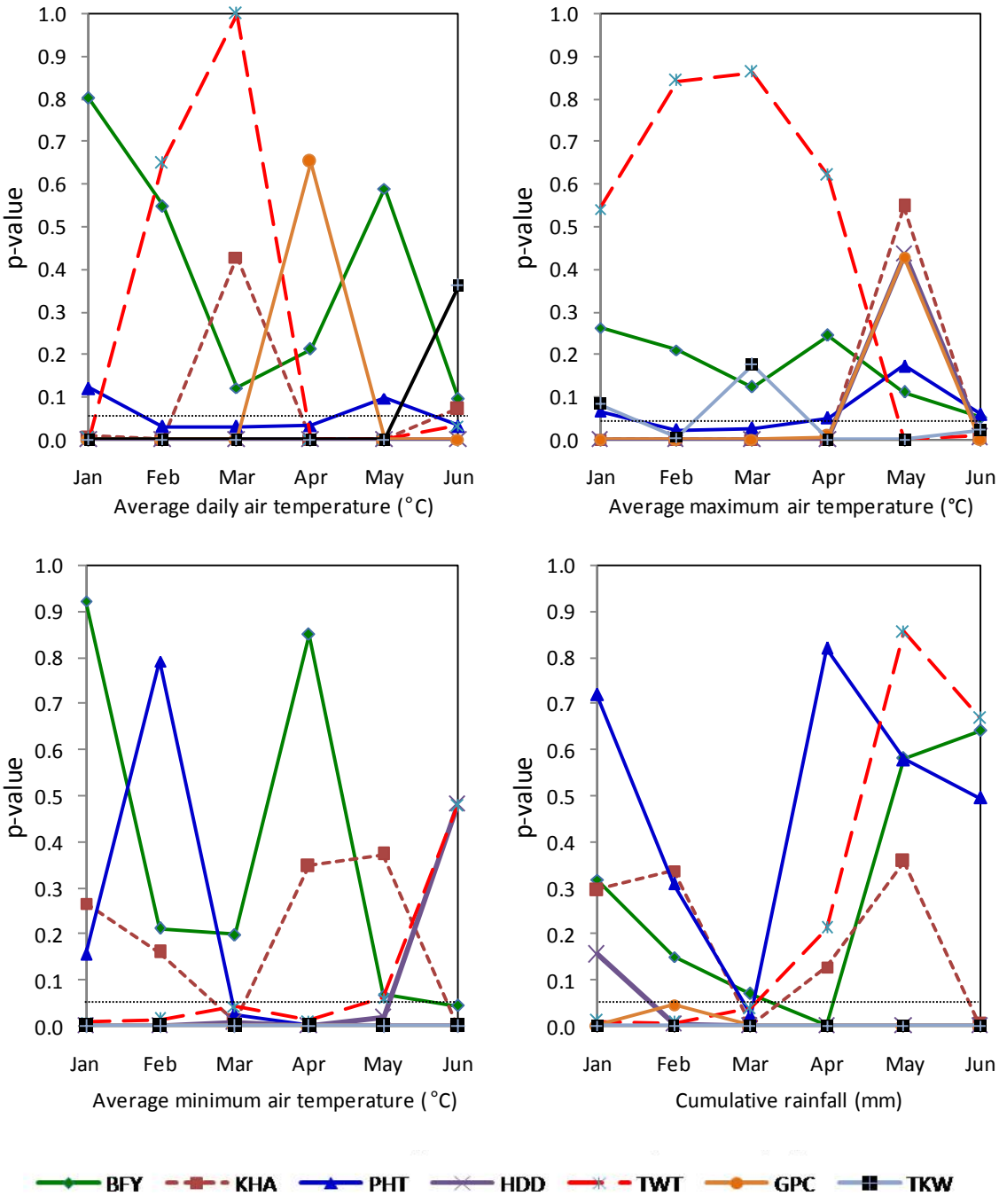

Figure 2.6 The effects of four weather parameters from January to June on seven traits. The dash line represents the significant level at $p<0.05$.

Table 2.1 Phenotypic means, range, and broad-sense heritabilities for ten traits of the parents (OS9A and QCB36) and the OS9XQ36 164-RIL population in six environments and across all environments.

Trait[†]	OS9A[++]	QCB36	RILs-mean	RILs- Range	Broad-sense Heritability (h^2)						
					Corvallis 2007	Corvallis 2008	Moro 2008	Pendleton 2008	Pullman 2008	Moscow 2008	Across environm
KHA (Index)	24.0a	12.4b	23.0	5.4-40.8	0.90	0.97	0.86	0.96	0.84	0.93	0.96
BFY (g kg^{-1})	134b	171a	141	112-178	-	0.68	0.56	0.76	0.59	-	0.84
BRN (g kg^{-1})	260b	283a	275	236-311	-	0.82	0.68	0.74	0.73	-	0.85
MID (g kg^{-1})	607a	545b	583	536-623	-	0.81	0.71	0.83	0.73	-	0.90
PHT (cm)	79a	72b	84	48-109	0.85	0.94	0.82	0.96	0.87	0.91	0.96
HDD (d)	156b	160a	158	149-163	0.52	0.87	0.74	0.91	-	0.57	0.93
TWT (kg m^{-3})	757a	750a	770	719-797	0.82	0.90	0.61	0.88	0.80	0.71	0.86
GPC (g kg^{-1})	109b	121a	118	104-146	0.75	0.78	0.35	0.64	0.52	0.61	0.80
TKW (g)	44.3a	39.5a	43.1	36.3-50.1	0.74	0.93	0.57	0.82	0.80	0.72	0.91
KDM (mm)	2.8a	2.4b	2.7	2.3-3.1	0.74	0.93	0.58	0.81	0.77	0.66	0.91

[†] KHA: kernel hardness; BFY: break flour yield; BRN: bran yield; MID: middling yield; PHT: plant height; HDD: days to heading; TWT; test weight; GPC: grain protein content; TKW: thousand-kernel weight; KDM: kernel diameter.

[++] Mean values are least square (LS) means across all environments. Means within rows sharing the same letter are not significantly different from each other ($p<0.05$) using Fisher's *F*-protected LSD test.

Table 2.2 Analysis of variance (ANOVA) and variance components for seven traits of the parents (OS9A and QCB36) and the OS9XQ36 RIL population across six different environments (Corvallis, Moro, Pendleton, OR; Pullman, WA; and Moscow, ID) in 2007 and 2008.

Source[†]	Kernel hardness		Plant height		Days to heading[††]		Test weight		Grain protein content		Thousand-kernel weight		Kernel diameter	
	df	F-value	df	F Value	df	F-value	df	F-value	df	F-value	df	F-value	df	F-value
E	**5**	**369.35*** **	**5**	**74.46** ***	**4**	**811.74** ***	**5**	**16.75*** **	**5**	**41.19** ***	**5**	**85.35** ***	**5**	**57.83** ***
G	**165**	**24.32** ***	**165**	**22.45** ***	**165**	**9.25** ***	**165**	**7.61*** **	**165**	**5.70** ***	**165**	**10.07** ***	**165**	**11.17** ***
RIL	163	23.71 ***	163	22.25 ***	163	9.02 ***	163	7.85 ***	163	5.76 ***	163	10.34 ***	163	10.95 ***
Parent	1	57.22 ***	1	13.24 *	1	84.53 ***	1	0.32	1	13.65 *	1	7.33 *	1	31.35 **
RIL vs. Parent	1	159.67 ***	1	95.74 ***	1	0.02	1	3.87	1	1.40	1	1.88	1	7.04 *
Block(E)	**6**	**6.81** ***	**6**	**29.15** ***	**6**	**25.29** ***	**6**	**29.47** ***	**6**	**18.01** ***	**6**	**19.09** ***	**6**	**20.57*** **
E *G	**809**	**2.23** ***	**809**	**2.49** ***	**809**	**1.58** ***	**801**	**2.16*** **	**803**	**1.39** ***	**809**	**1.71** ***	**809**	**1.49** ***
E *RIL	799	2.23 ***	799	2.51 ***	799	1.59 ***	791	2.06 ***	793	1.36 ***	799	1.65 ***	799	1.45 ***
E*Parent	5	3.61 **	5	1.36	5	0.95	5	1.09	5	1.61	5	5.79 ***	5	3.90 **
E*(RIL Vs.Parent)	5	0.76	5	1.26	5	1.24	5	19.80 ***	5	7.00 ***	5	6.74 ***	5	6.02***
σ_E^2 [§]	77.433		207.648		137.746		258.043		142.810		31.002		0.050	
σ_G^2	33.156		130.889		2.840		202.046		32.813		7.509		0.017	
σ_{EG}^2	4.617		21.517		0.619		94.098		11.434		2.022		0.003	

[†] E represents environment; G represents genotype; df represents degree of freedom; F-value represents F statistics; Statistical significance level: * p<0.05, * * p<0.01, ***p<0.001

[§] The variance components, where σ_E^2 environmental variance; σ_G^2 genotypic variance; σ_{EG}^2 genotype × environment (GE) interaction variance

[††] The ANOVA of days to heading was carried out excluding the dataset from Pullman (WA).

Table 2.3 Analysis of variance (ANOVA) and variance components for three yield-traits of the parents (OS9A and QCB36) and the OS9XQ36 RIL population across four environments (Corvallis, Moro, Pendleton, OR; and Pullman, WA) in 2008.

Source[†]	Break flour yield		Bran yield		Middling yield	
	df	F-value	df	F-value	df	F-value
E	**3**	**8.74** ***	**3**	**13.58** ***	**3**	**5.30** **
G	**165**	**6.36** ***	**165**	**6.58** ***	**165**	**10.36** ***
RIL	163	6.00 ***	163	6.59 ***	163	10.00 ***
Parent	1	116.73 **	1	80.57 **	1	772.21 ***
RIL vs. Parent	1	12.88 *	1	0.78	1	0.62
Block(E)	**4**	**7.62** ***	**4**	**18.35** ***	**4**	**25.00** ***
E *G	**489**	**1.27** **	**489**	**1.54** ***	**489**	**1.35** ***
E *RIL	483	1.28 **	483	1.54 ***	483	1.33 ***
E*Parent	3	0.56	3	0.29	3	0.16
E*(RIL Vs.Parent)	3	1.33	3	4.10**	3	2.10***
σ_E^2 [§]		30.628		121.350		69.730
σ_G^2		124.701		172.007		319.145
σ_{EG}^2		19.705		42.911		34.802

[†] E Represents environment; G represents genotype; df represents degree of freedom; F-value represents F statistics; Statistical significance level: * p<0.05, * * p<0.01, ***p<0.001

[§] The variance components, where σ_E^2 environmental variance; σ_G^2 genotypic variance; σ_{EG}^2 genotype × environment (GE) interaction variance

Table 2.4 Correlation coefficients for pair wise comparisons of nine traits based on a set of 164 RILs grown in four field environments (Corvallis, Moro, Pendleton and Pullman in 2008).

Trait[†]	Environment[‡]	KHA[§]	BFY	BRN	MID	PHT	HDD	TWT	GPC	TKW
BFY	Cob	**-0.502**								
	Cav	**-0.587**								
	Mor	**-0.465**								
	Pen	**-0.613**								
	Pul	**-0.464**								
BRN	Cob	**-0.273**	**-0.226**							
	Cav	**-0.219**	-0.075							
	Mor	**-0.278**	**-0.314**							
	Pen	**-0.215**	*-0.108*							
	Pul	**-0.391**	*-0.109*							
MID	Cob	**0.597**	**-0.512**	**-0.721**						
	Cav	**0.581**	**-0.650**	**-0.709**						
	Mor	**0.611**	**-0.466**	**-0.694**						
	Pen	**0.594**	**-0.600**	**-0.730**						
	Pul	**0.636**	**-0.632**	**-0.701**						
PHT	Cob	*0.083*	**0.193**	**-0.412**	**0.225**					
	Cav	**0.233**	-0.066	0.002	0.043					
	Mor	-0.032	0.081	-0.090	0.021					
	Pen	-0.033	0.041	**-0.343**	**0.247**					
	Pul	0.081	-0.003	**-0.231**	**0.180**					
HDD	Cob	**-0.237**	-0.058	**0.267**	**-0.193**	**-0.449**				
	Cav	**-0.257**	**0.181**	-0.008	-0.119	**-0.225**				
	Mor	-0.071	0.030	0.085	-0.103	*-0.113*				
	Pen	-0.091	*0.122*	**0.294**	**-0.322**	**-0.341**				
	Pul	-0.034	*-0.117*	0.010	0.076	-0.083				
TWT	Cob	**0.110**	0.023	**-0.383**	**0.321**	**0.256**	-0.021			
	Cav	**0.255**	-0.040	-0.086	0.091	**0.395**	**-0.415**			
	Mor	**0.184**	*0.116*	**-0.469**	**0.349**	0.075	**-0.264**			
	Pen	-0.040	*0.124*	**-0.470**	**0.293**	**0.634**	**-0.510**			
	Pul	**0.173**	0.087	**-0.310**	**0.178**	**0.526**	*-0.128*			
GPC	Cob	-0.032	**-0.075**	**0.307**	**-0.218**	**-0.122**	**0.261**	**-0.443**		
	Cav	0.070	**-0.180**	**0.152**	0.011	-0.038	0.038	-0.083		
	Mor	*0.121*	**-0.257**	**0.170**	0.036	**-0.184**	**0.235**	*-0.125*		
	Pen	0.105	**-0.177**	**0.406**	**-0.207**	**-0.151**	**0.353**	**-0.482**		
	Pul	0.026	*-0.118*	**0.202**	-0.074	-0.040	*0.112*	**-0.381**		
TKW	Cob	**0.127**	0.007	**-0.416**	**0.362**	**0.475**	**-0.536**	**0.479**	**-0.496**	
	Cav	-0.051	-0.062	*0.144*	-0.065	**0.277**	0.075	*-0.145*	*0.144*	
	Mor	0.049	-0.041	**-0.335**	**0.343**	-0.047	*-0.108*	**0.499**	0.096	
	Pen	**-0.151**	0.075	**-0.383**	**0.257**	**0.435**	**-0.360**	**0.643**	**-0.422**	
	Pul	0.037	-0.076	**-0.137**	**0.158**	**0.473**	-0.091	**0.563**	**-0.166**	
KDM	Cob	**0.187**	-0.029	**-0.415**	**0.386**	**0.493**	**-0.539**	**0.526**	**-0.464**	**0.955**
	Cav	0.077	**-0.171**	**0.178**	-0.014	**0.378**	-0.005	-0.004	0.106	**0.837**
	Mor	**0.146**	-0.084	**-0.349**	**0.388**	0.021	**-0.183**	**0.573**	0.059	**0.915**
	Pen	-0.052	0.022	**-0.381**	**0.292**	**0.531**	**-0.362**	**0.687**	**-0.343**	**0.912**
	Pul	**0.154**	-0.104	**-0.206**	**0.233**	**0.457**	-0.104	**0.620**	**-0.161**	**0.903**

[†] Abbreviation of traits is the same as in Table 2.1.

[‡] Across environments (Cob), Corvallis (Cav), Moro (Mor), Pendleton (Pen), and Pullman (Pul)

[§] Values represent the correlation coefficients between traits across environments, Corvallis, Moro, Pendleton, and Pullman in 2008, respectively. Bold and Italic numbers represent significant correlation coefficients at $p<0.01$ and $p< 0.05$ level, respectively.

CHAPTER 3

Three Chromosome Translocations Identified During Linkage Analysis in Common Wheat (*Triticum aestivum* L.)

Guomei Wang, Jeffrey M. Leonard, and Oscar Riera-Lizarazu

Abstract

With the development of inexpensive and high-throughput DNA marker platforms, high-density genetic mapping can be applied as a tool for the study of wheat genetics and breeding. Although linkage maps may be used for quantitative trait loci (QTL) mapping, they also provide useful information about the genetic background of the parental lines. This study shows the potential for identifying and characterizing chromosome translocations through linkage mapping. Three chromosome translocations, 1BL.1RS, 2N^{v}S-2AS.2AL and 5B:7B, were identified from a genetic map with 650 marker loci. The map was based on a mapping population of 164 F_6- derived recombinant inbred lines (RILs) derived from a cross between wheat lines OS9A and QCB36. Two translocations, the short arm of rye (*Secale cereale* L.) chromosome 1R (1RS) linked to 1BL and a segment of the short arm of *Ae. ventricosa* chromosome 2N^{v} (2N^{v}S) linked to 2AS were introduced into the mapping population from parent QCB36. As for the 5B:7B translocation, no significant marker segregation distortion was observed. The identification of karyotypic anomalies during linkage analysis of a mapping population and understanding their potential impacts on genetic map quality is a necessary pre-requisite if this resource is to be utilized in map-based studies.

Introduction

Genetic linkage maps are useful tools to study the structure of genomes and to localize factors that underlie phenotypic traits. In common wheat (*Triticum aestivum* L.), the development of linkage maps with adequate genome coverage, despite the complexity of the wheat genome, has become easier due to developments in molecular marker technology. Two marker systems, simple sequence repeat or microsatellite (SSR) based on polymerase chain reaction (PCR) and diversity array technology (DArT), a high throughput array hybridization-based genotyping platform, are now commonly used in wheat studies (Akbari et al., 2006; Francki et al., 2009; Mantovani et al., 2008; Peleg et al., 2008). In addition, various high-throughput platforms to assay single nucleotide polymorphisms (SNPs) are being developed and will become available shortly (Akhunov et al., 2009; Edwards et al., 2009; Paux et al., 2010). The availability of these marker platforms and the ease in linkage map construction have fostered intense activities in the areas of qualitative gene tagging, quantitative trait loci (QTL) mapping, marker-assisted selection, physical map construction, and map-based gene cloning (Gupta et al., 2008).

Karyotypic variation can lead to ambiguities in linkage map construction which in turn affect subsequent studies that depend on map-based approaches. This issue is particularly important in wheat since germplasm includes accessions carrying a variety of wheat-wheat and wheat-alien chromosomal translocations (Badaeva et al., 2007; Friebe et al., 1996). In the course of the development of a comprehensive linkage map based on a recombinant inbred line (RIL) population (OS9XQ36) derived from a cross between two elite soft white wheat lines, we discovered that three important chromosomal translocations (1BL.1RS, 2N^{v}S-2AS.2AL, and 5B:7B) were segregating in this population. Here, we report a comprehensive linkage map, and describe details about the identification of translocations, the characterization of these translocations using our linkage data, the separation of markers from translocation 5B:7B to

chromosomes 5B and 7B, and the effects of these translocations on the final map construction.

Materials and methods

Plant materials, DNA extraction and markers screening

The plant material for this study included 164 F_6-derived recombinant inbred lines (RILs) (the OS9XQ36 wheat mapping population) generated from a cross between OS9A (PI 658243), a single plant selection from the cultivar 'Stephens' [CI 17596; 'Nord Desprez' (Vilmorin 27/Hybride du Joncquois) / Pullman Sel. 101] and QCB36 (PI 658244), a single plant selection from the elite breeding line OR9900553 ('Arminda' /3/ VPM/ 'Moisson 951' // 2*'Hill' /5/ 'Kavkaz' /3/ 'Hybrid Delhi' / 'Olesen' // 'Bluebird' /4/ Pullman 101 // 'Omaha' / 178383 // 'Riebsel' /3/ 'Riebsel 1744' // 'Suweon' / 'Gaines' /5/ 'Stephens' // 'Aurora' / 'Yamhill') (Riera-Lizarazu et al., 2010). To develop a linkage map, genomic DNA of the OS9XQ36 mapping population and parents was extracted from young leaf tissue samples harvested from seedlings grown in the greenhouse following the protocol described by Riera-Lizarazu et al. (2000). A collection of over 1,300 SSR markers (Somers et al., 2004) was screened for polymorphisms between OS9A and QCB36. Subsequently, polymorphic markers were used to genotype the mapping population. PCR-based assays for the SSR markers were performed as described by Leonard et al. (2008). The mapping population was also genotyped at the allele-specific markers for the semi-dwarfing genes, *Rht-B1* and *Rht-D1,* and the vernalization response gene *Vrn-B1*. Assays for the gene-specific markers *Rht-B1* and *Rht-D1* were followed by protocols described by Ellis et al. (2002) and assays for *Vrn-B1* followed by the procedure described by Fu et al. (2005). The mapping population was also genotyped with a rye (*Secale cereale* L.)-specific repetitive element Ris-1 marker (Koebner, 1995) and a sequence-tagged version of marker *Xcmwg682* that detects *Aegilops ventricosa* Tausch. chromatin (Helguera et al., 2003). Finally, the OS9XQ36

mapping population and its parental lines were genotyped with DArT markers (Triticarte Pty Ltd., Yarralumla ACT, Australia) (Akbari et al., 2006; Jaccoud et al., 2001).

Genetic map construction

The construction of a linkage map was performed using Joinmap 4.0 (Ooijen, 2006). Linkage groups were identified and separated with a minimum logarithm of odds (LOD) score of 6.0, and only markers that could be ordered at a LOD score of ≥ 3.0 were included in linkage maps. Locus order and genetic distances (centiMorgan, cM) were calculated using regression mapping and the Kosambi mapping function (Kosambi, 1944). The assignment of a linkage group to a chromosome was deduced from other published wheat maps (Akbari et al., 2006; Nelson et al., 1995; Quarrie et al., 2005; Roder et al., 1998; Semagn et al., 2006; Somers et al., 2004). Linkage maps were drawn using MapChart v2.2 (Voorrips, 2002).

Results

Mapping

Of 1,320 SSR markers screened, 397 (30%) were polymorphic between the two parents of the mapping population (OS9A and QCB36). Of 593 DArT markers, 360 (61%) were polymorphic. Thus, 757 markers were used to genotype the mapping population and construct a linkage map. Of a total 757 defined loci, 650 (86%) were placed to specific locations on a comprehensive linkage map, 49 (6%) were assigned to an approximate position, and 58 (~8%) remained unlinked. The 650-locus map consisted of 326 SSR, 321 DArT, and three gene-specific marker loci, and was arranged in 46 linkage groups spanning 1,801 cM with an average of 31 marker loci per chromosome and an average inter-marker distance of 2.8 cM (Table 3.1; Figure 3.1). Overall, the linkage maps presented here show a high consistency in marker order and marker distribution

with previously published maps (Akbari et al., 2006; Peleg et al., 2008; Somers et al., 2004).

Comparison of our linkage map to published linkage maps indicates that our map covers nearly 60% to 80% of the wheat genome. Thirteen linkage groups totaling 583 cM cover the A genome with 200 loci and an average inter-marker distance of 2.9 cM. The B-genome has the highest number of loci (279) in 15 linkage groups spanning the shortest genetic distance (570 cM) with an average inter-marker distance of 2.0 cM. The number of loci for the 18 D-genome linkage groups was 171 spanning 648 cM with an average inter-marker distance of 3.8 cM. The total number of mapped SSR on each genome is similar but DArT markers were underrepresented in D genome linkage groups. A- and B-genome maps contained 106 and 166 DArT markers, respectively, but there were only 49 DArT loci assigned to the D-genome. Overall, DArT markers were complementary to other markers used and provided additional genome coverage (Table 1; Figure 3.1).

Translocation chromosomes

In the linkage map of chromosome 1B, there was a large terminal cluster of tightly linked marker loci, suggesting the absence of recombination in a significant portion of this chromosome (Figure 3.2). Map comparisons showed that the cluster of linked loci in our map corresponded to more than 60 cM in other linkage maps of wheat chromosome 1B (Semagn et al., 2006; Somers et al., 2004). Furthermore, this comparison indicated that this cluster of linked markers represented the entire short arm of wheat chromosome 1B. We reasoned that a likely explanation for this observation was the segregation of the 1BL.1RS translocation chromosome known to be relatively frequent in elite wheat germplasm (Lukaszewski, 1990; Zeller, 1973). In lines that carry 1BL.1RS, the short arm of chromosome 1B (1BS) has been substituted by the orthologous short arm of rye chromosome 1R (1RS). Thus, a population from a cross between a line carrying a normal chromosome 1B and a line carrying the 1BL.1RS

chromosome would show no recombination between the short arms of chromosomes 1B and 1R. To test this possibility, we assayed the parents of the mapping population with a rye-specific marker (RIS) (Koebner, 1995). Rye chromatin was absent in DNA from OS9A, but was present in DNA from QCB36. When the entire population was assayed with the rye-chromatin specific marker followed by linkage analysis, the cluster of linked SSR and DArT loci were also found to be linked to the rye-specific element RIS. Furthermore, majority markers from this cluster (54) only detected OS9A chromatin (OS9A dominant markers) but not chromatin from QCB36 (QCB36 null markers). Thus, assays with the rye-specific marker RIS and both SSR and DArT markers suggested that QCB36 carries rye chromatin (1RS) in the place of the short arm of chromosome 1B. Interestingly, three markers mapped to 1BS, *Xgwm264*, *Xbarc8* and *Xgwm273,* detected chromatin in both OS9A and QCB36 (co-dominant markers). This supports our conclusion that the rye chromatin in QCB36 is homoeologous to chromatin in 1BS. We were also able to map three SSR markers spanning 27 cM of the long arm of chromosome 1B. This suggests that QCB36 carries the long arm of chromosome 1B (1BL), although the level of polymorphism between the long arms of chromosome 1B in OS9A and QCB36 is apparently very low. Overall, our analysis suggests that QCB36 is the source of the 1BL.1RS chromosome translocation in our mapping population. This is consistent with QCB36's pedigree which includes Kavkaz and Aurora, two lines known to carry the 1BL.1RS translocation (Schlegel, 1997; Weng et al., 2007). OS9A, on the other hand does not have ancestors that carry this translocation.

In the linkage map of chromosome 2A, we also observed a terminal cluster of about 30 tightly linked loci in the short arm (Figure 3.3). Map comparisons showed that the cluster of linked loci in our map corresponded to 15-30 cM of the terminal end of the short arm of chromosome 2A (Somers et al., 2004; Song et al., 2005). A likely explanation for this marker cluster was the segregation for another wheat-alien translocation known as 2N^{v}S-2AS.2AL (Bariana and McIntosh, 1994; Bonhomme et al.,

1995). In lines that carry 2N^{v}S-2AS.2AL, a terminal end of the short arm of chromosome 2A (2AS) has been substituted by an orthologous segment from the short arm of *Ae. ventricosa* chromosome 2N^{v} (2N^{v}S). Thus, a population from a cross between a line carrying a normal chromosome 2A and a line carrying the 2N^{v}S-2AS.2AL chromosome would show no recombination between the terminal ends of the short arms of chromosomes 2A and 2N^{v}S. To test this possibility, we assayed the parents of the mapping population with the 2N^{v}S-specific allele of *Xcmwg682* (Helguera et al., 2000). The 2N^{v}S-specific marker was detected in DNA from QCB36 but was absent in OS9A. When the mapping population was assayed with this marker and coupled with linkage analysis, we found that the cluster of terminal markers was linked to the 2N^{v}S-specific marker. As was the case with the 1BL.1BS translocation, most of SSR and DArT markers in the linked cluster detected DNA from OS9A (OS9A dominant markers), but not from QCB36 DNA (QCB36 null markers). This suggested that QCB36 carries *Ae. ventricosa* chromatin (2N^{v}S) replacing a segment of the terminal end of the short arm chromosome 2A. We were also able to map 17 SSR and DArT markers spanning 87 cM representing the proximal end of the short arm of chromosome 2A as well as the entire long arm (2AL). This suggests that besides the terminal end of the short arm, QCB36 carries most of chromosome 2A. Thus, our analysis suggests that QCB36 is also the source of the 2N^{v}S-2AS.2AL chromosome translocation in our mapping population. This is consistent with QCB36's pedigree which includes a VPM1 derivative known to carry this translocation (Badaeva et al., 2007; Bariana and McIntosh, 1994). OS9A, on the other hand, does not seem to have ancestors known to carry this translocation.

Another anomaly that we observed during map construction was strong linkage (up to LOD 20) of 41 loci from chromosomes 5B and 7B (Figure 3.4). A likely explanation of this unexpected level of association of markers from different chromosomes is the segregation of a reciprocal translocation involving chromosomes 5B and 7B. Reciprocal translocations result in the statistical association or linkage between loci on the

chromosomes involved in the interchange due to the formation and resolution of a multivalent at meiosis (Livingstone et al., 2000). In order to resolve this pseudolinkage group involving loci from chromosomes 5B and 7B, we used QuadMap (Durrant et al., 2006) to construct multiple maps and used the variance in marker-pair distances among permuted maps to identify loci that belonged to the four segments of the translocation in question. Unfortunately, only two segments could be defined with this approach. One segment only included markers *Xgwm46* and *Xgwm16* belonging to chromosome 7B whereas the other segment included a collection of markers from both chromosomes. We attribute our difficulty in defining additional segments to very tight linkage between all of the markers involved (marker-pair distance variances being mostly 0 cM). Map comparisons showed that the loci involved in our pseudolinkage group were localized in chromosomal regions characterized by reduced recombination. Since this analytical approach yielded limited results, we opted to use information from other linkage maps to segregate markers from chromosomes 5B and 7B and reconstructed maps for these chromosomes in isolation (Figure 3.4 and 3.5). Marker *Xgwm213* was excluded from our analysis since this marker has been mapped to both chromosomes 5B and 7B (Somers et al., 2004). In any case, the resulting maps were comparable to those reported elsewhere. It was impossible to deduce the origin of the 5B:7B reciprocal translocation by inspecting the pedigrees of OS9A and QCB36 because both lines have ancestors that are known to carry this chromosome interchange. On the other hand, we have developed another population based on the cultivar 'Stephens' (the source of OS9A) that does not show a pseudolinkage group involving chromosomes 5B and 7B (data not shown). Thus, we believe that QCB36 is the likely source of the 5B:7B chromosome translocation in our population.

Marker segregation distortion (SD)

Evaluation of segregation distortion (SD) revealed areas on chromosomes 1B, 2A, 4D and 7A that favored the transmission of alleles from OS9A whereas regions on

chromosomes 2D, 3B, 4B and 7A favored alleles from QCB36 (Figure 3.1). The 1BL.1RS and the 2N^{v}S-2AS.2AL chromosome translocations were present at a lower frequency (40 and 44%, respectively) than expected from random segregation (50%). Thus, loci associated with these translocation chromosomes exhibited significant segregation distortion ($p<0.05$). In both cases, the wheat-alien translocation chromosomes were disfavored over normal chromosomes 1B and 2A. Two other regions displaying significant segregation distortion were observed on chromosomes 4B and 4D close to the semi-dwarfing genes *Rht-B1* and *Rht-D1* ($p<0.01$). These distortions probably resulted from negative selection against lines with extreme heights (tall or dwarf) during the development of the mapping population.

Discussion

Genetic maps are useful tools to dissect complex traits. In the case of wheat, an allohexaploid species with a large genome and a relatively high number of chromosomes (21), the construction of a linkage map requires substantial resources in terms of polymorphic markers and mapping platforms. Here, we describe a comprehensive linkage map of wheat with 650 loci covering 1,801 cM. This comprehensive linkage map provides around 60% to 80% coverage of wheat genome compared with other published maps (Akbari et al., 2006; Paillard et al., 2003; Quarrie et al., 2005; Song et al., 2005).

Wheat-wheat and wheat-alien chromosomal translocations have played an important role in wheat evolution and breeding (Friebe et al., 1996). In one study, a survey of germplasm from Europe, Asia and the USA (252 genotypes) revealed that over a quarter (27%) of accessions carried chromosome translocations (Badaeva et al., 2007). Thus, the fixation of spontaneous translocations in wheat suggests that these may have a beneficial or adaptive value. Similarly, spontaneous wheat-alien translocations have also been described. One example that has had a substantial impact

on wheat improvement is the introduction of alien chromosome 1RS from rye (*Secale cereale* L.) into wheat. The 1RS chromosome has been reported to carry genes for disease and insect resistance, yield enhancement, and other agronomic traits (Moreno-Sevilla et al., 1995; Zeller and Hsam, 1996). On the other hand, 1RS has a negative effect on wheat end-use product quality, such as milling and baking quality (Dhaliwal et al., 1987; Johnson et al., 1999; McKendry et al., 1996). Although alien chromosome 1RS segment has negative effects on the agronomic performance of wheat, the agronomic desirability is probably dependent on the size of the transferred chromosome segment and the degree of linkage drag. Villareal et al. (1998) argued that the superior agronomic performance of wheat lines with the translocation 1RS segment is probably because of the positive interaction between the genes of translocation chromosome 1RS and the genes of *Triticum aestivum* chromosomes. Less recombination was observed at the markers near RIS, probably due to the lack of pairing between the short arms of 1B and the 1RS.1BL chromosome which have led to the exclusive formation of rod bivalents (pairing of the long arms only). In contrast, no significant impact from translocation 1RS.1BL was observed on the recombination rate between markers on the long arm of chromosome 1B.

Another type of alien chromosome translocation carrying important disease resistance genes is the translocation $2N^vS$-2AS.2AL, which had incorporated chromatin from chromosome 2NS of the wild species *Aegilops ventricosa* (Bariana and McIntosh, 1994). The rust resistance gene cluster *Lr37-Yr17-Sr38* has been mapped onto the 2NS chromosome segment of translocation $2N^vS$-2AS.2AL (Seah et al., 2001). The cereal cyst nematode resistance gene *Cre5* is also located in this alien introgression (Jahier et al., 2001). Markers close to the translocation chromosome segment $2N^vS$-2AS.2AL show significant segregation distortion, and less recombination was observed in the chromosome region near the translocation breakpoint. The lack of pairing between the short arm of 2A and the translocation chromosome $2N^vS$-2AS.2AL reduced the

recombination in the proximal areas of the short arm of chromosome 2A due to the formation of rod bivalents (mostly pairing of the long arms). However, the recombination rate between markers on the long arm of chromosome 2A was not impacted, an observation that is contrary to what would have expected. This may be due to the fact that the terminal segment is small and located far away from the long arm of the chromosome.

Based on a study of around 500 wheat genotypes, B-genome chromosomes have been reported to be involved in wheat-wheat chromosome interchanges more frequently than A or D-genome chromosomes (Badaeva et al., 2007). Among these, the T5B:7B-1 reciprocal translocation, involving the short arm of chromosome 5B and the long arm of chromosome 7B, is abundant in germplasm from Western Europe, but rare in germplasm from Eastern Europe (Badaeva et al., 2009). This pattern of distribution has led to the suggestion that this translocation may have adaptive value. Thus, understanding the structure and organization of this reciprocal translocation is of importance. Chromosome banding studies suggest that the breakpoints for this translocation were at a fraction length (FL) of 0.4 in the short arm of chromosome 5B and an FL of 0.1 in the long arm of chromosome 7B. In our study, markers flanking these breakpoints on chromosomes 5B and 7B were tightly or completely linked. This observation is consistent with the cytological characterization of this reciprocal translocation and its expected impact on linkage mapping due to its behavior during meiosis. The strong linkage between markers of these chromosomes was exacerbated because these markers are apparently localized to areas of suppressed recombination despite being physically distant. Although the linkage maps for chromosomes 5B and 7B are comparable with other published maps, suppressed recombination and the reciprocal translocation effect may have affected our ability to determine accurate marker orders and distances. Thus, one should be cautious when using linkage maps for chromosomes 5B and 7B from mapping populations where the 5B:7B chromosome

translocation is segregating. Pseudo-linkage between loci from chromosomes 5B and 7B may result in marker-trait association uncertainties. Thus, additional research to evaluate the effect of this translocation on QTL mapping is warranted.

In this study, we characterized chromosome translocations discovered during linkage mapping using the OS9XQ36 mapping population. Also, the potential impact of chromosome translocations on genetic map construction, genetic analysis, marker segregation distortion, and recombination has been discussed. This information should allow better use of the OS9XQ36 mapping population in map-based studies.

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Figure 3.1 Genetic linkage groups of 650 marker loci analyzed on 164-RILs derived from the cross OS9 × Q36. The linkage groups are orientated with short arms at top for 21 chromosomes. Genetic distance in the linkage map is given in Kosambi centiMorgans (cM) on the left of each linkage group. Markers assigned to the same map location are boxed, and unmapped markers are shown at their most likely position in the genetic map (dashed line box). Marker loci showing statistical significant deviations from the expected 1:1 segregation ratio at $0.01<p<0.05$ and $p<0.01$ levels are indicated with * and **, respectively. Shaded chromosome segments indicate regions of significant ($p<0.01$) marker segregation distortion (grey boxes, OS9A alleles are favored; cross hatched, QCB36 alleles are favored). The black colored bar and triangle on the left of linkage maps 5B and 7B represent the approximate break regions of translocation 5B:7B.

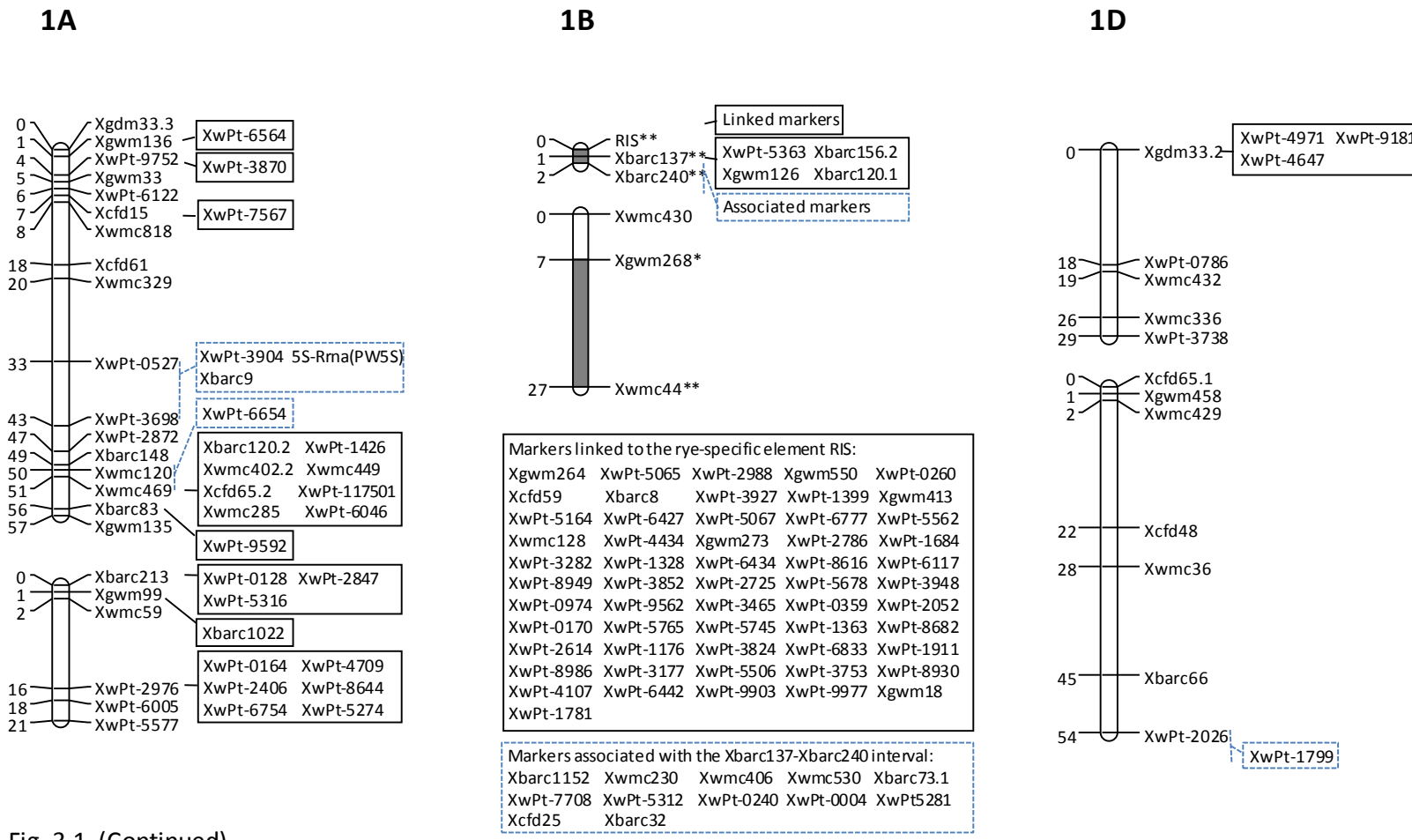
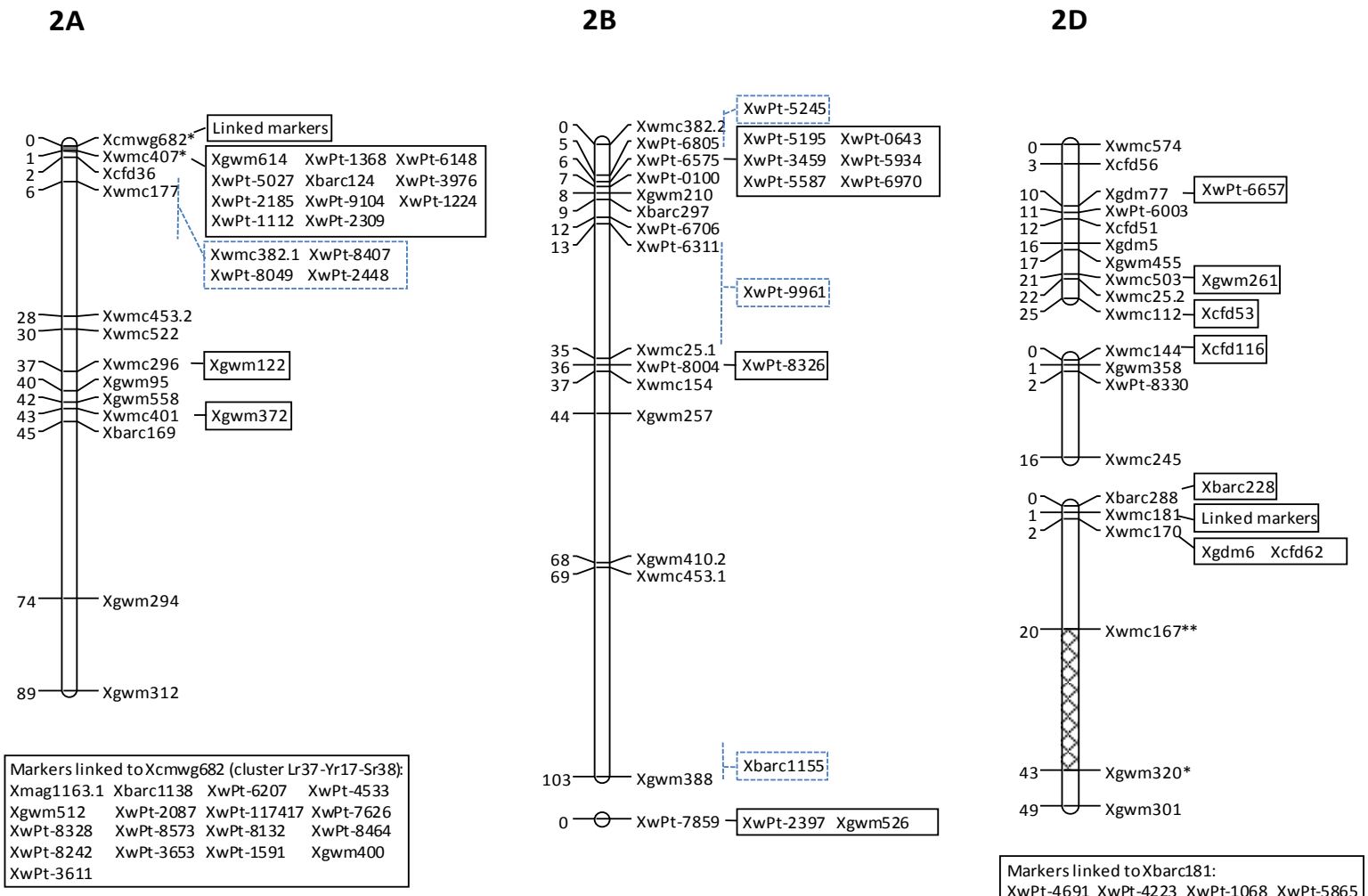


Fig. 3.1 (Continued)



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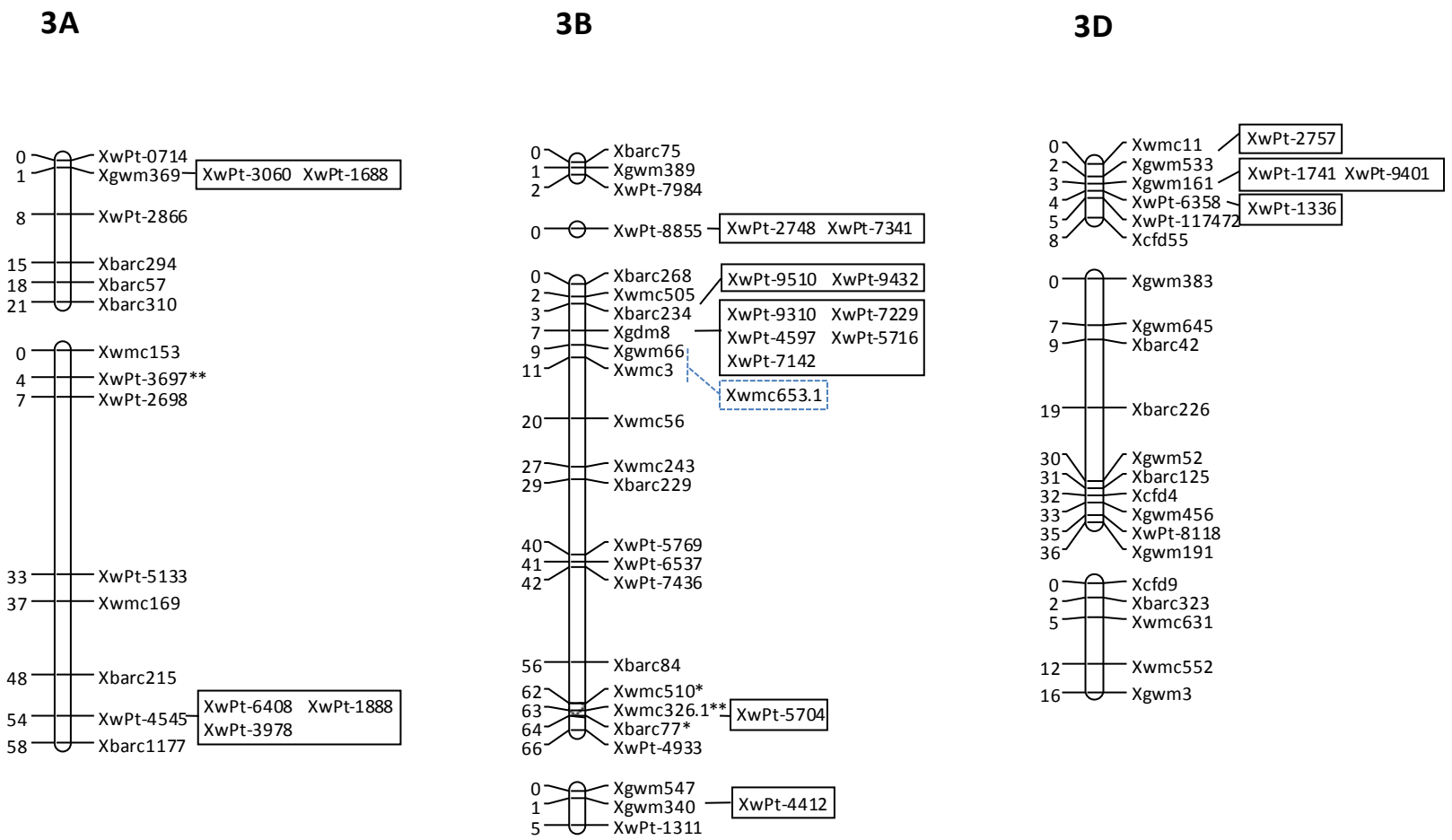

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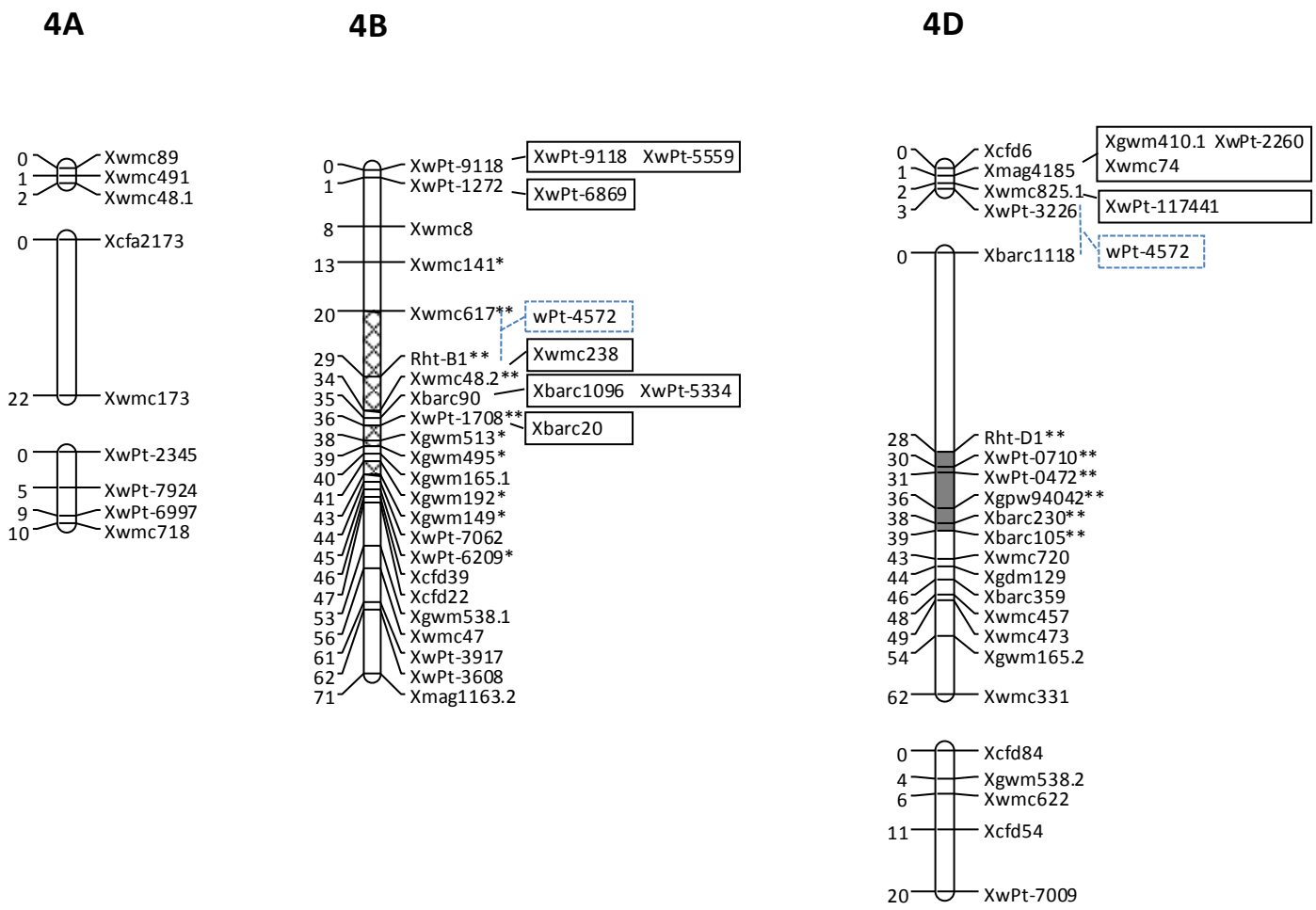

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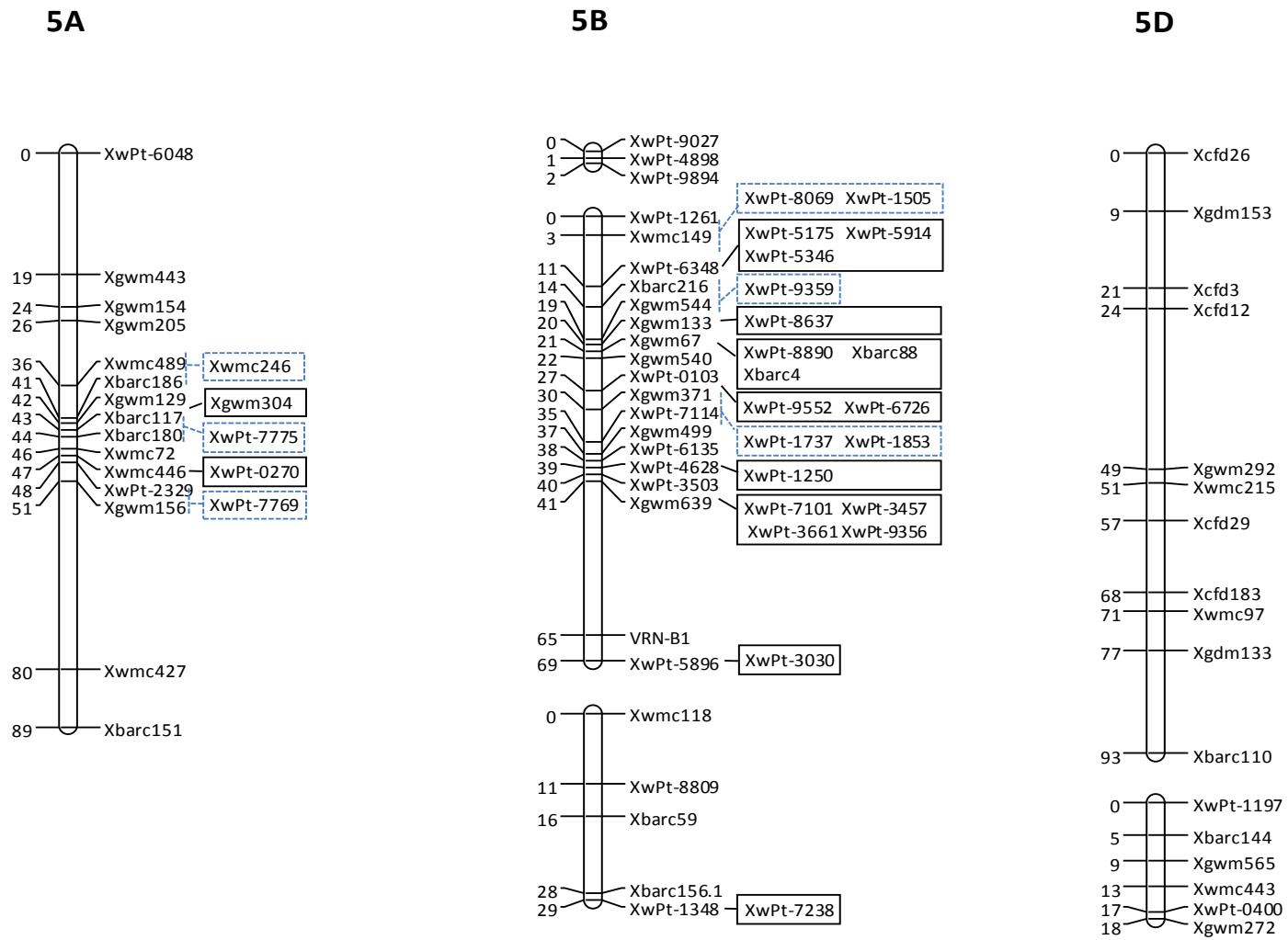

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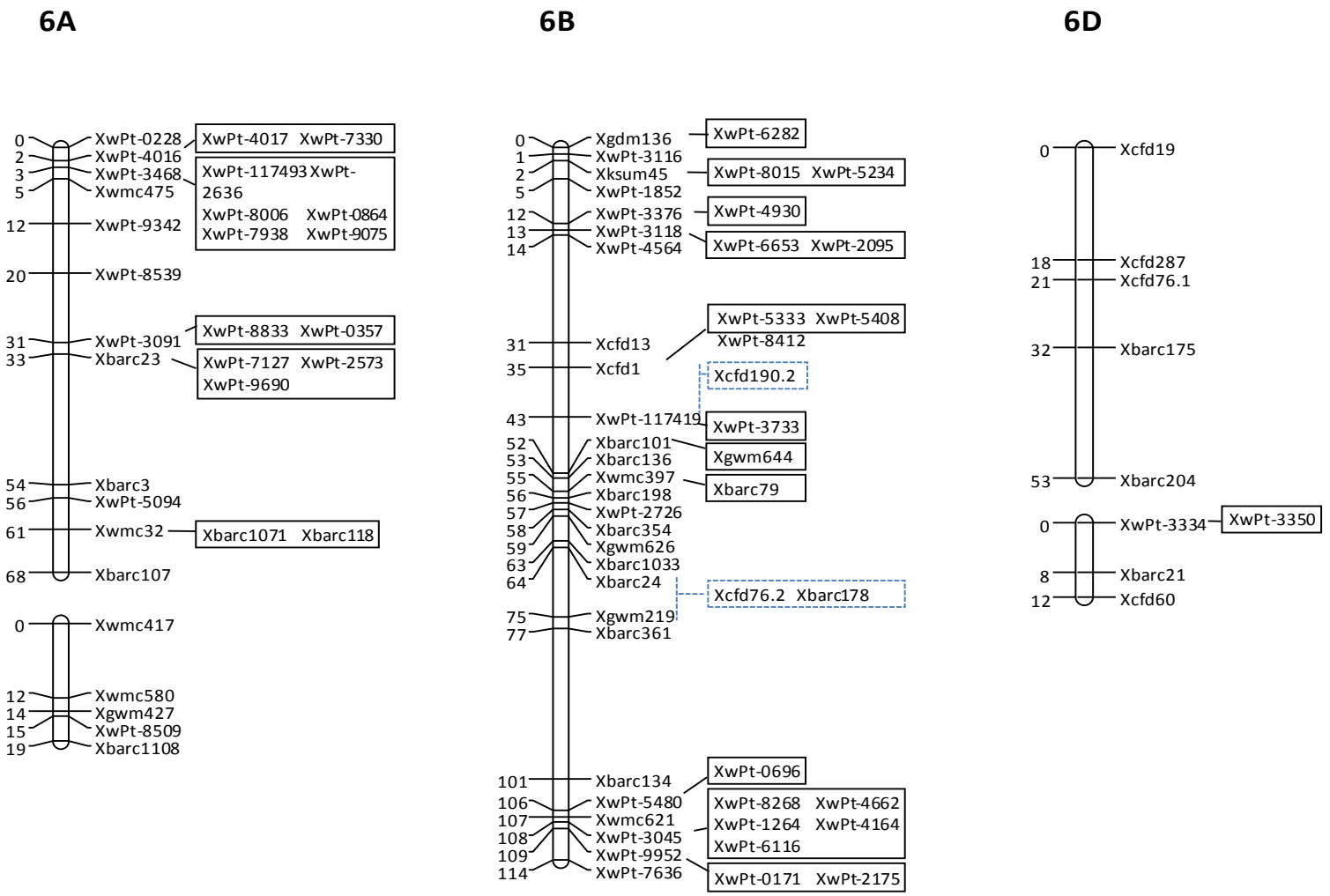

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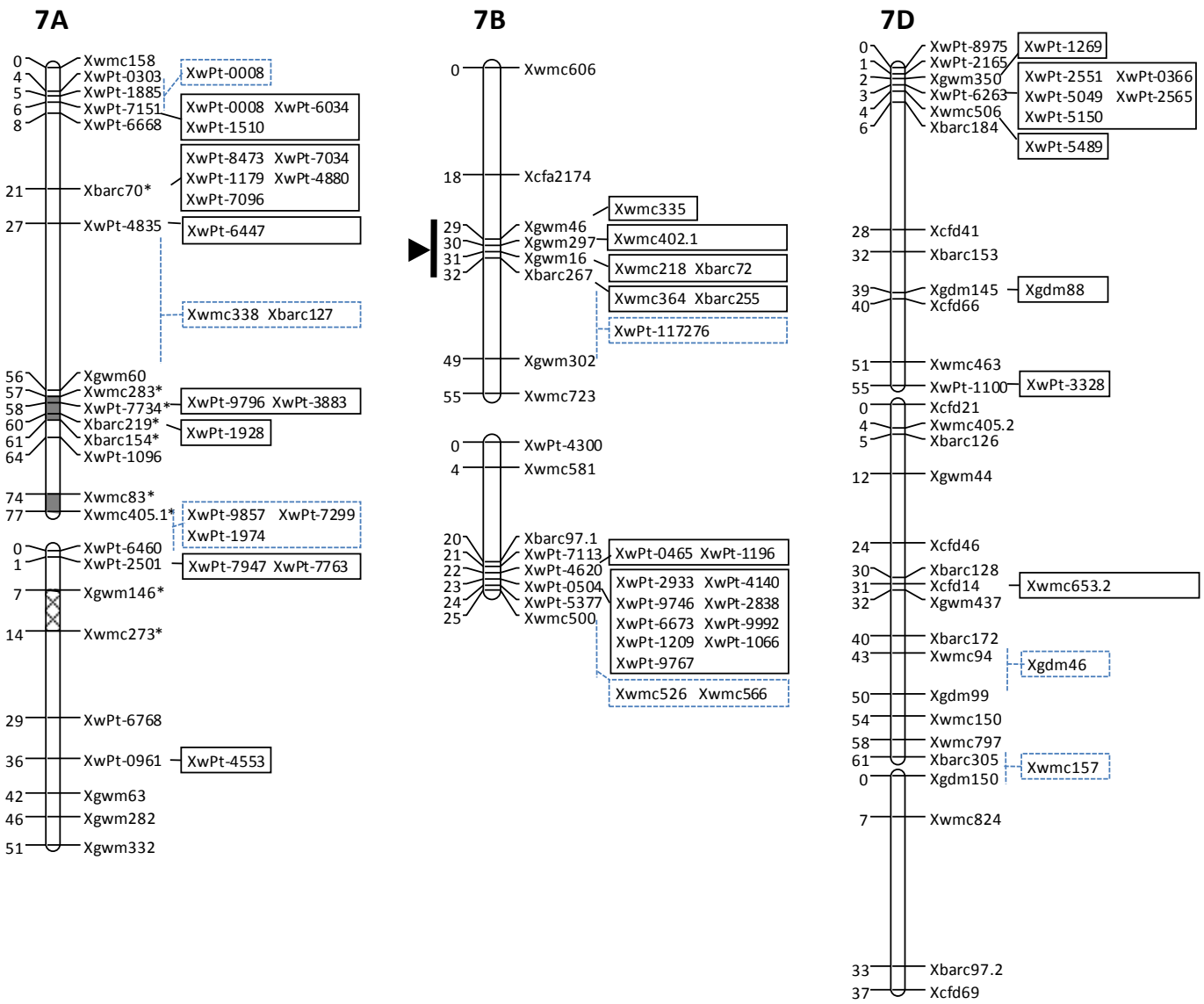
7A
0 Xwmc158
4 XwPt-0303
5 XwPt-1885
6 XwPt-7151
8 XwPt-6668
XwPt-0008
XwPt-0008 XwPt-6034
XwPt-1510
XwPt-8473 XwPt-7034
XwPt-1179 XwPt-4880
XwPt-7096
21 Xbarc70*
27 XwPt-4835
XwPt-6447
Xwmc338 Xbarc127
56 Xgwm60
57 Xwmc283*
58 XwPt-7734*
60 Xbarc219*
61 Xbarc154*
64 XwPt-1096
XwPt-9796 XwPt-3883
XwPt-1928
74 Xwmc83*
77 Xwmc405.1*
XwPt-9857 XwPt-7299
XwPt-1974
0 XwPt-6460
1 XwPt-2501
XwPt-7947 XwPt-7763
7 Xgwm146*
14 Xwmc273*
29 XwPt-6768
36 XwPt-0961
XwPt-4553
42 Xgwm63
46 Xgwm282
51 Xgwm332
7B
0 Xwmc606
18 Xcfa2174
29 Xgwm46
30 Xgwm297
31 Xgwm16
32 Xbarc267
Xwmc335
Xwmc402.1
Xwmc218 Xbarc72
Xwmc364 Xbarc255
XwPt-117276
49 Xgwm302
55 Xwmc723
0 XwPt-4300
4 Xwmc581
20 Xbarc97.1
21 XwPt-7113
22 XwPt-4620
23 XwPt-0504
24 XwPt-5377
25 Xwmc500
XwPt-0465 XwPt-1196
XwPt-2933 XwPt-4140
XwPt-9746 XwPt-2838
XwPt-6673 XwPt-9992
XwPt-1209 XwPt-1066
XwPt-9767
Xwmc526 Xwmc566
7D
0 XwPt-8975
1 XwPt-2165
2 Xgwm350
3 XwPt-6263
4 Xwmc506
6 Xbarc184
XwPt-1269
XwPt-2551 XwPt-0366
XwPt-5049 XwPt-2565
XwPt-5150
XwPt-5489
28 Xcfd41
32 Xbarc153
39 Xgdm145
40 Xcfd66
Xgdm88
51 Xwmc463
55 XwPt-1100
XwPt-3328
0 Xcfd21
4 Xwmc405.2
5 Xbarc126
12 Xgwm44
24 Xcfd46
30 Xbarc128
31 Xcfd14
32 Xgwm437
Xwmc653.2
40 Xbarc172
43 Xwmc94
Xgdm46
50 Xgdm99
54 Xwmc150
58 Xwmc797
61 Xbarc305
0 Xgdm150
Xwmc157
7 Xwmc824
33 Xbarc97.2
37 Xcfd69

Figure 3.2 Comparison between linkage map of the 1BL.1RS translocation and genetic maps of wheat chromosome 1B (Semagn et al., 2006; Somers et al., 2004). A total of 57 loci linked to a rye-specific element RIS was grouped into dominant or co-dominant (bold) markers. Genetic distance (Kosambi centiMorgans, cM) is indicated on the left hand side and locus name on the right-hand side.

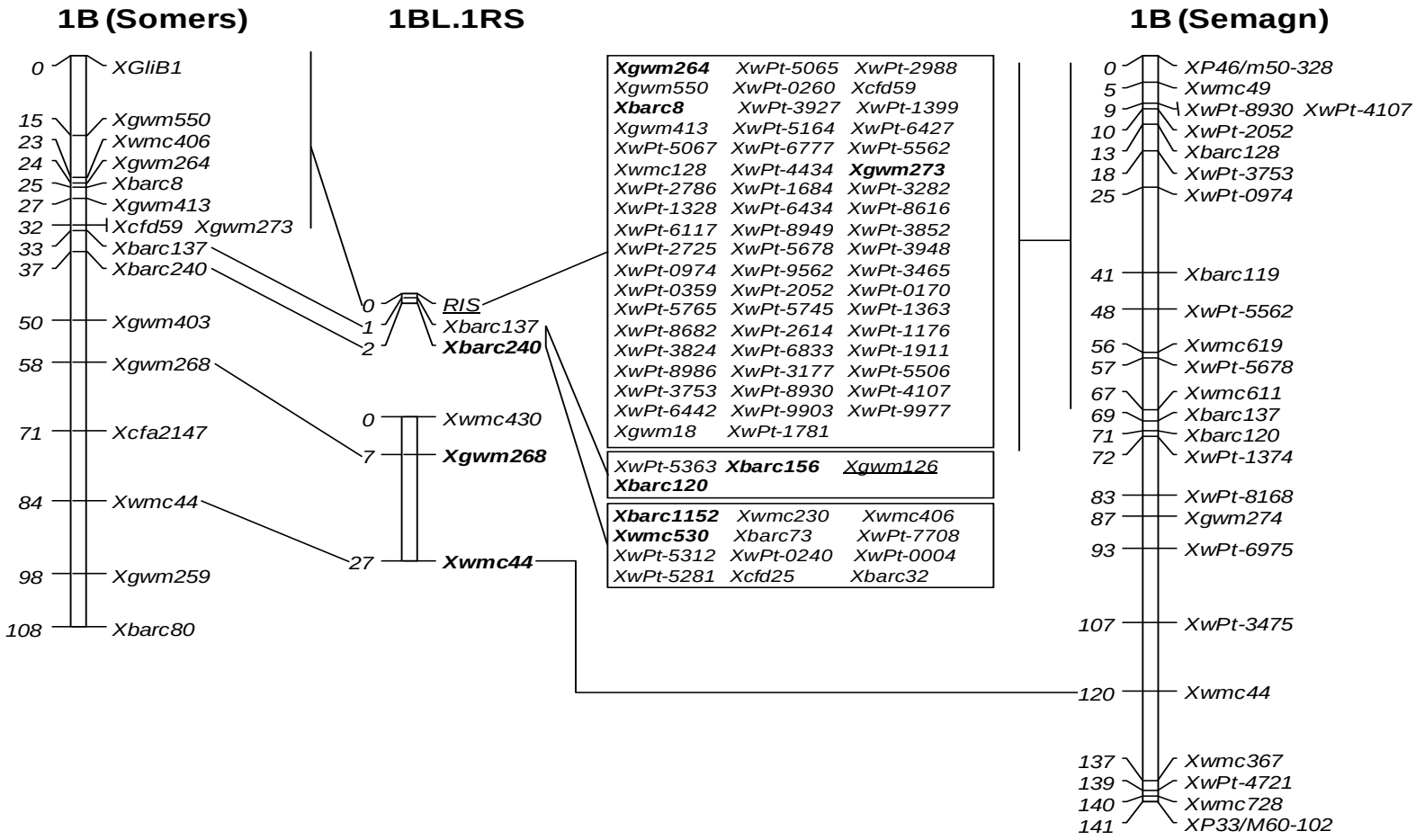

Figure 3.3 Comparison between linkage map of the 2N^{v}S-2AS.2AL translocation and genetic maps of wheat chromosome 2A (Francki et al., 2009; Somers et al., 2004). A total of 17 loci linked to N-alleles of *Xcmwg682*. Genetic distances (Kosambi centiMorgans, cM) are indicated on the left hand side and locus names on the right-hand side. The marker loci in black bold are the co-dominant markers for two parents OS9A and QCB36.

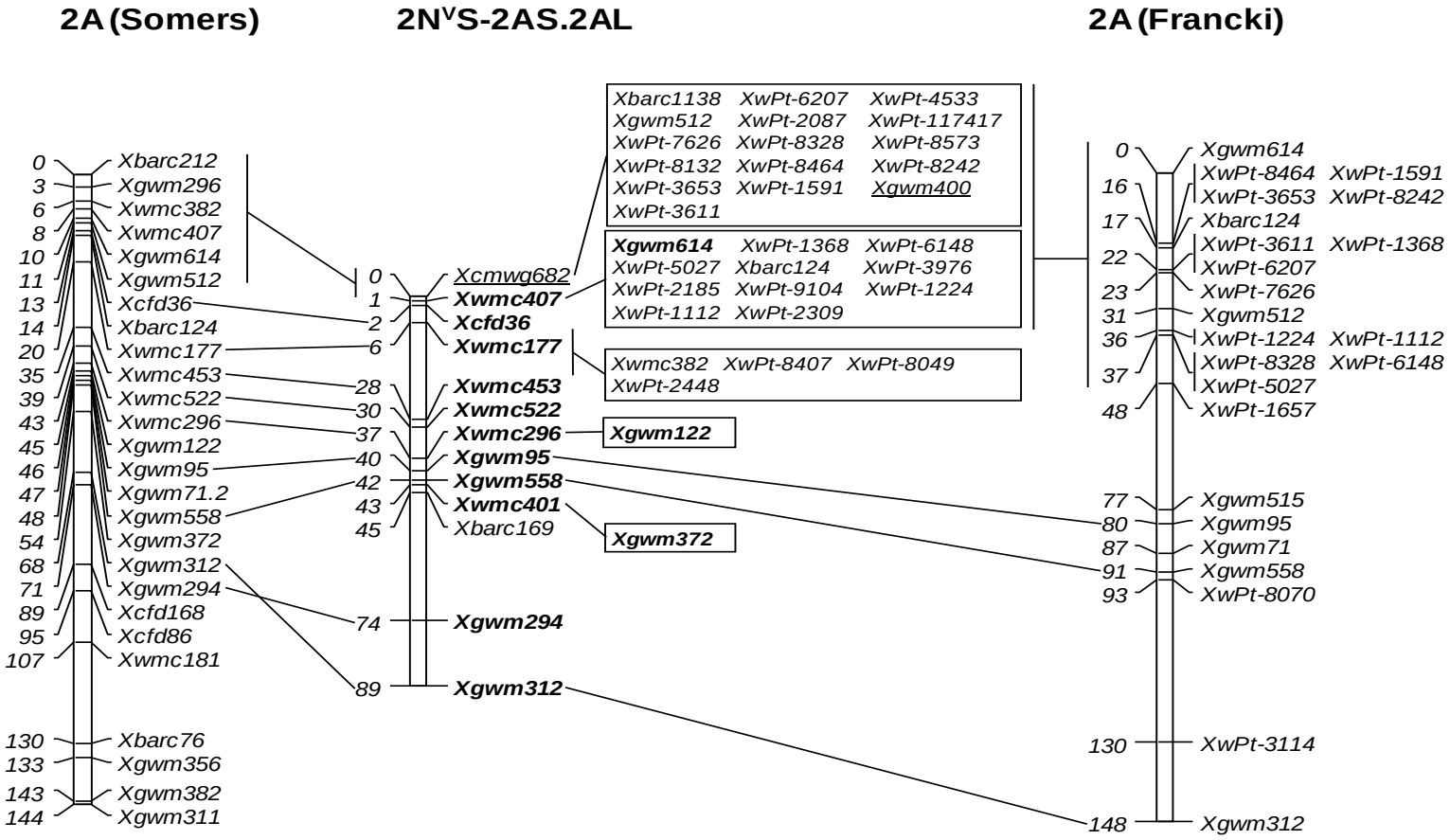

Figure 3.4 A linkage map of the 5B:7B translocation with 41 loci. Genetic distances (centiMorgans, cM) are indicated on the left hand side and locus names on the right-hand side. The loci in bold are from chromosome 7B, others are from chromosome 5B (blue Italic). Two linkages 5B and 7B are compared to the corresponding genetic linkages reported by Somers et al. (2004).

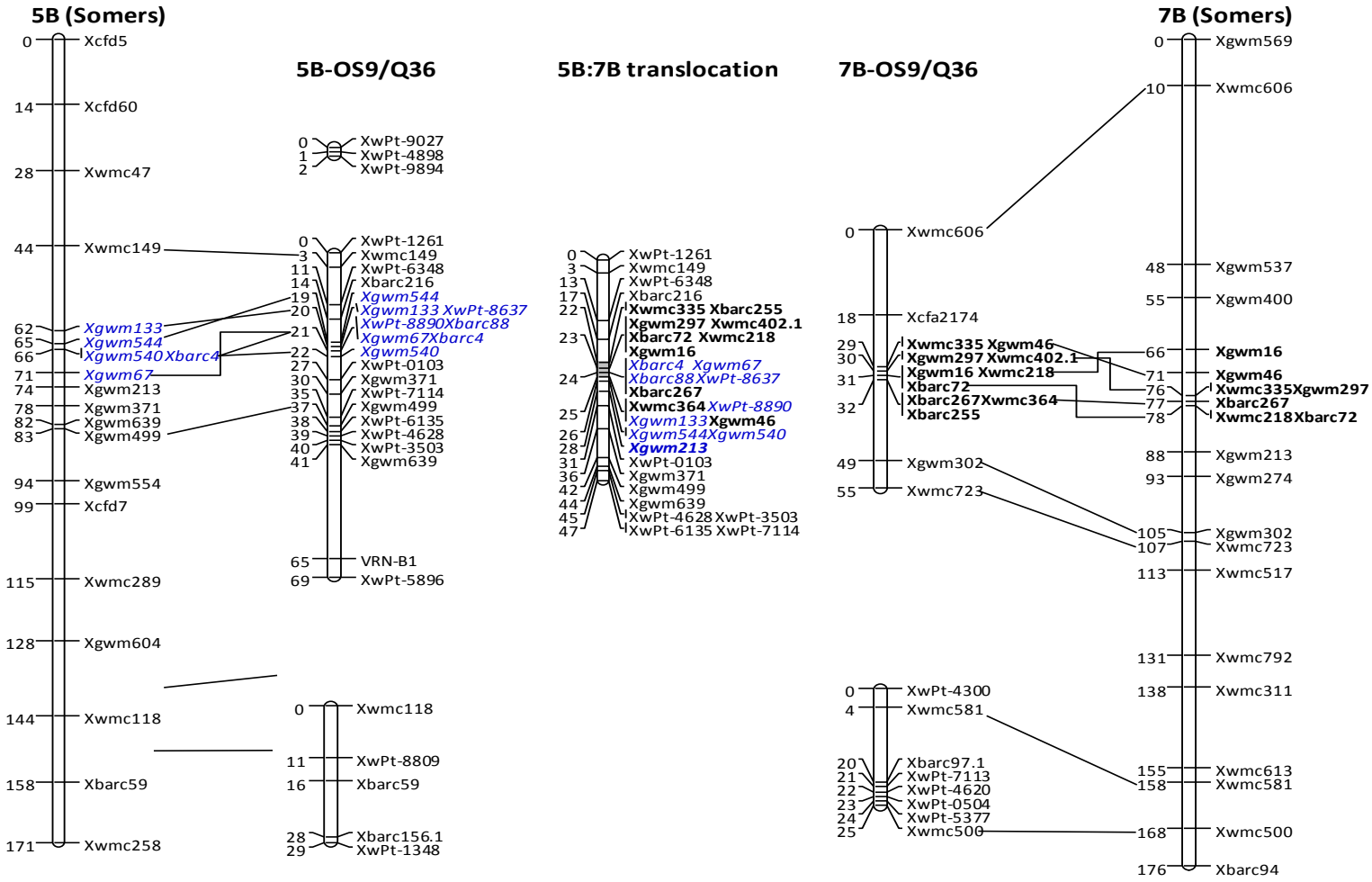

Figure 3.5 Comparison of the genetic maps of 5B and 7B with the physical maps (Somers et al. 2004). The approximate break regions of the translocation 5B:7B were indicated on genetic maps of 5B and 7B, respectively.

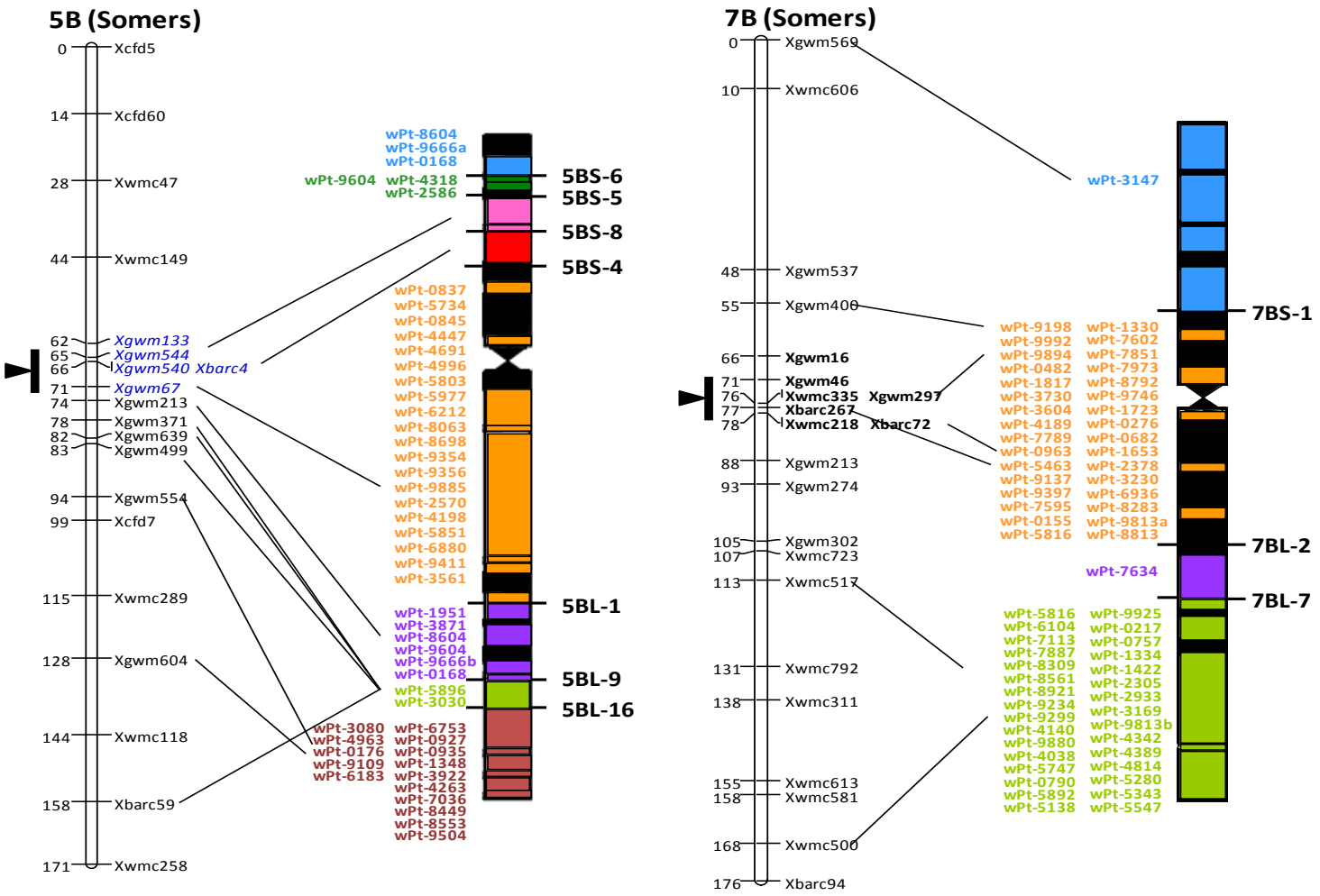

Table 3.1 Chromosome assignment and marker distribution on 46 linkage groups for the OS9×Q36 mapping population

Genome	Chromosome	No. of linkage groups	Genetic distance† (cM)	No. of loci on the comprehensive map‡	Number of markers			No. of loci on the framework linkage map‡	No. of loci linked to the framework linkage map	No. of loci assigned to an approximate position
					SSR	DArT	Gene-specific			
A	1A	2	79	45 (1.7)	21	24		23 (3.4)	22	4
	2A	1	89	43 (2.1)	21	22		13 (6.8)	30	4
	3A	2	79	19 (4.1)	8	11		14 (5.6)	5	0
	4A	3	34	9 (3.8)	6	3		9 (3.8)	0	0
	5A	1	89	17 (5.2)	14	3		15 (5.9)	2	3
	6A	2	87	32 (2.7)	11	21		17 (5.1)	15	0
	7A	2	128	35 (3.6)	13	22		24 (5.3)	11	6
	subtotal	**13**	**583**	**200 (2.9)**	**94**	**106**		**115 (5.1)**	**85**	**17**
B	1B	2	29	66 (0.4)	17	49		6 (4.8)	60	12
	2B	2	104	25 (4.2)	10	15		16 (6.5)	9	3
	3B	4	73	35 (2.1)	17	18		24 (3.0)	11	1
	4B	1	71	30 (2.4)	18	11	1	23 (3.1)	7	1
	5B	3	100	43 (2.3)	14	28	1	26 (3.8)	17	5
	6B	1	114	47 (2.4)	18	29		27 (4.2)	20	3
	7B	2	80	33 (2.4)	17	16		16 (5.0)	17	3
	subtotal	**15**	**570**	**279 (2.0)**	**111**	**166**	**2**	**138 (4.1)**	**141**	**28**
D	1D	2	83	15 (5.5)	9	6		12 (6.9)	3	1
	2D	3	90	38 (2.4)	24	14		20 (4.5)	18	0
	3D	3	60	25 (2.4)	18	7		21 (2.9)	4	0
	4D	3	85	27 (3.1)	20	6	1	23 (3.7)	4	1
	5D	2	112	17 (6.6)	15	2		17 (6.6)	0	0
	6D	2	65	9 (7.3)	7	2		8 (8.2)	1	0
	7D	3	152	40 (3.8)	28	12		30 (5.1)	10	2
	subtotal	**18**	**648**	**171 (3.8)**	**121**	**49**	**1**	**131 (4.9)**	**40**	**4**
	Total	***46***	***1801***	***650 (2.8)***	***326***	***321***	***3***	***384 (4.7)***	***266***	***49***

Note: † Genetic distance was calculated with regression mapping and Kosambi function.
‡ Numbers in brackets represent marker density (cM/marker).

CHAPTER 4

Identification of Genetic Factors Controlling Kernel Hardness and Related Traits in a Soft X 'Extra-soft' Wheat (*Triticum aestivum* L.) Cross

Guomei Wang, Jeffrey M. Leonard, Andrew S. Ross, C. James Peterson, and Oscar Riera-Lizarazu

Abstract

Wheat (*Triticum aestivum* L.) is classified as soft or hard based on kernel texture. Kernel hardness or softness is an important determinant of milling and baking quality. Soft wheat with superior soft texture and end-use quality is described as 'extra-soft' or 'super-soft'. The objective of this study was to identify the underlying genetic factors controlling the 'extra-soft' characteristic of wheat. A total of 47 significant quantitative trait loci (QTL) were identified for nine agronomic traits. Six QTL associated with kernel hardness and flour yield were detected on chromosomes 1BS, 4BS, 5BS, 2DS, 4DS and 5DL. The most important determinants for kernel hardness were QTL on orthologous regions in 4DS (*Xbarc1118-Rht-D1* interval) and 4BS (*Xwmc617-Rht-B1* interval). These results suggest that the 'extra-soft' characteristic was not controlled by Hardness (*Ha*) locus on chromosome 5DS. The QTL for break flour yield, bran yield, middling yield, days to heading, test weight, and thousand-kernel weight occupied a coincident location close to the semi-dwarfing gene *Rht-D1* on chromosome 4DS. Similarly, co-location QTL for break flour yield, bran yield, test weight, and thousand-kernel weight were identified on chromosome 4BS near the semi-dwarfing gene *Rht-B1*. The clustering of these QTL on chromosomes 4BS and 4DS for traits showing significant correlations suggests pleiotropic effects and represent the action of the semi-dwarfing genes *Rht-B1* and *Rht-D1* on chromosomes 4BS and 4DS, respectively. However, kernel hardness differences could not be entirely accounted for by either the *Rht-D1* or *Rht-B1* locus. Thus, the kernel hardness QTL on chromosomes 4B and 4D are genetically linked to these green-revolution genes and may not represent pleiotropic effects.

Introduction

Improvement of flour yield and milling quality is an important objective in wheat (*Triticum aestivum* L.) breeding programs. Kernel hardness or softness is used as the criterion to separate wheat into two market classes, hard and soft. Flour from soft-grained wheat is generally used for pastry-type end-use applications, such as cookies and cakes, rather than bread-based products. In addition to being a fundamental distinction between soft and hard wheat market classes, kernel hardness is a complex trait affecting milling, baking, and other end-use quality of wheat (Borner et al., 2002). Within the soft wheat class, genotypes with consistently softer grains are described as 'extra-soft'. 'Extra-soft' wheats have higher break flour yield and superior milling quality than soft wheat. This is consistent with a negative correlation reported between kernel hardness and flour yield (Parker et al., 1999).

This 'extra-soft' characteristic, which positively affects break flour yield and end-use quality relative to common soft wheat, has fostered interest in developing a novel market class of soft wheat. In turn, the economic potential of this new class of wheat has created an interest in understanding the genetic basis of the 'extra-soft' grain characteristic. Substantial efforts have been devoted to mapping and characterizing the underlying biochemical and genetic control of the variation of wheat grain texture. From a biochemical perspective, Greenwell and Schofield (1986) found that the protein friabilin could be useful as an indication for grain hardness. Friabilin, present in soft wheat but partially or completely absent in hard wheat grains, is encoded by the Hardness (*Ha*) locus located on the short arm of chromosome 5D (Jolly et al., 1996; Mattern et al., 1973). Friabilin is composed of two separate proteins, puroindolines, encoded by *pinA* and *pinB*. The puroindolines are associated with polar lipids and endosperm membranes (Jolly et al., 1996). Consequently, it has been suggested that the

puroindolines are the causal agents for the soft grain phenotype in wheat (Giroux and Morris, 1998). Studies using transgenetic wheat showed that the soft wheat phenotype is primarily controlled by the *pinB-D1b* allele (Beecher et al., 2002; Hogg et al., 2004).

In addition to the *Ha* locus and puroindoline genes, a number of quantitative trait loci (QTL) have been detected for wheat grain hardness in different mapping populations (Arbelbide and Bernardo, 2006; Breseghello et al., 2005; Campbell et al., 1999; Campbell et al., 2001; Nelson et al., 2006; Perretant et al., 2000; Pshenichnikova et al., 2008; Sourdille et al., 1996; Zanetti et al., 2001). Sourdille et al. (1996) reported four additional regions on chromosomes 2A, 2D, 5B and 6D that contribute to the degree of hardness, while three additional genes having an indirect effect on kernel hardness are located on chromosomes 5A, 6D and 7A. Additionally, two significant QTLs for kernel hardness on chromosomes 2B and 6B were coincident with the QTLs for grain protein content (Galande et al., 2001). Most recently, a major QTL for grain hardness was mapped onto chromosome 1BL, accounting for 28% of the phenotypic variance of kernel hardness, while only 8% of the phenotypic variance was explained by the QTLs mapped closely to the *Ha* locus and the puroindoline genes on 5DS (Li et al., 2009). Ten out of 19 QTLs for grain hardness were located on the same chromosome regions as the QTL for grain protein content, wet gluten content or water absorption (Li et al., 2009). These results indicate that kernel hardness is controlled by many QTL, and affected by QTL for other related traits.

Most of genetic studies on wheat kernel hardness have focused on the genetic difference between soft and hard grain, but little work has been done on the genetic factors controlling the difference between soft and 'extra-soft' grain. To better understand the genetic control of 'extra-soft' characteristic, we developed a $F_{5:6}$ recombinant inbred line (RIL) mapping population derived from a cross between the soft white wheat cultivar 'Stephens' (hardness index ~24) and ' OR9900553' (hardness index ~12), an elite breeding line with the 'extra-soft' grain characteristic. Although the

objective of this study was to identify the underlying genetic factors controlling the 'extra-soft' characteristic by detecting QTL for kernel hardness, the mapping population was also used to identify and locate QTL associated with three quality traits, break flour yield, bran yield, and middling flour yield; and five other agronomic traits. We also determined whether semi-dwarfing genes *Rht-B1* and *Rht-D1* influenced wheat endosperm texture, in order to clarify the genetic relationship between these semi-dwarfing genes and kernel hardness.

Materials and methods

Plant materials and experimental design

The OS9XQ36 wheat mapping population consists of 164 $F_{5:6}$-derived RILs generated from a cross between soft white winter (SWW) wheat OS9A (Stephens) and 'extra-soft' white winter wheat QCB36 (OR9900553) made in 1999 at Oregon State University. The parents contributed alleles to two gibberellic acid (GA)-insensitive, semi-dwarfing genes, *Rht-Bb1* (OS9A) and *Rht-D1b* (QCB36). As one of 20 mapping populations in the WheatCAP consortium, the OS9XQ36 population was registered as a mapping population, and the development and properties of this population have been described (Riera-Lizarazu et al., 2010).

The OS9XQ36 mapping population and its parents were grown at Hyslop farm (Corvallis, OR; environment abbreviation CR07) in 2007. In 2008, they were again planted in Corvallis (CR08), Moro (MR08), Pendleton (PE08), OR, Pullman, WA (PU08), and Moscow, ID (MC08).The RILs and its two parents were arranged in a randomized complete blocks design (RCBD) with two replications in each location. Phenotypic traits including days to heading (HDD), plant height (PHT), test weight (TWT), and grain protein content (GPC), were collected for RILs in all six field environments. The kernel hardness (KHA), thousand-kernel weight (TKW), and kernel diameter (KDM) were collected for the mapping population from the greenhouse and Corvallis farm in 2007

and the other five field environments in 2008. The milling related traits including break flour yield (BFY), bran recovered flour (BRN), and unground middling flour (MID), were evaluated for the population only from 2008 grow-outs in Corvallis, Moro, Pendleton and Pullman. Least square (LS) means for the various traits of each RIL in the individual environment and combined field environments (CB) were calculated using SAS 9.2.

Genotyping, linkage mapping, and QTL analysis

The two parents and 164-RILs were genotyped with simple sequence repeat (SSR) and diversity array technology (DArT) markers. In addition, all RILs were genotyped with markers specific for semi-dwarfing alleles at the *Rht-B1* and *Rht-D1* loci on chromosomes 4BS and 4DS, respectively (Ellis et al., 2002). The two parental lines were assayed for puroindoline alleles at the *pinA* and *pinB* loci on 5DS. However, both parents were monomorphic for alleles at the *pinA-D1* and *pinB-D1* loci. The procedures for genotyping molecular markers on RILs and the development of the genetic linkage map were described in Chapter 3. A genetic map with a length of 1,821 centimorgans (cM) and an average density of one marker per 5.5 cM was constructed with only one marker per locus. The genetic map used in QTL analysis is composed of 229 SSR markers, 38 DArT markers and 3 gene-specific markers arranged in 45 linkage groups anchored to the 21 chromosomes of wheat. The order of marker loci in each linkage group is consistent with previous reports (Somers et al., 2004).

The genetic linkage map and LS mean values of phenotypic traits were used in QTL analysis using interval mapping and multiple-QTL model (MQM) mapping implemented in MapQTL 5.0 (Van Ooijen, 2004). The significant likelihood-odds (LOD) threshold corresponding to the genome wide significance at 0.05 levels was estimated with 1,000 permutations, resulting in LOD scores of 3.0 to 3.5 for all the investigated traits. Interval mapping was first performed to identify significant QTL at a 1 cM interval. The marker closest to the significant LOD peak at each linkage was selected as a cofactor, and then all the selected markers were used as genetic background controls in

MQM analysis. If the inclusion of cofactors led to the identification of new significant QTL, the new cofactor(s) was included in subsequent MQM analysis. This process continued until no new significant QTL were detected and the final MQM model was obtained. Thus, significant QTL were declared from the final MQM model. The final linkage maps with approximate 1-LOD QTL intervals were drawn using MapChart 2.2 (Voorrips, 2002).

Results

Distribution of phenotypic traits

Continuous variation and transgressive segregation were observed for all the traits evaluated at each of the environments and across environments among the 164 RILs. The LS means for KHA among RILs varied from 5.4 to 40.6 in combined environments, showing that wheat kernel texture ranged from values typically associated with 'extra-soft' wheat to values typically associated with common soft wheat. Similarly, a continuous distribution was observed for BFY (range from 112 to 178 g/kg) in the RIL population. Compared to other environments, HDD were generally longer in the PU08 and MC08 environments.

QTL results

A total of 47 significant QTL, with a LOD score higher than the LOD threshold calculated for the respective trait, were detected for the nine traits (Figure 4.1; Tables 4.1 and 4.2). Among these, 24 QTL were detected in at least two environments, of which seven were significant in all environments. The number of QTL detected per trait ranged from three for MID to nine QTL for TKW. The majority of significant QTL mapped onto the B or D genomes distributed on 16 wheat chromosomes in total. Only chromosomes 1D, 3A, 4A, 5A and 6D lacked mapped QTL. The QTL peak position, the corresponding R^2 value (proportion of phenotypic variance explained by QTL), 1-LOD QTL support limit,

and the additive effect, are reported for each QTL (Table 4.2). Of these 47 QTL, five QTL for KHA, MID, PHT, and TWT explained more than 30% of the phenotypic variance, four QTL explained 20-30% and 12 QTL explained 10-20% of the phenotypic variance. In addition, both parents contributed positive and negative alleles to all traits except BRN and MID, and the direction of the additive effect of each QTL was consistent across different environments.

Kernel hardness (KHA) QTL

Five significant QTL were detected on four chromosomes (4BS, 4DS, 5DL and 7DS) and explained 7.1-33.8% of the phenotypic variance each with a LOD of 3.0 to 11.3 (Table 4.1, Figure 4.1). Three QTL, *Qkha.orr-4B*, *Qkha.orr-4D*, and *Qkha.orr-5D*, were detected in at least three environments. The most significant QTL, *Qkha.orr-4D,* was identified on 4DS in the interval between *Xbarc1118* and *Rht-D1* in all seven environments and across environments with LOD scores ranging from 5.8 to 11.3. This QTL explained 14.7-33.8% of the phenotypic variance in grain hardness with an additive effect of 2.7 to 4.6. Another prominent QTL, *Qkha.orr-4B,* mapped to the interval between *Xwmc617* and *Rht-B1* in five environments and across environments, accounted for 8.0 to 20.2% of the phenotypic variance with additive effects of 2.1-3.1. The *Qkha.orr-5D* interval on chromosome 5DL was significant in the PU08 and MC08 environments and across environments, explaining 7.6-10.2% of the phenotypic variance with negative additive effects from 1.2 to 2.2. A QTL on 4D and *Qkha.orr-7D* were detected only at CR07. Except for *Qkha.orr-5D*, other QTL have positive additive effects, indicating that KHA was increased by alleles from OS9A at these QTL loci.

Break flour yield (BFY) QTL

Six QTL were detected on chromosomes 1BS, 4BS, 5BS, 7BL, 2DS and 4DS for BFY (Table 4.1, Figure 4.1). Both *Qbfy.orr-1B* and *Qbfy.orr-5B* were identified in three environments and across environments. The major QTL, *Qbfy.orr-1B,* was mapped to chromosome 1BS with a very narrow 1-LOD support limit interval between *RIS* and

marker *Xbarc240*. It explained 9.1-15.6 % of the phenotypic variance with additive effects of 4.3 to 5.9. Another QTL, *Qbfy.orr-5B,* was consistently detected near marker locus *XwPt-0103* on 5BS with LOD scores of 3.6 to 10.8, and explained 8.9-19.9% of the phenotypic variance. The *Qbfy.orr-4D* QTL was mapped between semi-dwarfing gene *Rht-D1* and marker *Xbarc1118* on 4DS with negative additive effects ranging from 6.2 to 8.9. Three QTL *Qbfy.orr-4B*, *Qbfy.orr-7B,* and *Qbfy.orr-2D* were detected only in a single environment, and accounted for 9.9%, 11.9% and 7.3% of the phenotypic variance, respectively.

Bran recovered flour (BRN) QTL

A total of five QTL were identified on chromosomes 6AL, 1BS, 4BS, 5BL and 4DS for BRN (Table 4.1, Figure 4.1). The most significant QTL, *Qbrn.orr-1B,* was detected in all environments and across environments with LOD scores ranging from 6.7-12.2. This QTL explained 6.9-23.6% of the phenotypic variance with additive effects ranging from 4.9 to 8.1. Another major QTL, *Qbrn.orr-4D,* was detected at three environments and across environments with LOD scores ranging from 5.3 to 11.2. This QTL was consistently located in the interval between markers *Xbarc1118* and *Rht-D1* and the phenotypic variance explained by this QTL ranges from 15.5 to 26.2% with negative additive effects from 8.2 to 10.7. Other QTL on chromosome 6AL, 4BS and 5BL were only detected at CB (across locations), CR08, and CR08, and accounted for 5.8%, 6.9%, and 6.1% of the phenotypic variance, respectively. The parent Q36 contributed the lower value allele to all the five QTL, indicating that QCB36 possessed alleles decreasing BRN.

Unground middling flour (MID) QTL

Three significant QTL were identified on chromosomes 5BL, 4DS and 4DL for MID (Table 4.1, Figure 4.1). The QTL *Qmid.orr-4D* on 4DS was detected in all four environments and across environments with LOD scores of 8.6-16.2. It explained 18.3-47.6% of the phenotypic variance with additive effects ranging from 9.0 to 18.2. The *Qmid.orr-5B* QTL was detected at CR08 and across environments, accounting for 14.5%

and 11.9% of the phenotypic variance with additive effects of 7.8 and 6.9, respectively. In contrast to the QTL for BRN, all major and minor QTL for MID were contributed by OS9A alleles that increased MID.

Plant height (PHT) QTL

Among four significant QTL detected for PHT, three QTL on chromosomes 6AL, 4BS and 4DS were detected in all environments, while *Qpht.orr-3D* was detected only in two environments (Table 4.2, Figure 4.1). Two major QTL were mapped onto semi-dwarfing genes *Rht-B1* and *Rht-D1* with LOD scores up to 36.1 and 41.6, respectively. These two QTL explained up to 80% of the total phenotypic variance in plant height across six field environments. The *Qpht.orr-4B* explained 15.3-36.1% of the phenotypic variance with a negative additive values ranging from 3.7 to 11.1. *Qpht.orr-4D* accounted for 28.0-50.3% of phenotypic variance with additive effects ranging from 3.9 to 12.9. Two minor QTL on chromosomes 6AL and 3DL explained 4.3-9.8% and 3.7-5.1% of the phenotypic variance, respectively. The negative additive effects of *Qpht.orr-4B* and *Qpht.orr-3D* indicate that PHT was decreased by the alleles from QCB36, while OS9A contributed alleles increasing PHT.

Days to heading (HDD) QTL

Five QTL were identified on chromosomes 1BL, 6BS, 6BL, 4DS and 7DS, but no significant loci were detected at MR08 and PU08 (Table 4.2, Figure 4.1). The QTL *Qhdd.orr-4D* was mapped onto the interval between *Rht-D1* and *Xgpw94042.* The phenotypic variance explained by this QTL ranged from 11.3-21.6% with negative additive effects ranging from 0.6 to 1.3 in three environments and across environments. The *Qhdd.orr-6B* QTL, mapped to the interval between *XwPt-5480* and *Xwmc621* at PE08 and across environments, explained 10.4% and 7.5% of the phenotypic variance with negative additive effects of -0.7 and -0.4, respectively. Both *Qhdd.orr-1B* and *Qhdd.orr-7D* were significant only at CR08, accounting for 9.0% and 9.9% of the phenotypic variance with additive effects of 0.8 and 0.9, respectively. Although HDD

showed transgressive segregation, over 50% of the RILs headed within three days in all environments except at CR08. This result indicates that no major locus with a large effect for HDD segregated in the OS9XQ36 population.

Test weight (TWT) QTL

Four QTL were identified on chromosomes 7AL, 4BS, 4DS and 5DL (Table 4.2, Figure 4.1). *Qtwt.orr-4D*, mapped to the interval between *Rht-D1* and *Xgpw94042*, was detected in six environments and across environments with LOD scores ranging from 4.0 to 15.8. This QTL explained 10.0-30.9% of the phenotypic variance with additive effects ranging from 6.1 to 18.6. *Qtwt.orr-4B,* located in the interval between *Xwmc617* and *Rht-B1*, was detected in five environments and across environments, accounting for 8.3 to 30.7% of the phenotypic variance with negative additive values ranging from 5.2 to 18.3. Both *Qtwt.orr-7A* and *Qtwt.orr-5D* were identified only at MC08, and explained 9.6% and 9.3% of the phenotypic variance, respectively.

Grain protein content (GPC) QTL

A total of six significant QTL were mapped to chromosomes 1AL, 2AS, 3BL, 6BS, 5DL, and 7DL (Table 4.2, Figure 4.1). Of these, *Qgpc.orr-7D* was detected at two environments, while other QTL were significant only at a single environment and no significant QTL was detected at PE08 and PU08. The *Qgpc.orr-7D* explained 9.8% and 7.6% of the phenotypic variance with negative additive effects of 2.3 and 2.8 at CR07 and CR08, respectively. Besides *Qgpc.orr-1A*, all six QTL showed negative effects with QCB36 alleles decreasing GPC.

Thousand-kernel weight (TKW) QTL

A total of nine QTL were detected on chromosomes 6AL, 7AL, 1BS, 2BL, 4BS, 4BL, 3DL and 4DS (Table 4.2, Figure 4.1). The major QTL, *Qtkw.orr-6A,* mapped to the interval between *XwPt-5094* and *Xwmc3*, was detected in six environments and across environments. The *Qtkw.orr-4B* on chromosome 4BL explained 10.3 to 18.5% of the

phenotypic variance with negative additive effects ranging between 1.2 and 1.5. *Qtkw.orr-2B* was mapped to the interval between *Xbarc1155* and *Xgwm388*, explaining 7.7 to 9.3% of the phenotypic variance. *Qtkw.orr-7A* and *Qtkw.orr-3D* were detected only at PE08 and CR08, and accounted for 5.4% and 6.9% of the phenotypic variance, respectively. Parent OS9A contributed positive alleles at all identified QTL except those QTL on chromosome 4B.

Effects of semi-dwarfing genes on kernel hardness

Due to the identification of major QTL near the semi-dwarfing genes *Rht-D1* and *Rht-B1* for most traits, the possible effects of *Rht* alleles on the variation in grain hardness were analyzed. RILs of the OS9XQ36 population were classified as semi-dwarf (carrying one dwarfing gene *Rht-B1b* or *Rht-D1b*), dwarf (carrying both dwarfing genes) or tall (carrying neither dwarfing gene) (Figure 4.2). Even though the four combinations of *Rht* alleles produced three distinct groups of RILs based on PHT, no significant correlation was observed between hardness and *Rht* alleles among RILs. Data was subsequently analyzed in an attempt to identify genetic associations between *Rht-D1* and hardness after accounting for the effects of QTL *Qkha.orr-4B* (*Xgwm617-Rht-B1*). No significant correlation between hardness and *Rht-D1* alleles was observed. This was due to the fact that both the tall allele *Rht-B1a* and the semi-dwarf allele *Rht-D1b* contributed by QCB36 decreased hardness because of linkage of the plant height alleles and QTL alleles decreasing hardness or pleiotropic effects of the plant height alleles, as confirmed by pairwise t-tests on hardness of the four genotype groups classified based on the alleles at *Rht-B1* and *Rht-D1* among the RILs population. The genotype class of 36 RILs (*Rht-B1a* and *Rht-D1b* alleles) had a mean of hardness index of 17.97 that was highly significant ($P<0.005$) lower than that of anyone of the other three genotype classes with a hardness index of 23.30, 23.51, and 25.46, respectively.

Discussion

Stable QTL for hardness and milling yield

Most of the QTL detected for nine traits investigated in this study were in agreement with previous QTL studies. As expected, two major QTL for PHT were confirmed near the well-known semi-dwarfing gene *Rht-B1* and *Rht-D1* on chromosomes 4BS and 4DS, respectively (Börner et al., 1997; Ellis et al., 2002). Another minor QTL *Qpht.orr-6A* coincides with a previously reported QTL (Spielmeyer et al., 2007). Our study is consistent with other studies where the variation for PHT is largely explained by the effect of *Rht* genes but it is also under polygenic control. The above three QTL were consistently detected in different environments. The consistent detection of identical QTL from trial to trial suggests little genotype X environment (GE) interaction.

Previous studies identified the *Ha* locus and puroindoline genes on 5DS as the major genes controlling the variation between hard and soft wheat. The continuous distribution of OS9XQ36 population for kernel hardness is different from the bimodal distribution typically seen in the RIL populations developed from a soft × hard wheat cross (Bergman et al., 1998; Campbell et al., 1999). By eliminating variation at *Ha* locus in the choice of our parental lines, we were able to focus on other loci that influence and determine the difference between soft and 'extra-soft' grain hardness. By eliminating the contribution of major genes on chromosome 5DS, we were able to identify two major QTL on chromosomes 4BS (*Qkha.orr-4B*) and 4DS (*Qkha.orr-4D*) close to the semi-dwarfing genes *Rht-B1* and *Rht-D1* that explained up to 20% and 34% of the phenotypic variance, respectively. The QTL on chromosome 4DS is most likely identical to the previously reported QTL location for grain hardness (Li et al., 2009; Zanetti et al., 2001). Additionally, two QTL with minor effects on hardness were found on chromosomes 5DL and 7DS. Therefore, QTL analysis revealed that inheritance of the soft

kernel characteristic is complex. To our knowledge, this is the first report of a major QTL for hardness on chromosome 4BS. Based on map comparisons, the QTL on chromosomes 4BS and 4DS (*Qkha.orr-4B* and *Qkha.orr-4D*) appear to be orthologous loci.

Several genetic factors are probably involved in the determination of grain hardness and the three milling yield traits in our study. Two coincident QTL were found on chromosomes 4DS and 5BS for BFY, BRN, and MID, even though the effect of the QTL on chromosome 4DS for BFY is much smaller than the effect on hardness. Similarly, the QTL mapped to chromosome 4BS for BFY and BRN, was also coupled with a QTL for KHA, but its effect on these two traits was less than that for hardness. Another co-located QTL for BFY and BRN was mapped to chromosome 1BS, explaining up to 15% of the phenotypic variance. The presence of the QTL supported the idea that the 1RS.1BL translocation is associated with wheat grain yield traits, but it is also likely that the translocation is associated directly with other endosperm characteristics rather than hardness per se (Kim et al., 2004). Three other QTL detected in this study, *Qbfy.orr-4B, Qbfy.orr-2D* and *Qbfy.orr-4D,* were in agreement with previously reported QTL for flour yield of hard wheat (Christopher et al., 2008; McCartney et al., 2006; Schmidt et al., 2004). Also, both *Qbfy.orr-7B* and *Qbfy.orr-5B* were reported previously for grain yield (Kuchel et al., 2007). None of the QTL associated with BRN and MID flour yield has been previously reported. The significant QTL on chromosomes 4BS and 4DS here coincided with hardness and gave an indication that there exist two potential QTL accounting for the variation of kernel hardness. Of interest is whether the common QTL loci influencing KHA, BFY, BRN, and MID on chromosomes 4BS and 4DS are directly linked to the semi-dwarfing genes *Rht-B1* and *Rht-D1*, respectively.

QTL for agronomic traits

Three QTL identified for HDD in this study were coincident with QTL identified in previous studies. However, we were unable to identify any significant QTL near the

vernalization gene *Vrn-B1* on chromosome 5B despite of the variation for HDD among the RILs of this population. Our inability to detect the influence of vernalization alleles may be due to complete vernalization of materials in the field and confounding effects of alleles at earliness per se loci. The minor QTL we identified on chromosome 7DS for HDD may be identical to the QTL identified in previous studies (Borner et al., 2002; Sourdille et al., 2000). *Qhdd.orr-4D* and *Qhdd.orr-6B* were reported to be associated with earliness per se (Hoogendoorn, 1985), suggesting that our population is segregating for alleles at these loci. The QTL on chromosome 4DS coincident with hardness suggests that HDD or factors affecting HDD influence kernel hardness or endosperm texture through unknown underlying mechanisms.

Most of TKW QTL reported here had not been reported elsewhere. However, three QTL, one each on chromosome 6A (*Qtkw.orr-6A* between markers *Xbarc3* and *Xbarc107)*, 1B (*Qtkw.orr-1B* near marker *RIS*) and 2B (*Qtkw.orr-2B* near *Xgwm388)* were in agreement with QTL identified previously (Groos et al., 2003; Huang et al., 2004; Snape et al., 2007). As for TWT, the QTL on chromosome 2DL was consistent with a previously identified QTL as well (Narasimhamoorthy et al., 2006). Taking into consideration the influence of HDD on grain filling, some concurrent genetic factors for HDD, TKW and TWT may exist. Therefore, it is not surprising that coincident QTL on chromosome 4DS were mapped for HDD, TKW, and TWT in the present study.

The majority of GPC QTL identified in this study appeared similar to those reported previously in wheat. For example, *Qgpc.orr-6B* between markers *Xbarc101* and *Xbarc103,* and *Qgpc.orr-2A* between markers *Xcmwg682* and *Xcfd36* occupy similar chromosome regions to QTL identified elsewhere (Groos et al., 2003; Joppa et al., 1997; Khan et al., 2000). However, four minor GPC QTL on chromosomes 1AL, 3BL, 5DL, and 7DL were identified only in this study. All QTL for GPC could only be detected in a single environment except *Qgpc.orr-7D,* and no significant QTL was detected at PE08 and PU08. It seems that the limited phenotypic variation for this trait in the mapping

population is the primary reason for our inability to detect QTL for GPC across environments.

Co-location of QTL

A total of seven coincident QTL occurred for at least two different traits on chromosomes 1B, 4B, 5B, 4D, 5D, 6A, and 7A. Of these, the most significant QTL for eight out of nine investigated traits was mapped to the same chromosome region near semi-dwarfing gene *Rht-D1* on chromosome 4DS. GPC was the only trait investigated that lacked QTL in this chromosomal region. Similarly, another coincident QTL for six investigated traits was detected on the chromosomal region near *Rht-B1* on chromosome 4BS. These findings are generally consistent with the QTL identified in other studies, in which both semi-dwarfing genes *Rht-B1* and *Rht-D1* have been reported to be associated with some agronomic traits and disease resistance in wheat, including TWT, TKW, and *Fusarium* crown rot (Collard et al., 2005; McCartney et al., 2006; Singh et al., 2001; Wallwork et al., 2004). Besides these, *Qbfy.orr-5B* was coincident with QTL for MID and BRN on chromosome 5B. Coincident QTL for three traits (BFY, BRN and TKW) were detected in the same marker interval *RIS-Xbarc240* on chromosome 1BS. Both *Qbrn.orr-6B* and *Qmid.orr-6B* were coincident with the QTL for HDD on chromosome 6BL. A QTL for BRN was located on the same region of chromosome 3B as a QTL for GPC. These coincident QTL for multiple traits were consistent across environments, suggesting that these traits may be correlated with each other and may share common genetic factors. If this is the case, all traits measured in this study except GPC are correlated with KHA. This correlation has two possible explanations. One interpretation suggests that pleiotropic effects of single genes on multiple phenotypes. The other possibility is that multiple closely linked genes are not easily distinguished in a QTL study. For example, the relationship between PHT and KHA in this population is not due to plant height per se, but rather to either linked genes

affecting KHA or a pleiotropic effect of the dwarfing allele at the *Rht-B1* or *Rht-D1* locus. At present, we cannot distinguish those two possible explanations for QTL coincidence.

Selection of soft wheat cultivars with high flour yield and end-use quality has been successful because of the important negative correlation between milling flour yield and KHA. However, until now, the genetic control of the 'extra-soft' characteristic has not received much attention. This may reflect the fact that texture variation from soft to 'extra-soft' is small relative to the much larger difference between soft and hard wheat. In addition, the large effect of the *Ha* locus is more amenable to qualitative genetic studies. The quantitative nature of the genetic determinants of the 'extra soft' trait and smaller phenotypic variance make the objectives of this study more challenging. Nonetheless, we have been able to show that at least five QTL directly contribute to the 'extra-soft' trait.

Relationship of *Rht* genes and kernel hardness

We found an association of both *Rht-B1* and *Rht-D1* genes with kernel hardness. However, our study suggested that KHA is not influenced directly by *Rht* genes. Also, there is no pleiotropic effect of the dwarfing gene *Rht-B1* based on analysis of lines carrying different alleles of *Rht-D1* and *Rht-B1*. Path coefficient analysis supported the contention that KHA and BFY are not highly associated with PHT (Richards, 1992). In contrast, both KHA and BFY were observed to be highly correlated with other agronomic traits, such as HDD and TWT in our study. Recent studies have revealed that GPC is positively correlated with KHA. This is supported by the fact that softer wheat grain has lower protein content than hard wheat (Gaines, 1985; Nelson et al., 2006; Yamazaki and Donelson, 1983).

This study identified several QTL for wheat KHA and BFY, along with significant QTL for other agronomic traits. It increases our understanding of the underlying genetic factors controlling kernel hardness, break flour yield, and other agronomic traits. The QTL reported here could orient marker-assisted selection strategies to breed for high

market value ‘extra-soft’ wheat lines. The identification of significant QTL is also the first step in identifying the specific genetic factors that underline phenotypes of interest. In this case, we have identified two QTL that contribute to the ‘extra-soft’ phenotype. Fine mapping of these QTL near *Rht* genes would be the next step towards isolating and identifying the relevant genes.

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Figure 4.1 Genetic linkage map of wheat showing quantitative trait loci (QTL) mapped to 16 chromosomes in the OS9XQ36 RIL mapping population. The approximate 1-LOD supported intervals for QTL are indicated by vertical bars. The additive effects contributed by 'Stephens' and 'OR9900553' are indicated by solid black boxes and open boxes on the left side of each linkage group, respectively. Chromosome segments shown in black indicate the approximate position of the centromere inferred from the wheat microsatellite consensus map (Somers et al., 1996). QTL abbreviations for traits: *Qkha.orr* Kernel hardness; *Qbfy.orr* Break flour yield; *Qbrn.orr* Bran recovered yield; *Qmid.orr* Middling flour yield; *Qpht.orr* Plant height; *Qhdd.orr* Days to heading; *Qtwt.orr* Test weight; *Qgpc.orr* Grain protein content; *Qtkw.orr* Thousand-kernel weight. Abbreviations of environments in which the QTL were detected are given in brackets: 1, Corvallis (OR), 2007; 2, Corvallis (OR), 2008; 3, Moro (OR), 2008; 4, Pendleton (OR), 2008; 5, Pullman (WA), 2008; 6, Moscow (ID), 2008; 7, Greenhouse, 2007; and C, Combined across field environments.

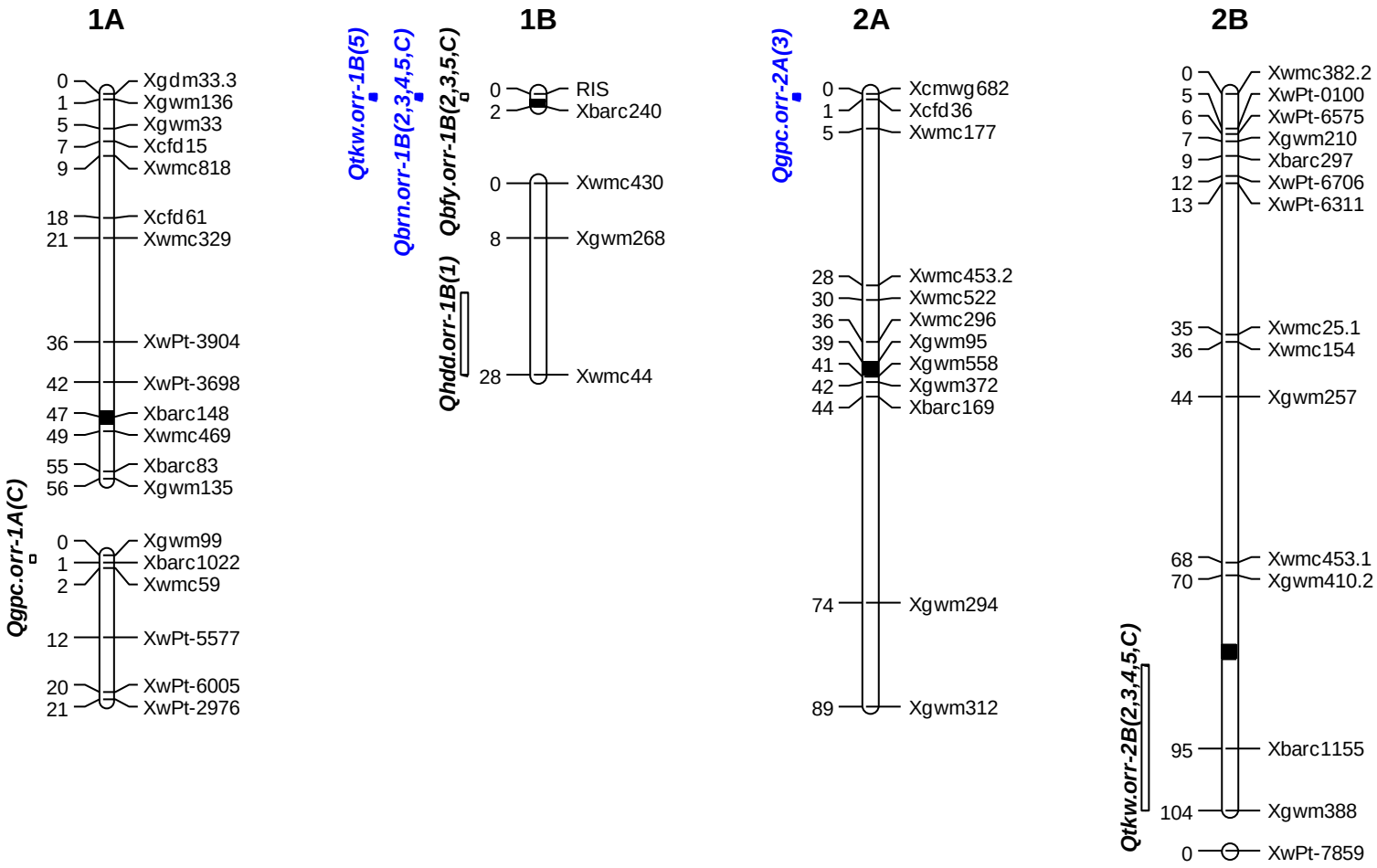
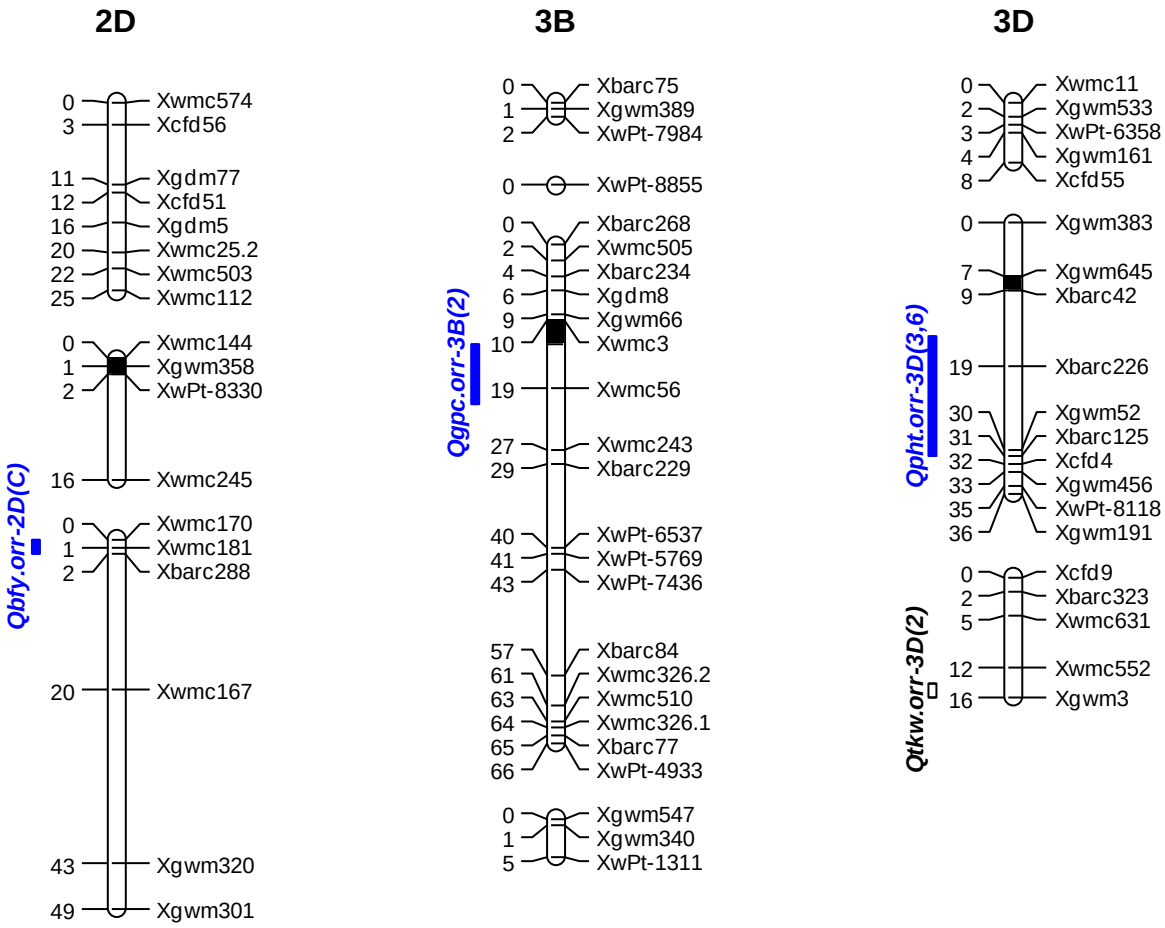


Figure 4.1 (Continued)



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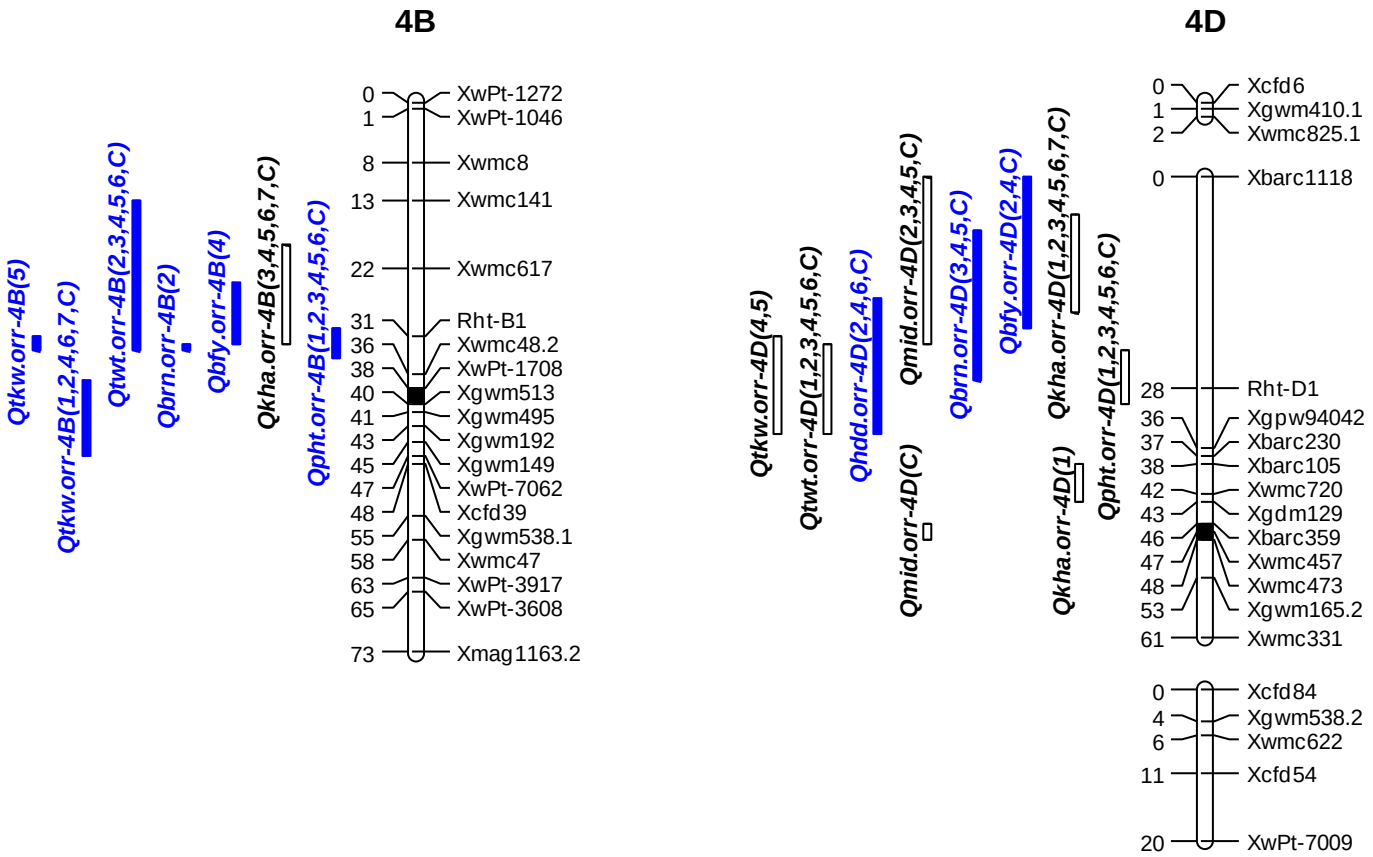

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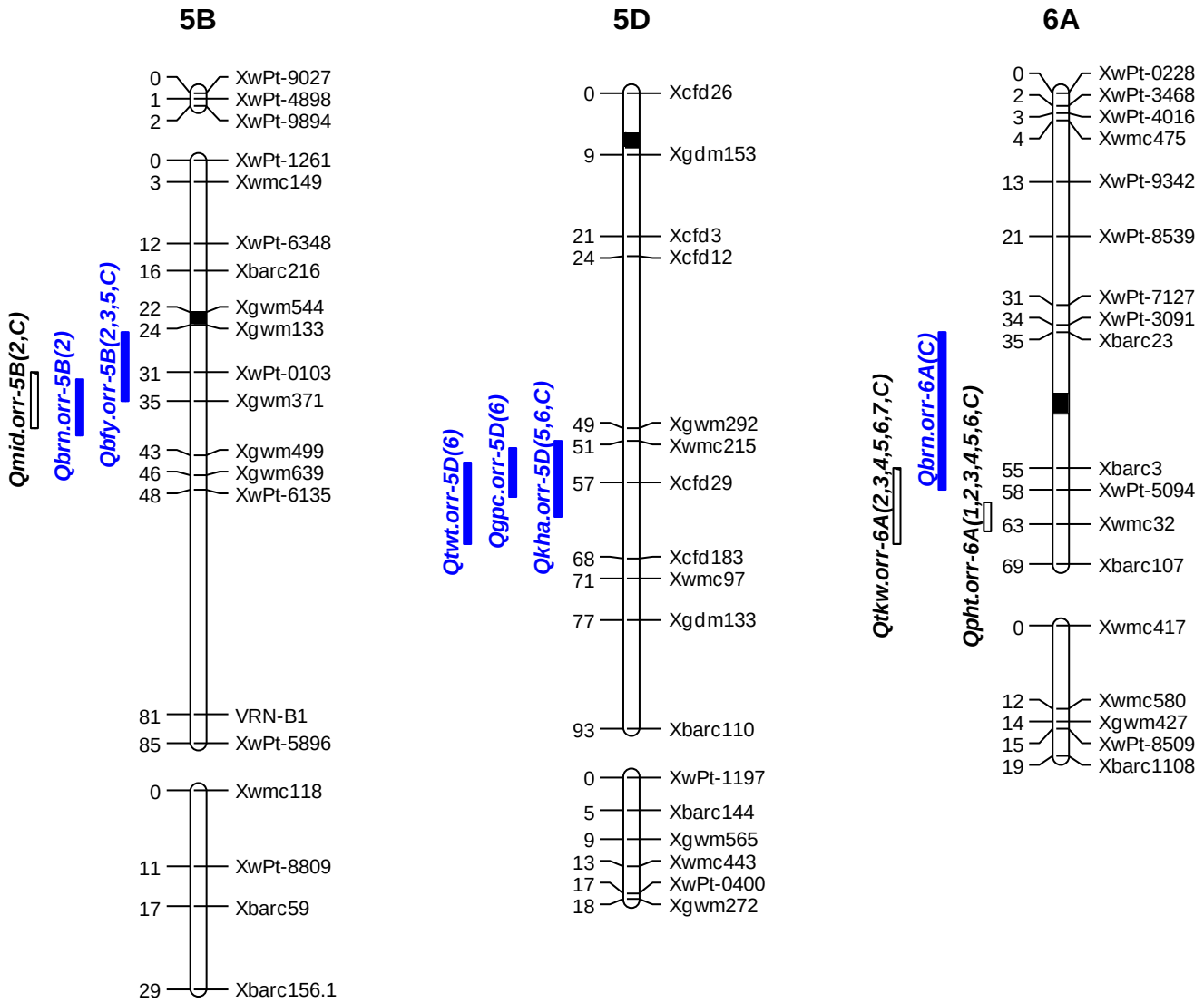

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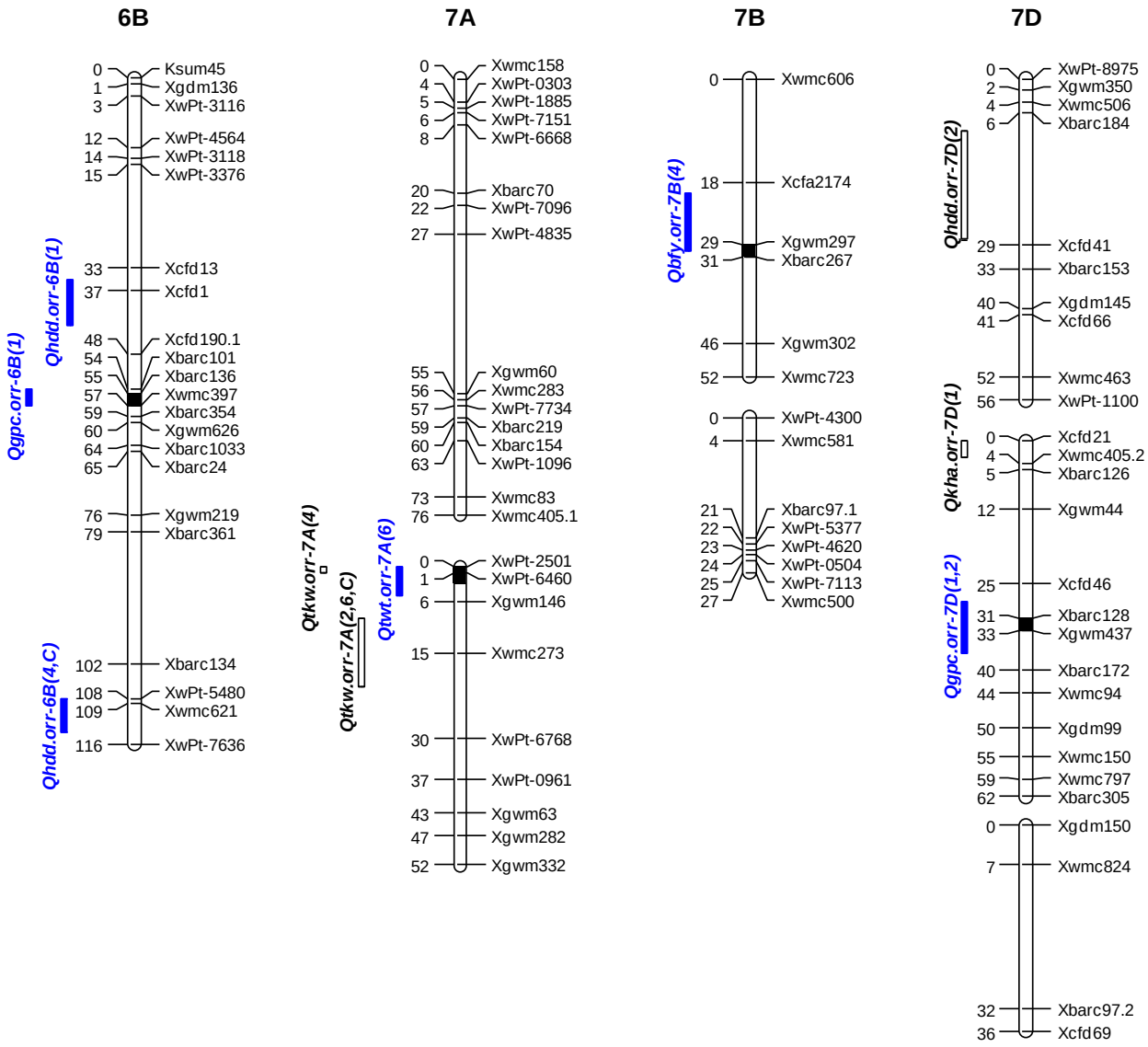
6B
0 Ksum45
1 Xgdm136
3 XwPt-3116
12 XwPt-4564
14 XwPt-3118
15 XwPt-3376
33 Xcfd13
37 Xcfd1
48 Xcfd190.1
54 Xbarc101
55 Xbarc136
57 Xwmc397
59 Xbarc354
60 Xgwm626
64 Xbarc1033
65 Xbarc24
76 Xgwm219
79 Xbarc361
102 Xbarc134
108 XwPt-5480
109 Xwmc621
116 XwPt-7636
Qgpc.orr-6B(1)
Qhdd.orr-6B(1)
Qhdd.orr-6B(4,C)
7A
0 Xwmc158
4 XwPt-0303
5 XwPt-1885
6 XwPt-7151
8 XwPt-6668
20 Xbarc70
22 XwPt-7096
27 XwPt-4835
55 Xgwm60
56 Xwmc283
57 XwPt-7734
59 Xbarc219
60 Xbarc154
63 XwPt-1096
73 Xwmc83
76 Xwmc405.1
0 XwPt-2501
1 XwPt-6460
6 Xgwm146
15 Xwmc273
30 XwPt-6768
37 XwPt-0961
43 Xgwm63
47 Xgwm282
52 Xgwm332
Qtkw.orr-7A(4)
Qtkw.orr-7A(2,6,C)
Qtwt.orr-7A(6)
7B
0 Xwmc606
18 Xcfa2174
29 Xgwm297
31 Xbarc267
46 Xgwm302
52 Xwmc723
0 XwPt-4300
4 Xwmc581
21 Xbarc97.1
22 XwPt-5377
23 XwPt-4620
24 XwPt-0504
25 XwPt-7113
27 Xwmc500
Qbfy.orr-7B(4)
7D
0 XwPt-8975
2 Xgwm350
4 Xwmc506
6 Xbarc184
29 Xcfd41
33 Xbarc153
40 Xgdm145
41 Xcfd66
52 Xwmc463
56 XwPt-1100
0 Xcfd21
4 Xwmc405.2
5 Xbarc126
12 Xgwm44
25 Xcfd46
31 Xbarc128
33 Xgwm437
40 Xbarc172
44 Xwmc94
50 Xgdm99
55 Xwmc150
59 Xwmc797
62 Xbarc305
0 Xgdm150
7 Xwmc824
32 Xbarc97.2
36 Xcfd69
Qhdd.orr-7D(2)
Qk'ha.orr-7D(1)
Qgpc.orr-7D(1,2)

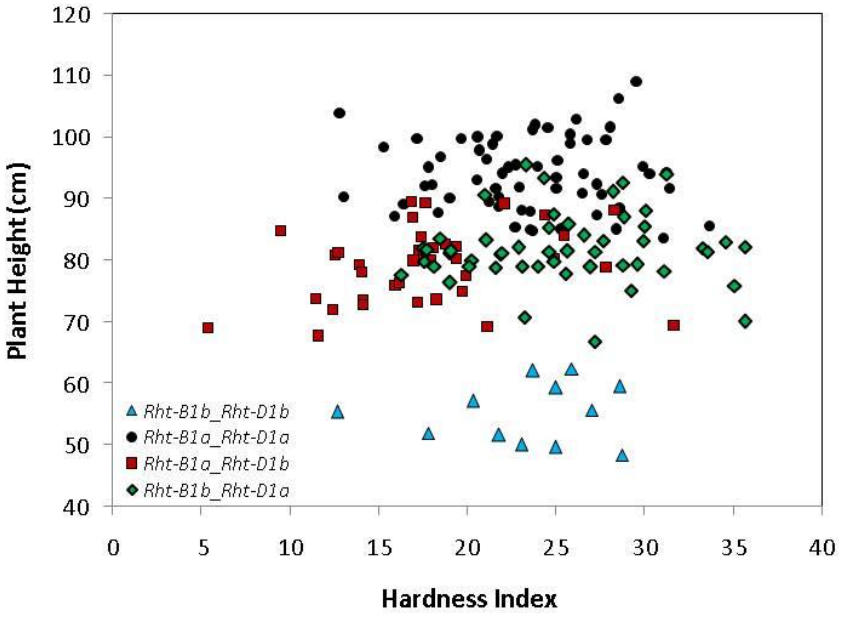

Figure 4.2 Relationship between kernel hardness and plant height in the OS9XQ36 RIL mapping population derived from the cross between OS9A (soft, *Rht-B1b Rht-D1a*) and QCB36 (extra-soft, *Rht-B1a Rht-D1b*). Symbols indicate allele status at the tow *Rht* loci.

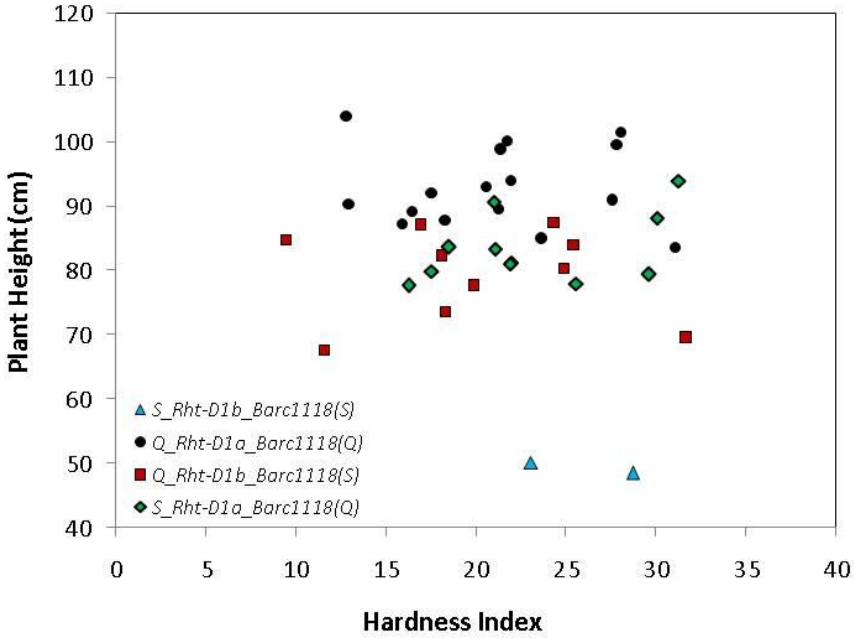

Figure 4.3 Relationship between kernel hardness and plant height in RILs after accounting for the QTL effects on chromosome 4BS (*Xgwm617-Rht-B1*). The capital letter S represents OS9A (Stephens) and Q represents QCB36 (OR9900553).

Table 4.1 Summary of QTL for kernel hardness (KHA), break flour yield (BFY), bran yield (BRN), and middling yield (MID) using the OS9XQ36 RIL population.

Trait & QTL Symbol	Environment abbreviation	Chromo-some arm [a]	QTL peak[b]	LOD[c]	1-LOD support limit [d]	R^2 [e] (%)	Additive effect [f]
KHA	GH07	4BS	26*(Xwmc617)*	3.7	19-31 *(Xwmc617-Rht-B1)*	9.3	2.3
Qkha.orr		4DS	13 *(Xbarc1118)*	10.9	7-18 *(Xbarc1118-Rht-D1)*	33.8	4.6
	CR07	4DS	6 *(Xbarc1118)*	5.8	0-16 *(Xbarc1118-Rht-D1)*	14.7	2.7
		4DS	40 *(Xwmc720)*	3.8	38-43 *(Xbarc105-Xwmc720)*	8.5	2.0
		7DS	0 *(Xcfd21)*	3.4	0-3 *(Xcfd21-Xwmc405.2)*	7.1	1.7
	CR08	4DS	17 *(Rht-D1)*	11.3	11-22 *(Xbarc1118-Rht-D1)*	33.8	4.2
	MR08	4BS	25 *(Rht-B1)*	3.0	22-29 *(Xwmc141-Rht-B1)*	8.0	2.1
		4DS	16 *(Rht-D1)*	8.8	10-22 *(Xbarc1118-Rht-D1)*	27.5	4.1
	PE08	4BS	27 *(Rht-B1)*	7.9	25-30 *(Xwmc617-Rht-B1)*	20.2	3.1
		4DS	11 *(Xbarc1118)*	7.8	4-17 *(Xbarc1118-Rht-D1)*	22.7	3.3
	PU08	4BS	27 *(Rht-B1)*	4.1	24-32 *(Xwmc617-Rht-B1)*	10.8	2.4
		4DS	12 *(Xbarc1118)*	7.2	3-18 *(Xbarc1118-Rht-D1)*	20.9	3.4
		5DL	54 *(Xwmc215)*	3.4	50-57 *(Xgwm292-Xcfd29)*	8.0	-1.2
	MC08	4BS	28 *(Rht-B1)*	4.9	23-32 *(Xwmc617-Rht-B1)*	12.2	2.5
		4DS	11 *(Xbarc1118)*	6.6	2-18 *(Xbarc1118-Rht-D1)*	18.7	3.2
		5DL	54 *(Xcfd29)*	4.6	51-60 *(Xwmc215-Xcfd29)*	10.2	-2.2
	CB	4BS	27 *(Rht-B1)*	5.4	24-30 *(Xwmc617-Rht-B1)*	13.3	2.4
		4DS	12 *(Xbarc1118)*	9.4	5-18 *(Xbarc1118-Rht-D1)*	25.7	3.4
		5DL	55 *(Xcfd29)*	3.5	51-62 *(Xwmc215-Xcfd29)*	7.6	-1.7
BFY	CR08	1BS	0 *(RIS)*	4.2	0-1*(RIS-Xbarc240)*	9.1	4.3
Qbfy.orr		5BS	31*(XwPt-0103)*	4.1	27-35 *(Xgwm133-Xgwm371)*	8.9	-4.1
		4DS	11 *(Xbarc1118)*	6.9	3-18 *(Xbarc1118-Rht-D1)*	20.0	-7.0
	MR08	1BS	0 *(RIS)*	6.1	0-1 *(RIS-Xbarc240)*	14.0	5.9
		5BS	29*(XwPt-0103)*	6.5	25-33 *(Xgwm133-XwPt-0103)*	15.7	-6.3

	PE08	4BS	28 *(Rht-B1)*	3.9	24-32 *(Xwmc617-Rht-B1)*	9.9	-5.5
		7BL	25 *(Xgwm297)*	4.8	20-30 *(Xcfa2174-Xgwm297)*	11.9	-5.8
		4DS	11 *(Xbarc1118)*	4.2	0-20 *(Xbarc1118-Rht-D1)*	12.2	-6.2
	PU08	1BS	0 *(RIS)*	4.4	0-2 *(RIS-Xbarc240)*	10.9	4.3
		5BS	33 *(XwPt-0103)*	3.6	25-35 *(Xgwm133-Xgwm371)*	9.0	-3.9
	CB	1BS	0 *(RIS)*	8.9	0-1 *(RIS-Xbarc240)*	15.6	4.9
		5BS	30 *(XwPt-0103)*	10.8	27-34 *(Xgwm133-Xgwm371)*	19.9	-5.4
		2DS	1 *(Xwmc181)*	4.4	0-2 *(Xwmc170-Xbarc288)*	7.3	-3.3
		4DS	11 *(Xbarc1118)*	8.9	4-17 *(Xbarc1118-Rht-D1)*	19.2	-8.9
BRN	CR08	1BS	0 *(RIS)*	12.1	0-2 *(RIS-Xbarc240)*	20.9	-7.3
Qbrn.orr		4BS	35 *(Xwmc48.2)*	4.3	32-38 *(Rht-B1-XwPt-1708)*	6.9	-4.2
		5BL	35 *(Xgwm371)*	4.0	32-40 *(XwPt-0103-Xgwm499)*	6.1	-3.8
	MR08	1BS	0 *(RIS)*	6.9	0-2 *(RIS-Xbarc240)*	16.3	-8.1
		4DS	15 *(Rht-D1)*	5.3	7-23 *(Xbarc1118-Rht-D1)*	15.5	-8.9
	PE08	1BS	0 *(RIS)*	3.7	0-2 *(RIS-Xbarc240)*	6.9	-4.9
		4DS	19 *(Rht-D1)*	9.2	13-27 *(Xbarc1118-Rht-D1)*	25.7	-10.7
	PU08	1BS	0 *(RIS)*	6.7	0-2 *(RIS-Xbarc240)*	14.7	-6.0
		4DS	15 *(Rht-D1)*	9.8	8-21 *(Xbarc1118-Rht-D1)*	26.2	-9.0
	CB	6AL	47 *(Xbarc3)*	3.1	35-58 *(Xbarc23-Xbarc3)*	5.8	-3.7
		1BS	0 *(RIS)*	12.2	0-2 *(RIS-Xbarc240)*	23.6	-7.1
		4DS	16 *(Rht-D1)*	11.2	10-21 *(Xbarc1118-Rht-D1)*	25.4	-8.2
MID	CR08	5BL	33 *(XwPt-0103)*	7.6	31-35 *(XwPt-0103-Xgwm371)*	14.5	7.8
Qmid.orr		4DS	3 *(Xbarc1118)*	8.6	0-11 *(Xbarc1118-Rht-D1)*	18.3	9.0
		4DS	42 (Xwmc720)	3.6	40-43 (Xbarc106-Xgdm129)	6.4	5.2
	MR08	4DS	16 *(Rht-D1)*	10.4	10-22 *(Xbarc1118-Rht-D1)*	33.4	14.0
	PE08	4DS	17*(Rht-D1)*	16.2	12-20 *(Xbarc1118-Rht-D1)*	47.6	18.2
	PU08	4DS	15 *(Rht-D1)*	15.6	11-20 *(Xbarc1118-Rht-D1)*	45.3	14.5
	CB	5BL	34 *(Xgwm371)*	6.5	32-39 *(XwPt-0103-Xgwm371)*	11.9	6.9

	4DS	12 *(Rht-D1)*	14.2	7-19 *(Xbarc1118-Rht-D1)*	34.7	12.9
	4DL	47 *(Xwmc457)*	4.5	46-48 *(Xbarc359-Xwmc473)*	7.6	5.2

Note: *CR07* Corvallis (OR), 2007; *CR08* Corvallis (OR), 2008; *MR08* Moro (OR), 2008; *PE08* Pendleton (OR), 2008; *PU08* Pullman (WA), 2008; *MC08* Moscow (ID), 2008; *CB* Combined across field environments.

[a] The letter S represented the short arm of chromosome, and L indicated the long arm of chromosome.

[b] Position of QTL peak is expressed in centiMorgans (cM), nearest locus to QTL peak is indicated in brackets.

[c] Logarithm of the odds ratio (LOD) of QTL peak that exceeded the significant LOD threshold from 1,000 permutations.

[d] The flanking loci of 1-LOD support limit are indicated in brackets.

[e] R^2 is the proportion of the phenotypic variance explained by the QTL after accounting for co-factors.

[f] Positive additive values indicate that higher value alleles are from Stephens (OS9A) and the negative values indicate that the higher value alleles are from OR9900553 (QCB36)

Table 4.2 Summary of QTL for plant height (PHT), days to heading (HDD), test weight (TWT), grain protein content (GPC), and thousand-kernel weight (TKW) using the OS9XQ36 RIL population.

Trait & QTL Symbol	Environment abbreviation	Chromo-some arm[a]	QTL peak[b] (cM)	LOD[c]	1-LOD support limit[d]	R^2 [e] (%)	Additive Effect[f]
PHT	CR07	6AL	61 *(Xwmc32)*	5.4	59-64 *(XwPt-5094-Xwmc32)*	6.3	4.2
Qpht.orr		4BS	31 *(Rht-B1)*	21.1	30-32 *(Rht-B1-Xwmc48.2)*	30.8	-9.2
		4DS	28 *(Rht-D1)*	25.1	23-29 *(Rht-D1-Xgpw94042)*	39.0	10.9
	CR08	6AL	62 *(Xwmc32)*	11.2	60-64 *(XwPt-5094-Xwmc32)*	8.6	5.0
		4BS	31 *(Rht-B1)*	34.2	31-32 *(Xwmc617-Rht-B1)*	38.6	-11.1
		4DS	28 *(Rht-D1)*	39.8	27-29 *(Xbarc1118-Rht-D1)*	47.8	12.9
	MR08	6AL	63 *(Xwmc32)*	3.8	59-64 *(XwPt-5094-Xwmc32)*	5.4	1.6
		4BS	32 *(Rht-B1)*	15.3	30-34 *(Rht-B1-Xwmc48.2)*	26.8	-3.7
		3DL	28 *(Xgwm52)*	3.4	22-31 *(Xbarc226-Xgwm52)*	5.1	-1.6
		4DS	28 *(Rht-D1)*	16.1	23-29 *(Xbarc1118-Rht-D1)*	28.0	3.9
	PE08	6AL	63 *(Xwmc32)*	6.2	60-65 *(XwPt-5094-Xwmc32)*	4.3	3.0
		4BS	31 *(Rht-B1)*	36.1	31-32 *(Xwmc617-Rht-B1)*	40.0	-9.9
		4DS	28 *(Rht-D1)*	41.6	26-28 *(Xbarc1118-Rht-D1)*	50.3	11.6
	PU08	6AL	61 *(Xwmc32)*	8.1	59-63 *(XwPt-5094-Xwmc32)*	9.8	3.2
		4BS	31 *(Rht-B1)*	19.4	31-32 *(Rht-B1-Xwmc48.2)*	27.1	-5.4
		4DS	28 *(Rht-D1)*	25.4	25-30 *(Xbarc1118-Rht-D1)*	38.7	6.8
	MC08	6AL	63 *(Xwmc32)*	7.9	60-64*(XwPt-5094-Xwmc32)*	8.2	3.2
		4BS	31 *(Rht-B1)*	22.2	30-33 *(Xwmc617-Rht-B1)*	29.3	-6.2
		3DL	24 *(Xbarc226)*	3.6	15-30 *(Xbarc226-Xgwm52)*	3.7	-2.2
		4DS	28 *(Rht-D1)*	28.1	26-29 *(Xbarc1118-Rht-D1)*	40.5	7.7
	CB	6AL	62 *(Xwmc32)*	10.4	60-64 *(XwPt-5094-Xwmc32)*	8.1	3.4
		4BS	31 *(Rht-B1)*	34.1	31-32 *(Rht-B1-Xwmc48.2)*	38.6	-7.6

		4DS	28 *(Rht-D1)*	38.3	26-28 *(Xbarc1118-Rht-D1)*	46.2	8.9
HDD	CR07	6BS	37 *(Xcfd1)*	3.4	35-43 *(Xcfd13-Xcfd1)*	8.3	-0.6
Qhdd.orr	CR08	1BL	28 *(Xwmc44)*	4.2	16-28 *(Xgwm268-Xwmc44)*	9.0	0.8
		4DS	28 *(Rht-D1)*	9.4	21-31 *(Rht-D1-Xgpw94042)*	21.6	-1.3
		7DS	18 *(Xcfd41)*	3.9	9-28*(Xbarc184-Xcfd41)*	9.9	0.9
	MR08	No significant QTL					
	PE08	6BL	109*(Xwmc621)*	5.1	109-112 *(XwPt-5480 -Xwmc621)*	10.4	-0.7
		4DS	28 *(Rht-D1)*	8.2	20-32 *(Rht-D1-Xgpw94042)*	17.8	-1.0
	PU08	No significant QTL					
	MC08	4DS	25 *(Rht-D1)*	5.1	16-32 *(Rht-D1-Xgpw94042)*	14.8	-0.7
	CB	6BL	109 *(Xwmc621)*	3.2	108-114 *(XwPt-5480-Xwmc621)*	7.50	-0.4
		4DS	28 *(Rht-D1)*	4.6	19-34 *(Rht-D1-Xgpw94042)*	11.3	-0.6
TWT	CR07	4DS	28 *(Rht-D1)*	6.0	23-32 *(Rht-D1-Xgpw94042)*	16.3	6.7
Qtwt.orr	CR08	4BS	27 *(Rht-B1)*	4.1	24-30 *(Xwmc617-Rht-B1)*	11.6	-5.2
		4DS	30 *((Rht-D1)*	7.1	25-33 *(Rht-D1-Xgpw94042)*	18.0	6.6
	MR08	4BS	24 *(Xwmc617)*	3.1	13-29 *(Xwmc141-Rht-B1)*	8.3	-5.5
		4DS	28 *(Rht-D1)*	4.0	22-32 *(Rht-D1-Xgpw94042)*	10.0	6.1
	PE08	4BS	29 *(Rht-B1)*	14.8	27-32 *(Xwmc617-Rht-B1)*	30.7	-18.3
		4DS	28 *(Rht-D1)*	15.8	26-30 *(Rht-D1- Xgpw94042)*	30.9	18.6
	PU08	4BS	30 *(Rht-B1)*	11.0	28-33 *(Xwmc617-Rht-B1)*	23.6	-9.9
		4DS	28 *(Rht-D1)*	12.5	26-32 *(Rht-D1-Xgpw94042)*	25.8	10.6
	MC08	7AL	2 *(XwPt-6460)*	4.7	0-5 *(XwPt-2501-Xgwm146)*	9.6	-4.4
		4BS	26 *(Rht-B1)*	3.7	22-30 *(Xwmc617-Rht-B1)*	8.3	-4.3
		4DS	29 *(Rht-D1)*	5.5	23-34 *(Rht-D1-Xgpw94042)*	11.2	5.2
		5DL	60 *(Xcfd29)*	4.5	54-66 *(Xcfd29-Xcfd183)*	9.3	-4.5
	CB	4BS	28 *(Rht-B1)*	11.3	26-30 *(Xwmc617-Rht-B1)*	24.2	-8.3
		4DS	28 *(Rht-D1)*	14.7	26-31 *(Rht-D1-Xgpw94042)*	30.3	9.3

GPC Qgpc.orr	CR07	6BS	55*(Xbarc136)*	5.1	54-57 *(Xbarc101-Xwmc397)*	13.0	-2.6
		7DL	32 *(Xbarc128)*	3.9	28-33 *(Xcfd46-Xgwm437)*	9.8	-2.3
	CR08	3BL	16 *(Xwmc56)*	4.7	13-21 *(Xwmc3-Xwmc56)*	13.7	-3.9
		7DL	33 *(Xgwm437)*	3.0	31-37 *(Xbarc128-Xgwm437)*	7.6	-2.8
	MR08	2AS	0 *(Xcmwg682)*	3.4	0-1 *(Xcmwg682-Xcfd36)*	9.2	-2.5
	PE08	No significant QTL					
	PU08	No significant QTL					
	MC08	5DL	57 *(Xcfd29)*	4.1	52-59 *(Xwmc215-Xcfd29)*	10.9	-3.1
	CB	1AL	0 *(Xgwm99)*	4.1	0-1 *(Xgwm99-Xbarc1022)*	10.6	5.2
TKW Qtkw.orr	GH07	6AL	61 *(Xwmc32)*	3.8	58-64 *(XwPt-5094-Xwmc32)*	9.3	1.8
		4BS	36 *(Xwmc48.2)*	6.8	34-38 *(Xwmc48.2-XwPt-1708)*	16.5	-2.3
	CR07	4BL	45 *(Xgwm149)*	4.7	44-47 *(Xgwm192-XwPt-7062)*	12.9	-1.2
	CR08	6AL	62 *(Xwmc32)*	5.1	59-66 *(XwPt-5094-Xwmc32)*	8.2	1.1
		7AL	15 *(Xwmc273)*	5.8	12-20 *(Xgwm146-XwPt6168)*	8.9	1.1
		2BL	98 *(Xbarc1155)*	5.1	87-103*(Xbarc1155-Xgwm388)*	8.3	1.1
		4BL	41 *(Xgwm495)*	8.6	40-43 *(Xgwm513-Xgwm495)*	13.8	-1.5
		3DL	16 *(Xgwm3)*	4.6	14-16 *(Xwmc552-Xgwm3)*	6.9	1.0
	MR08	6AL	59 *(XwPt-5094)*	4.1	56-63 *(Xbarc3-XwPt-5094)*	10.4	1.0
		2BL	98 *(Xgwm388)*	3.7	95-104*(Xbarc1155-Xgwm388)*	9.7	1.0
	PE08	6AL	60 *(XwPt-5094)*	3.6	55-63 *(Xbarc3-XwPt-5094)*	5.8	0.9
		7AL	0 *(XwPt-2501)*	3.5	0-1 *(XwPt2501-XwPt6460)*	5.4	0.9
		2BL	86 *(Xbarc1155)*	4.1	77-100 *(Xgwm410.2-Xbarc1155)*	7.7	1.2
		4BS	38 *(XwPt-1708)*	14.3	37-39 *(Xwmc48.2-XwPt1708)*	26.0	-2.0
		4DS	28 *(Rht-D1)*	4.4	21-34 *(Rht-D1-Xgpw94042)*	7.1	1.1
	PU08	6AL	60 *(XwPt-5094)*	6.0	57-62 *(Xbarc3-XwPt-5094)*	12.0	1.2
		1BS	0 *(RIS)*	3.3	0-2 *(RIS-Xbarc240)*	5.5	-0.8
		2BL	99 *(Xbarc1155)*	4.3	86-103 *(Xbarc1155-Xgwm388)*	8.8	1.0

	4BS	32 *(Rht-B1)*	11.8	31-33 *(Rht-B1-Xwmc48.2)*	24.7	-1.8
	4DS	28 *(Rht-D1)*	4.2	22-31 *(Rht-D1-Xgpw94042)*	7.7	1.0
MC08	6AL	62 *(Xwmc32)*	4.7	59-66 *(XwPt-5094-Xbarc107)*	10.7	1.2
	7AL	15 *(Xwmc273)*	3.6	10-21 *(Xgwm146-Xwmc273)*	7.9	1.1
	4BL	44 *(Xgwm192)*	4.5	43-46 *(Xgwm192-Xgwm149)*	10.3	-1.2
CB	6AL	61 *(Xwmc32)*	5.5	59-66 *(XwPt-5094-Xwmc32)*	10.2	1.0
	7AL	15 *(Xwmc273)*	3.1	9-20 *(Xgwm146-Xwmc273)*	5.3	0.7
	2BL	93 *(Xbarc1155)*	4.3	83-104 *(Xbarc1155-Xgwm388)*	7.7	0.9
	4BL	41 *(Xgwm495)*	9.9	40-43 *(Xgwm513-Xgwm192)*	18.5	-1.3

Note: *CR07* Corvallis (OR), 2007; *CR08* Corvallis (OR), 2008; *MR08* Moro (OR), 2008; *PE08* Pendleton (OR), 2008; *PU08* Pullman (WA), 2008; *MC08* Moscow (ID), 2008; *CB* Combined across field environments.

[a] The letter S represented the short arm of chromosome, and L indicated the long arm of chromosome.

[b] Position of QTL peak is expressed in centiMorgans (cM), nearest locus to QTL peak is indicated in brackets.

[c] Logarithm of the odds ratio (LOD) of QTL peak that exceeded the significant LOD threshold from 1,000 permutations.

[d] The flanking loci of 1-LOD support limit are indicated in brackets.

[e] R^2 is the proportion of the phenotypic variance explained by the QTL after accounting for co-factors.

[f] Positive additive values indicate that higher value alleles are from Stephens (OS9A) and the negative values indicate that the higher value alleles are from OR9900553 (QCB36).

CHAPTER 5

Association Analysis of Kernel Hardness and Related Agronomic Traits in a Diverse Collection of Wheat Lines

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Abstract

Kernel hardness is an important determinant of milling and end-use quality of wheat (*Triticum aestivum* L.). The objective of this study was to test the associative mapping to identify marker-trait associations for kernel hardness, thousand-kernel weight, grain protein content, test weight, and plant height. A diversity panel of 94 wheat lines was genotyped with a total of 487 diversity array technology (DArT) and simple sequence repeat (SSR) markers. A linear mixed-effects model (MLM) was used to detect marker-trait associations incorporating covariance of population structure and relative kinship. A total of five markers were found significantly associated with kernel hardness, test weight and plant height after the correction of false discovery rate (α_c=0.05). The gene *pinB,* previously reported as a major determiant of kernel hardness along with *pinA* (part of the *Ha* locus) on chromosome 5DS, was highly associated with kernel hardness. We also identified associations between marker *XwPt-7187* on chromosome 2A and kernel hardness, two DArT markers *XwPt-1250* and X*wPt-4628* and test weight, and *Xgwm512* and plant height. Our results demonstrated that association analysis can be an effective approach for identifying and validating quantitative trait locus (QTL) for kernel hardness and other complex traits of interest in wheat.

Introduction

Association mapping, or linkage disequilibrium (LD) mapping, is an approach to genetically dissect complex traits based on the non-random association between alleles at a locus (or loci) and phenotypic traits of interest across a diverse germplasm set (Weir, 1996). Association mapping was initially developed and applied in human genetics to identify causal mutations for common complex human genetic disorders, such as cystic fibrosis (Kerem et al., 1989). Due to the potential of identifying causal polymorphisms underlying complex traits, association mapping has been recently applied in plant genetics studies, such as *Arabidopsis,* maize, rice, barley, wheat, and potato (Breseghello et al., 2005; Flint-Garcia et al., 2003; Kraakman et al., 2004; Nordborg et al., 2002; Remington et al., 2001). Recently, association mapping has been used to identify associations between molecular marker loci and complex traits of interest in wheat, such as kernel size and milling quality (Breseghello and Sorrells, 2006).

The genetic factors controlling wheat kernel hardness (KHA) are complex and to a large extent poorly understood. KHA is mainly controlled by the *Ha* locus and two puroindoline genes, *pinA* and *pinB,* on the short arm of chromosome 5D (Jolly et al., 1996; Morris et al., 1994). Additionally, a number of quantitative trait loci (QTL) were identified for KHA in different bi-parental mapping populations (Arbelbide and Bernardo, 2006; Breseghello et al., 2005; Campbell et al., 1999; Campbell et al., 2001; Nelson et al., 2006; Perretant et al., 2000; Pshenichnikova et al., 2008; Sourdille et al., 1996; Zanetti et al., 2001). However, QTL were not consistently identified across the different mapping populations.

Association mapping has two main advantages over traditional QTL mapping. First, association mapping does not require the development of bi-parental progenies, which saves time and resources (Kraakman et al., 2004). Secondly, association mapping

has potentially higher resolution power for mapping QTL and greater capacity for detecting additional alleles than traditional QTL mapping procedures based on bi-parental populations (Yu and Buckler, 2006). In wheat, there has been increased interest in identifying novel marker-trait associations using associative mapping (Gupta et al., 2005; Zhu et al., 2008). Recently, Breseghello et al. (2006) demonstrate that association mapping is a valid alternative approach to traditional linkage based QTL mapping with the successful mapping of kernel size and milling quality traits within a set of soft winter wheat cultivars using simple sequence repeat (SSR) markers and a linear mixed-effects model (MLM).

The objectives of this study were to identify new marker-trait associations and to validate QTL previously found by linkage mapping. The five traits of interest included KHA, thousand-kernel weight (TKW), grain protein content (GPC), test weight (TWT), and plant height (PHT). A collection of 94 wheat accessions including hard, soft, and 'extra-soft' genotypes was used to detect the marker-trait associations.

Materials and methods

Plant materials and phenotypic data

A panel of 94 diverse wheat (*Triticum aestivum* L.) lines was selected from the wheat breeding program in the Department of Crop and Soil Science at Oregon State University, Corvallis, Oregon. The diversity panel consisted of hard, soft and 'extra-soft' genotypes, including 'Stephens' (soft grain) and 'OR9900553' (extra-soft grain), which are the parental lines of the OS9XQ36 mapping population used for QTL analysis (Riera-Lizarazu et al., 2010). In 2009, the diversity panel was planted in outside beds of the greenhouse at Oregon State University (GOB) and Hyslop field (HYS), Corvallis, OR, in a completely randomized design (CRD). Five traits (KHA, TKW, GPC, TWT, and PHT) were measured and evaluated as described by Riera-Lizarazu et al. (2010).

Genotypic data

For genomic DNA extraction, the 94 genotypes were planted in the greenhouse. Two to three weeks after planting, young leaves were harvested from five plants per genotype, frozen in liquid nitrogen, and stored at -80 ˚C prior to DNA extraction. Genomic DNA was extracted from young leaf tissue following the methods described by Riera-Lizarazu et al. (2000). The population was genotyped using diversity array technology (DArT) and simple sequence repeat (SSR) markers. One micro gram of DNA from each sample was sent to Triticarte Pty Ltd (Yarralumla ACT, Australia) for whole-genome screening and genotyping using DArT markers (Akbari et al., 2006). The panel was also genotyped with primers specific for semi-dwarfing genes *Rht-B1* and *Rht-D1*, hardness genes *pinA* and *pinB*, and an additional 120 SSR markers. Reverse primers labeled with FAM, TET, NED, or HEX fluorescent dyes were paired with unlabeled forward primers. PCR was performed using an MJ Research PTC-100 thermal cycler following the protocol described by Leonard et al. (2008). The PCR products were separated by size using an ABI 3100 capillary electrophoresis system (Applied Biosystems, Foster City, CA) at Washington State University, Pullman, WA. Results were analyzed using GeneMarker V3.0 software. In the case of SSR markers that produced more than one band, each band was scored independently as a different locus, provided that the size ranges were clearly separated. Markers were ordered according to previously published wheat consensus maps (Akbari et al., 2006; Nelson et al., 1995; Quarrie et al., 2005; Roder et al., 1998; Semagn et al., 2006; Somers et al., 2004).

Population structure and association analysis

DArT and SSR markers with a minor allele frequency of 5% or greater and a missing allele frequency less than 15% were used in the association analysis. The underlying population structure was estimated with all markers using the software

program STRUCTURE 2.3 (Pritchard et al., 2000). The admixture model was applied with correlated allele frequencies. The number of subpopulations (k) was estimated by setting k from one to 12 in four runs with replications 500, 000 and 50, 000 burn-in cycles. The ad hoc statistics was then used to determine the number of subpopulations (Evanno et al., 2005).

Marker-trait associations were estimated with a MLM method described by Yu et al. (2005) incorporating the population structure Q-matrix and the relative kinship K-matrix as covariates using software package TASSEL 2.1. The Q-matrix of three subpopulations was derived from STRUCTURE and the K-matrix was produced by TASSEL. The phenotypic traits measured in environments GOB and HYS were used for marker-trait association analysis. Significant associations between marker loci and traits were determined based on the q-value from the correction of multiple testing using false discovery error (FDR, α_c=0.05) using the software package R. A general linear model (GLM) with 5,000 permutations was used to estimate the amount of phenotypic variance explained by significant marker loci.

Results

Phenotypic data and marker polymorphism

The panel showed phenotypic variation for all five traits (Figure 5.1). KHA displayed a bimodal distribution, while both TKW and GPC were continuously distributed. The distribution of TWT was left-skewed, and PHT was right-skewed. Least square (LS) means and the ranges of phenotypes are summarized in Table 5.1.

The 94-line panel, including the parents Stephens and OR9900553 from the QTL mapping population (Riera-Lizarazu et al., 2010), were genotyped with 487 markers. A total of 382 DArT markers and 425 alleles generated from 105 SSR markers were used in

marker-trait association analysis. The number of alleles per SSR marker locus varied from 2 to 12, with an average of 4.1 alleles per locus. An additional 305 SSR alleles and 16 DArT markers had a low frequency of polymorphism (≤5%). The rare alleles, which may reflect genotyping or sampling error, were excluded from association analysis.

Population structure

The average logarithm of the probability of likelihood LnP(D) showed constant increase with increasing subpopulation number k, and no significantly clear cut-off was observed based on the LnP(D) plot. The ad hoc statistic of Evanno et al. (2005) was used to determine the number of subpopulations, which resulted in k=3. Figure 5.2 shows three clusters in a neighbor-joining tree created from all marker loci using Tassel 2.1. The structure of the population is consistent with known origins and pedigrees. The three marker-based clusters reflect groupings made on the basis of release locations or origins: sub-group A includes 38 genotypes from the Pacific Northwest area, 37 genotypes in sub-group B have a French genetic background (some admixtures of Oregon and French background), and the remaining 19 genotypes in sub-group C are from the Nebraska area (some admixtures of Oregon with Nebraska background). Some wheat lines are known to be reselections of one another, for example, 'Brundage96' is reselected from 'Brundage' and both are closely related to each other in the neighbor-joining tree. As expected, QCB36 and OR9900553 are clustered together since QCB36 is a reselected line from the breeding line OR9900553.

Association mapping

Associations between marker alleles and mean phenotypic values were tested by two MLM models: one incorporated the K-matrix and another incorporated both the Q-matrix and the K-matrix. There was no significant difference between the two models with or without the K-matrix on the marker-trait association analysis. Additionally, two

Q-matrices with subpopulations equal to 3 or 4 were included in MLM analysis together with the K-matrix, separately. Whether three or four subpopulations were included, no differences in marker-trait associations were observed when using the two different structure covariate matrices. Thus, our correction for origin of germplasm in the form of population structure (k=3) was incorporated in marker-trait association analysis with the MLM approach.

Significant marker-trait associations were identified for KHA, TWT, and PHT, but not for TKW and GPC (Table 5.2). Only two out of 487 markers, DArT marker *XwPt-7187* on chromosome 2A and the gene *pinB* on 5D*,* were found highly associated with KHA after FDR correction at α_c= 0.05, accounting for 7.3-12.2% and 19.8-28.5% of the phenotypic variance, respectively. Gene *pinB* is a major determinant of KHA along with *pinA* (part of the *Ha* locus) on the short arm of chromosome 5D (Jolly et al., 1996). Two tightly linked DArT markers on chromosome 5B, *XwPt-1250* and *XwPt-4628,* were associated with TWT in environment GOB, but not in HYS. These two markers explained 34.3% and 39.4% of the phenotypic variance of TWT, respectively. Two alleles of marker *Xgwm512* on chromosome 2A were associated with PHT in environment HYS, explaining 32.5% and 26.1% of the phenotypic variance.

Discussion

Population structure can lead to spurious marker-trait associations in association analysis if ignored (Pritchard et al., 2000). Therefore, incorporating population structure in association analysis leads to more robust analysis results. Our genotypes were not convergent based on the structure analysis using three datasets, 49 randomly selected unlinked DArT markers, the whole dataset of 487 markers, or 382 DArT markers. In the 94-line mapping panel, several highly related lines and/or admixed accessions were included. The relationships among lines were confirmed by the neighbor-joining tree. The non-convergence of LnP(D) is probably due to the complex genetic background of

some mixed lines in the germplasm panel. Other factors contributing to non-convergence or clustering of the population might include a bad exploration of space, and equiprobable classification of individuals in some groups (Pritchard et al., 2000). Clustering of cultivars might change if a subset of marker data was included in structure analysis, but most of the wheat lines were correctly assigned to clusters. In our study, the 94-line diversity panel was grouped into three subpopulations based on ad hoc statistics (Evanno et al., 2005).

Association mapping is a method for high-resolution mapping of QTL and is useful for dissecting complex traits controlled by multiple QTL. Of five identified markers for phenotypic traits, only *pinB* was in a chromosome region where QTL associated with KHA have been previously identified (Jolly et al., 1996). Thus, association mapping is an alternative approach for identifying new marker-trait associations. We observed an association between gene *pinB* and KHA which explained 29% of the phenotypic variance in two environments, providing further evidence that the *pinB* gene contributes to KHA. However, the *pinB* gene only explained part of the variation for kernel hardness, indicating that there are other genetic factors contributing to KHA. Both semi-dwarfing genes *Rht-B1* and *Rht-D1* were found linked to KHA QTL in Chapter four, but no significant associations were identified in this study. This result suggests that there might not have been sufficient power to detect the associations between KHA and markers used in this study.

In our study, five out of 487 markers were found associated with three phenotypic traits after correction for FDR. Compared to traditional QTL mapping, our association mapping study had less power to identify significant markers for KHA and, not surprisingly, only two markers were found for KHA. The low resolution of our analysis is likely due to small sample size, genetic distance, population admixture, and limited variation among genotypes used in this study. The genetic difference between

hard and soft wheat is controlled by the *Ha* locus and two puroindoline genes: *pinA* and *pinB* (Jolly et al., 1996). Although hard, soft, and 'extra-soft' wheat lines were included in this wheat diversity panel, the original purpose of this panel was not primarily to detect factors related to kernel texture. In order to identify marker loci associated with the 'extra-soft' characteristic of wheat kernel, a subset of 52 soft and five 'extra-soft' wheat lines were selected from the 94 wheat lines. Association analysis was performed using this new wheat panel, however, no significant marker loci were identified for KHA. It seems probable that the variation of KHA among soft genotypes may be insufficient for the identification of new marker-trait associations for this trait. Therefore, a larger population with more 'extra-soft' wheat lines may be required for association analysis to obtain better results.

Compared to traditional QTL mapping, association mapping is less time-consuming because no segregating offsprings need to be produced. The association approach is relatively complex in methodology, but modestly demanding with respect to genotyping. This is in contrast to the genotyping and methodological demands for QTL mapping. To improve the power of association analysis, it is necessary to obtain a large phenotypic dataset from multiple environments for a diverse population. This could be achieved through multiple field trials with replicates and a large collection of wheat lines.

Although few significant marker loci were identified in this study, our association approach identified markers that may be of use for marker-assisted selection. Furthermore, we aim to expand the current marker dataset with additional SSR markers, as well as candidate genes. To further improve our methodology, we propose to add more soft and 'extra-soft' wheat lines to the diversity panel, and investigate a more appropriate statistical model for association analysis.

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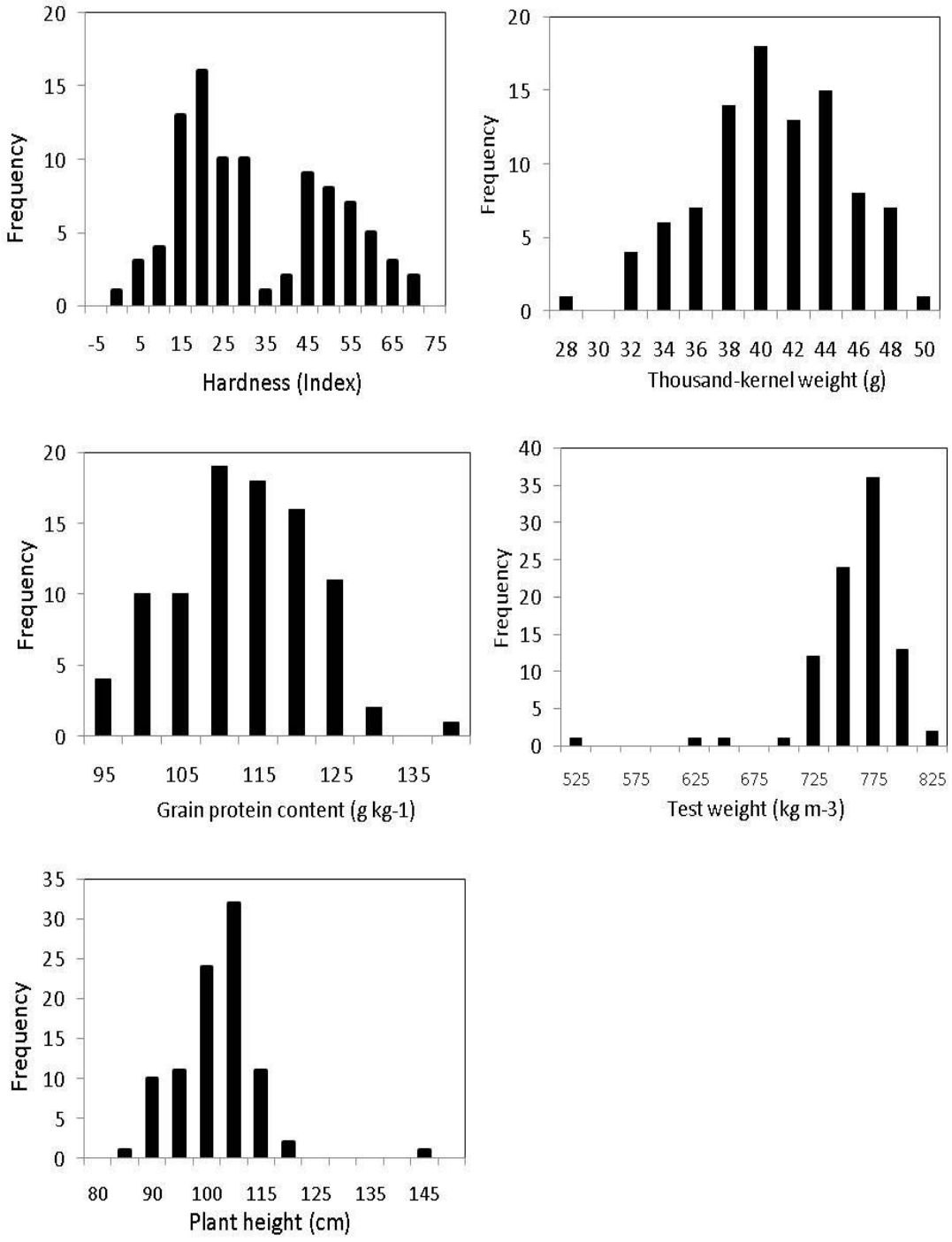

Figure 5.1 Frequency distribution of least square (LS) means of kernel hardness, thousand-kernel weight, grain protein content, test weight, and plant height for the 94-line diversity panel. Measurements represent means of two environments, GOB and HYS.

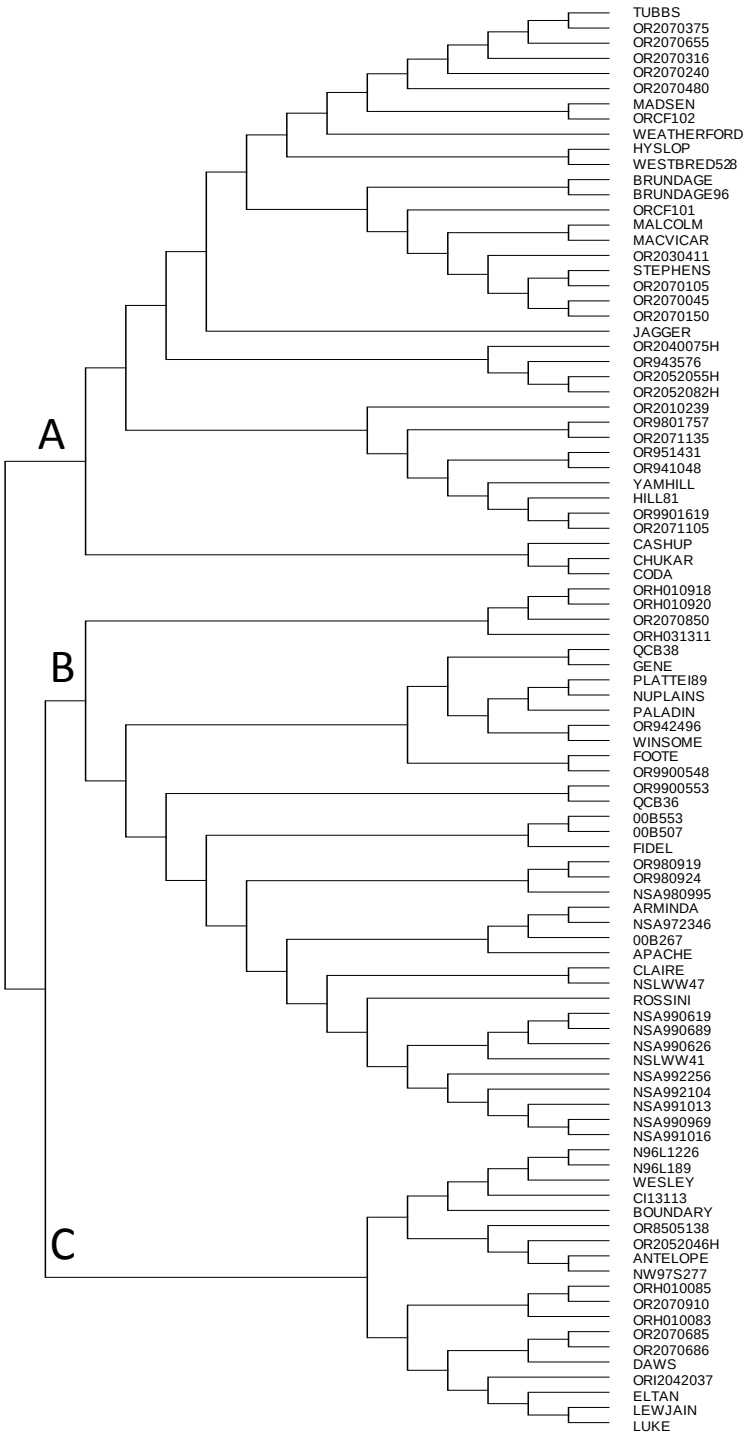

Figure 5.2 A neighbor joining tree of the 94-line diversity panel based on 487 marker. Wheat lines in the same cluster share the release location or origin, including (A) Pacific Northwest area (some admixtures of Oregon and Washington wheat lines), (B) French background (some admixtures of Oregon and French background), and (C) Nebraska (some admixtures of Oregon and Nebraska background).

Table 5.1 Summary statistics of five agronomic trait least square (LS) mean, standard error (SD) of mean, and the range of the 94-line diversity panel.

Trait	Environment	LS Mean	Range	SD
Kernel hardness	Hyslop	36.7	-0.7 - 78.8	20.6
	Corvallis	25.9	-8.4 - 69.5	16.5
Thousand-kernel weight (g)	Hyslop	34.9	18.2 - 46.7	5.2
	Corvallis	45.9	30.1 -58.0	5.1
Grain protein content (g kg^{-1})	Hyslop	111	92 - 136	9.0
Test weight (kg m^{-3})	Hyslop	747	515– 820	39.2
Plant height (cm)	Hyslop	102	85- 142	9.0

Table 5.2 Chromosome regions and significant marker alleles associated with kernel hardness, test weight, and plant height. The association analysis was performed with linear mixed-effects model (MLM) incorporating the structure Q-matrix and kinship K-matrix on the basis of 487 DArT and SSR markers using TASSEL 2.1.

Trait	Marker alleles	Chromosome	Position (cM)	P-value †		R^{2} §	
				Corvallis‡	Hyslop	Corvallis	Hyslop
Hardness	*XwPt-7187*	2A	17	**	0.0074	12.2	7.3
	pinB	5D	0	*	**	19.8	28.5
Test weight	*XwPt-1250*	5B	93	**	-	34.3	-
	XwPt-4628	5B	93	**	-	39.4	-
Plant height	*Xgwm512.179*	2A	11	-	**	-	32.5
	Xgwm512.209	2A	11	-	**	-	26.1

† *, ** indicate marker-trait association is significant at α_c =0.05 and 0.001, respectively.

‡ - indicates missing value.

§ R^2 is the percentage of phenotypic variance explained by the marker.

CHAPTER 6

Conclusions

Guomei Wang

Wheat (*Triticum aestivum* L.) varieties with higher market value than commodity wheat is of significant interest to growers and therefore to wheat breeders. Since good end-use quality and high flour yield are paramount to this concern, interest in understanding the genetic factors that control quality has never been greater than at present. Kernel texture is an important determinant of milling and baking quality in common wheat. Wheat is sorted into two market classes, soft or hard according to kernel texture. Within the soft wheat market class, varieties with superior soft texture and end-use quality are described as 'extra-soft' or 'super-soft.' Although the genetics of the difference between the two market classes of wheat is now well established, little is known about the variation within each class of hardness and its underlying genetic basis.

The goal of this study was to lay the groundwork for understanding both the genetic factors and relationships between traits that control the difference between soft and 'extra-soft' wheat varieties. This question was addressed in a variety of approaches, most of which employed a large bi-parental mapping population segregating for the phenotypes mentioned. To confirm and enhance the results of this study, the traditional QTL mapping approach was supplemented with an association mapping study of the same traits in a diverse collection of elite wheat lines in this research.

Prior to genetic study, path coefficient analysis was performed on an F_6- derived recombinant inbred line (RIL) mapping population with164 lines (Chapter 2). This population was developed from a cross between two elite Pacific Northwest wheat lines, OS9A (soft kernel) and QCB36 ('extra-soft' kernel). The purpose of this initial study was to determine the interrelationship of a variety of phenotypic traits through analysis of correlations. Besides direct quality measurements such as kernel hardness (KHA) and flour yield, agronomic traits, such as heading date, which had previously been reported correlated with flour quality, were included. As an example of results, KHA is negatively

associated with break flour yield (BFY), and is correlated with other agronomic traits as well. This may provide some easily quantified and selected phenotypes breeders can employ to develop 'extra-soft' varieties. Significant environmental effects and genotype-environment interactions for seven agronomic traits and BFY were also found. Weather in June significantly affected wheat grain filling and development. Because the environmental effects on KHA and BFY were significant, a wheat breeder may consider also selecting for both heading and maturity dates during the development of 'extra-soft' wheat varieties.

Measuring flour yield is a slow and costly method to evaluate lines in a breeding program. By identifying the genetic and environmental factors that significantly affect the desired phenotype, this study offers the promise of a breeding approach that is less resource-intensive and more economical than direct measurement of kernel quality. There is an obvious inverse relationship between resources required and number of lines that can be screened during a breeding cycle. When more lines can be screened, effective selection can be applied earlier in the program to increase the number of lines with desired attributes that can be carried through the entire cycle.

Our development of a genetic linkage map was intended to support the QTL mapping of the genetic factors that directly contribute to the 'extra-soft' kernel phenotype. Mapping anomalies discovered during that process led us to identify three chromosome translocations, 1BL.1RS, 2N^{v}S-2AS.2AL, and 5B:7B within the population (Chapter 3). QCB36 was the donor of all three translocations. Wide use of marker-assisted selection in wheat breeding has only begun recently. Knowledge of the translocations present in any line has largely been dependent on precise record keeping and the ability of a breeder to discern phenotypes. As translocations often carry a number of desired traits, it is important to be able to identify them in wheat varieties. In addition, the presence of a translocation is likely to inhibit recombination in that chromosomal region, making it difficult or impossible to introduce wheat genes from

other varieties in that region. Our identification of these translocations, their characterization, and the markers that can be used to identify them in PNW varieties, adds a useful tool for thoughtful design of new varieties.

The commonly used approach of QTL mapping was used to identify chromosomal regions underlying the 'extra-soft' characteristic (Chapter 4). Although the complexity of factors contributing to the kernel hardness phenotype complicated the study, the bi-parental cross yielded a genetic map with a good resolution. We verified the accuracy of the QTL study by clearly identifying the semi-dwarfing loci *Rht-B1* and *Rht-D1* for plant height, which were segregating within the mapping population. A total of 47 significant QTL were subsequently detected for KHA and other related traits, including BFY, bran flour yield (BRN), middling yield (MID), plant height (PHT), days to heading (HDD), test weight (TWT), grain protein content (GPC), and thousand-kernel weight (TKW). The large number of QTL identified substantiated the methodology and offers an inroad to manipulating these quantitative traits.

Six QTL for KHA and BFY were detected on identical chromosomal regions. At this point, we cannot distinguish whether this is the result of pleiotropy or closely linked genes. Only by isolation of these QTL and further investigation can these questions be resolved. The most important QTL for KHA were on 4DS (*Xbarc1118-Rht-D1* interval) and 4BS (*Xwmc617-Rht-B1* interval) in our study. The results suggest that the 'extra-soft' characteristic is not controlled by the Hardness (*Ha*) locus on 5DS, the primary determinant of hard versus soft grain in wheat. Analysis of the effect of allelic status at *Rht-D1* revealed that the KHA QTL is not directly affected by *Rht-D1* but by a gene(s) tightly linked to this green-revolution gene. Therefore, it will be possible to manipulate the KHA QTL while retaining the preferred allele at *Rht-D1* locus. The major QTL for BFY, BRN, MID, HDD, TWT, and TKW occupied a coincident location close to semi-dwarfing gene *Rht-D1* on chromosome 4DS. Similarly, QTL for BFY, BFY, TWT, and TKW were identified on 4BS near semi-dwarfing gene *Rht-B1*. The identification of these markers

provides a tool that can be used immediately for selecting lines with the high-value 'extra-soft' trait.

Association mapping was used as an alternate approach and complementary tool to QTL mapping and to identify other marker-trait associations (Chapter 5). Although only two environments of phenotypic data were available, we validated the approach by identifying gene *pinB* as highly associated with KHA. This gene has been reported previously as one of the major genes controlling kernel hardness. Since the diversity panel was not originally assembled for the study of the 'extra-soft' trait, it contained a number of hard wheat lines that allowed identification of *pinB*. A total of five markers were found significantly associated with KHA, TWT and PHY.

The path from QTL detection to identification of gene candidates to candidate gene validation in wheat is very long. The discovery of significant QTL and marker-trait associations for KHA and other agronomic traits in this work is an exciting first step towards dissecting the QTL. Nonetheless, the potential to apply QTL mapping results to wheat breeding programs through marker-assisted selection strategies is an immediate benefit of this endeavor. As the potential value of the 'extra-soft' market class could be significant, hopefully the work detailed in this dissertation provides both valuable practical tools and inroads to address basic questions about the genetics of flour quality.

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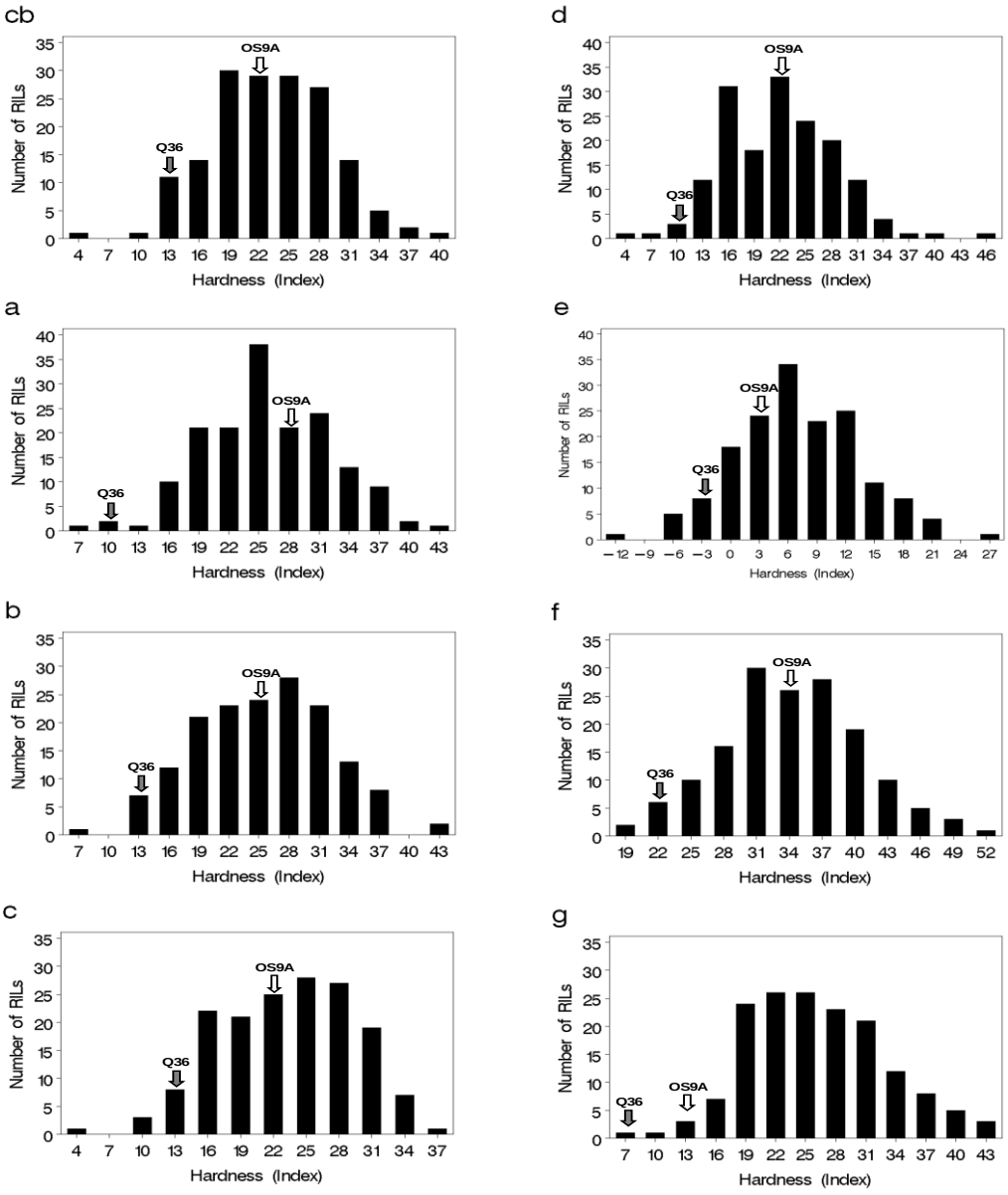
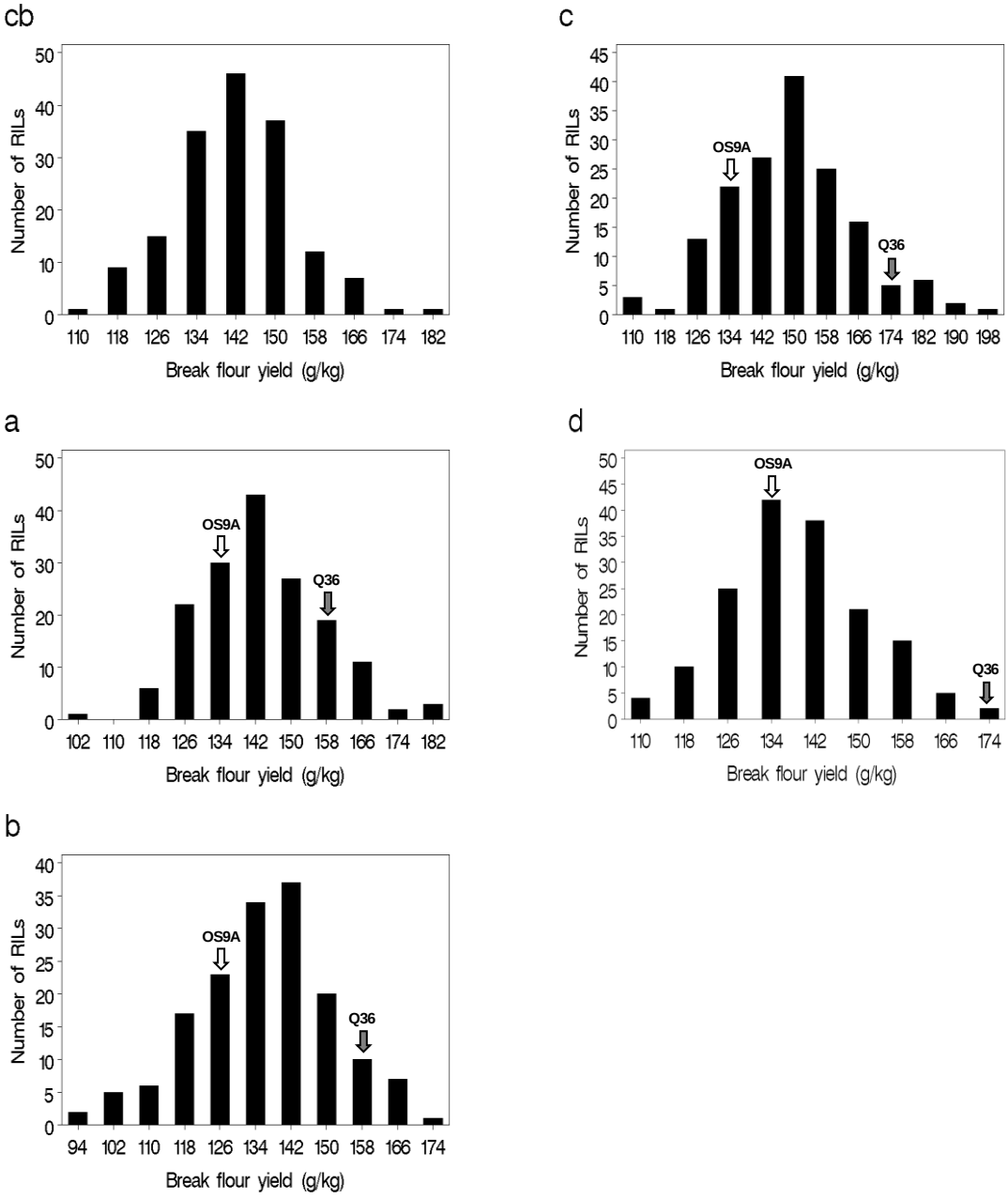
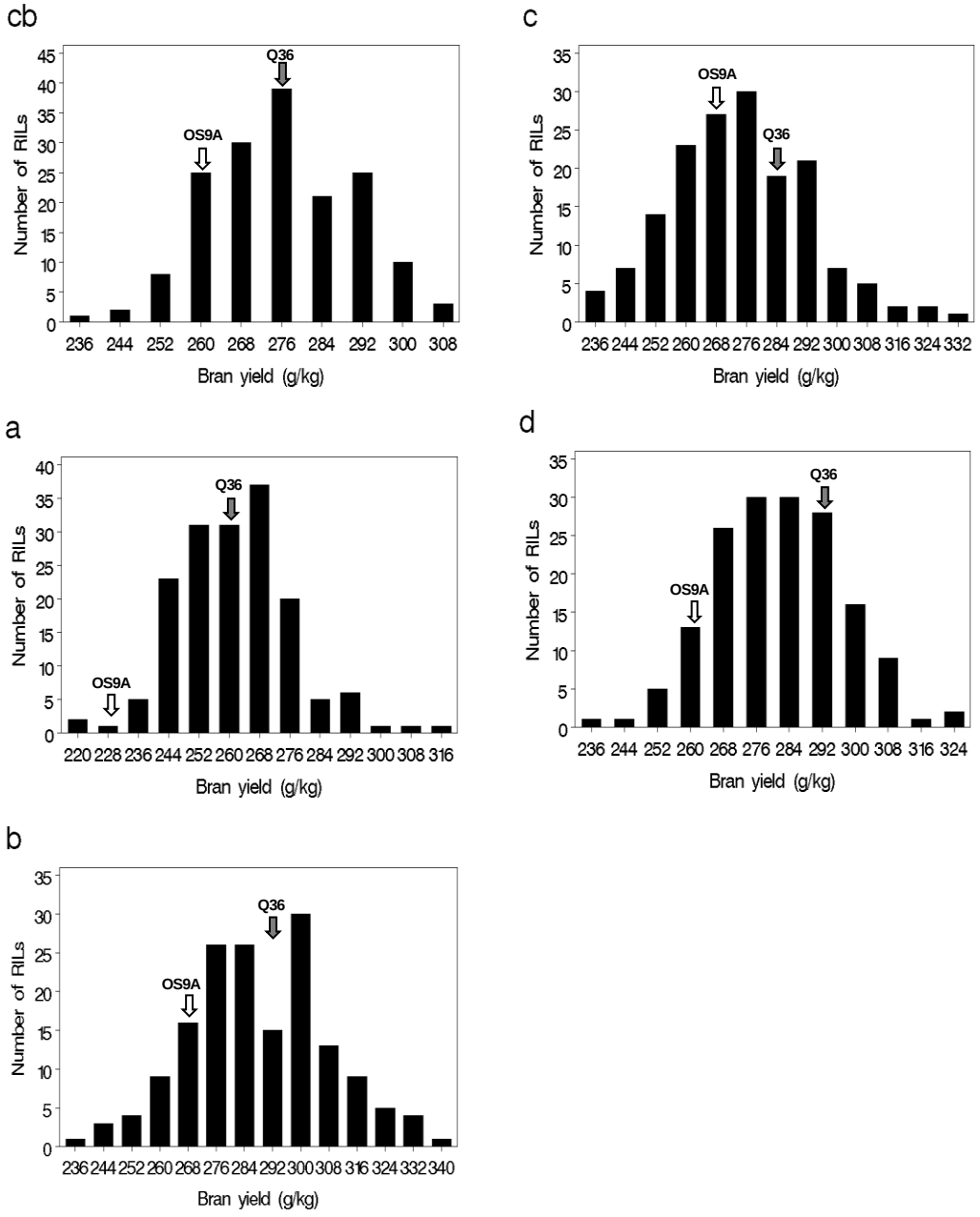


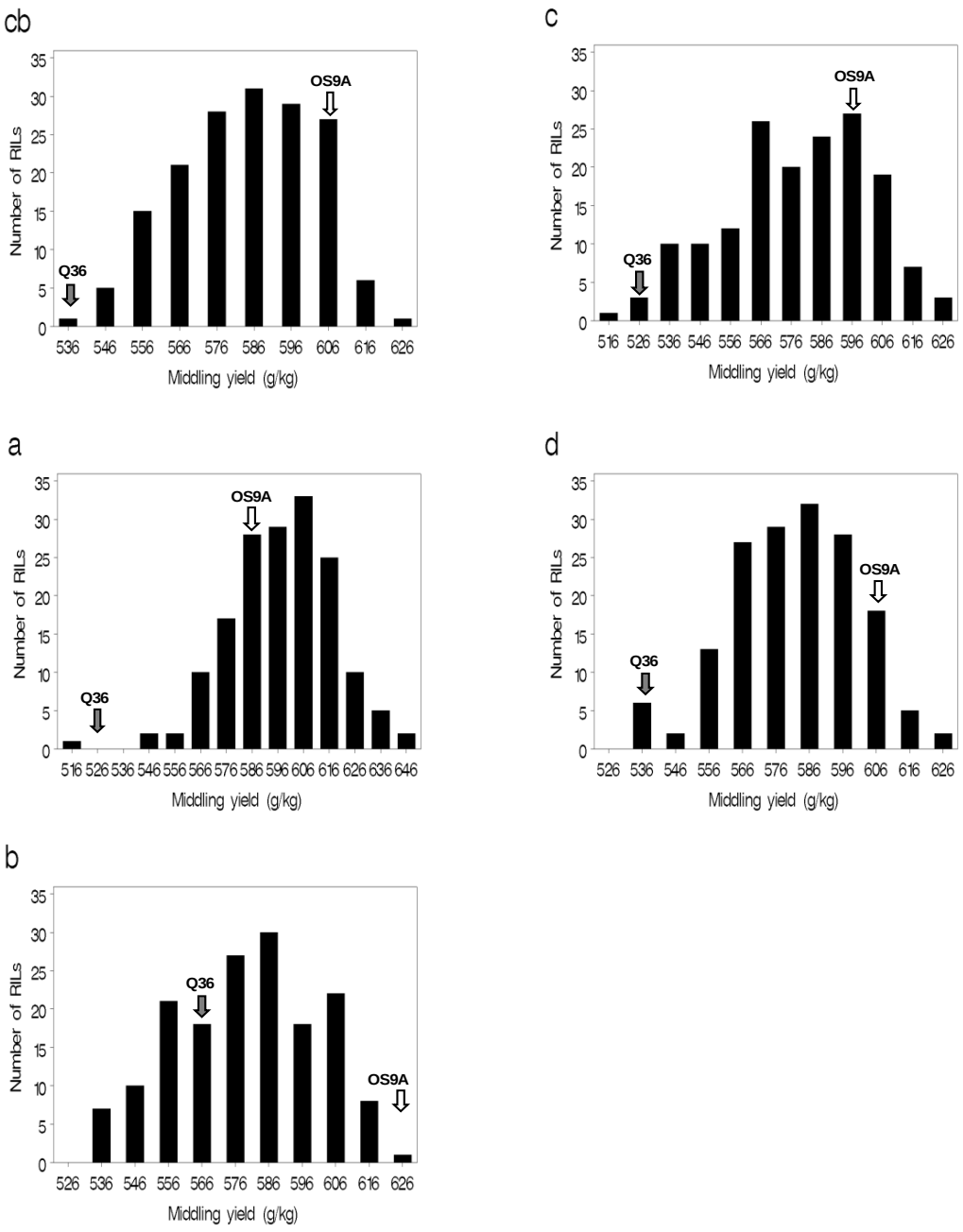
Supplemental Figure 4.1S Frequency distribution of kernel hardness (index) of the 164 F_6-derived recombinant inbred lines (RILs) grown at six field environments in 2007 and 2008. The data sets were obtained from the trials (cb) Across six field environments (a) Corvallis (OR), 2008, (b) Moro (OR), 2008, (c) Pendleton (OR), 2008, (d) Pullman (WA), 2008, (e) Moscow (ID), 2008, (f) Corvallis (OR), 2007, and (g) Greenhouse (OR), 2007. Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



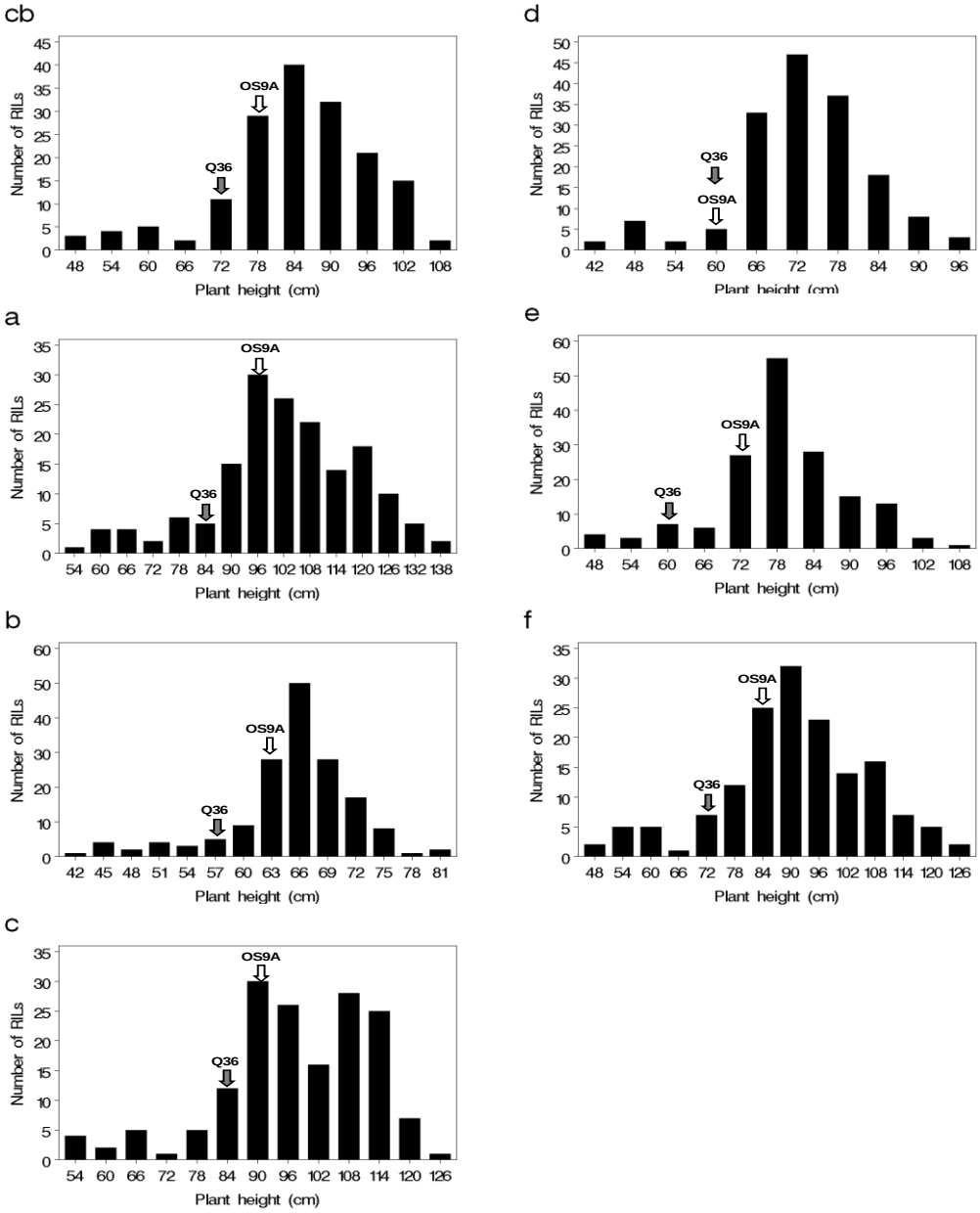
Supplemental Figure 4.2S Frequency distribution of break flour yield (g/kg) of the 164 F_6-derived recombinant inbred lines (RILs) grown at four different field environments in 2008. The data sets were obtained from the trials (cb) Across four environments, (a) Corvallis (OR), (b) Moro (OR), (c) Pendleton (OR), and (d) Pullman (WA). Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



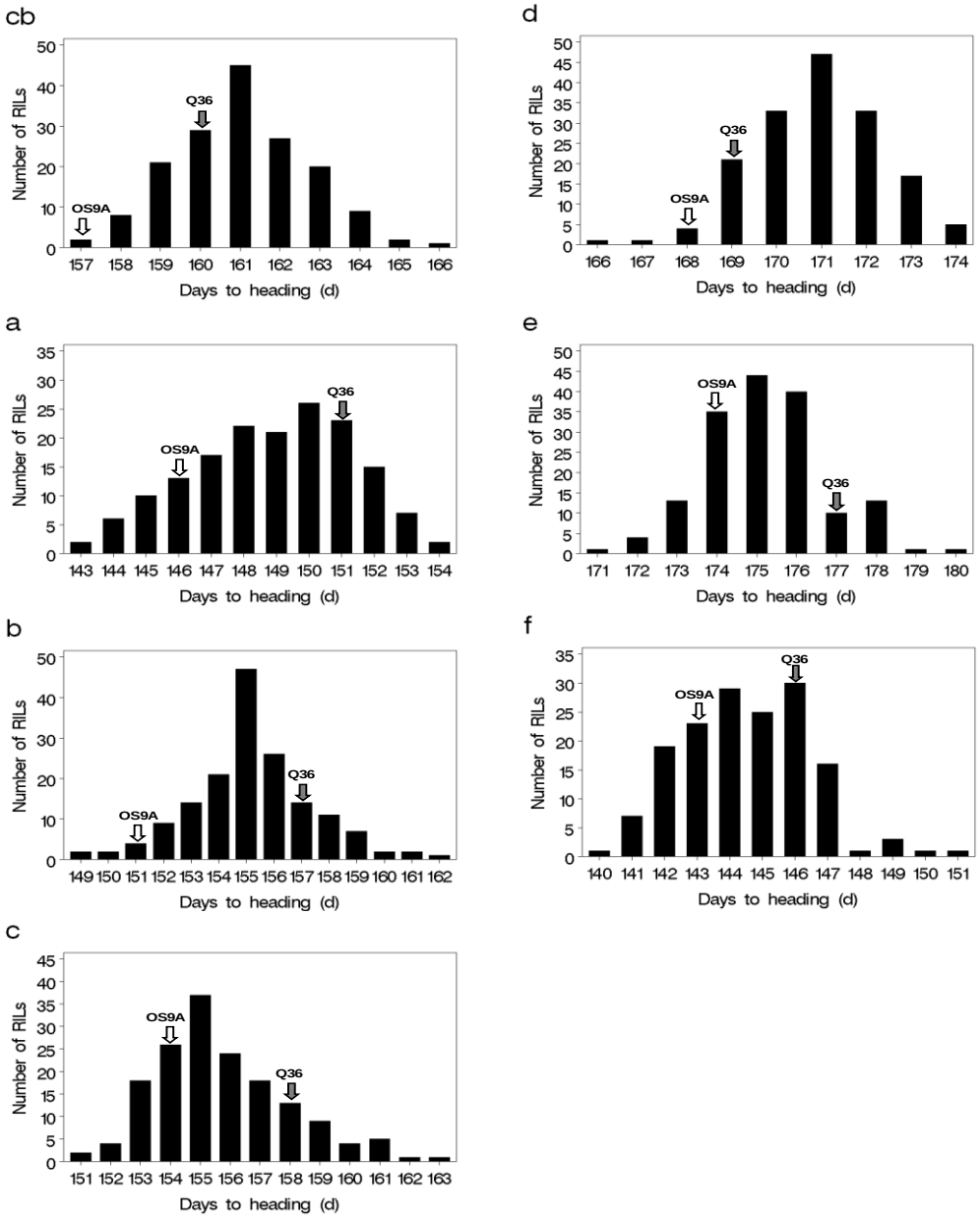
Supplemental Figure 4.3S Frequency distribution of bran yield (g/kg) of the 164 F_6-derived recombinant inbred lines (RILs) grown at four field environments in 2008. The data sets were obtained from the trials (cb) Across four environments, (a) Corvallis (OR), (b) Moro (OR), (c) Pendleton (OR), and (d) Pullman (WA). Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



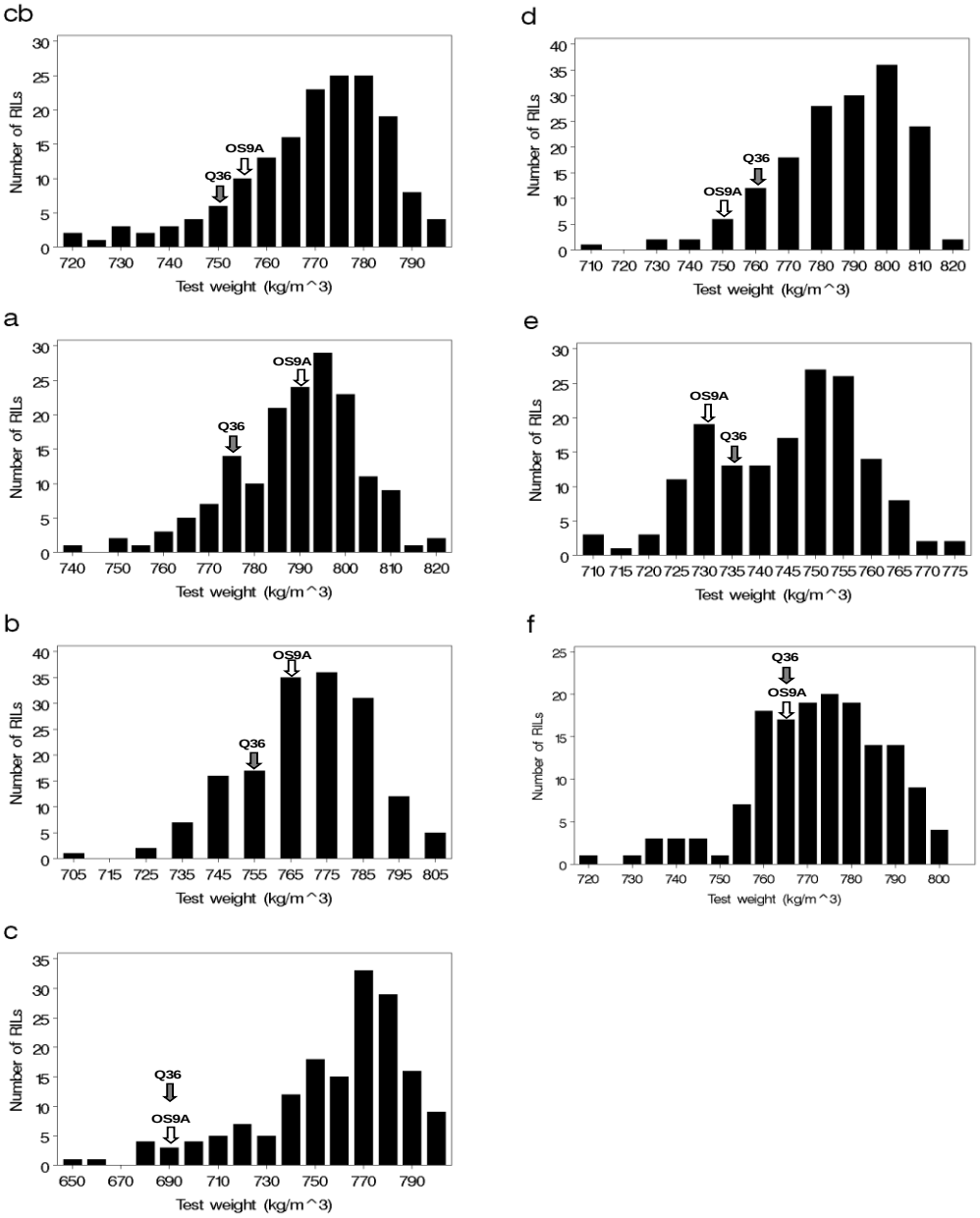
Supplemental Figure 4.4S Frequency distribution of middling yield (g/kg) of the 164 F_6-derived recombinant inbred lines (RILs) grown at four field environments in 2008. The data sets were obtained from the trials (cb) Across four environments, (a) Corvallis (OR), (b) Moro (OR), (c) Pendleton (OR), and (d) Pullman (WA). Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



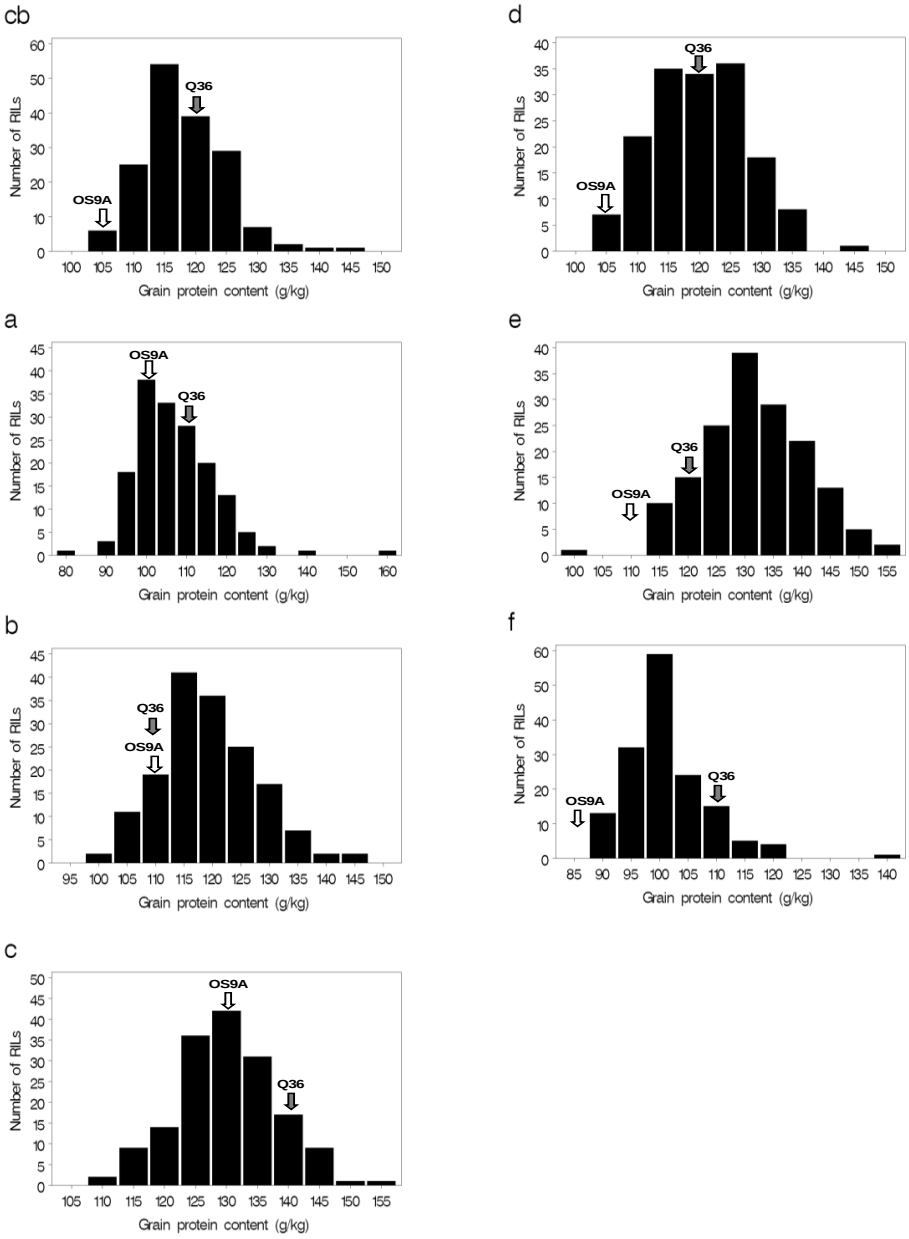
Supplemental Figure 4.5S Frequency distribution of plant height (cm) of the 164 F_6-derived recombinant inbred lines (RILs) grown at six field environments in 2007 and 2008. The data sets were obtained from the trials (cb) Across six field environments, (a) Corvallis (OR), 2008, (b) Moro (OR), 2008, (c) Pendleton (OR), 2008, (d) Pullman (WA), 2008, (e) Moscow (ID), 2008, and (f) Corvallis (OR), 2007. Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



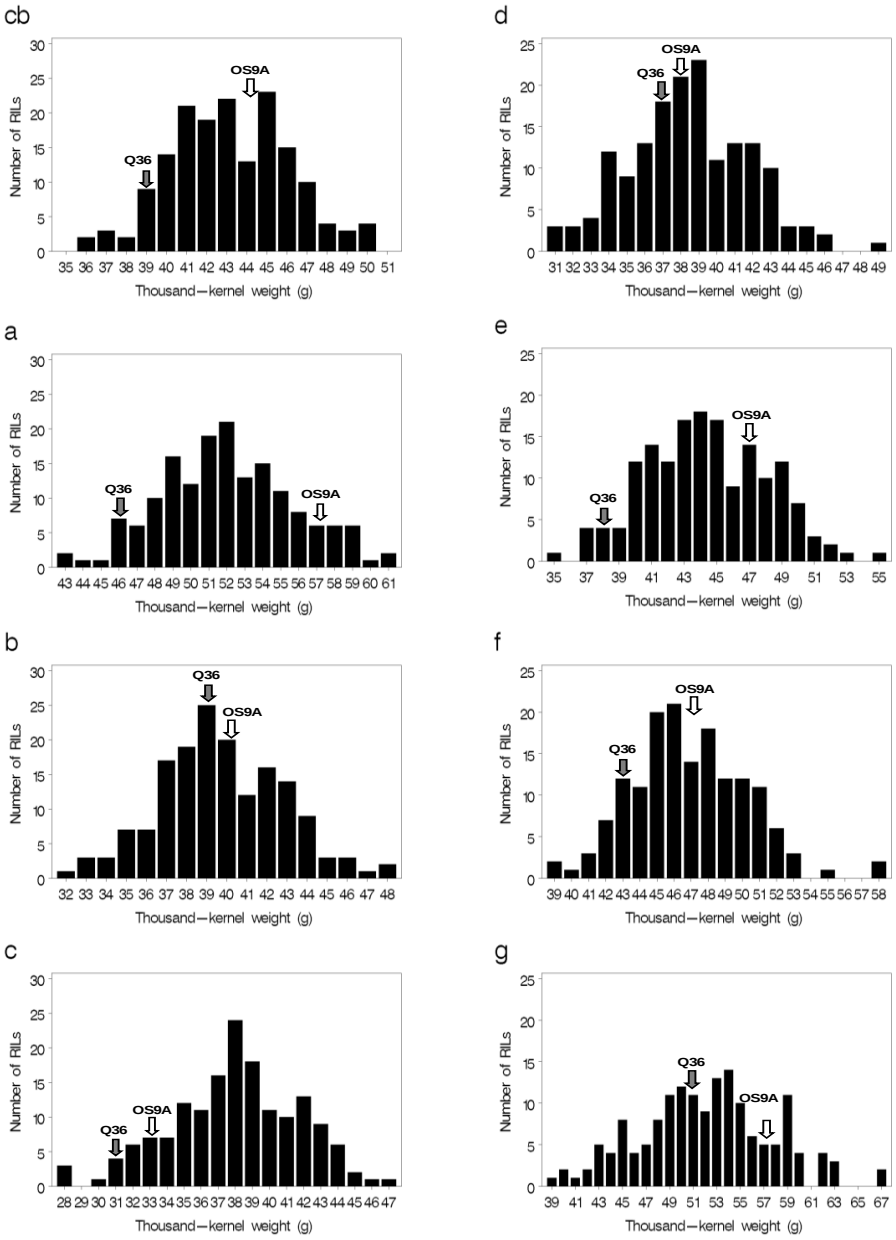
Supplemental Figure 4.6S Frequency distribution of days to heading (d) of the 164 F_6-derived recombinant inbred lines (RILs) grown at six different environments in 2007 and 2008. The data sets were obtained from the trials (cb) Across six field environments, (a) Corvallis (OR), 2008, (b) Moro (OR), 2008, (c) Pendleton (OR), 2008, (d) Pullman (WA), 2008, (e) Moscow (ID), 2008, and (f) Corvallis (OR), 2007. Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



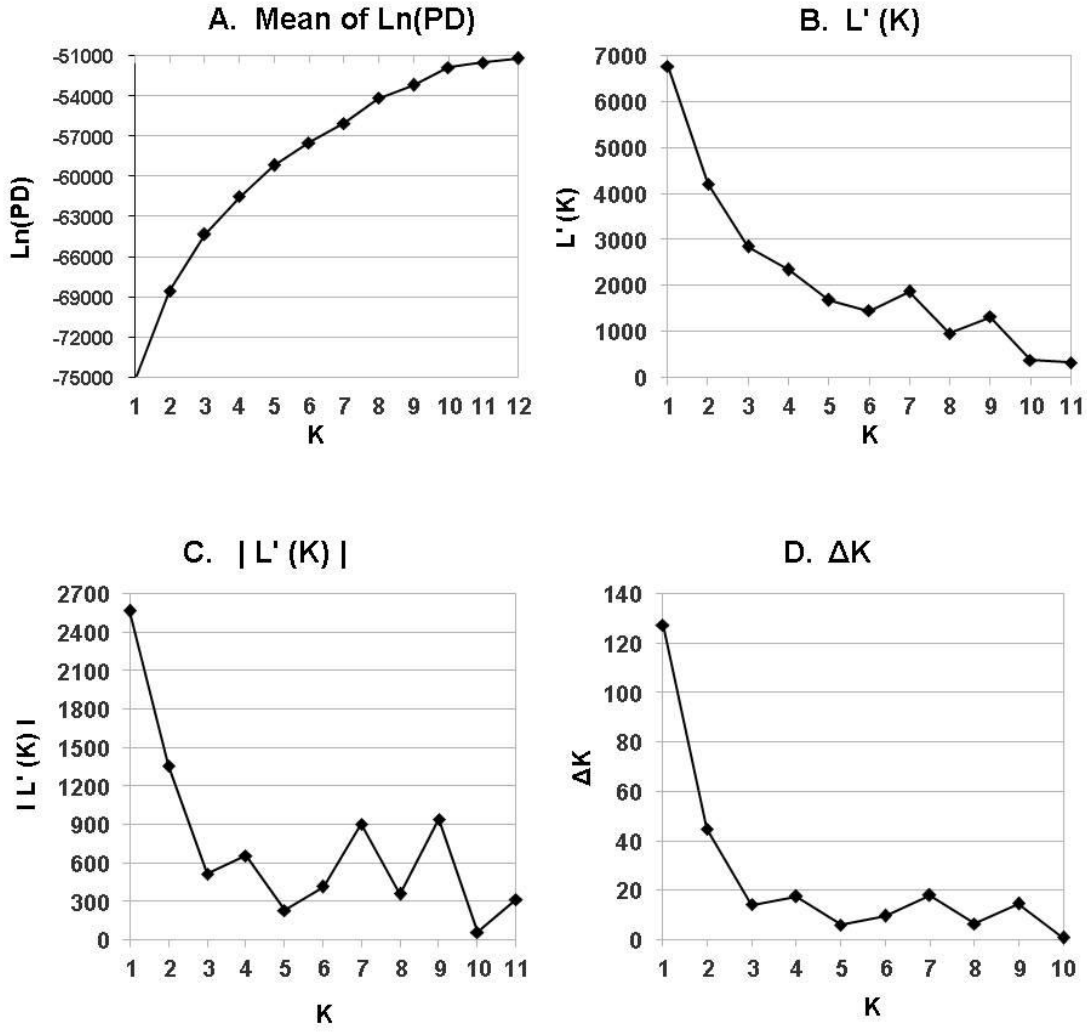
Supplemental Figure 4.7S Frequency distribution of test weight (kg/m³) of the 164 F_6-derived recombinant inbred lines (RILs) grown at six different environments in 2007 and 2008. The data sets were obtained from the trials (cb) Across six field environments, (a) Corvallis (OR), 2008, (b) Moro (OR), 2008, (c) Pendleton (OR), 2008, (d) Pullman (WA), 2008, (e) Moscow (ID), 2008, and (f) Corvallis (OR), 2007. Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



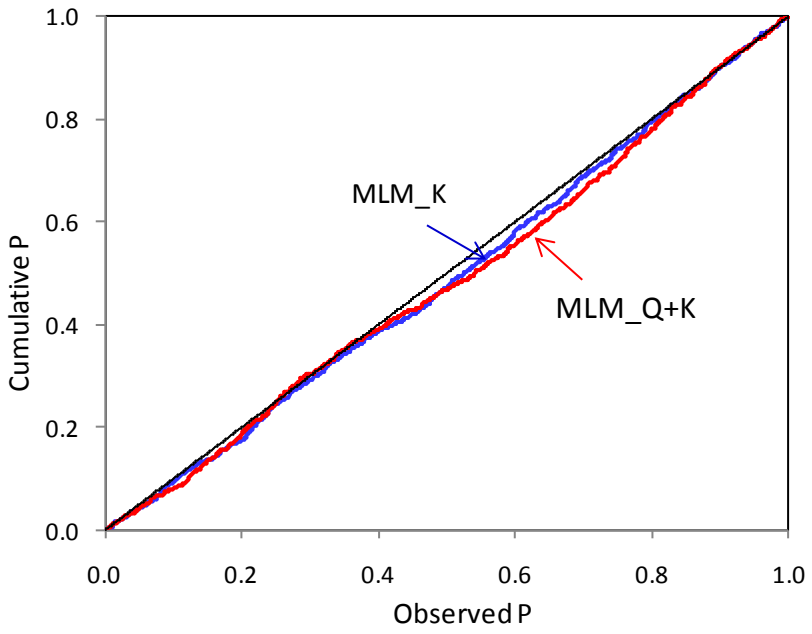
Supplemental Figure 4.8S Frequency distribution of grain protein content (g/kg) of the 164 F_6-derived recombinant inbred lines (RILs) grown at six different environments in 2007 and 2008. The data sets were obtained from the trials (cb) Across six field environments (a) Corvallis (OR), 2008, (b) Moro (OR), 2008, (c) Pendleton (OR), 2008, (d) Pullman (WA), 2008, (e) Moscow (ID), 2008, and (f) Corvallis (OR), 2007. Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



Supplemental Figure 4.9S Frequency distribution of thousand-kernel weight (g) of the 164 F_6-derived recombinant inbred lines (RILs) grown at six different environments in 2007 and 2008. The data sets were obtained from the trials (cb) Across six field environments, (a) Corvallis (OR), 2008, (b) Moro (OR), 2008, (c) Pendleton (OR), 2008, (d) Pullman (WA), 2008, (e) Moscow (ID), 2008, (f) Corvallis (OR), 2007, and (g) Greenhouse (OR), 2007. Mean trait value of the parental lines OS9A and QCB36 on each environment was indicated by white and black arrow, respectively.



Supplemental Figure 5.1S Identification of the appropriate sub-population number (K) with a total of 487 DArT and SSR markers using the ad hoc statistics (Evanno et al. 2005). (A) Ln(PD) again sub-population (K); (B) L'(K) again sub-population (K); (C) |L'(K)| again sub-population (K); and (D) ΔK again sub-population number (K).



Supplemental Figure 5.2S Plot of cumulative probability versus observed probability from association analysis using two different statistical models. MLM_K represents the mixed-effects model with kinship K-matrix as covariate in the association analysis, and MLM_Q+K represents the mixed-effects model with structure Q-matrix (k=3) and K-matrix incorporated.