GENETICS OF DROUGHT TOLERANCE IN HARD RED SPING WHEAT IN THE NORTHERN UNITED STATES OF AMERICA

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Drought affects about 50% of wheat (*Triticum aestivum* L.) globally and is a major threat for sustainable wheat production. This dissertation discusses three studies carried out to dissect drought tolerance in hard red spring wheat (HRSW) in the northern United States of America (USA). The first study used a bi-parental mapping approach with a recombinant inbred line (RIL) population developed from a cross between a drought-tolerant cultivar, 'Reeder' (PI613586), and a drought-susceptible cultivar, 'Albany.' The RILs were evaluated in different locations in North Dakota (ND) over three years. Data were collected on plant height (PH), days to heading (DH), yield (YLD), test weight (TW), and thousand kernel weight (TKW). A high-density genetic map was constructed using Illumina's Infinium 90K single nucleotide polymorphism (SNP) genotypic data. In the second study, the same RIL population was evaluated for PH, DH, YLD, TKW, number of tillers (TIL), number of spikes (SPK), canopy dry weight (CDW), and wilting score (WS) in the greenhouse rather than the field. The third study used association mapping (AM) approach with an association panel comprised of ≥350 genotypes which were evaluated for PH, DH, YLD, TW, and TKW in different locations in ND. The bi-parental mapping study identified a total of 38 QTL each in the field and the greenhouse experiment. Among those, a total of six and eight QTL respectively, were identified under drought conditions. A total of five and eight QTL respectively were identified for both control and drought conditions. Besides this, the bi-parental mapping study identified six QTL for Drought susceptibility index (DSI) in the greenhouse experiment. In contrast, AM study identified a total of 69 QTL where 16 QTL were identified under drought conditions and 50 QTL were identified under both drought and control conditions. Further, 12 genomic regions associated with drought tolerance were repeated across two and sometimes all three studies. Ten

novel QTL on chromosomes 2D, 3D, 4A, 4D, 5B, 7A, and 7B were identified. The QTL identified exclusively under drought conditions, under both drought and normal conditions and for DSI could be helpful for developing drought-tolerant wheat cultivars through marker-assisted selection (MAS).

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DEDICATION

This dissertation is dedicated to my father, Md. Afaz Uddin Shah, and my mother, Mrs. Hasina Begum.

PREFACE

This dissertation has five chapters. Chapter1 provides the general introduction to and objectives of the study. Chapter 2 contains the literature review. Chapters 3, 4, and 5 present three articles written for submission to the appropriate journals. Each article has its own abstract, introduction, material methods, results, discussions, conclusions, and references. A general abstract at the start of the dissertation and a general conclusions section and appendices at the end are also included.

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LIST OF ABBREVIATIONS

AM……....	Association mapping
ANOVA……	Analysis of variance
CDW……..	Canopy dry weight
CI…….....	Confidence interval
CIM……...	Composite interval mapping
cM…….....	Centi-Morgan
CV…….....	Coefficient of variation
DH…….....	Days to heading
DSI…….....	Drought susceptibility index
GS…….....	Genome studio
GWAS……...	Genome-wide association study
HRSW……...	Hard red spring wheat
K…….....	Kinship
LOD……...	Log of odds
LSD……...	Least significant difference
MLM…….	Mixed linear model
MTA……..	Marker-trait association
ND…….....	North Dakota
NDAWN……	North Dakota Agricultural Weather Network
NDSU……	North Dakota State University
PC……	Principal component
PH…….....	Plant height

PV…….....	Phenotypic variation
QTL……...	Quantitative trait loci
RCBD……	Randomized complete block design
RIL…….....	Recombinant inbred line
SAS……...	Statistical analysis system
SNP……...	Single nucleotide polymorphism
SPK……...	Number of spikes
SSD……...	Single seed descent
SSR……...	Simple sequence repeat
TIL……...	Number of tillers
TKW……..	Thousand kernel weight
TW…….....	Test weight
WS…….....	Wilting score
YLD……...	Yield

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CHAPTER 1. INTRODUCTION

1.1. General Introduction

Wheat (*Triticum aestivum* L.) has an evolutionary history spanning 50-70 million years (Inda et al., 2008), which after a series of hybridizations, natural selections, artificial selections, and mutations, became the present bread wheat (*T. aestivum*, 2n = 6x = 42, genome AABBDD) (Huang et al., 2002; Dvorak and Akhunov, 2005). Currently, bread wheat can be categorized into five different types. They are: hard red winter wheat (HRWW), hard red spring wheat (HRSW), soft red winter wheat (SRWW), soft white wheat, and durum wheat (Vocke and Ali, 2013). Among these types, HRSW, which represents 25% of total wheat production in the USA (Vocke and Ali, 2013), is especially important for the state of North Dakota (ND). This state accounts for about half of the total HRSW production in the USA. HRSW has great importance to the growers of ND as the wheat is exported to more than 70 countries and used for making some of the world's finest baked goods due to its higher protein content and superior overall quality (ND Wheat Commission, 2016).

HRSW experiences drought frequently in ND, especially in the semi-arid western part of the state, causing enormous economic loss (Climate change and the economy, 2008). Even in the larger global context, drought is a very critical issue, affecting about 50% of the world HRSW production area regularly (Pfeiffer et al., 2005)**.** However, the development of HRSW cultivars with drought tolerance can save this cash crop and even make its cultivation more profitable. Both drought and drought tolerance should be addressed to go to the direction of developing HRSW cultivars with drought tolerance.

Drought refers to the reduction of accessible water in the soil and atmospheric conditions that cause plants to lose water by transpiration or evaporation. In contrast, drought tolerance

means the ability of the plant to grow, reproduce satisfactorily, and give a harvestable yield under short-term or prolonged water-deficient conditions (Turner, 1979). Developing wheat cultivars with improved drought tolerance will be a sustainable and economically-viable approach to resolving drought problems. Understanding the genetics of drought tolerance in wheat is a prerequisite to achieving this goal.

Drought tolerance in crop plants is associated with a wide range of morpho-physiological traits, with the genetics of drought tolerance having a quantitative inheritance (Blum, 1988). Each trait associated with drought tolerance is controlled by many genes or gene complexes; a number of these traits are identified as heritable. These traits have additive variance and show continuous variation, indicating a good scope to improve drought tolerance (Tuberosa and Salvi, 2006). Genomic locations controlling drought tolerance can be identified through the analysis of quantitative trait loci (QTL).

1.2. Objectives

1.2.1. General objective

The general objective of this study is to understand the genetics of drought tolerance in HRSW in the northern USA.

1.2.2. Specific objectives

The specific objectives of this study are to:

- Identify QTL for drought tolerance in HRSW in the northern USA through bi-parental mapping using field experiments.
- Identify QTL for drought tolerance in HRSW of in the northern USA through bi-parental mapping using greenhouse experiments.

- Identify QTL for drought tolerance in HRSW in the northern USA through association mapping.

1.3. References

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Boca Raton.

Climate change and the economy. 2008. Natl. Conf. State Legis. Available at http://www.ncsl.org/print/environ/ClimateChangeND.pdf (verified 15 December 2016).

Dvorak, J., and E. Akhunov. 2005. Tempos of gene locus deletions and duplications and their relationship to recombination rate during diploid and polyploid evolution in the Aegilops-Triticum alliance. Genetics 17: 323–332.

Huang, S., A. Sirikhachornkit, X. Su, J. Faris, B. Gill, R. Haselkorn, and P. Gornicki. 2002. Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the Triticum/Aegilops complex and the evolutionary history of polypoid wheat. Proc. Natl. Acad. Sci. USA. 99: 8133–8138.

Inda, L.A., J.G. Segarra-Moragues, J. Müller, P.M. Peterson, and P. Catalán. 2008. Dated historical biogeography of the temperate Loliinae (Poaceae, Pooideae) grasses in the northern and southern hemispheres. Mol. Phylogenet. Evol. 46(3): 932–957.

North Dakota Wheat Commission. 2016. Build bigger better Mark. Available at http://www.ndwheat.com/buyers/default.asp?ID=294 (verified 15 December 2016).

Pfeiffer, W.H., R.M. Trethowan, M.V. Ginkel, M.I. Ortiz, and S. Rajaram. 2005. Breeding for abiotic stress tolerance in wheat. pp. 401–489. *In* Harris, M.A. and P.J.C. (eds.), Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches. The Haworth Press, NY, USA.

Tuberosa, R., and S. Salvi. 2006. Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci. 11(8): 405–412.

Turner, N.C. 1979. Drought resistance and adaptation to water deficits in crop plants. pp. 343–372. *In* H. Mussell, C.R.S. (eds.), Stress physiology in crop plants. John Wiley & Sons, NY, USA.

Vocke, G., and M. Ali. 2013. U.S. Wheat production practices, costs, and yields: Variations across regions. Economic information bulletin (116). Economic Research Service, United States Department of Agriculture.

CHAPTER 2. LITERATURE REVIEW

2.1. Bread Wheat

2.1.1. Evolution of wheat

The evolution of the family Poaceae (grasses) occurred around 50 to 70 million years ago (Kellogg, 2001; Huang et al., 2002). The sub-family Pooideae, which includes wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa*), diverged from Poaceae around 20 million years ago (Inda et al., 2008). Around 300,000 to 500,000 years before present (BP), wild emmer wheat (*T. dicoccoides*, 2n = 4x =28, genome AABB) was produced after a hybridization event between wild diploid wheat (*T. urartu*, 2n = 2x = 14, genome AA) and goat grass (*Aegilops speltoides*, 2n = 2x = 14, genome BB) (Huang et al., 2002; Dvorak and Akhunov, 2005). Hunters started to cultivate wild emmer wheat about 10,000 BP. They engaged in plant selection subconsciously until a more developed cultivated emmer wheat emerged (*T. dicoccum*, 2n = 4x =28, genome AABB). This cultivated emmer wheat hybridized spontaneously with another goat grass (*Ae. tauschii* (2n = 2x = 14, genome DD) around 9,000 BP, producing an early spelt wheat (*T. spelta*, 2n = 6x = 42, genome AABBDD). The ears of both emmer and early spelt wheat were changed to easily-threshed types by a natural mutation about 8,500 bp; these types then evolved into free-threshing type (*T. durum*, 2n = 4x =28, genome AABB) and bread wheat (*T. aestivum*, 2n = 6x = 42, genome AABBDD) (Fig. 2.1).

2.1.2. The wheat classes of the USA

Wheat can be categorized into five major classes (Vocke and Ali, 2013), which are described in the following five subsections:

2.1.2.1. Hard red winter wheat (HRWW)

This type of wheat comprises about 40% of total USA production and is mainly grown in the Great Plains (from Texas in the south through Montana in the north). Hard red winter wheat is high in protein, which makes it suitable for bread flour.

2.1.2.2. Hard red spring wheat (HRSW)

This type of wheat comprises about 25% of the total USA production and is mainly grown in the Northern Plains (ND, Montana, Minnesota, and South Dakota). Hard red spring wheat has high protein levels and is primarily used for making specialty bread and pasta and for blending with lower-protein HRWW.

2.1.2.3. Soft red winter wheat (SRWW)

This type of wheat accounts for 15 to 20% of the total USA production and is grown primarily in states along the Mississippi River and in the East. Soft red winter wheat flour is used for making cakes, cookies, crackers, and other wheat products made from low-protein flour.

2.1.2.4. Soft wheat

This type of wheat, which accounts for 10 to 15% of the total USA production, is grown in Washington, Oregon, Idaho, Michigan, and New York. Flour from this wheat is used for making cakes, cookies, crackers, and other wheat products made from low-protein flour.

2.1.2.5. Durum wheat

This type of wheat, accounting for 3 to 5% of the total USA production, is grown primarily in ND and Montana and is used to make pasta.

2.1.3. The wheat genome

The genome size of hexaploid wheat (*T. aestivum* L., 2n=42, AABBDD) is approximately 1.7 x 10^{10} bp, which is about a hundred times bigger than that of the Arabidopsis genome, forty times than that of rice, and about six times than that of maize (Bennett and Smith., 1976; Amuruganathan and Earle, 1991). The wheat genome is big because of polyploidy and extensive duplication. To illustrate the latter wheat has an over 80% DNA sequence repetition (Smith and Flavell, 1974). The average wheat chromosome measures around 810 MB, which is about 25 times bigger than the average rice chromosome. Studies indicate that wheat has approximately 30,000 genes, only a few hundred of which have been identified, mapped, and their primary and pleiotropic effects described (Farag, 2004).

2.2. Drought

2.2.1. Importance of drought tolerance

Drought, which often corresponds with high temperatures, is the main natural hazard threatening wheat production in many parts of the world (Araus et al., 2008). It regularly affects about 50% of the wheat production area worldwide (Pfeiffer et al., 2005) and represents a potential threat to plant growth and development by stressing plants. Drought stress affects species differently, and even affects members within species differently. Long-term data show that drought is becoming more common in some areas. For example, droughts in Morocco used to occur once every decade at the start of the 20th century, but now occur every other year (Forester et al., 2004). It is predictable that droughts may get even worse in many areas due to

diminishing water availability and global climatic changes. Irrigation has thus become a widely-used method to reduce the impact of drought. The Food and Agriculture Organization of the United Nations (FAO) has predicted a 14% increase in water use for irrigation between 2002 and 2030 in developing countries. This increase will contribute to water shortages in one out of five developing countries during that time frame (FAO, 2002). In addition to its impact on water supplies, irrigation has many other associated costs, including fuel costs. Further, irrigation might add salts to the soil, making it saline and hence less suitable for crop production (Rhoades and Loveday, 1990). Treatment of that soil would further increase the cost of production. To conclude, changes in weather patterns will ultimately lead to less available water for irrigated wheat production. Given these expected conditions, a better understanding of drought tolerance could be helpful to select or develop adapted varieties of wheat with better productivity under water-stress conditions. However, breeding for extreme drought is difficult and not practical.

2.2.2. Plant strategies for drought tolerance

2.2.2.1. Morphological mechanisms

Different types of morphological responses that plants can exhibit under drought conditions are described below:

Drought escape: Plants shorten their life cycle to reproduce before acute dryness occurs. Flowering time is very important for plant adaptation to drought as a plant with a short life cycle that flowers early can escape terminal drought (Araus et al., 2002). Therefore, the development of short-duration varieties has been very useful for minimizing yield loss from terminal drought (Kumar and Abbo, 2001). However, the shorter life cycle may reduce yield due to a corresponding reduction in photosynthates.

Drought avoidance: Some plants can control transpiration loss through stomatal tissue and maintain a high tissue-water potential to resist drought. Also, such plants have extensive and prolific root systems to uptake more water (Turner et al., 2001). Glaucousness and waxy bloom on leaves also help plants to maintain a high tissue-water potential (Richards, 1986).

Phenotypic flexibility: Plants shorten the number of leaves and leaf surface area to cope with drought, saving on water loss and, ultimately, yield loss (Schuppler et al., 1998). Hairy leaves increase the degree of light reflection and reduce leaf temperatures and transpiration (Sandquist and Ehleringer, 2003), and thick and proliferated root systems could allow plants to capture more water and hence could be a key factor in drought resistance (Kavar et al., 2007).

2.2.2.2. Physiological mechanisms

Osmotic adjustment: The overproduction of low-molecular-weight, highly soluble, nontoxic compounds even at high cytosolic concentrations (e.g., soluble sugars, sugar alcohols, glycine betaine, organic acids, calcium, potassium, and chloride ions) lower the cell's osmotic potential and attract water into the cell. Osmoregulators can maintain the turgor pressure of plant cells and minimize the harmful effects of drought (Morgan, 1990; Serraj and Sinclair, 2002).

Plant growth regulators: Plant growth regulators, or phytohormones, play a major role in drought tolerance. Drought decreases the endogenous content of auxins, gibberellins, and cytokinins and increases abscisic acid and ethylene (Nilsen and Orcutte, 1996). This change favors stomatal closure and minimizes transpiration during drought (Morgan, 1990). Also, increased abscisic acid changes the relative growth rates of various plant parts, such as increasing the root-to-shoot dry weight ratio and inhibiting leaf area development and the production of prolific and deeper roots (Sharp et al., 1994).

2.3. Studies on Drought Tolerance

2.3.1. Historical background

Breeders have been trying to improve drought tolerance in wheat for decades, starting in Australia in the late 1800s. At that time, cultivars commonly used in England were not performing well in the more arid regions of Australia. Breeders started visually selecting for traits such as earliness to avoid stress: this ultimately improved yield under drought conditions. They also selected for some morphological traits, such as short straw, smaller leaf area, and fewer tillers (Marshall, 1987). Decreasing whole plant surface minimizes transpiration, and it also minimizes the amount of vegetative biomass, which is correlated with yield (Babu et al., 2003).

2.3.2. Complexity of the study

Breeders have debated whether to select for yield potential to increase yield under drought tolerance (Alexander et al., 2012). They have also debated whether the selection is more effective in stressed or unstressed conditions (Alexander et al., 2012). Much research has been done to try to answer these questions, but breeders have not come to a consensus (Srivastava, 1987). The quantitative nature of drought-stress tolerance with its low heritability and high genotype × environment (G × E) interaction has contributed to the lack of consensus.

Breeding for drought tolerance is complicated further by the fact that several types of abiotic stresses can challenge crop plants at the same time. The remedy for one stress may have the opposite effect under a different abiotic stresses. For example, some plants avoid heat stress by increasing transpiration, and in effect, creating their own evaporative cooling systems. This mechanism increases water loss, and makes a plant drought-susceptible. An opposite effect is when the stomata closes to help to decrease water loss and maintain turgor under less-available

soil moisture (Fleury et al., 2010). The problem arises when high temperatures and drought occur simultaneously and the two mechanisms oppose each other. Osmo-protectant amino acid proline is another example of when a similar conflict can occur. This compound is good for drought tolerance, but has a toxic effect under heat stress. Therefore, it may not offer an appropriate tolerance mechanism in field conditions when heat and drought stresses occur at the same time (Rizhsky et al., 2004).

Further, plant morphology sometimes makes the selection confusing, as with the traits plant height (PH) and tillering. Small plants with fewer tillers can show higher water-use efficiency (WUE) than tall multi-tillered plants (Fleury et al., 2010). Therefore, the selection for high WUE may lead to smaller plants with a lower yield under drought conditions (Blum, 2005).

2.3.2. Traits which can be considered for drought tolerance study

Many morphological characteristics, such as root length, tillering, spike number per m^2, grain number per spike, the number of fertile tillers per plant, one thousand grain weight, peduncle length, spike weight, stem weight, awn length, and grain weight per spike can affect wheat tolerance to drought (Blum, 2005). However, yield stability and relative yield performance under both drought-stressed and favorable environments have been proposed for effective selection of drought-tolerant genotypes (Pinter et al., 1990). Stability of grain yield for each genotype can be estimated by the drought-susceptibility index (DSI), which measures the yield difference between stressed and non-stressed environments (Blum et al., 1989). Passioura (2007) indicated that floral sterility caused by water stress could be a promising target trait for improvement. However, no QTL studies for this trait have been published so far for wheat.

Along with yield components, root traits should not be ignored as they have a crucial role in harvesting water from the soil (Tuberosa and Salvi, 2006). A simulation analysis of root

system modification indicated that an extra one mm of water extracted during grain filling of wheat would increase yield by 55 kg per ha in Australia (Manschadi et al., 2006). Yet, the root has not attracted much attention in genetic studies (Fleury et al., 2010). No QTL/genes controlling root architecture under drought conditions have been discovered in wheat to date.

Leaf wilting could be considered a fundamental indicator of drought response, which would also make the drought-tolerance evaluation of crops easier. This is because the International Board on Plant Genetic Resources (IBPGR) developed a straightforward 1-9 scale for scoring leaf wilting. According to the scale, 1 indicates normal and 9 indicates dead and dry plants under moisture stress (IBPGR, 1983). This scale has been used to screen drought-tolerant germplasms (Nkouannessi, 2005).

2.4. Modern Approaches

2.4.1. Recent roles of molecular markers

Marker-assisted selection (MAS) provides a very useful tool to improve the efficiency of transferring traits, especially quantitative traits, to desired genotypes. Marker assisted selection uses identified QTL and allows breeders to select desirable germplasms without field testing. Quantitative trait loci have been studied for drought-tolerant traits in many species, including rice (*Oryza sativa*), barley, maize (*Zea mays),* and wheat (Alexander et al., 2012). Recently, some studies focused on identifying QTL for yield and its components under drought conditions. Among these, Kirigwi et al. (2007) identified one QTL on chromosome 4 AL for yield and yield components under drought stress in spring wheat. Alexander et al. (2012) identified a new QTL for drought tolerance on chromosome 7B. But, any major QTL for drought tolerance in spring wheat in the northern USA have yet to be discovered. As different regions have different types of drought, most likely different types of drought-tolerance mechanisms have to be discovered.

Therefore, drought-tolerance QTL could be different for different regions. To discover the target QTL for drought tolerance in a given region, the adapted drought-tolerant wheat germplasm of that region should be used as the plant materials for the study.

2.4.2. QTL mapping

Quantitative trait loci can be identified using bi-parental QTL mapping or association mapping (AM). The principle of bi-parental QTL mapping is to associate the phenotypic traits with molecular markers using statistical tools. The detection and location of the loci underlying quantitative trait variation include three basic steps. The first step is the creation of a bi-parental population and its characterization with molecular markers. This will lead to the construction of a genome-wide genetic map of the population. The second step is a phenotypic evaluation of the same population for the traits of interest. The final step is analyzing the association of the molecular markers with the phenotypic trait data using appropriate statistical methods. Whereas, AM is an alternative approach to QTL mapping for identifying an association between genotype and phenotype (Yu and Buckler, 2006). Association mapping is based on linkage disequilibrium (LD). Association mapping detects correlations between genotypes and phenotypes in a sample of unrelated individuals. In contrast to bi-parental mapping, AM exploits a broader population and consequently samples multiple alleles and maps at a higher resolution (Yu and Buckler, 2006).

2.4.3. SNP markers

Among the different kinds of molecular markers available, single nucleotide polymorphisms (SNP) can be used for QTL mapping as SNPs are highly abundant and distributed throughout the genome in various species, including plants (Garg et al., 1999; Drenkard et al., 2000; Nasu et al., 2002). The abundance of these polymorphisms in plant

genomes makes the SNP marker system an attractive tool for mapping, marker-assisted breeding, and map-based cloning (Batley et al., 2003). Abundantly available genome sequence information of wheat has led to the discovery of thousands of SNPs, like in many other species, including *Arabidopsis thaliana* (Schmid et al., 2003), soybean (Hyten et al., 2008), and maize (McMullen et al., 2009).

2.5. References

Alexander, L.M., F.M. Kirigwi, A.K. Fritz, and J.P. Fellers. 2012. Mapping and quantitative trait loci analysis of drought tolerance in a Spring wheat population using amplified fragment length polymorphism and diversity array technology markers. Crop Sci. 52(1): 253–261.

Amuruganathan, E., and E.D. Earle. 1991. Nuclear DNA content of some important plants species. Plant Mol. Biol. Rep. 9: 208–218.

Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C3 cereals: What should we breed for? Ann. Bot. 89(SPEC. ISS.): 925–940.

Araus, J.L., G. a. Slafer, C. Royo, and M.D. Serret. 2008. Breeding for yield potential and stress adaptation in cereals. CRC. Crit. Rev. Plant Sci. 27(6): 377–412.

Babu, R.C., B.D. Nguyen, V. Chamarerk, P. Shanmugasundaram, P. Chezhinan, P. Jeyaprakash, and E. Al. 2003. Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance. Crop Sci. 43: 1457–1469.

Batley, J., R. Mogg, D. Edwards, H. O'Sullivan, K.J. Edwards, and M. Zoric. 2003. A high-throughput SNuPE assay for genotyping SNPs in the flanking regions of Zea mays sequence tagged simple sequence repeats. Mol. Breed. 11: 111–120.

Bennett, M.D., and J.B. Smith. 1976. Nuclear DNA amounts in angiosperms. Phil. Trans. Roy. Soc. Lond. B. 274: 227–274.

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Boca Raton.

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 56: 1159–1168. Available at www.publish.csiro.au/journals/ajar.

Drenkard, E., B.G. Richter, S. Rozen, L.M. Stutius, N.A. Angell, M. Mindrinos, R.J. Cho, P.J. Oefner, R.W. Davis, and F.M. Ausubel. 2000. A simple procedure for the analysis of single nucleotide polymorphisms facilitates map-based cloning in Arabidopsis. Plant Physiol. 124: 1483–1492.

Dvorak, J., and E. Akhunov. 2005. Tempos of gene locus deletions and duplications and their relationship to recombination rate during diploid and polyploid evolution in the Aegilops-Triticum alliance. Genetics 17: 323–332.

FAO. 2002. World agriculture 2030: Main findings. Available at http://wwwfao.org/english/newsroom/news/2002/7833-en. html (verified 22Sept 2011). Rome, Italy.

Fleury, D., S. Jefferies, H. Kuchel, and P. Langridge. 2010. Genetic and genomic tools to improve drought tolerance in wheat. J. Exp. Bot. 61(12): 3211–3222.

Forester, B.P., R.P. Dllis, J. Moir, V. Talame, M.C. Sanguineti, R. Tuberosa, D. This, and et al. 2004. Genotype and phenotype associations with drought tolerance in barley tested in North Africa. Ann. Appl. Biol. 144: 157–168.

Garg, K., P. Green, and D.A. Nickerson. 1999. Identification of candidate coding region single nucleotide polymorphisms in 165 human genes using assembled expressed sequence tags. Genome Res. 9(206): 1087–1092.

Huang, S., A. Sirikhachornkit, X. Su, J. Faris, B. Gill, R. Haselkorn, and P. Gornicki. 2002. Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the Triticum/Aegilops complex and the evolutionary history of polypoid wheat. Proc. Natl. Acad. Sci. USA. 99: 8133–8138.

Hyten, D., Q. Song, and I. Choi. 2008. High-throughput genotyping with the GoldenGate assay in the complex genome of soybean. Theor. Appl. Genet. 116: 945–952.

IBPGR. 1983. Descriptors for cowpea. Rome, Italy: International Board for Plant Genetic Resources.

Inda, L.A., J.G. Segarra-Moragues, J. Müller, P.M. Peterson, and P. Catalán. 2008. Dated historical biogeography of the temperate Loliinae (Poaceae, Pooideae) grasses in the northern and southern hemispheres. Mol. Phylogenet. Evol. 46(3): 932–957.

Kavar, T., M. Maras, M. Kidric, J. Sustar-Vozlic, and V. Meglic. 2007. Identification of genes involved in the response of leaves of Phaseolus vulgaris to drought stress. Mol Breed. 2: 159–172.

Kellogg, E.A. 2001. Evolutionary history of the grasses. Plant Physiol. 125: 1198–1205.

Kirigwi, F.M., M. Van Ginkel, G. Brown-Guedira, B.S. Gill, G.M. Paulsen, and A.K. Fritz. 2007. Markers associated with a QTL for grain yield in wheat under drought. Mol. Breed. 20(4): 401–413.

Kumar, J., and S. Abbo. 2001. Genetics of flowering time in chickpea and its bearing on productivity in the semi-arid environments. Adv. Agron. 72: 107–138.

Manschadi, A.M., J. Christopher, P. Devoil, and G.L. Hammer. 2006. The role of root architectural traits in adaptation of wheat to water-limited environments. Funct. Plant Biol. 33(9): 823–837.

Marshall, D.R. 1987. Australian plant breeding strategies for rainfed areas. pp. 89–99. *In* Srivastava, J.P., Al., E. (eds.), Drought tolerance in winter cereals: Proc. Int. Workshop, Capri, Italy. 27-31 October 1985. John Wiley & Sons, Chishester, West Sussex, UK.

McMullen, M., S. Kresovich, and H. Villeda. 2009. Genetic properties of the maize nested association mapping population. Science 325: 737–740.

Morgan, P.W. 1990. Effects of abiotic stresses on plant hormone systems. pp. 113–146. *In* Stress Responses in plants: Adaptation and acclimation mechanisms. Wiley-Liss, Inc.

Nasu, S., J. Suzuki, R. Ohta, K. Hasegawa, R. Yui, N. Kitazawa, L. Monna, and Y. Minobe. 2002. Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (Oryza satva, Oryza rufipogon) and establishment of SNP markers. DNA Res. 9: 163–171.

Nilsen, E.T., and D.M. Orcutte. 1996. Phytohormones and plant responses to stress. pp. 183–198. *In* Nilsen, E., Orcutte, D. (eds.), Physiology of plant under stress: Abiotic factors. John Wiley and Sons, New York, NY.

Nkouannessi, M. 2005. The genetic, morphological and physiological evaluation of African cowpea genotypes. Dissertation.

Passioura, J. 2007. The drought environment: Physical, biological and agricultural perspectives. J. Exp. Bot. 58: 113–117.

Pfeiffer, W.H., R.M. Trethowan, M.V. Ginkel, M.I. Ortiz, and S. Rajaram. 2005. Breeding for abiotic stress tolerance in wheat. pp. 401–489. *In* Harris, M.A. and P.J.C. (eds.), Abiotic stresses: Plant resistance through breeding and molecular approaches. The Haworth Press, New york, NY.

Pinter, J.P.J., G. Zipoli, R.J. Reginato, R.D. Jackson, and S.B. Idso. 1990. Canopy temperature as an indicator of differential water use and yield performance among wheat cultivars. Agric. Water Manag.: 18:35–48.

Rhoades, J.D., and J. Loveday. 1990. Salinity in irrigated agriculture. pp. 1089–1142. *In* Stewart, B.A., Nielsen, D.R. (eds.), Irrigation of agricultural crops. Agron. Monogr. 30. ASA, CSSA, SSSA, Madison, WI.

Richards, R.A. 1986. Defining selection criteria to improve yield under drought. Plant Growth Regul. 20: 157–166.

Rizhsky, L., H.J. Liang, J. Shuman, V. Shulaev, S. Davletova, and R. Mittler. 2004. When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiol. 134: 1683–1696.

Sandquist, D.R., and J.R. Ehleringer. 2003. Population- and family-level variation of brittlebush (Encelia farinosa, Asteraceae) pubescence: Iits relation to drought and implications for selection in variable environments. Am. J. Bot. 90: 1481–1486.

Schmid, K., T.R. Sorensen, O. Stracke, T. Torjek, T. Altmann, M.l Olds, and B. Weisshaar. 2003. Large scale identification and analysis of genome wide single nucleotide polymorphisms for mapping in Arabidopsis thaliana. Genome Res. 13: 1250–1257.

Schuppler, U., P.H. He, P.C.L. John, and R. Munns. 1998. Effects of water stress on cell division and cell-division-cycle-2-like cell-cycle kinase activity in wheat leaves. Plant Physiol. 117: 667–678.

Serraj, R., and T.R. Sinclair. 2002. Osmolyte accumulation: Can it really help increase crop yield under drought conditions? Plant Cell Environ. 25: 33–341.

Sharp, R.E., Y. Wu, G.S. Voetberg, I.N. Soab, and M.E. LeNoble. 1994. Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. J. Exp. Bot. 45: 1743–1751.

Srivastava, J.P. 1987. Barley and wheat improvement for moisture limiting areas in West Asia and North Africa. pp. 65–78. *In* Srivastava, J.P., Al., E. (eds.), Drought tolerance in winter cereals: Proc. Int. Workshop, Capri, Italy. 27-31 Oct. 1985. John Wiley & Sons, .

Tuberosa, R., and S. Salvi. 2006. Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci. 11(8): 405–412.

Turner, N.C. 1979. Drought resistance and adaptation to water deficits in crop plants. pp. 343–372. *In* H. Mussell, C.R.S. (eds.), Stress physiology in crop plants. John Wiley & Sons, New York, NY.

Turner, N.C., G.C. Wright, and K.H.M. Siddique. 2001. Adaptation of grain legumes (pulses) to water-limited environments. Adv. Agron. 71(September 2016): 193–231.

Vocke, G., and M. Ali. 2013. U.S. wheat production practices, costs, and yields: Variations across regions. Economic Information Bulletin(116). Economic Research Service, United States Department of Agriculture.

Yu, J., and E.S. Buckler. 2006. Genetic association mapping and genome organization of maize. Curr. Opin. Biotechnol. 17: 155–160

CHAPTER 3. IDENTIFYING QTL FOR DROUGHT TOLERANCE IN SPRING WHEAT IN THE NORTHERN USA USING FIELD EXPERIMENTS

3.1. Abstract

Drought can potentially hinder plant growth and development, causing a significant decrease in wheat productivity and quality. Understanding the genetics of drought tolerance in wheat is a prerequisite for developing drought-tolerant cultivars. Here, the genetics of drought tolerance in spring wheat in the USA was analyzed using a population of 149 RILs (Recombinant inbred lines) developed from a cross between a drought-tolerant cultivar, 'Reeder' (PI613586), and a drought-susceptible cultivar, 'Albany.' The RIL population was evaluated at three locations for three years, and phenotypic data was collected for plant height (PH), days to heading (DH), yield (YLD), test weight (TW), and thousand kernel weight (TKW). The RIL population was genotyped using Illumina's Infinium 90K SNP (single nucleotide polymorphism) assay. A whole genome high-density genetic map was produced using 10,657 polymorphic SNP markers, with an average density of 1 marker per 0.36 cM. The markers were mapped onto 28 linkage groups representing 21 wheat chromosomes. These markers represented 2,057 unique loci. A total of 11 consistent important QTL for drought tolerance were identified. Among these QTL, six QTL were exclusively identified in drought-prone environments, and five QTL were constitutive QTL (QTL present both in drought and non-drought conditions). One major QTL located on chromosome 7B was identified exclusively in drought environments and explained 13.6% of YLD. Two major QTL located on chromosomes 7B and 2B were identified in drought-prone environments. They controlled 14.86% and 13.94% of phenotypic variation (PV) for TW and YLD, respectively. By comparison, the constitutive QTL contributed 13.44 to 38.36% of PV for associated traits, and their Log of odds (LOD) scores ranged from 5.43 to 20.17. One novel

QTL with drought tolerance was identified on chromosome 2D. All these QTL, with significant drought tolerance could assist in marker-assisted selection (MAS) for drought tolerance in spring wheat in the Northern Plains. Also, they can be a starting point for map-based cloning of the QTL/genes for drought tolerance.

3.2. Introduction

Hard red spring wheat (HRSW), comprising about 25% of the total USA wheat production, is unique for its high protein content (Vocke and Ali, 2013). But this important crop often experiences drought, which is one of the main natural hazards harming wheat production worldwide (Araus et al., 2008). It regularly affects about 50% of wheat producing areas (Pfeiffer et al., 2005). Drought refers to reduced accessible water in the soil and atmospheric **c**onditions that cause plants to wilt or even die by losing water through transpiration. However, drought tolerance enables plants to yield satisfactorily under limited or periodic water-deficient conditions (Turner, 1979). Therefore, developing wheat cultivars with improved drought tolerance is the key to resolving drought problems.

Drought tolerance in wheat can be achieved through developing cultivars capable of maintaining high water potential in drought conditions (Turner et al., 2001). Also, plants could escape from late-season drought through the development of early wheat cultivars (Araus et al., 2002). Understanding the genetics of drought tolerance in wheat is a prerequisite to achieving it. Past observations indicate that drought tolerance in crop plants is quantitatively inherited, or controlled by many genes or gene complexes (Blum, 1988), which can in turn be traced through QTL mapping methods.

Breeders have frequent debates over the appropriate phenotypic approaches for QTL analysis (Alexander et al., 2012). Many morphological traits, such as root length, tillering, spike

number per m^2, grain number per spike, number of fertile tillers per plant, one thousand grain weight, peduncle length, spike weight, stem weight, awn length, and grain weight per spike, can be affected by drought (Blum, 2005). However, yield stability under both drought-stressed and favorable environments has been proposed for the effective selection of drought-tolerant genotypes (Pinter, et al., 1990). From a breeder's perspective, yield and yield-related traits comprise the best morphological traits to screen for in drought-tolerant plants.

An efficient tool for genotyping is needed as most of the QTL mapping studies on drought tolerance in wheat have been conducted using low-resolution maps composed of only several hundred molecular markers (Kirigwi et al., 2007; Muchero et al., 2009; Peleg et al., 2009; Sayed, 2011; Alexander et al., 2012; Ibrahim et al., 2012a; Kumar et al., 2012; Malik et al., 2015). Because of the size of the bread wheat genome **(**~17 Gb), greater marker coverage is also needed to generate a dense genetic linkage map, which could help to identify tightly-linked markers associated with traits of interest. This is very important for the successful introgression of target loci in MAS programs. Precise identification of QTL will also facilitate easier positional cloning of those QTL (Kumar et al., 2016). The recently-developed Infinium iSelect 90K assay, with 81,587 transcriptome-based SNPs (Wang et al., 2014), can be an excellent tool for investigating the genetic basis of drought tolerance in wheat. Therefore, in this study, an attempt was made to decipher the genetics of drought tolerance in spring wheat using the Infinium iSelect 90K assay.

3. 3. Materials and Methods

3.3.1. Plant materials

The cultivars 'Reeder' (PI613586) and 'Albany' were used to develop a population of 149 RILs. Reeder is a drought-tolerant HRSW cultivar released by the North Dakota Agricultural

Experiment Station at North Dakota State University (NDSU) in 1999. It is a semi-dwarf cultivar best adapted to western North Dakota (ND), a semi-arid region of the state. Reeder has good milling and baking qualities and also possesses resistance to the Upper Midwest races of stem and leaf rusts. The other parent, Albany, developed by Trigen Seed LLC, is a very high yielding, semi-dwarf HRSW cultivar adapted to intensive-input management and better adapted to the eastern area of the Northern Plains spring wheat region, where drought is not prevalent. A single seed descent (SSD) method was used to advance the RIL populations to the F_8 generation. The study also included the checks, 'Glenn'(Mergoum et al., 2006), 'SY Tyra' (Agripro® wheat variety, USA), 'Faller'(Mergoum et al., 2008), 'Steele-ND'(Mergoum et al., 2005), 'Alsen' (Frohberg, et al., 2006), 'Mott,' 'Elgin,' 'RB07' (Anderson et al., 2009), 'Dapps' (Mergoum et al., 2005), 'Prosper'(Mergoum et al., 2013), 'ND901CLPlus' (Mergoum et al., 2009) (PI655233), 'Velva' (Mergoum et al., 2014), 'SY Soren' (Agripro® wheat variety, USA), 'Duclair' (Lanning et al., 2011), 'ND819' (experimental line), 'Polaris,' 'Saturn,' and 'Granite' (PI619072). The checks ND819, Dapps, and Steele-ND are tolerant to drought stress. The genotypes SY Soren, Glenn, Alsen, ND901CLPlus, Saturn, and Velva show moderate tolerance, whereas Granite, Elgin, RB07, Duclair, Prosper, Mott, Faller, and SY Tyra show susceptibility to drought.

3.3.2. Field experiments

The evaluation of agronomic performances of the RIL, their parents, and 18 checks was carried out under non-irrigated field conditions at different locations in ND. The plant materials were evaluated in: Prosper, Carrington, and Minot in 2012; Prosper, Carrington, and Williston in 2013; and Prosper, Carrington, and Hettinger in 2014. Prosper is located in the eastern region of ND (46.9630° N, 97.0198° W). Carrington is located in the east-central region of ND (47.4497°

N, 99.1262° W). Minot sits between semi-arid grassland in the west and central ND's sub-humid grassland (48.2330° N, 101.2923° W). Williston is located in northwestern ND (48.1470° N, 103.6180° W), and Hettinger in southwestern ND (46.0014° N, 102.6368° W). The total rainfall in Prosper during the 2012, 2013, and 2014 growing periods (seed sowing to ripening) was 120.1 mm, 269.9 mm, and 176.8 mm, respectively (Table 3.1). Carrington had total rainfall of 171.2 mm, 159.8 mm, and 190.5 mm during the 2012, 2013, and 2014 growing periods, respectively. And, during the same growing periods, Minot, Williston, and Hettinger had total rainfall of 162.2 mm, 320.4 mm, and 200.3 mm, respectively (Table 3.1) (NDAWN, 2015). The available soil moisture of the experimental sites based on soil types is presented in Table 3.1 (Frazen, 2003). Each experiment was conducted in a randomized complete block design (RCBD) with two replicates. In 2012 and 2013, each genotype was planted in a 2.44 m × 1.22 m plot containing seven rows with a 15.24 cm gap between rows. The plot size was slightly large in 2014, at 2.44m × 1.42m, with the same number of rows (seven), but a larger gap of 17. 78 cm between them.

Table 3.1. Soil types, plant-available water (water-holding capacity of soil), and total rainfall for nine environments.

Environments	Soil type	Plant-available water (mm water/30.48 cm soil)	Rainfall (mm)
Prosper 12	Fine silty loam	45.72-63.5	120.1
Carrington 12	Coarse loamy	19.09-31.75	171.2
Minot 12	Fine sandy loam	31.75-45.72	162.2
Prosper 13	Fine silty loam	45.72-63.5	269.9
Carrington 13	Coarse loamy	19.05-31.75	159.8
Williston 13	Fine sandy loam	31.75-45.72	320.4
Prosper 14	Fine silty loam	45.72-63.5	176.8
Carrington 14	Coarse loamy	19.05-31.75	190.5
Hettinger 14	Fine sandy loam	31.75-45.72	200.3

3.3.3. Data collection

Each year, the phenotypic data were recorded for DH, PH, YLD, TW, and TKW at each site. The heading data were taken when more than 50% of the plants in the plot were heading. Plant height was measured from base to tip excluding the awn for plants in the middle of the plot. Yield per plot was converted to yield/ha for further analysis. Similarly, Kg/0.5 pint cup was converted to Kg/m^3 as the TW for further analysis. A thousand kernels were counted using a seed counter (Model U, International Marketing and Design Co.) and weighed.

3.3.4. Phenotypic data analysis

The statistical analysis system used for analyzing the phenotypic data was ANOVA Proc MIXED (SAS Institute, 2004). The RILs, their parents, and the checks were considered as fixed effects, whereas environments and blocks were considered as random effects. The mean values were separated using the *F*-protected least significant difference (LSD) value at the $P \leq 0.05$ level of significance. Pearson correlations between traits for each environment were calculated using the SAS's CORR procedure (SAS Institute, 2004). Only the locations whose data exhibited a low

coefficient of variation (CV) value and a significant difference among entries are reported in this study.

3.3.5. Genotyping

Genomic DNA from each genotype was isolated from lyophilized young leaves using the DNeay Plant Mini Kit (Qiagen, Valencia, CA, cat. no. 69106). This DNA was run on 0.8% agarose gel to check its quality. The NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) was used to check DNA concentration. The RIL population, parents, and checks were genotyped using the Illumina 90K iSelect wheat SNP assay in the Small Grains Genotyping Lab, USDA-ARS, Fargo, ND. The genotyping module GenomeStudio V2011.1 (www.illumina.com, verified 18 Dec. 2015) was used to analyze the SNP data.

3.3.6. Map construction

The Illumina iSelect 90K assay (Wang et al., 2014) produced data for 81,587 SNPs. Out of these markers, 12,151 SNP polymorphic markers between parental genotypes were identified. Out of those polymorphic loci, 1,391 markers were discarded because they had 1) an allele frequency of <0.4 for any of the parental genotypes, 2) inconsistent results in five replicates of each parental genotype, 3) overlapping clusters for RILs, and 4) >20% missing data. The remaining 10,760 markers were used for map construction using a combination of MapMaker 3.0 (Lander and Botstein, 1989) and CarthaGène v.1.2.3R (de Givry et al., 2005) software. At first, five to nine polymorphic markers from each chromosome covering the whole genome were selected as anchors based on available mapping information in multiple populations (Wang et al., 2014). Using MapMaker 3.0 (Lander and Botstein, 1989) and the nine anchor markers, 10,657 polymorphic markers were placed onto 21 wheat chromosomes using a minimum LOD score of 5.0 and a maximum distance of 40 cM. The linkage maps were then developed using CarthaGène

V.1.2.3R (de Givry et al., 2005). Kosambi's mapping function (Kosambi, 1944) was used to determine the genetic distance among markers on the linkage groups.

3.3.7. QTL mapping

Composite interval mapping (CIM) was used to identify QTL for each trait in each environment as well as across environments (AE) using QTL Cartographer V2.5_011 (Wang et al., 2012). In QTL Cartographer, Model 6 (standard model), forward and backward regression, five control markers (co-factors), window size of 10 cM, and walk speed of 1 cM were used. A total of 1000 permutations were used to determine the LOD threshold for identifying the significant QTL. Confidence intervals (CI) were estimated by ± 2 LOD (from the peak) method. The QTL with overlapping CIs or QTL located within 10 cM regions were considered as the same QTL. Only the significant QTL detected (those above the threshold LOD score) were included in this study. If any such QTL were identified with an LOD score below the threshold, but >2.5 in other environments, the QTL were also included in the results as supporting information. The QTL identified in at least two environments or associated with at least two traits were also reported in this study. The QTL regions were drawn using the Mapchart 2.3 program (Voorrips, 2002). Map locations of the associated markers were used to see if the QTL identified in this study have been reported in earlier studies.

3.4. Results

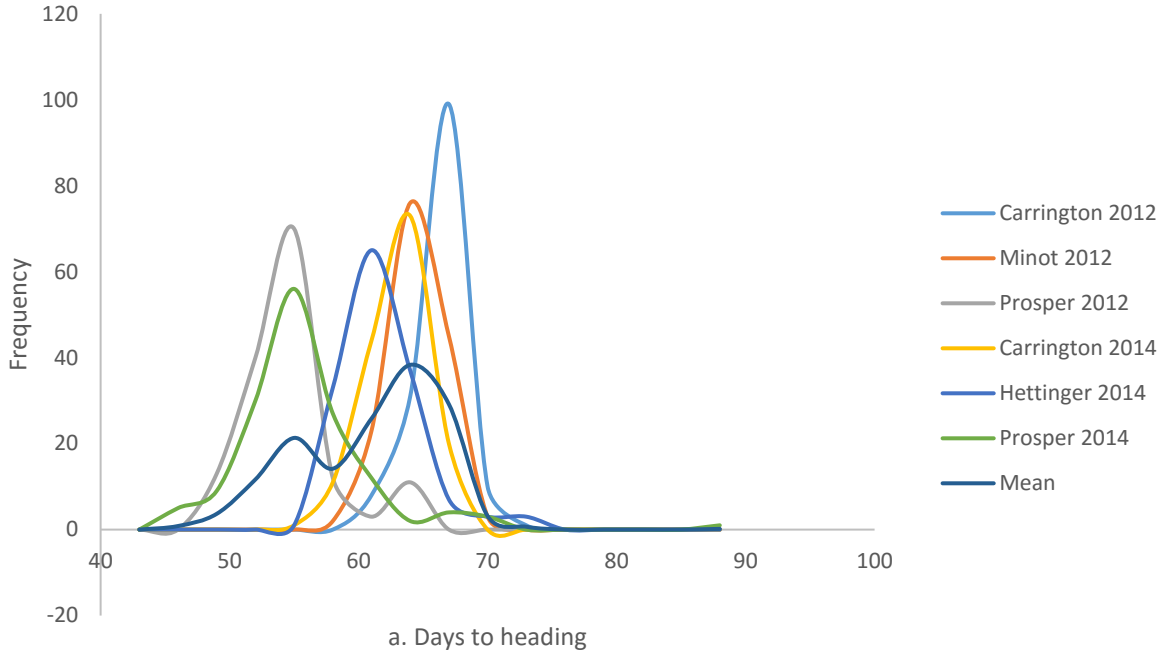
3.4.1. Phenotypic analyses

In the 2012 and 2014 field trials, significant differences among genotypes for most of the agronomic traits were found. The genotypes did not show any significant differences for the agronomic traits in the 2013 trials (Table 3.2). Therefore, given the criteria described earlier, only the data from the six environments planted in 2012 and 2014 were used for analysis in this

study. The data on DH from Carrington in 2014 did not show a significant difference (Table 3.2), and thus, was not used for further analysis.

The RIL population showed continuous variation for all of the agronomic traits (Fig. 3.1). The parent Reeder showed a higher PH, and Albany had delayed heading in all six locations. Albany had a higher YLD in all of the environments except for Prosper in 2012, and again in 2014. Albany had a greater TW in Carrington in 2012 and 2014, and in Prosper in 2012, whereas Reeder had a greater TW in Minot in 2012, Hettinger in 2014, and Prosper in 2014. Reeder had a greater TKW in all of the environments in both 2012 and 2014. Transgressive segregations in both directions were also observed for all of the traits (Table 3.3).

The heading date had a highly significant negative correlation with YLD, TW, and TKW in all of the environments. Late-heading plants tended to be taller in two of the environments and also with the mean value of all the environments. Plant height did not show any significant association with any of the traits except DH. The higher yielding plants gave a higher TW in every environment. Also, higher-yielding plants had a higher TKW in all of the environments except Carrington in 2014. Again, the plants with a higher TW tended to have a higher TKW in all of the environments except Carrington in 2014 (Table 3.4).



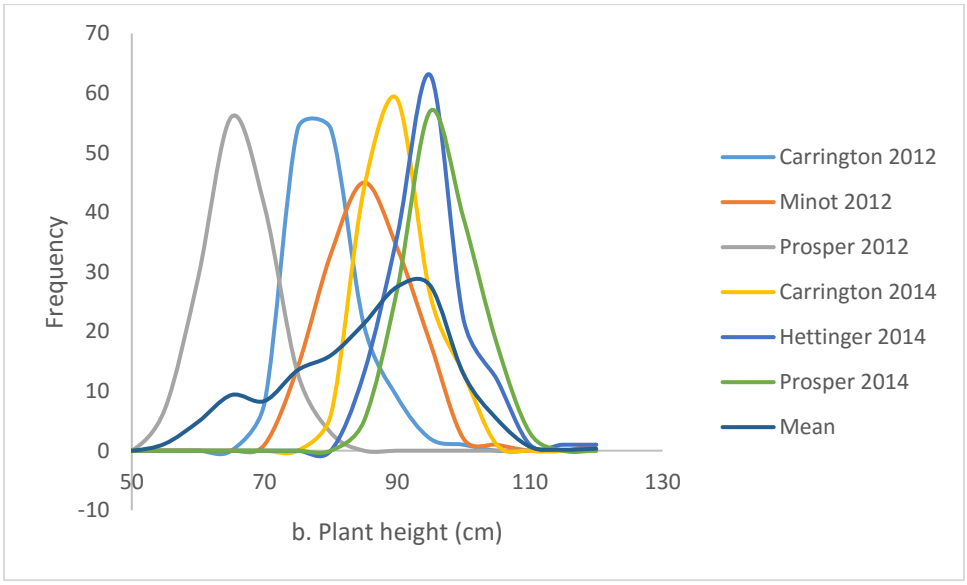
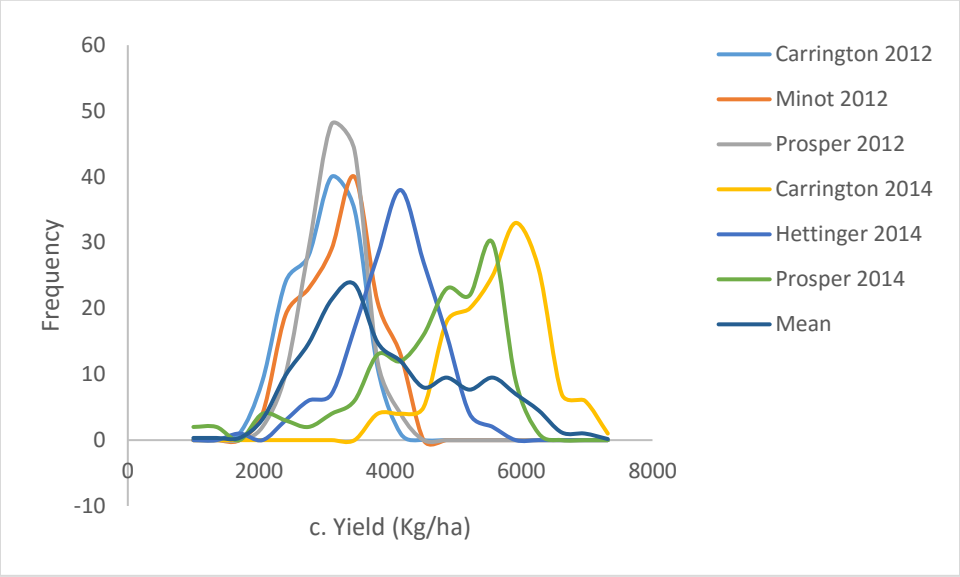


Fig. 3.1. Frequency distribution of the agronomic traits of 149 RILs of the cross of Reeder and Albany (a. days to heading, b. plant height).



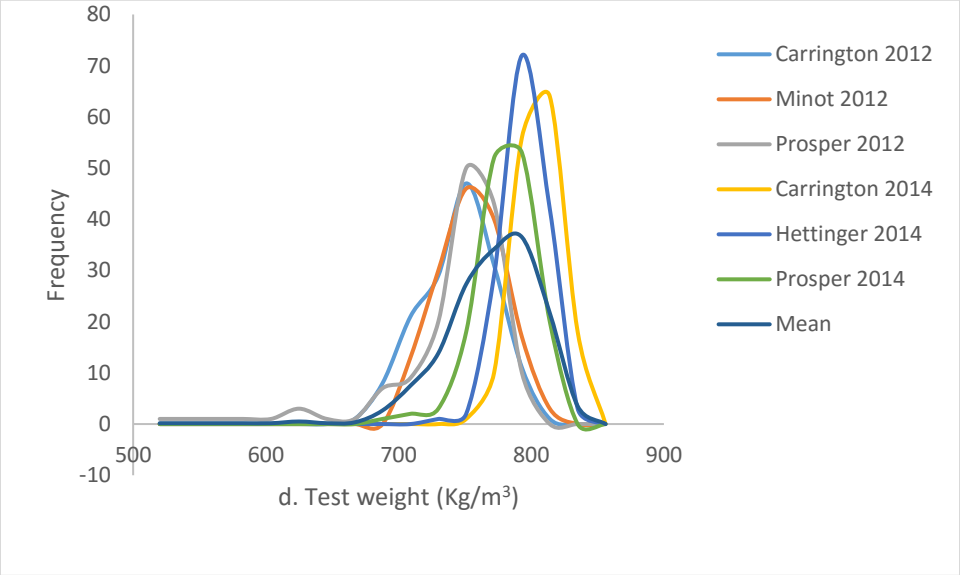


Fig. 3.1. Frequency distribution of the agronomic traits of 149 RILs of the cross Reeder and Albany (c. yield, d. test weight) (continued).

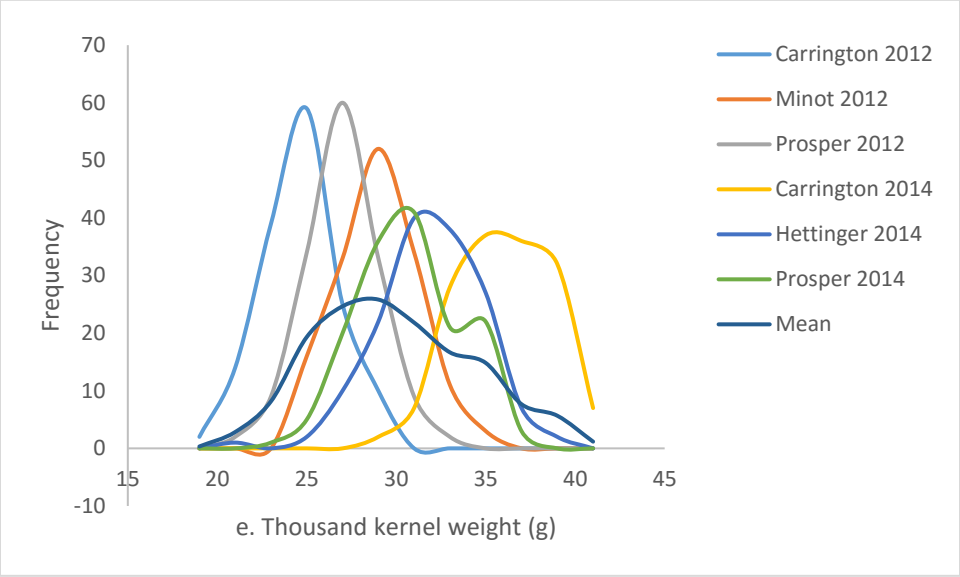


Fig. 3.1. Frequency distribution of the agronomic traits of 149 RILs of the cross of Reeder and Albany (e. thousand kernel weight) (continued).

3.4.2. Genetic linkage map

Out of the 10,760 markers (see materials and methods section for details) selected for linkage mapping, 10,657 markers were mapped onto 28 linkage groups found on 21 wheat chromosomes (Table 3.5). The 10,657 markers represented 2,057 unique loci (19.3%), and 8,600 markers (80.7%) co-segregated with other loci. The B-genome contained the most number of markers, followed by the A-genome and the D-genome (Table 3.4). The number of markers on individual linkage groups ranged from five (1D1, 5D2) to 1,221 (2B), while for individual chromosomes, the number of markers ranged from 48 (chromosome 3D) to 1,221 (chromosome 2B) (Table 3.4). The average number of markers mapped per chromosome was 507.48, while the average number of unique loci per chromosome was 97.95.

Table 3.2. Analysis of variance for the agronomic traits in nine environments.

Environment and sources[ɬ]	df	HD[‡]	PH[§]	YLD[¶]	TW[#]	TKW[ǂ]
Carrington 2012						
Treatment	168	17.51***	4.41***	8.52***	13.13***	9.52***
Error	168	0.53	12.07	52396	124.22	0.93
CV%		1.11	4.52	8.12	1.51	4.04
Minot 2012						
Treatment	168	11.5***	2.11***	3.49***	8***	4.31***
Error	168	0.92	41.07	167980	158.39	2.43
CV%		1.51	7.7	13.56	1.69	5.51
Prosper 2012						
Treatment	168	18.67***	1.65***	2.49***	13.6***	10.5***
Error	168	1.49	39.67	131705	297.05	0.88
CV%		2.285	9.74	12.13	2.35	3.56
Carrington 2013						
Treatment	167	0.88	1.36*	1.09	0.74	0.96
Error	155	8.21	20.82	273310	361.09	8.12
CV%		5.15	6.27	10.96	2.2	9.19
Williston 2013						
Treatment	168	1.24	0.97	1.13	0.78	1.07
Error	168	10.1	68	334357	182.46	6.83
CV%		5.12	14.7	22	1.64	8.67
Prosper 2013						
Treatment	168	0.91	1.3*	1.14	0.7	0.74
Error	168	16.88	50.79	1577173	342.73	8.81
CV%		7.93	9.63	26.6	2.28	9.08
Carrington 2014						
Treatment	168	0.93	2.38***	1.75***	7.58***	5.09***
Error	168	12.75	22.7	553814	65.75	2.78
CV%		5.89	5.47	13.44	1.03	4.78
Hettinger 2014						
Treatment	168	3.91***	4.38***	4.31***	10.24***	8.05***
Error	168	4.97	16.11	187916	51.83	2.11
CV%		3.701	4.361661	11.1163	0.917296	4.629367
Prosper 2014						
Treatment	168	5.49***	4.49***	8.85***	9.18***	7.34***
Error	168	9.4	12.89	259910	95.36	2.6
CV%		5.66	3.83	11.32	1.27	5.35

*Significant at 0.05, ***Significant at 0.001 probability level

[ɬ]HD = days to heading, [§]PH = height, [¶]YLD = yield, [#]TW = test weight, [ǂ]TKW = thousand kernel weight

Table 3.3. Phenotypic performances of Reeder and Albany, RIL population and checks in different environments (Env.).

Env.ǂ	Parental lines		RIL population			Checks	LSD (0.05)
	Reeder	Albany	Min	Max	Mean		
	……Plant height, cm……..…….						
1	77.47	68.58	67.31	97.79	76.82	78.32	6.81
2	87.63	80.01	67.31	115.57	83.10	84.53	12.56
3	62.23	57.15	52.07	77.47	64.19	69.36	12.34
4	83.82	83.82	76.20	104.14	86.79	86.22	9.34
5	92.71	88.90	80.01	119.38	92.07	91.86	7.87
6	95.25	90.17	81.28	106.68	93.83	93.20	7.04
M	83.19	78.11	74.30	101.39	82.80	83.91	9.33
	……Days to heading, days……						
1	63.00	65.50	59.50	71.00	65.23	64.22	1.42
2	61.50	64.00	57.50	69.50	63.27	63.00	1.88
3	50.50	54.50	46.50	64.00	53.58	51.28	2.39
4	.	.	.	.	.	.	.
5	56.50	60.50	54.00	72.00	60.48	58.50	4.37
6	50.50	55.50	45.50	87.50	54.59	50.47	6.01
M	56.40	60.00	53.80	67.90	59.43	57.49	3.21
	……Yield, Kg/ha……..						
1	2657.17	2984.96	1664.03	3913.01	2823.10	2793.97	448.65
2	2864.88	2915.50	1761.58	4072.12	3043.44	2840.95	803.31
3	2773.05	2558.44	1920.18	4121.90	2996.84	2996.80	711.31
4	4490.20	6080.77	3650.85	7016.44	5457.19	5026.72	1458.61
5	3791.92	3881.20	1610.86	5366.24	3864.81	4194.71	849.65
6	4886.96	4384.69	654.42	6078.55	4416.39	5225.90	999.23
M	3577.36	3800.93	2562.57	4461.78	3766.96	3846.51	878.46
	……..Test weight, Kg/m^3……						
1	722.47	747.17	662.17	798.93	733.79	750.45	21.85
2	773.14	747.53	690.14	807.10	743.76	757.54	24.67
3	748.62	773.87	501.81	790.76	731.27	753.48	33.78
4	780.59	799.66	743.54	827.45	794.53	794.64	15.89
5	799.84	784.95	720.84	818.91	784.07	790.59	14.11
6	785.67	783.86	687.06	809.65	769.87	785.99	19.14
M	768.39	772.84	678.61	803.02	759.55	772.11	21.57

Table 3.3. Phenotypic performances of Reeder and Albany, RIL population, and checks in different environments (Env.) (continued).

Env[ŧ]	Parental lines		RIL population			Checks	LSD (0.05)
	Reeder	Albany	Min	Max	Mean		
	……Thousand kernel weight, g……....						
1	25.25	22.00	18.00	29.00	23.71	25.56	1.89
2	32.25	24.00	23.25	34.25	28.10	29.79	3.05
3	27.00	25.50	20.75	32.00	26.18	28.26	1.84
4	35.00	25.50	27.25	40.00	35.12	35.56	3.27
5	35.00	29.50	21.00	38.00	31.09	33.47	2.85
6	33.75	29.00	22.25	37.00	29.80	33.25	3.16
M	31.38	25.92	22.63	34.38	29.00	30.98	2.68

[ŧ]1 = Carrington 2012, 2 = Minot 2012, 3 = Prosper 2012, 4 = Carrington 2014, 5 = Hettinger 2014, 6 = Prosper 2014, 7 = Mean across environments

The 10,657 (2,057 loci) markers mapped in this study covered a total genetic map length of 3,793.1 cM, with an average distance of 0.36 cM between any two markers (Table 3.4). The A-genome chromosomes covered a total length of 1,542.2 cM, with an average distance of 0.37 cM between two markers. The B-genome had a total map length of 1,259.1 cM, with an average distance of 0.35 cM between two markers. The D-genome covered a total map length of 991.8 cM, with an average distance of 1.52 cM between two markers. Individually, chromosome 5A was the longest, with a total map length of 299 cM. Chromosome 6D was the shortest, with a total map length of 51.5 cM. Overall, observed marker order was consistent when compared with earlier published genetic maps (Wang et al., 2014).

Table 3.4. Correlation coefficients between five agronomic traits in the RIL population (Reeder × Albany) in different environments (Env.) and the overall mean across environments (M).

Trait[ᵗ] and Env.[ǂ]	PH	DH	YLD	TW
DH				
1	0.04 ns	-	-	-
2	0.32***	-	-	-
3	0.07 ns	-	-	-
4	.	-	-	-
5	0.13 ns	-	-	-
6	0.31***	-	-	-
M	0.24**	-	-	-
YLD				
1	0.00 ns	-0.58***	-	-
2	-0.03 ns	-0.47***	-	-
3	0.18*	-0.38***	-	-
4	0.29***	.	-	-
5	0.06 ns	-0.44***	-	-
6	-0.27***	-0.68***	-	-
M	-0.07 ns	-0.59***	-	-
TW				
1	-0.02 ns	-0.62***	0.62***	-
2	-0.05 ns	-0.57***	0.53***	-
3	-0.19*	-0.72***	0.51***	-
4	0.029 ns	.	0.18*	-
5	0.01 ns	-0.33***	0.29***	-
6	-0.21**	-0.55***	0.61***	-
M	-0.14 ns	-0.6***	0.49***	-
TKW				
1	0.17*	-0.45***	0.56***	0.47***
2	0.2**	-0.33***	0.34***	0.45***
3	0.2*	-0.28***	0.23**	0.29***
4	0.19*	.	0.12 ns	0.03 ns
5	0.08 ns	-0.48***	0.25**	0.36***
6	-0.06 ns	-0.5***	0.47***	0.52***
M	0.13 ns	-0.4***	0.29***	0.30***

*Significant at p<0.05, **Significant at p<0.01, ***Significant at p<0.001 level

[ᵗ]PH = Plant height, DH = Days to heading, YLD = Yield, TW = Test weight, TKW = Thousand kernel weight

[ǂ]1 = Carrington 2012, 2 = Minot 2012, 3 = Prosper 2012, 4 = Carrington 2014, 5 = Hettinger 2014, 6 = Prosper 2014, 7 = Mean across environments

Table 3.5. Distribution of markers across linkage groups in the genetic map developed using the Reeder × Albany RIL population.

Linkage groups	No. of markers	No. of unique loci	Map length	Average map density	Average map density
				cM/marker	cM/locus
1A	567	126	174.90	0.31	1.39
2A	439	101	223.50	0.51	2.21
3A	659	123	213.90	0.32	1.74
4A	560	114	218.90	0.39	1.92
5A	605	163	299.00	0.49	1.83
6A	590	117	176.70	0.30	1.51
7A	905	168	235.30	0.26	1.40
1B	629	86	107.50	0.17	1.25
2B	1221	160	181.80	0.15	1.14
3B	1115	213	250.20	0.22	1.17
4B	244	78	120.90	0.50	1.55
5B1	565	125	209.40	0.37	1.68
5B2	25	8.00	18.00	0.72	2.25
6B	426	101	158.10	0.37	1.57
7B	723	134	213.20	0.29	1.59
1D1	5	2	0.30	0.06	0.15
1D2	254	40	87.80	0.35	2.20
1D3	91	26	126.10	1.39	4.85
2D	653	46	180.40	0.28	3.92
3D	48	18	162.90	3.39	9.05
4D	53	23	129.90	2.45	5.65
5D1	25	8	47.50	1.90	5.94
5D2	5	4	24.90	4.98	6.23
5D3	130	21	31.50	0.24	1.50
6D1	10	5	3.00	0.30	0.60
6D2	23	19	44.50	1.93	2.34
6D3	22	6	4.00	0.18	0.67
7D	65	22	149.00	2.29	6.77
A genome	4,325	912	1,542.20	0.37	1.72
B genome	4,948	905	1,259.10	0.35	1.52
D genome	1,384	240.00	991.80	1.52	3.84
Whole genome	**10,657**	**2,057**	**3,793.10**	**0.36**	**1.84**

3.4.3. QTL analysis

3.4.3.1. QTL for DH

Composite interval mapping (CIM) for DH identified nine QTL located on five different chromosomes. These QTL explained from 4.12 to 38.36% of phenotypic variation (PV) (Table 3.6; Fig 3.2). Four QTL explained >10% of PV, and therefore, can be considered as major QTL. The QTL with the greatest and consistent effect for DH was identified on chromosome 5A in all of the environments except one and explained up to 38.36% of PV. The second major QTL was identified on chromosome 5D in all of the environments except one and explained up to 29.93% of PV. The third major QTL explained 17.4% of PV and was identified on 7B in all of the environments. The fourth major QTL was identified on chromosome 4A in all of the environments except one and explained up to 13.44% of PV. The alleles for reduced DH on 5A and 4A were contributed by the parent Reeder, while the alleles for reduced DH on the other two major QTL were contributed by the parent Albany.

3.4.3.2. QTL for PH

Eight QTL identified for PH were located on seven different chromosomes (Table 3.6; Fig.3.2). Two of them were considered major QTL (PV>10%). The QTL found on chromosome 2D had the largest effect, explaining up to 17.2% of PV. This QTL was identified in three different environments and in the overall mean. The second major QTL found on chromosome 6A was also identified in three different environments and explained up to 11.37% of PV. Besides these, three more QTL explained almost 10% of PV. Two of them were identified on chromosome 7B, and another one on chromosome 5B. The QTL in the QTL region 26 of chromosome 7B was identified in three environments and in the overall mean. Another QTL in the QTL region 24 of chromosome 7B was identified in two of the environments and in the

overall mean. The QTL on chromosome 5B was identified in two environments only. The alleles for reduced PH for the above-mentioned QTL on chromosomes 2D, 6A, and 7B were contributed by the parent Albany. The allele for reduced PH on chromosome 5B was contributed by the parent Reeder (Table 3.5).

3.4.3.3. QTL for yield

The six QTL identified for yield were located on six different chromosomes (Table 3.6; Fig.3.2). Four of these QTL explained greater than 10% of PV and were considered as major QTL. The major QTL located on chromosome 2B had a PV (Phenotypic variation) up to 13.94%; that on 5A had a PV up to 22.35%; and that on 5D had a PV up to 22.83%. All three QTL were identified in three of the environments and in the overall mean, and thus, could be considered as consistent or stable QTL. The fourth major QTL on chromosome 7B was identified in one location and in the overall mean, explaining up to 13.6% of PV. The alleles for higher yield for the QTL on chromosomes 5D, 2B, and 7B were contributed by the parent Albany, whereas the allele for the major QTL on chromosome 5A was contributed by the parent Reeder (Table 3.6).

3.4.3.4. QTL for TW

Seven QTL located on six different chromosomes were identified for TW (Table 3.6; Fig.3.2). Five QTL among them were considered as major QTL. The QTL with the greatest effect (PV of up to 24.47%) was located on chromosome 5D and identified in two different environments and in the overall mean. The second major QTL, with up to 17.79% PV, was on chromosome 5A and identified in two of the environments. The major QTL on chromosome 2B had the third greatest and consistent effect as it was identified in four different environments, with a PV of up to 16.5%. The fourth major QTL was located on 2A (with a PV of up to 15.93%)

and was identified in three of the environments and in the overall mean. A fifth major QTL on chromosome 7B, explaining up to 14.86% of PV, was identified in three different environments and in the overall mean. The alleles for a higher test weight for the major QTL on chromosomes 5D, 2B, 2A, and 7B were contributed by the parent Albany. The allele for the remaining major QTL on chromosome 5A was contributed by the parent Reeder (Table 3.6).

3.4.3.5. QTL for TKW

The eight QTL identified for TKW were located on seven different chromosomes (Table 3.6; Fig.3.2). The QTL with the largest phenotypic effect (with a PV of up to 15.22%) was located on chromosome 6A; it also had a consistent effect as it was identified in five different environments and in the overall mean. The second major QTL was located on chromosome 4A, explaining 14.18% of PV, but it was identified in only a single environment. Another QTL explaining up to 9.66% of PV was located on chromosome 2A and identified in two different environments and in the overall mean. The alleles for increased TKW for the major QTL on 6A were contributed by the cultivar Reeder (Table 3.6).

3.4.4. Co-localized or pleiotropic QTL

Co-localized QTL could be used for the simultaneous improvement of more than one trait when the desirable alleles come from the same parent. A total of 38 QTL were identified in this study for five agronomic traits (Table 3.6; Fig.3.2). Many of those QTL had overlapping confidence intervals (CI). The QTL with overlapping CI or located within 10 cM of each other were considered as the same QTL region. Overall, these 38 QTL were located in 26 different genomic regions on 13 different chromosomes. A total of 21 co-localized or pleiotropic QTL were located in nine genomic regions. Individual genomic regions were associated with two to three traits. Genomic region 7 was associated with DH, YLD, and TW. The QTL for YLD

(*QYL.ndsu.2B)* and TW (*QTW.ndsu.2B)* had a major effect, whereas that for DH (*QDH.ndsu.2B.2*) had a minor effect. The genomic region 20 located on chromosome 5D also harbored major QTL for the same three traits. The desirable alleles in both regions (7 and 20) were contributed by the parent Albany. Genomic region 17 on chromosome 5A also harbored major QTL (*QDH.ndsu.5A.3, QYL.ndsu.5A,* and *QTW.ndsu.5A)* for the same three traits, where Reeder contributed the desirable alleles.

Six QTL regions harbored QTL for two traits. QTL region 12 harbored QTL for TKW (*QTKW.ndsu.4A)* and DH (*QDH.ndsu.4A.1)*. The QTL for DH had a minor effect, whereas the QTL for TKW had a major effect. Reeder contributed the desirable alleles in both cases. QTL region 13 harbored QTL for DH (*QDH.ndsu.4A.2)* and TW (*QTW.ndsu.4A)*. The QTL for DH was a major QTL, while that for TW was minor. Desirable alleles for both traits were contributed by Reeder. QTL region 25 was also associated with DH (*QDH.ndsu.7B)* and TW *(QTW.ndsu.7B).* Both QTL had major effects, with the desirable alleles contributed by Albany. The QTL for PH (*QPH.ndsu.2D*) and TKW (*QTKW.ndsu.2D.2*) were associated with QTL region 9. The QTL for PH had a major effect, while that for TKW had a minor effect. Desired alleles from the QTL were contributed by different parents. QTL region 24 harbored QTL for PH (*QPH.ndsu.7B.2*) and YLD (*QYL.ndsu.7B*), where both QTL had major effects and the desired alleles came from Albany. QTL region 3 harbored QTL for TW (*QTW.ndsu.2A.2*) and TKW

Table 3.6. QTL identified for the agronomic traits in a RIL population derived from the cross between Reeder and Albany.

QTL and trait	QTL region	Other associated traits[ŧ]	Env.[ɨ]	Position[§]	LOD[¶]	Additive effect	R^2 (%)
Days to heading							
QDH.ndsu.2B.1	6	_	1, 2*,3*	26.81-30.11	3.82	0.78	5.2
QDH.ndsu.2B.2	7	YLD, TW	1	76.11	4.25	0.52	5.74
QDH.ndsu.4A.1	12	TKW	2	47.51	4.56	-0.66	8.24
QDH.ndsu.4A.2	13	TW	1, 2, 3, 5, 6	133.91-143.11	9	-1.66	13.44
QDH.ndsu.5A.1	15	_	1*, 6	109.51-112.61	3.48	-0.61	4.12
QDH.ndsu.5A.2	16	_	1*,2, 3	131.91-142.01	4.09	-0.92	6.22
QDH.ndsu.5A.3	17	YLD, TW	1, 3,4, 5, 6,	205.71-208.31	20.17	-2.84	38.36
QDH.ndsu.5D2	20	TW, YLD	2, 3, 4, 5, 6	11.91-20.91	15.16	2.29	29.93
QDH.ndsu.7B	25	TW	1, 2, 3, 4, 5*, 6	27.41-31.11	10.25	1.43	17.41
Plant height							
QPH.ndsu.2A	5	_	1, 4*	128.41-133.11	3.60	1.49	7.68
QPH.ndsu.2D	9	TKW	1, 3, 4, 7	151.11-165.71	7.31	2.04	17.2
QPH.ndsu.3B	10	_	3, 6*, 7*	184.31-187.71	4.33	1.53	8.55
QPH.ndsu.4A	14	_	2*, 7*	175.01-176.01	3.23	-1.70	6.73
QPH.ndsu.5B1	18	_	5,6	32.41-33.21	4.5	-1.81	9.01
QPH.ndsu.6A	22	_	2*, 3, 4	85.51-90.61	5.28	1.83	11.37
QPH.ndsu.7B.1	26	_	1, 3*, 6, 7	129.41-130.31	4.94	1.54	9.44
QPH.ndsu.7B.2	24	YLD	4*, 5, 7*	24.21-26.21	3.69	1.81	9.36
Test weight							
QTW.ndsu.2A.1	4	_	1, 2, 5	100.71-104.31	4.53	-7.73	8.16
QTW.ndsu.2A.2	3	TKW	4, 5, 6*,7*	80.11-82.11	7.14	-6.45	15.93
QTW.ndsu.2B	7	YLD, HD	1, 2, 5, 6, 7	84.31-95.61	8.02	-12.25	16.5
QTW.ndsu.4A	13	DH	6	139.91	3.79	5.8	7.22
QTW.ndsu.5A	17	YLD, DH	3, 6	207.01	9.43	20.77	17.79
QTW.ndsu.5D2	20	DH, YLD	3, 6, 7	11.91	12.38	-25.22	24.47
QTW.ndsu.7B	25	DH	1, 2, 3, 7	29.11-40.11	8.95	-13.28	14.86

Table 3.6. QTL identified for the agronomic traits in a RIL population derived from the cross between Reeder and Albany (continued).

QTL and trait	QTL region	Other associated traitsɪ	Env.‡	Position§	LOD¶	Additive effect	R^2(%)
Thousand kernel weight							
QTKW.ndsu.1A	1	_	4*, 6*	87.61-94.01	3.43	-0.77	7.08
QTKW.ndsu.2A	3	TW	3,4, 7	76.51-78.21	4.36	0.82	9.66
QTKW.ndsu.2D.1	8	_	2, 3*,7*	110.21-111.21	3.73	0.63	7.69
QTKW.ndsu.2D.2	9	PH	1, 4	155.31-155.61	4.06	0.72	8.47
QTKW.ndsu.4A	12	DH	3	58.81	6.82	0.84	14.18
QTKW.ndsu.5B1	19	_	1*, 5*	152.01-153.01	2.72	-0.69	5.61
QTKW.ndsu.6A	21	_	1, 2, 3, 4*, 5, 7	65.41-68.21	5.43	0.89	15.22
QTKW.ndsu.7A	23	_	1*, 3*	53.71	2.58	0.49	5.36
Yield							
QYL.ndsu.1B	2	_	3,5*,7*	64.21-71.91	3.99	-259.69	8.57
QYL.ndsu.2B	7	TW, HD	1,2, 3*,7	81.31-83.31	7.22	-209.44	13.94
QYL.ndsu.3B	11	_	4*,7*	202.21-213.81	3.17	-189.99	7.3
QYL.ndsu.5A	17	DH, TW	3, 6, 7	198.61-206.51	11.12	192.14	22.35
QYL.ndsu.5D2	20	TW, DH	3, 5*, 6,7	11.91-14.91	10.49	-466.60	22.83
QYL.ndsu.7B	24	PH	1,2*	22.21-25.21	5.87	-178.75	13.6

ɪPH = Plant height, DH = Days to heading, YLD = Yield, TW = Test weight, TKW = Thousand kernel weight

‡Env. = environment, 1 = Carrington 2012, 2 = Minot 2012, 3 = Prosper 2012, 4 = Carrington 2014, 5 = Hettinger 2014, 6 = Prosper 2014, 7 = Mean across environments

§Position represents the peak point of the QTL interval

¶For log of odds (LOD) score

* The QTL in that environment was detected above a 2.5 LOD score, but below the threshold score.

(*QTKW.ndsu.2A*). Both had major effects, but the desired alleles were contributed by different parents (Table 3.6; Fig.3.2).

3.4.5. The QTL important for drought tolerance

A total of 11 consistent QTL important for drought tolerance were identified. Among these, six QTL were exclusively for drought-prone environments (Table 3.7; Fig. 3.2). The QTL *QTW.ndsu.7B,* which is also associated with DH, had a major effect on TW and a LOD score of up to 8.95. The QTL *QYL.ndsu.2B* and *QYL.ndsu.7B* had major effects on yield. Surprisingly, the desired alleles from these three major QTL were contributed by the parent Albany, which was

considered the susceptible parent. The QTL *QDH.ndsu.2B.1*, which had a LOD score of up to 3.82, controlled 5.2% of PV for DH. In this QTL, the desirable allele was also contributed from the parent Albany. Another minor QTL for DH, *QDH.ndsu.5A.2*, had an LOD score of up to 4.09; the desired allele was contributed by the resistant parent Reeder. The third minor QTL, *QTKW.ndsu.2D.1*, controlled TKW up to 7.69% with a LOD score of up to 3.73; Reeder contributed the desired allele. Five major constitutive QTL identified in both water regimes were very consistent across locations (Table 3.7).

Table 3.7. QTL for drought tolerance in a RIL population derived from the cross between Reeder and Albany.

QTL	Trait[ᵗ]	QTL region	Other associated traits[ɪ]	Env.[ǂ]	Position[§]	LOD[¶]	Additive effect	R^2 (%)
QDH.ndsu.2B.1	DH	6	_	1, 2*, 3*	26.81-30.11	3.82	0.78	5.2
QDH.ndsu.4A.2	DH	13	TW	1, 2, 3, 5, 6	133.91-143.11	9	-1.66	13.44
QDH.ndsu.5A.2	DH	16	_	1*, 2, 3	131.91-142.01	4.09	-0.92	6.22
QDH.ndsu.5A.3	DH	17	YLD, TW	1, 3, 4, 5, 6	205.71-208.31	20.17	-2.84	38.36
QDH.ndsu.5D2	DH	20	TW, YLD	2, 3, 4, 5, 6	11.91-20.91	15.16	2.29	29.93
QTW.ndsu.2B	TW	7	YLD, HD	1, 2, 5, 6, 7	84.31-95.61	8.02	-12.25	16.5
QTW.ndsu.7B	TW	25	DH	1, 2, 3, 7	29.11-40.11	8.95	-13.28	14.86
QTKW.ndsu.2D.1	TKW	8	_	2, 3*,7*	110.21-111.21	3.73	0.63	7.69
QTKW.ndsu.6A	TKW	21	_	1, 2, 3, 4*, 5, 7	65.41-68.21	5.43	0.89	15.22
QYL.ndsu.2B	YL	7	TW, HD	1,2, 3*,7	81.31-83.31	7.22	-209.44	13.94
QYL.ndsu.7B	YL	24	PH	1,2*	22.21-25.21	5.87	-178.75	13.6

[ᵗ]PH = Plant height, DH = Days to heading, YLD = Yield, TW = Test weight, TKW = Thousand kernel weight

[ǂ]Env. = environment, 1 = Carrington 2012, 2 = Minot 2012, 3 = Prosper 2012, 4 = Carrington 2014, 5 = Hettinger 2014, 6 = Prosper 2014, 7 = Mean across environments

[§]Position represents the peak point of the QTL interval

[¶]For log of odds (LOD) score

* The QTL in that environment was detected above a 2.5 LOD score, but below the threshold score.

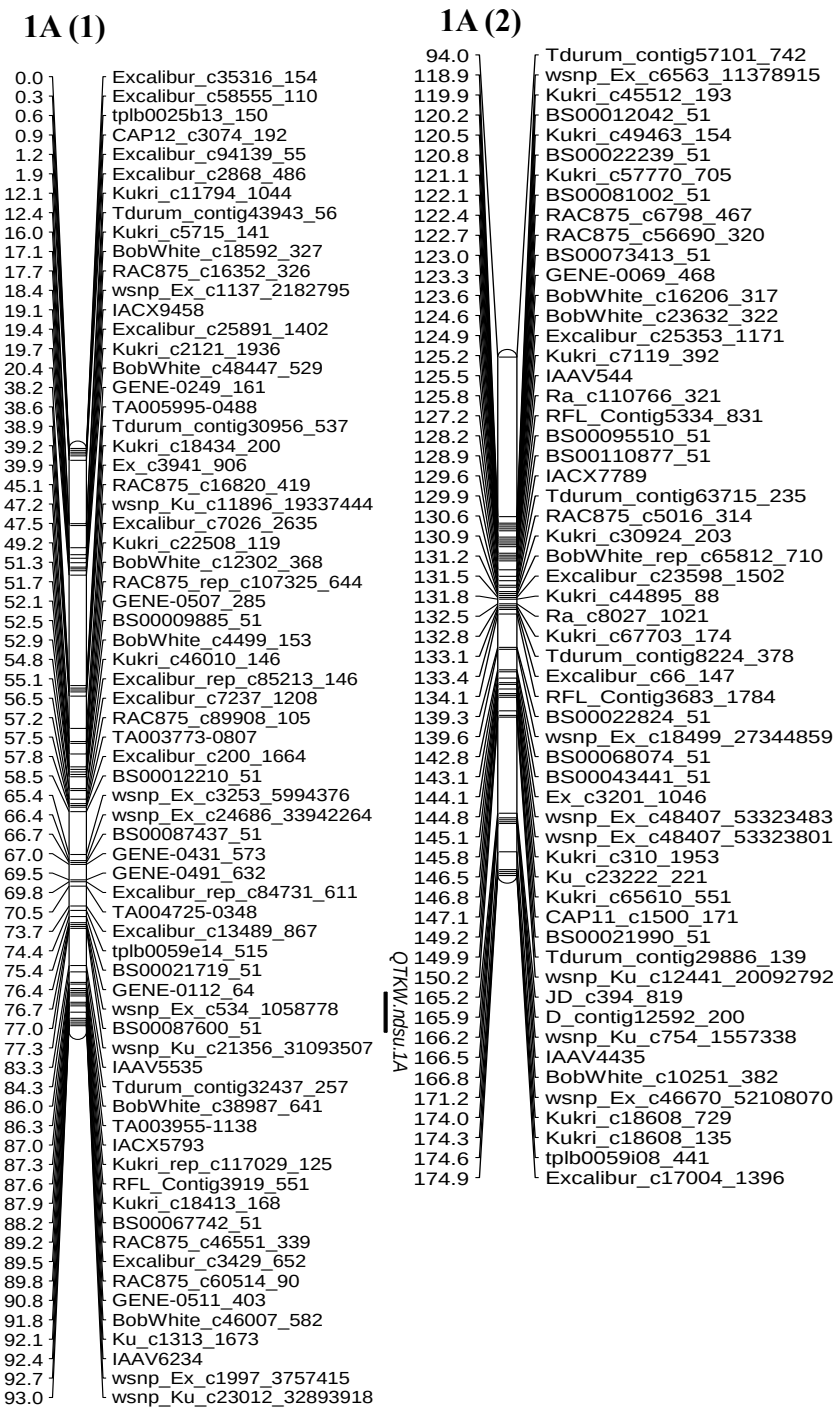


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps.

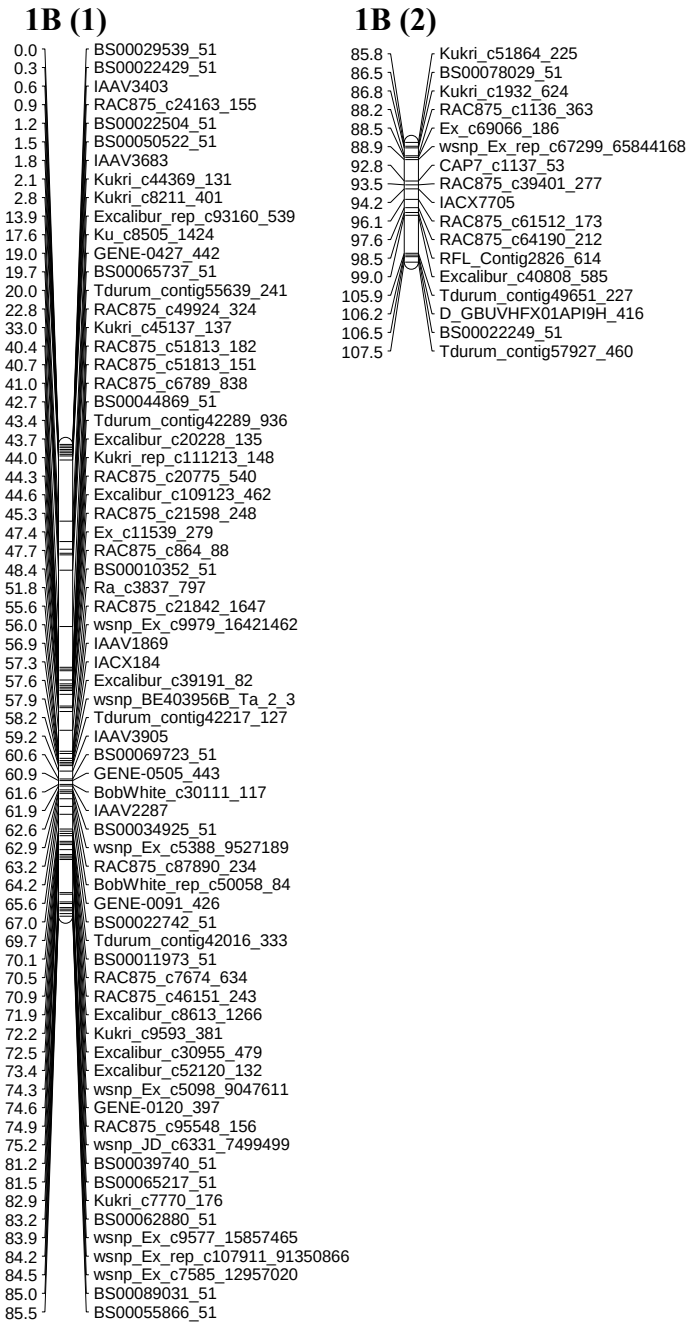


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

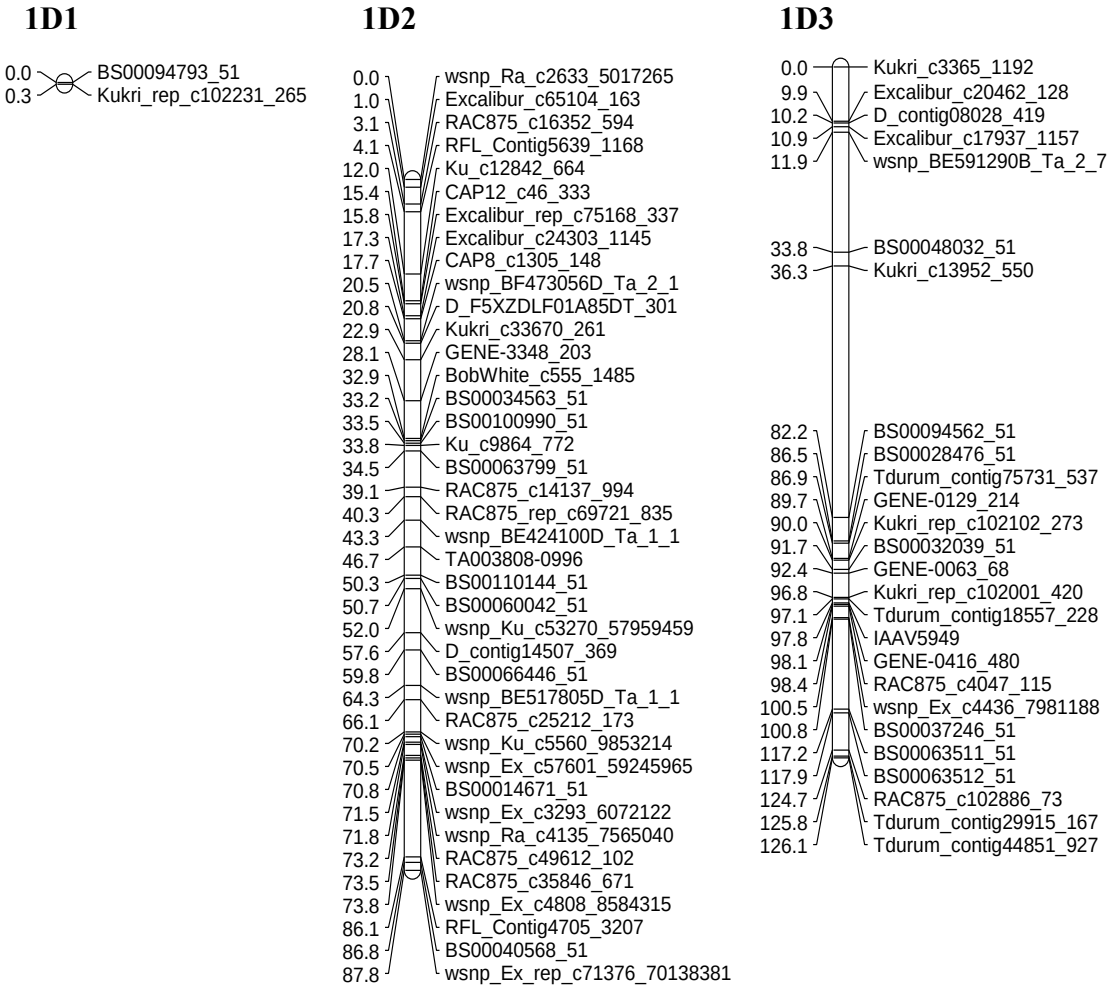


Fig. 3.2 Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

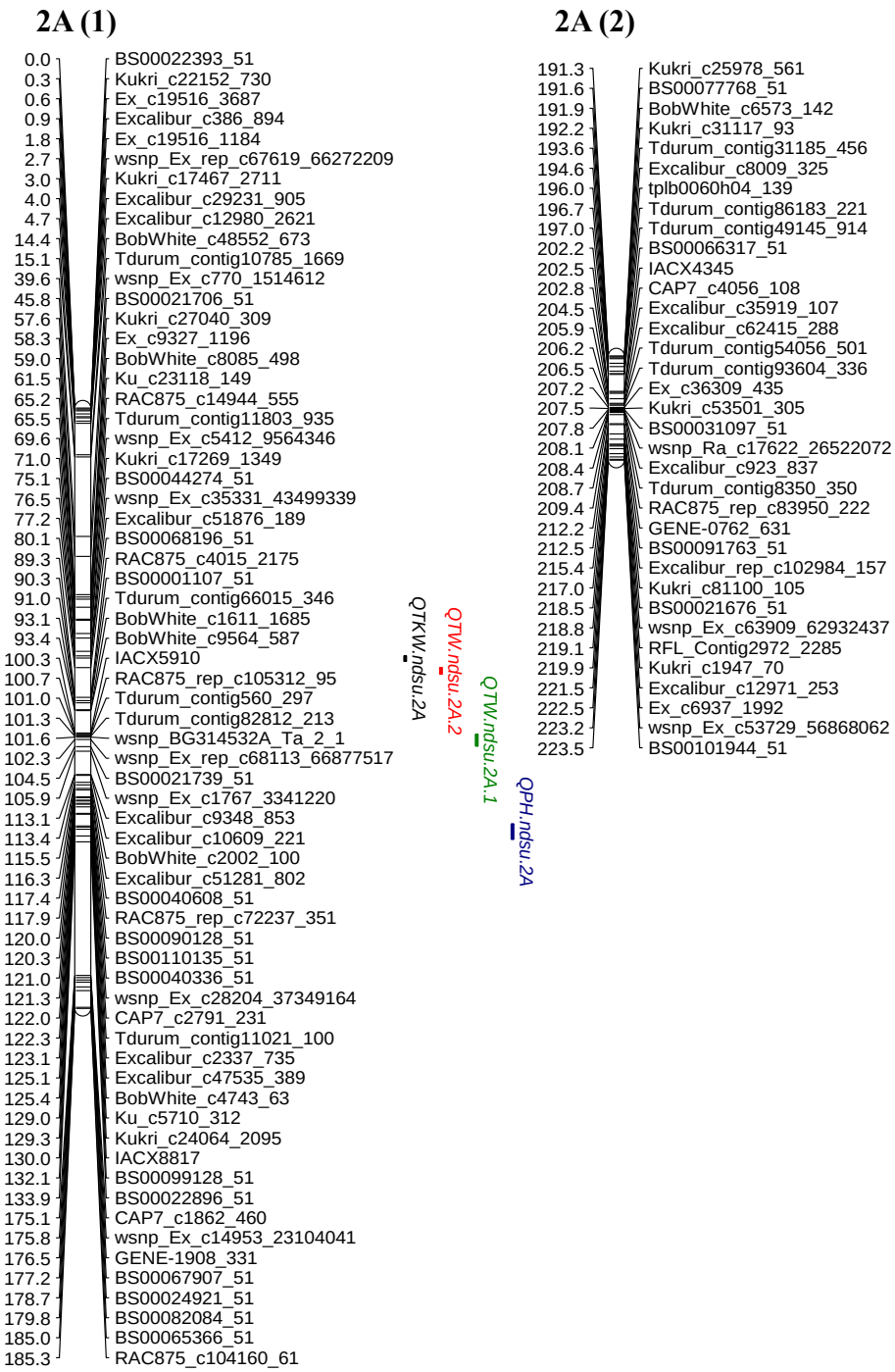


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

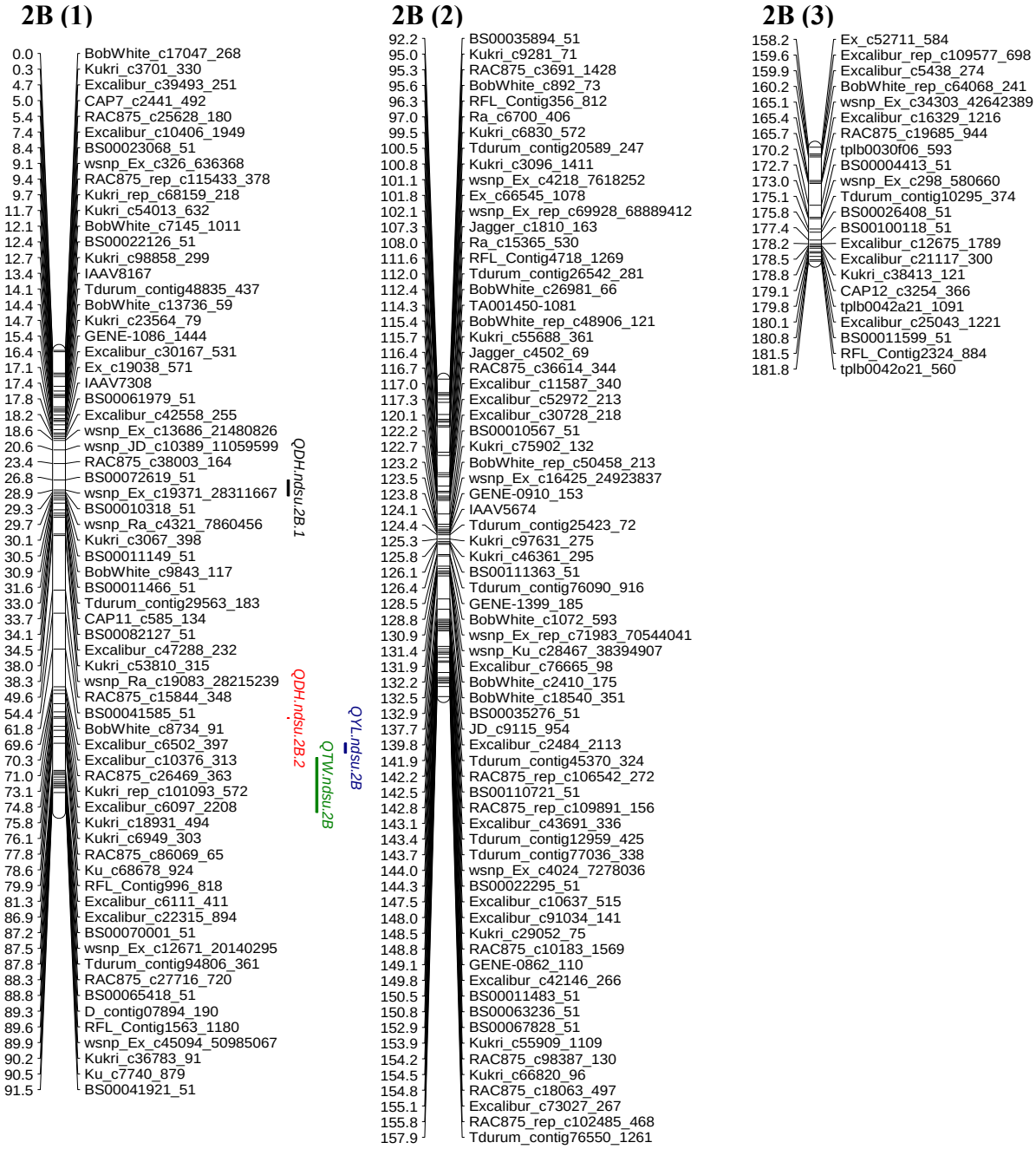


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

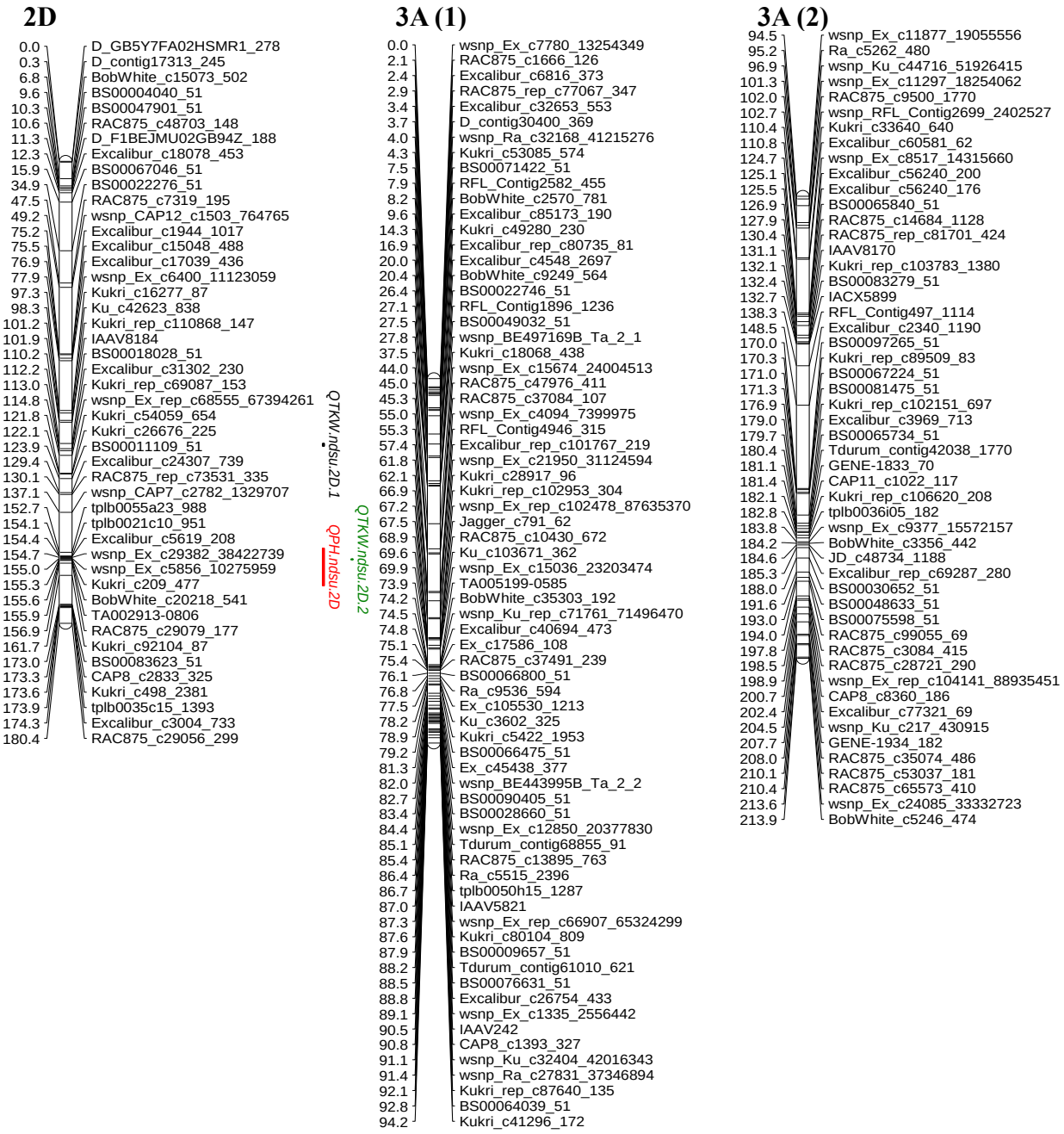


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

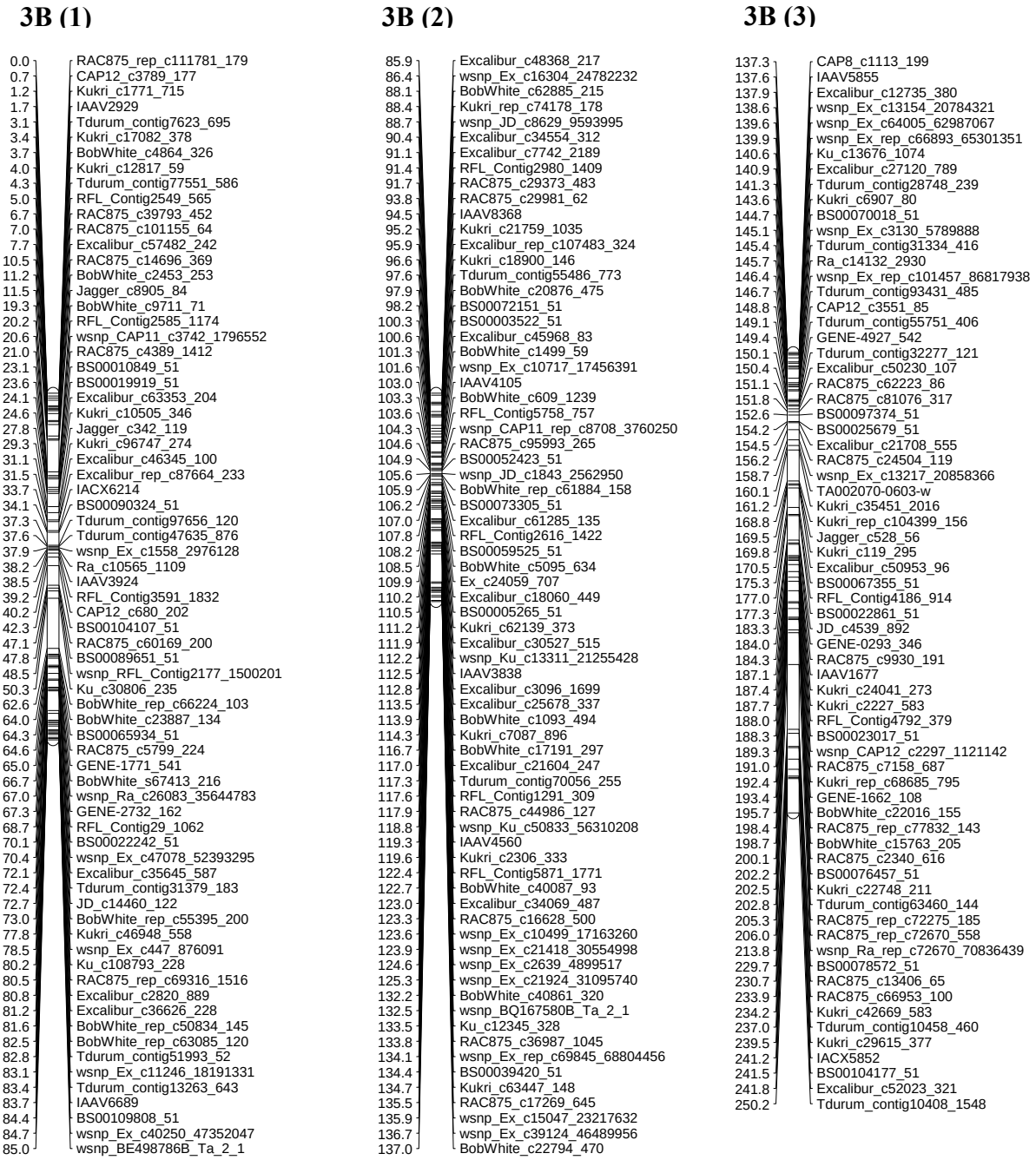


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

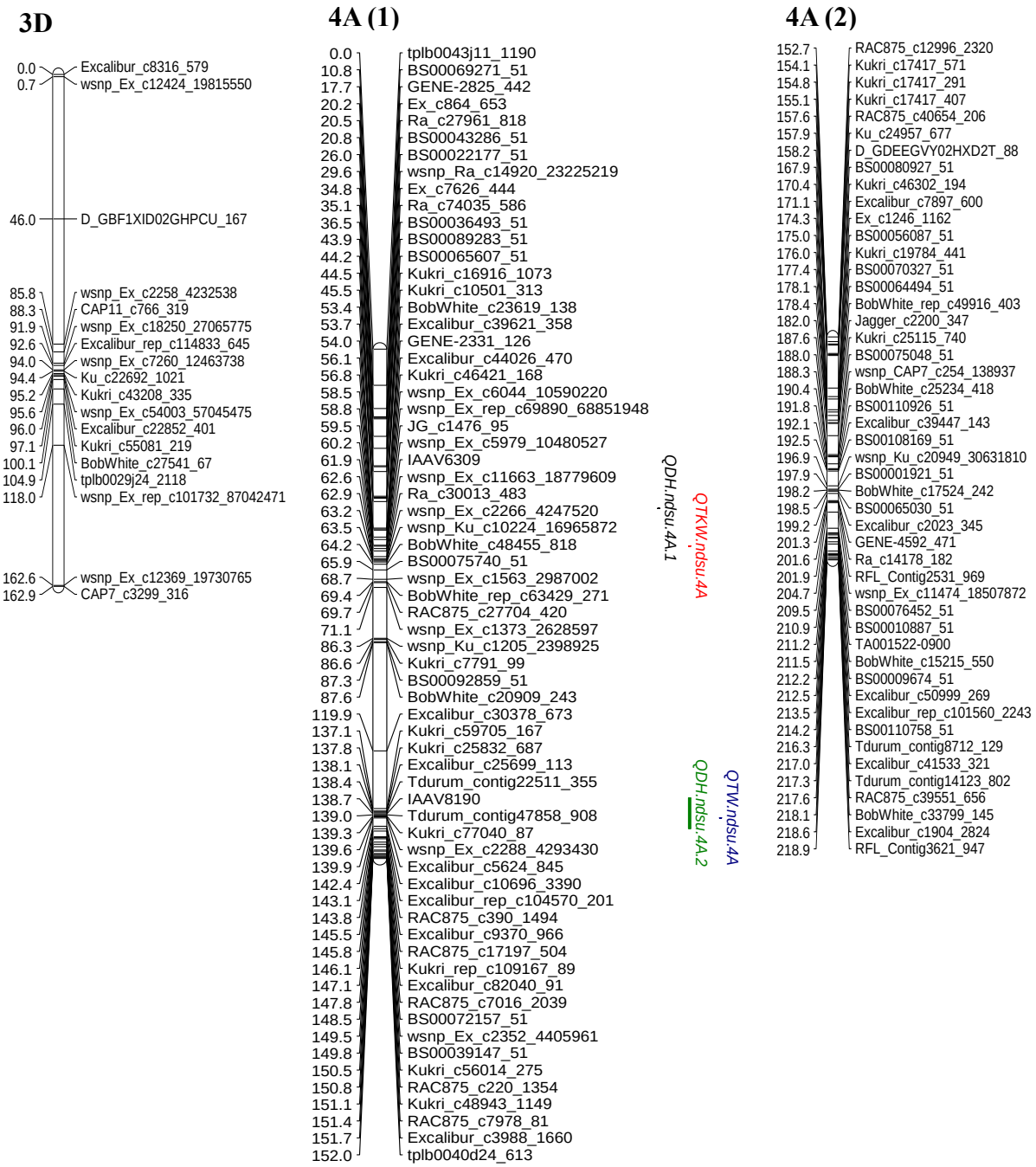


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

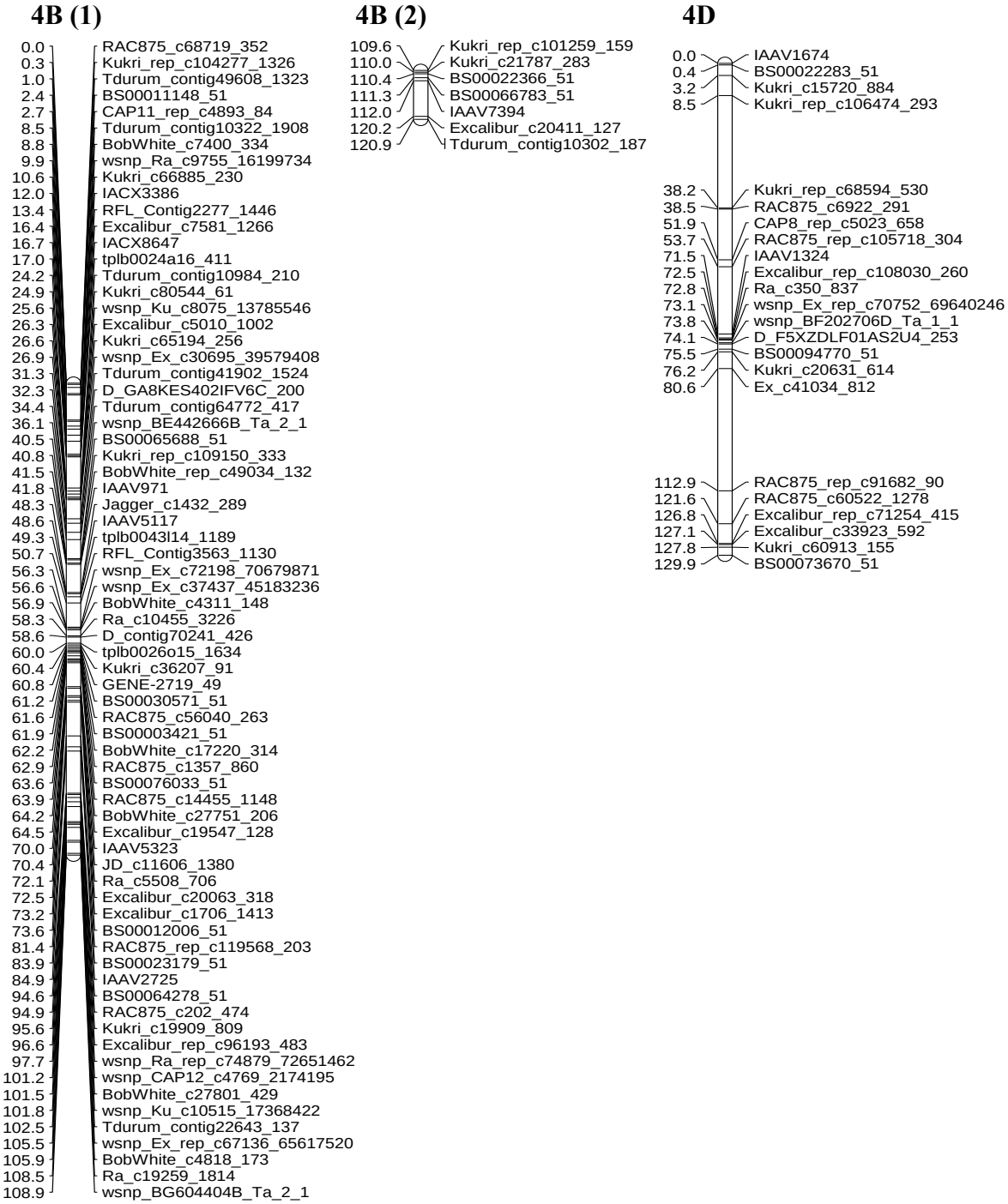


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

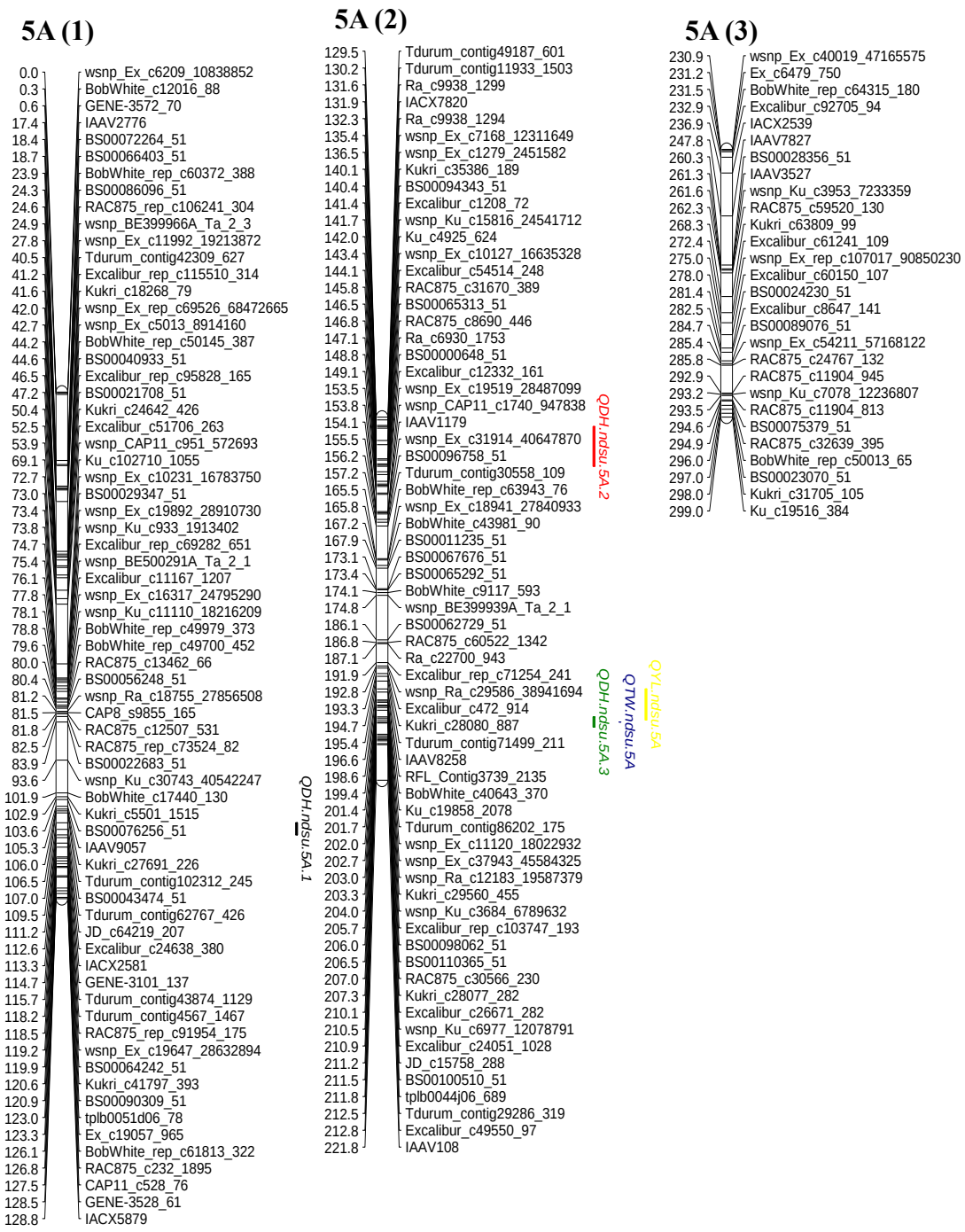


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

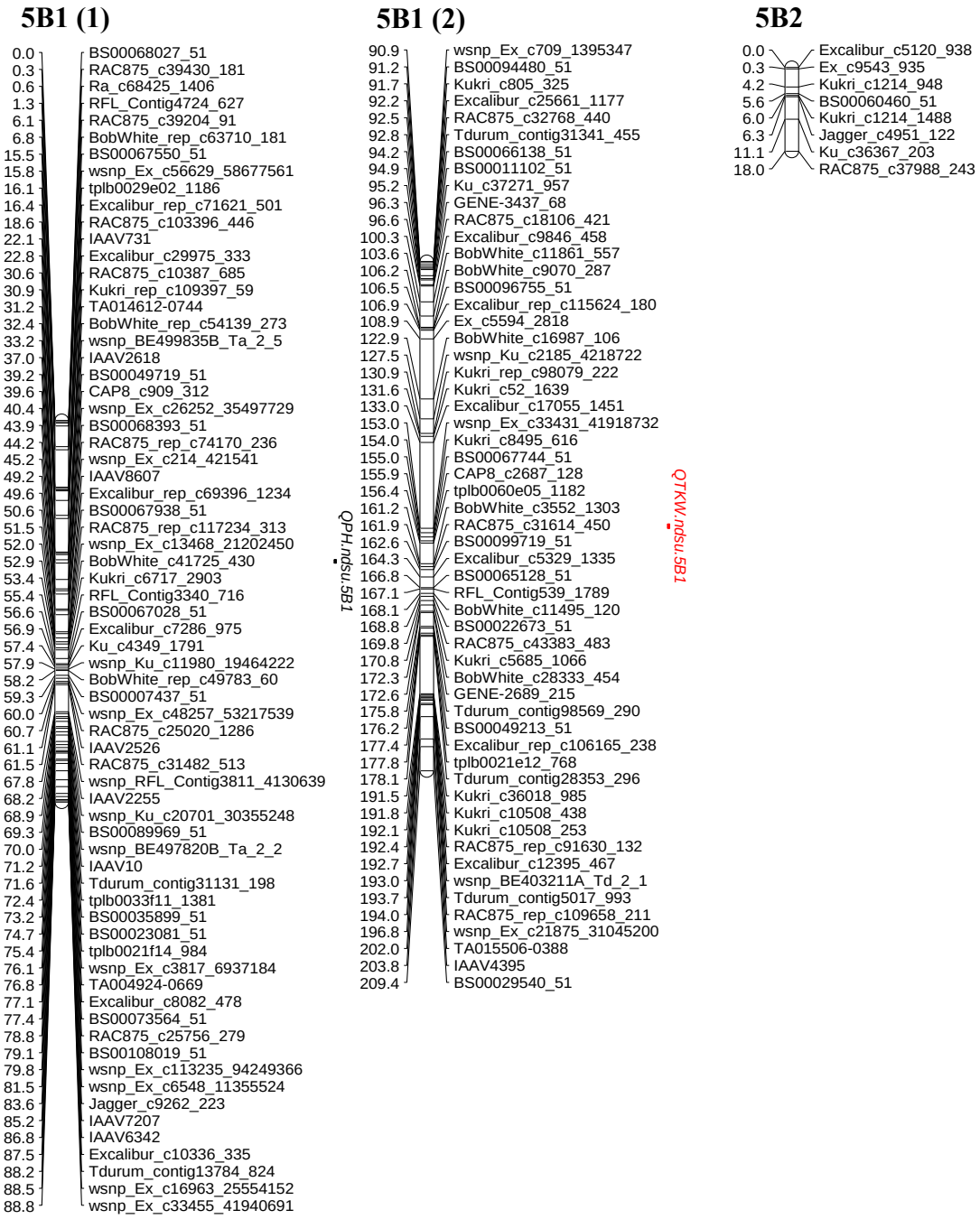


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

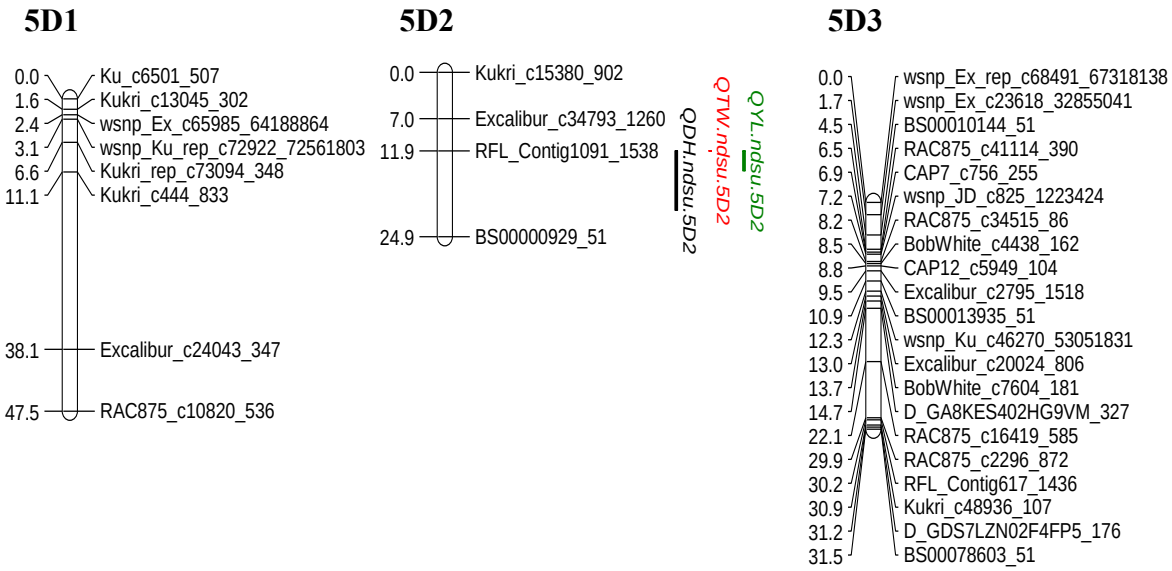


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

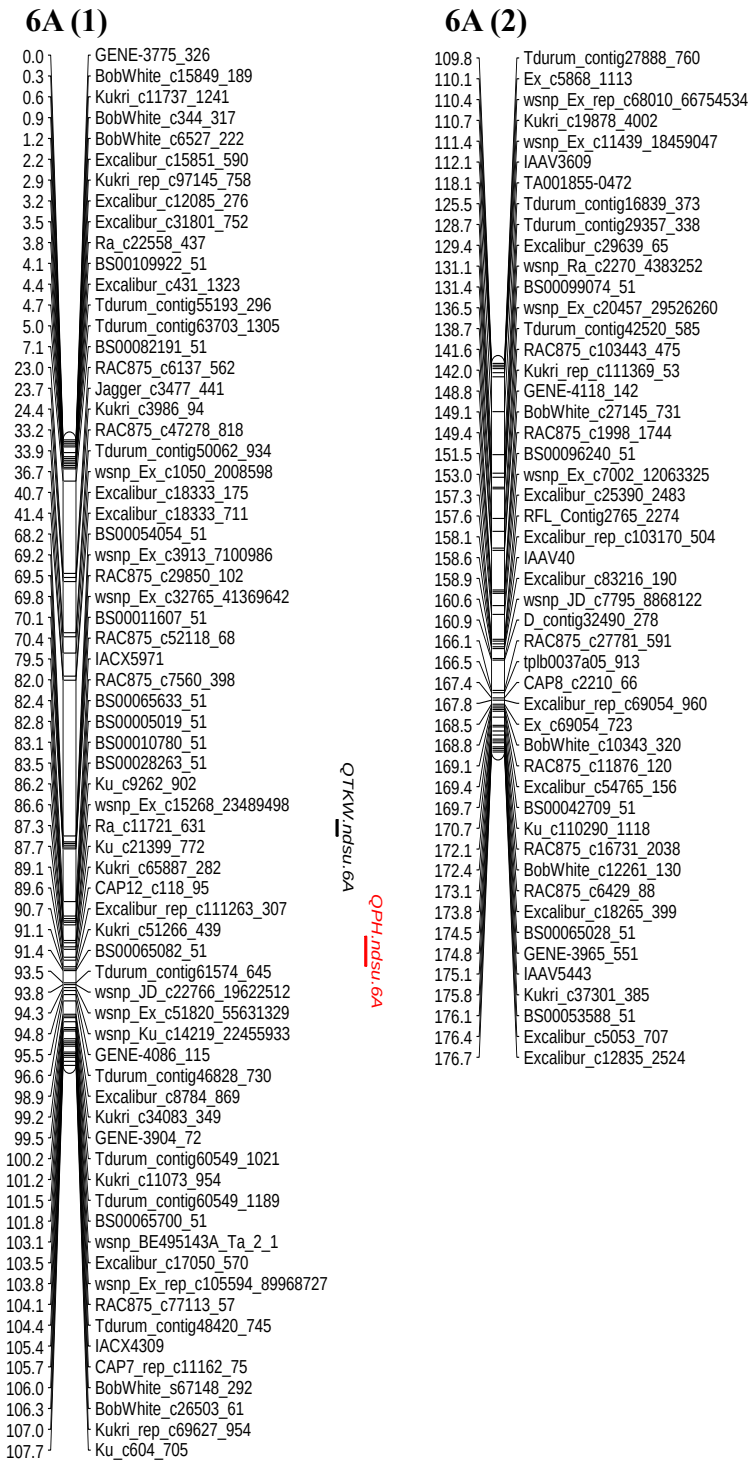


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

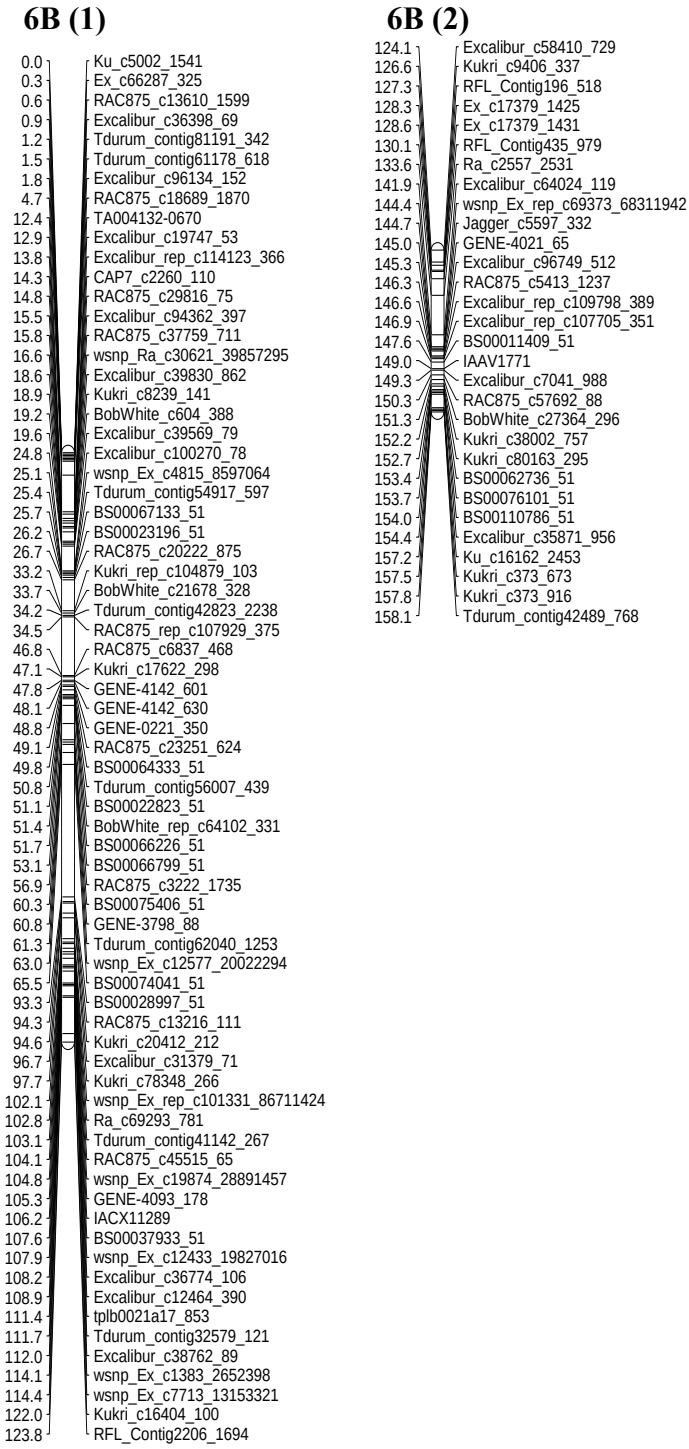


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

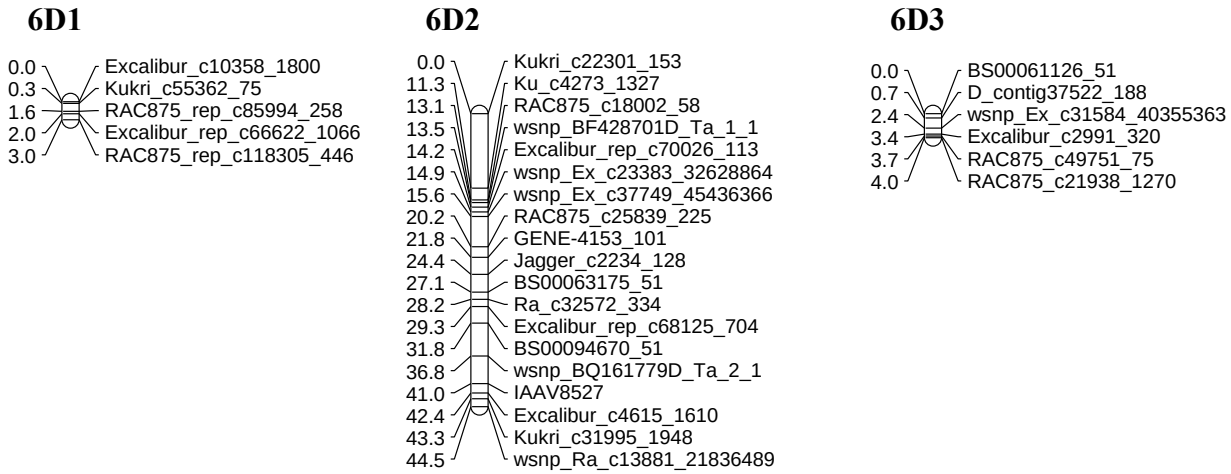


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

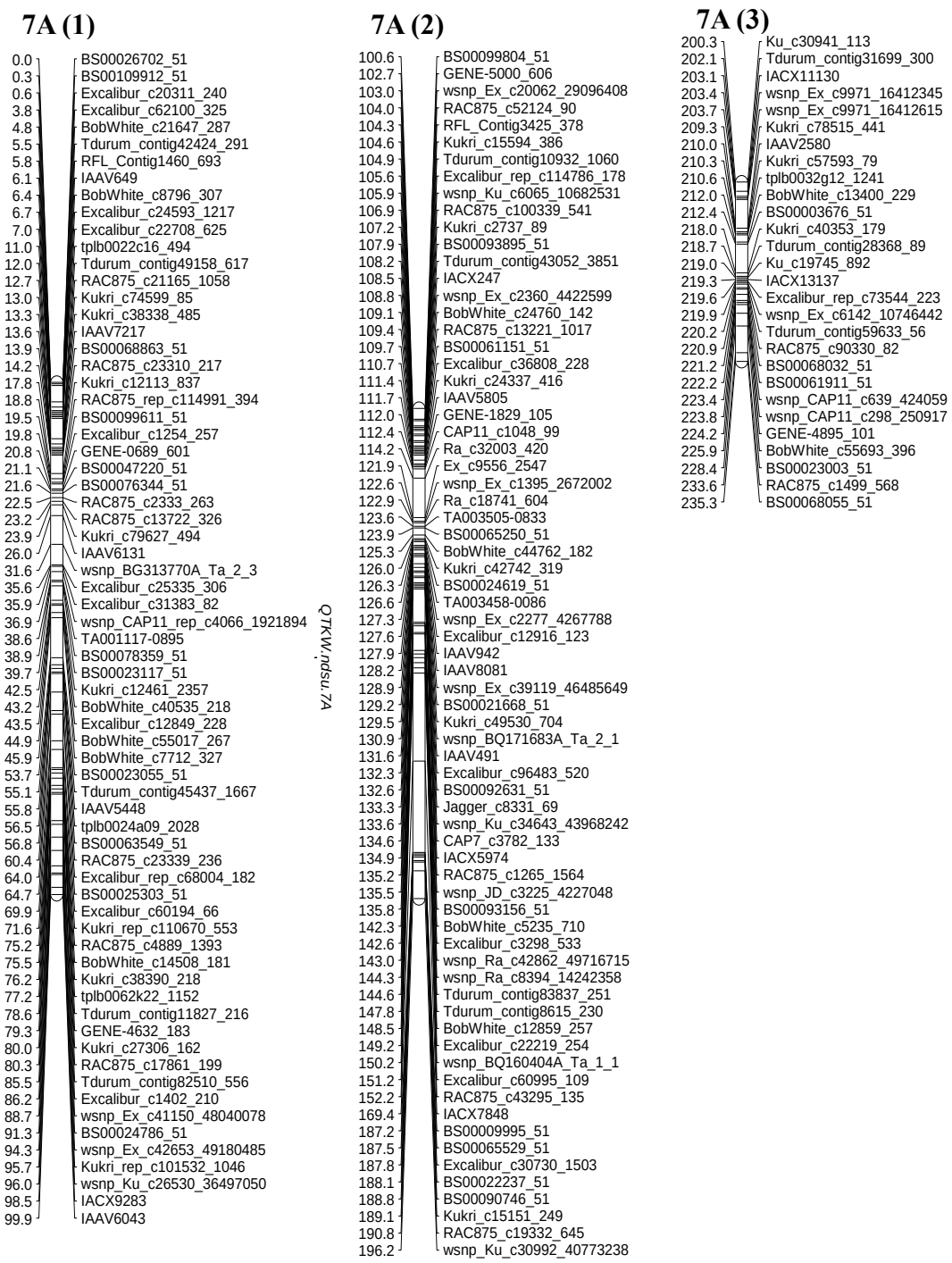


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

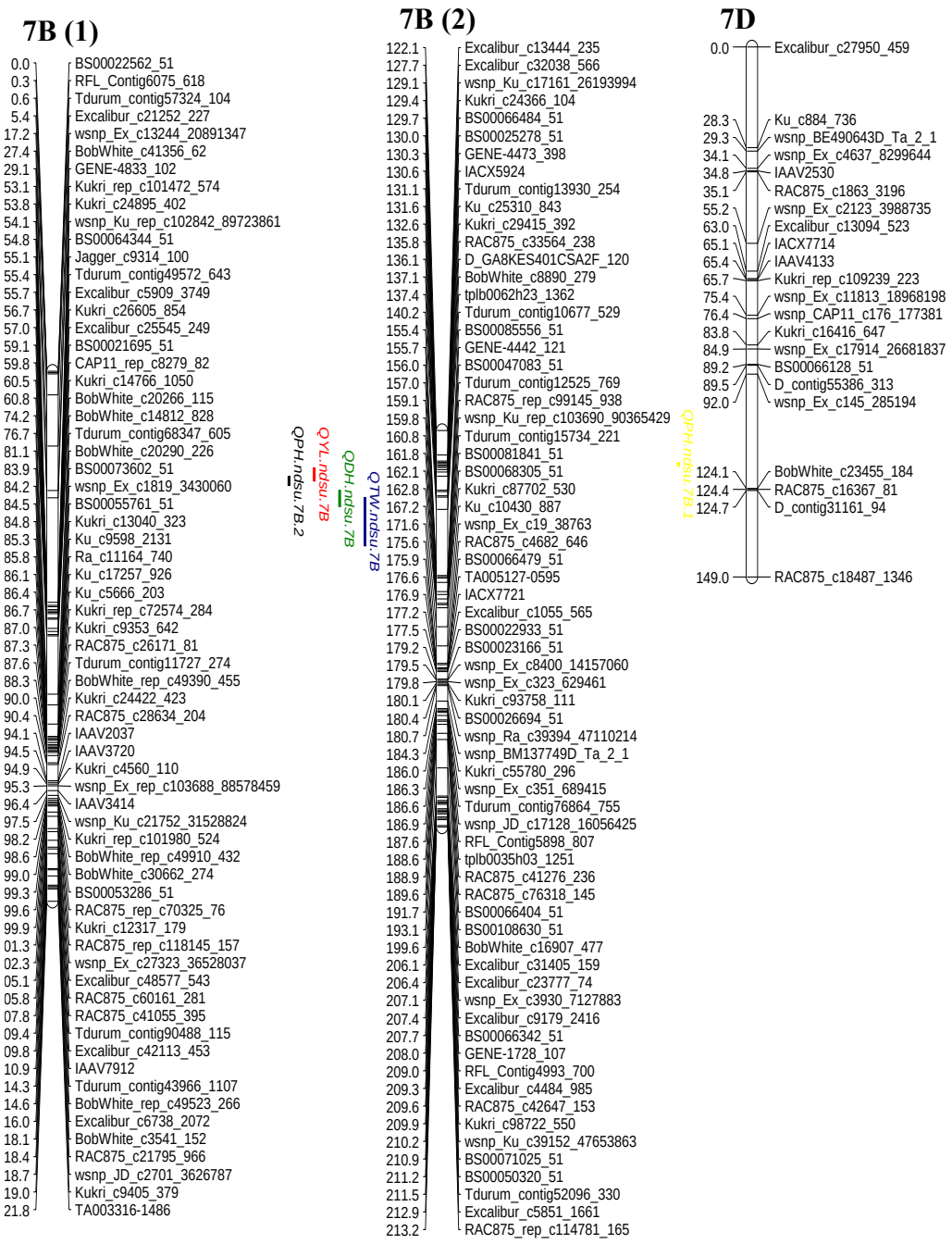


Fig. 3.2. Complete genetic linkage maps of the RIL population derived from the cross of Reeder and Albany. The location of the QTL associated with various agronomic traits is shown with a line on the right side of the linkage maps (continued).

3.5. Discussion

3.5.1. Linkage map

High-density single nucleotide polymorphism (SNP) genotyping arrays explore genomic diversity and marker-trait associations very efficiently (Wang et al., 2014). The Infinium iSelect 90K assay (Wang et al., 2014) uses > 81,000 gene-associated SNPs to assess polymorphism in allohexaploid and allotetraploid wheat populations (Wang et al., 2014; Wu et al., 2015; Kumar et al., 2016; Liu et al., 2016). Use of this genotyping tool offers a higher genome coverage and resolution in the dissection of wheat's agronomic traits than those used in previous studies (Kirigwi et al., 2007; Muchero et al., 2009; Sayed, 2011; Alexander et al., 2012; Ibrahim et al., 2012b; Kumar et al., 2012; Milner et al., 2016). The marker density (0.36cM/marker) or unique locus density (1.84 cM/locus) and genetic map length (3,793.1 cM) found in this study corresponded to the studies that used the 90K Infinium iSelect assay for genome mapping (Wang et al., 2014; Kumar et al., 2016). The A genome was found to be the longest, while the D genome was the shortest, which also corresponds with previous studies (Kumar et al., 2016). The marker order strongly corresponded with several linkage maps developed using the Infinium iSelect 90K SNP assay, as well (Desiderio et al., 2014; Russo et al., 2014; Wang et al., 2014; Kumar et al., 2016).

Four of the chromosomes (1D, 5B, 5D, and 6D) had more than one linkage group. Chromosome 5B had two, and chromosomes 1D, 5D, and 6D had three linkage groups. Probable reasons for the fragmentation could be the repeated elements that reside between gene-rich regions or the use of stringent mapping parameters (LOD score > 5 and distance < 40 cM) (Kumar et al., 2016). This fragmentation mostly occurred on the D-genome chromosomes as the Infinium iSelect 90K assay had a poor representation of the D genome (Wang et al., 2014).

Further, the D genome is the newest inclusion in the hexaploid wheat genome (dating to around 10,000 years ago) and exhibits fewer polymorphisms than the other genomes (Dubcovsky and Dvorak, 2007).

3.5.2. Use of secondary data to assess drought conditions

According to Lanceras et al. (2004), drought can be assessed by variables like weather conditions, soil moisture, and crop conditions over a particular growing season. Rainfall data, which impacts soil moisture, was collected to assess drought conditions for this study. It was obtained from the NDAWN database. The total amount of rainfall was collected from the date of planting to the date of plant physiological maturity. The date of physiological maturity was calculated by adding 30 days to DH (Simmons, 1914). The year 2012 had less rainfall than 2014 in all of the environments (the details are in the materials and methods section). Comparatively, the conditions in 2012 can be considered as drought, whereas, those in 2014 can be considered as normal. The yield data also support this categorization as all of the environments in 2012 had a smaller yield than in 2014.

3.5.3. Use of agronomic data to assess drought tolerance

Several studies suggested that drought tolerance can be incorporated into a breeding program most effectively by identifying QTL for YLD or YLD-related traits (Lanceras et al., 2004; Alexander et al., 2012). The agronomic traits used in this study are DH, PH, YLD, TW, and TKW. Yield is the trait of ultimate interest to breeders. In this study, YLD had a negative significant correlation with DH and a positive significant correlation with the rest of the traits. In general, more DH gives a plant the opportunity to produce more photosynthates (the product of photosynthesis) and hence a greater YLD. However, in this study, we observed that YLD was higher with reduced DH. This may be due to terminal drought in the experimental environments.

As snow is a major source of soil moisture in this region, and this soil moisture depletes with time. Therefore, the plants with more DH were affected by drought, which resulted in reduced YLD. Except for PH, increased values were desirable for the rest of the agronomic traits as they have a positive correlation with YLD. A bigger plant has the potential to produce more photosynthates, and therefore, should give more yield, but it often tends to lodge and compromises yield.

3.5.4. QTL for DH

The QTL for heading date has been identified in many studies (Kato et al., 1999; Sourdille et al., 2000; Shindo et al., 2003; Xu et al., 2005; Griffiths et al., 2009; Alexander et al., 2012; Kamran et al., 2013; Bogard et al., 2014; Zanke et al., 2014a; Guedira et al., 2016; Milner et al., 2016). According to these studies, the genetic factors controlling DH are vernalization sensitivity, photoperiod sensitivity, and earliness *per se* (Shindo et al., 2003). Generally, vernalization divides wheat cultivars into two groups. Winter wheat needs cold temperatures (vernalization) to initiate flowering, while spring wheat does not need cold temperatures. Wheat is usually photosensitive and a long-day plant. Therefore, ear emergence is very late unless a plant experiences long days. However, some genotypes can flower even with short days. On the other hand, earliness *per se* is the only environment-independent genetic factor controlling earliness (Shindo et al., 2003).

The present study revealed several major and minor QTL controlling the traits that confirm the quantitative nature of inheritance. Four major QTL (*QDH.ndsu.5A.3, QDH.ndsu.5D2, QDH.ndsu.7B,* and *QDH.ndsu.4A.2*) were found consistently in both drought and non-drought conditions. The earliness *per se* QTL *QEet.ocs.5A.2* (Kato et al., 1999) on chromosome 5AL and the QTL in this study,*QDH.ndsu.5A.3* at 205.71-208.31 cM, could occupy

the same location and represent the same QTL. The QTL *QDH.ndsu.4A.2* on chromosome 4A corresponded with the QTL reported by McCartney et al., (2005). However, a relatively minor QTL,*QDH.ndsu.4A.1,* was identified at 47.51 cM, which could be comparable to *QFlt.dms-4A.1* (Kamran et al., 2013). Sourdille et al. (2000) reported a QTL for earliness *per se* on chromosome 7BS, explaining 7.3 to 15.3% of PV, and the QTL identified in this study on chromosome 7B could represent the same QTL due to their sharing the same genomic region. Sourdille et al. (2000) reported a QTL on the long arm of chromosome 5D for earliness *per se*, which coincided with the QTL *QDH.ndsu.5D2* identified in this study.

3.5.5. QTL for YLD

Grain YLD is considered to be the most significant trait to plant breeders. It is the result of all the phases of vegetative and reproductive development. It is also influenced by edaphic and aerial environments (Quarrie et al., 2006). Yield QTL in wheat have been reported in several studies (McCartney et al., 2005; Quarrie et al., 2006; Kirigwi et al., 2007; Li et al., 2007, 2015; Maccaferri et al., 2008; Azadi et al., 2014; Cui et al., 2014; Edae et al., 2014; Narjesi et al., 2015; Gao et al., 2015; Milner et al., 2016). This study revealed six QTL for yield, both major and minor, indicating their quantitative nature of inheritance. The QTL *QYL.ndsu.2B* on chromosome 2B at 81.31-83.31 cM identified in all the drought-prone environments could be the same QTL (*QGy.ubo-2B*) that Milner et al. (2016) identified. This QTL can be called a drought-tolerant QTL as it contributed to YLD in all of the environments with less rainfall. Narjesi et al. (2015) reported a YLD QTL at 8.5 cM on chromosome 5D. However, the QTL *QYL.ndsu.5D2* identified in this study on the same chromosome, but at 11.91-12.91 cM on the second linkage group, seemed to be a different one. Considering the gaps between the linkage groups on the chromosome, the position of the QTL should be around the middle of the chromosome.

Maccaferri et al. (2008) identified a YLD QTL (*QYld.idw-7B*) at 0 cM on chromosome 7B that could be the same QTL as *QYL.ndsu.7B* identified at 22.21-25.21 cM on the same chromosome. The closest reported QTL of *QYL.ndsu.1B* on chromosome 1B at 64.21-71.91 cM was *QYd-1B.1,* identified on the same chromosome at 23-28 cM (Cui et al., 2014). The QTL *QYld.abrii-3B.4* (Azadi et al., 2014) identified on chromosome 3B at 92.3 cM seemed to be different than the QTL *QYL.ndsu.3B* in this study. Also, the QTL *QYL.ndsu.5D2* and *QYL.ndsu.5A* were most likely to be novel QTL as no reported QTL were found around their positions.

3.5.6. QTL for PH

Plant height is crucial in wheat breeding programs as it relates to lodging resistance and a high harvest index. For example, the dwarfism gene from Nonglin-10 played a vital role in wheat breeding programs during the Green Revolution of the 1960s (Liu et al., 2011). This study showed that PH had a positive correlation with DH, whereas DH had a negative correlation with YLD. Therefore, it could be stated that reduced PH is desirable for higher YLD. Quantitative trait loci for PH have been reported in several studies (McCartney et al., 2005; Pushpendra et al., 2007; Liu et al., 2011; Huang et al., 2012; Zanke et al., 2014b; Gao et al., 2015; Li et al., 2015; Narjesi et al., 2015; Milner et al., 2016; Singh et al., 2016). Eight QTL were identified in this study for PH, similar to Huang et al. (2012), who identified seven QTL for the trait. The QTL they identified on chromosomes 2D at 144 cM and 5B at 64.67 cM could represent the same QTL identified in this study on chromosome 2D at 151.11-165.71 cM (*QPH.ndsu.2D*) and on chromosome 5B at 32.41-33.21 cM (*QPH.ndsu.5B1*), respectively. The QTL *QPH.ndsu.2D* for PH was identified in two drought-prone environments, indicating its potential to tolerate drought. Milner et al. (2016) identified a QTL (*Qph.ubo-7B*) for PH on chromosome 7B at 138.4 cM, which could be same QTL (*QPH.ndsu.7B.1)* identified in this study on the same chromosome at

129.41-130.31 cM. This QTL was expressed in the drought-prone environments and thus could be useful for drought tolerance. Zanke et al. (2014b) identified a QTL for PH at 93.5 cM on chromosome 6A that could be comparable with this study's *QPH.ndsu.6A* at 85.51-90.61 cM on the same chromosome. This QTL was also identified in the two drought-prone environments. Zanke et al.(2014b) identified another QTL at 36 cM on chromosome 7B for the same trait that could be comparable to QTL *QPH.ndsu.7B.2* identified in this study on the same chromosome at 24.21-26.21 cM. They identified a QTL at 176.5 cM on chromosome 3B for PH, whereas this study identified a QTL at 184.31-187.71 cM for it on the same chromosome. They also identified a QTL at 117.2 cM on chromosome 2A, whereas this study identified the QTL *QPH.ndsu.2A* on the same chromosome at 128.41-133.11 cM.

3.5.7. QTL for TKW

Thousand kernel weight is one of the three major components of YLD; it is important for grain quality as larger and uniformly-sized kernels are visually attractive and command a higher market price (Ramya et al., 2010). Several studies have reported QTL related to wheat TKW (McCartney et al., 2005; Huang et al., 2006; Breseghello and Sorrells, 2007; Kuchel et al., 2007; Li et al., 2007, 2015; Zhang et al., 2008; Sun et al., 2009; Ramya et al., 2010; Azadi et al., 2014; Wei et al., 2014; Simmonds et al., 2014; Tadesse et al., 2015; Zanke et al., 2015). This study revealed eight QTL having both major and minor effects for the trait, indicating its quantitative nature of inheritance. McCartney et al. (2005) identified the QTL *QGwt.crc-2A* occupying the same position as the QTL *QTKW.ndsu.2A.* The QTL *qTgw2A* (Wei et al., 2014) and *QTgw.abrii-4A.2* (Zhang et al., 2008) also occupied the same location. The QTL *QTgw.abrii-2D1.3* (Azadi et al., 2014) and *QTKW.ndsu.2D.2* seemed to be the same QTL, occupying the same position on chromosome 2D. Also, the QTL *QTgw.abrii-4A.2* (Azadi et al., 2014) and *QTKW.ndsu.4A*

occupied the same location on chromosome 4A. The QTL *QTKW.ndsu.6A* was identified in all of the drought-prone environments, indicating its tolerance to drought; it occupied the same location as the QTL*qTgw6A2* (Wei et al., 2014). Another QTL, *QTKW.ndsu.7A,* was also identified in the two drought-prone environments and could be comparable to *qTgw7A* (Wei et al., 2014) due to their proximity. The QTL *QTKW.caas-1A.1* (Li et al., 2015) and *QTKW.ndsu.1A* were most likely to be the same QTL since they were found in the same genomic region. No reported QTL corresponded with the QTL *QTKW.ndsu.2D.1* and *QTKW.ndsu.5B1,* indicating the probability that they were novel QTL. The QTL *QTKW.ndsu.2D.1,* could be very important for drought-tolerance breeding as it was identified in two of the drought-prone environments.

3.5.8. QTL for TW

Test weight is an important trait to wheat breeders as it impacts flour yield during milling (Rustgi et al., 2013). Quantitative trait loci for TW were reported in several studies (McCartney et al., 2005, 2007; Huang et al., 2006; Narasimhamoorthy et al., 2006; Breseghello and Sorrells, 2007; Kuchel et al., 2007; Zhang et al., 2008; Sun et al., 2009; Rustgi et al., 2013; Hill et al., 2015; Tadesse et al., 2015). This study revealed seven QTL with both major and minor effects, indicating their quantitative nature of inheritance. The QTL identified in this study on chromosome 7B (*QTW.ndsu.7B)* at 29.11-40.11 cM was identified in all of the drought-prone environments, indicating its potential for drought tolerance. This QTL seemed to be the same QTL Sun et al. (2009) identified (*QTw.sdau-7B*). McCartney et al. (2005) identified a QTL, *QTwt.crc-2B,* linked with the marker *Xbarc183* at 96.7 cM on chromosome 7B that, according to the GrainGenes database, seemed to be the same as the QTL *QTW.ndsu.2B* identified in this study at 84.31-95.61 cM. This QTL was identified in two of the drought-prone environments.

McCartney et al. (2005) identified another QTL, (*QTwt.crc-5D*), between SSR markers *Xgdm63–Xwmc765* and positioned between 95-214.26 cM, according to the GrainGenes database. The QTL in this study, *QTW.ndsu.5D2,* could be the same as their QTL as it is also located in the same genomic region. The nearest reported QTL to *QTW.ndsu.5A* was *QTw.hwwgr-5AS* (Li et al., 2016), which seemed to be a different QTL. The QTL *QTw.sdau-2A* (Sun et al., 2009) located between SSR markers *Xwmc181a-Xubc840c* seemed to be the same QTL as the QTL *QTW.ndsu.2A.2* identified in this study. No reported QTL corresponded with the QTL *QTW.ndsu.4A.*

3.5.9. Pleiotropic QTL

The associations between traits in correlation studies could be justified by the co-localized or pleiotropic QTL (Table 3.4). These co-localized QTL could be of great value to breeders if the desirable alleles come from the same parent. Desirable alleles from three genomic regions (7, 20, and 25) came from parent Albany (Table 3.6; Fig.3.2). These QTL primarily have a major effect on YLD and YLD-related traits, making them even more important to breeders. The parent Reeder contributed all of the desirable alleles in three genomic regions (13, 17, and 24) (Table 3.6; Fig.3.2). Most of these QTL also had the major effect on YLD and YLD-related traits. The remaining co-localized QTL from three genomic regions did not contain desirable alleles from the same parents.

3.5.10. QTL for drought tolerance

The QTL identified on chromosome 7B (*QTW.ndsu.7B)* at 29.11-40.11 cM seemed to have drought tolerance as it was identified in all of the environments with drought conditions (Table 3.7). This QTL seemed to be the same QTL that Sun et al. (2009) identified (*QTw.sdau-7B*). The putative drought-tolerant QTL, *QYL.ndsu.7B,* was identified very close to another

major QTL, *QTW.ndsu.7B,* which also had drought tolerance, indicating the potential of this genomic region to control drought tolerance. This finding corresponds with Alexander et al. (2012), who found a QTL, *Qdt.ksu-7B,* located on chromosome 7B at 34.7 cM with significant drought tolerance. Another putative major QTL, *QYL.ndsu.2B,* corresponded with the QTL *QCrs-* (Ibrahim et al., 2012a), which was reported to deteriorate the trait of interest under both drought and control conditions. In the current study, however, the QTL was identified only in the environments with drought conditions. The QTL *QDH.ndsu.5A.2* occupied the same location as the QTL *QHea+* (Ibrahim et al., 2012b). In the latter study, the QTL *QHea+* improved the trait of interest in both well-watered and drought conditions. However, in the current study, *QDH.ndsu.5A.2* improved the trait of interest only under drought conditions. Ibrahim et al. (2012a) reported four QTL on chromosome 2D around 50 cM that improved the trait of interest under drought conditions. However, none of these reported QTL seemed to correspond with the QTL *QTKW.ndsu.2D.1* identified in this study.

The QTL for DH, *QDH.ndsu.5A.3*, could be a constitutive QTL for drought tolerance since it was identified consistently in both drought and non-drought condition environments. This QTL could occupy the same genomic region as the earliness *per se* QTL, *QEet.ocs.5A.2* (Kato et al., 1999). Another constitutive QTL for drought tolerance, *QDH.ndsu.5D2*, corresponded with a QTL for earliness *per se* located on the long arm of chromosome 5D (Sourdille et al., 2000). A constitutive QTL for drought tolerance through TKW was identified on chromosome 6A, which most likely represents the QTL *qTgw6A2* (Wei et al., 2014). Also, a constitutive drought-tolerant QTL, *QTW.ndsu.2B,* was identified for TW, which could be the same QTL as *QTwt.crc-2B* (McCartney et al., 2005).

3.6. Conclusions

Understanding the genetic basis of drought tolerance in wheat is of immense value for developing drought-tolerant wheat varieties. In this study, a high-density SNP-based genetic map was developed and used to elucidate the genetic factors involved in the control of drought tolerance in HRSW in the northern USA. Secondary data were used to assess drought conditions, and agronomic data on YLD and related traits were used to determine the QTL associated with drought tolerance.

Nine QTL for DH, eight QTL for PH, seven QTL for TW, eight QTL for TKW, and six QTL for YLD were identified in this study. Among these, 11 consistent QTL important for drought tolerance were identified; these included six QTL exclusively for drought environments and five constitutive QTL. The QTL identified on chromosomes 7B, 2B, 5A, 5D, and 6A had the greatest effect on drought tolerance. One novel QTL for drought tolerance was identified on chromosome 2D.

The closely-linked markers associated with the major QTL identified in this study could be immensely valuable in marker-assisted breeding programs aimed at improving drought tolerance in wheat. The high-density maps that were developed also offer a better starting platform for the fine mapping and ultimately map-based cloning of major and stable loci identified in this study. Further studies directed towards cloning these important QTL will help breeders to gain a greater understanding of the traits studied. More importantly, desirable alleles for several major loci were found to be contributed by the parent that was apparently susceptible to drought. This event suggests the potentiality of exploring drought susceptible germplasms in the development of drought-tolerant cultivars.

3.7. References

Alexander, L.M., F.M. Kirigwi, A.K. Fritz, and J.P. Fellers. 2012. Mapping and quantitative trait loci analysis of drought tolerance in a Spring wheat population using amplified fragment length polymorphism and diversity array technology markers. Crop Sci. 52(1): 253–261.

Anderson, J.A., G.L. Linkert, R.H. Busch, J.J. Wiersma, J.A. Kolmer, Y. Jin, R. Dill-Macky, J. V. Wiersma, G.A. Hareland, and D. V. McVey. 2009. Registration of "RB07" Wheat. J. Plant Regist. 3(2): 175. Available at https://www.crops.org/publications/jpr/abstracts/3/2/175.

Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C3 cereals: What should we breed for? Ann. Bot. 89(SPEC. ISS.): 925–940.

Araus, J.L., G. A. Slafer, C. Royo, and M.D. Serret. 2008. Breeding for yield potential and stress adaptation in cereals. CRC. Crit. Rev. Plant Sci. 27(6): 377–412.

Azadi, A., M. Mardi, E.M. Hervan, S.A. Mohammadi, F. Moradi, M.T. Tabatabaee, S.M. Pirseyedi, M. Ebrahimi, F. Fayaz, M. Kazemi, S. Ashkani, B. Nakhoda, and G. Mohammadi-Nejad. 2014. QTL mapping of yield and yield components under normal and salt-stress conditions in bread wheat (Triticum aestivum L.). Plant Mol. Biol. Report. (April 2014).

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Boca Raton, FL.

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 56: 1159–1168. Available at www.publish.csiro.au/journals/ajar.

Bogard, M., C. Ravel, E. Paux, J. Bordes, F. Balfourier, S.C. Chapman, J. Le Gouis, and V. Allard. 2014. Predictions of heading date in bread wheat (Triticum aestivum L.) using QTL-based parameters of an ecophysiological model. J. Exp. Bot. 65(20): 5849–5865.

Breseghello, F., and M.E. Sorrells. 2007. QTL analysis of kernel size and shape in two hexaploid wheat mapping populations. F. Crop. Res. 101(2): 172–179.

Cui, F., X. Fan, C. Zhao, W. Zhang, M. Chen, and J. Li. 2014. A novel genetic map of wheat: Utility for mapping QTL for yield under different nitrogen treatments. BMC Genet. 15: 57–73.

Desiderio, F., D. Guerra, D. Rubiales, L. Piarulli, M. Pasquini, A.M. Mastrangelo, R. Simeone, A. Blanco, L. Cattivelli, and G. Vale. 2014. Identification and mapping of quantitative trait loci for leaf rust resistance derived from a tetraploid wheat Triticum dicoccum accession. Mol. Breed. 34(4): 1659–1675.

Dubcovsky, J., and J. Dvorak. 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316 (5833): 1862–1866. Available at http://science.sciencemag.org/content/316/5833/1862.abstract.

Edae, E.A., P.F. Byrne, S.D. Haley, M.S. Lopes, and M.P. Reynolds. 2014. Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. Theor. Appl. Genet. 127(4): 791–807.

Frazen, D.W. 2003. North Dakota soil and fertilizer handbook. NDSU Extension Service, North Dakota State University, Fargo, ND.

Frohberg, R.C., R.W. Stack, T. Olson, J.D. Miller, and M. Mergoum. 2006. Registration of "Alsen." Crop Sci. 46: 2311–2312.

Gao, F., W. Wen, J. Liu, A. Rasheed, G. Yin, X. Xia, X. Wu, and Z. He. 2015. Genome-wide linkage mapping of QTL for yield components, plant height and yield-related physiological traits in the Chinese wheat cross Zhou 8425B/Chinese Spring. Front. Plant Sci. 6(December): 1099. Available at http://www.ncbi.nlm.nih.gov/pubmed/26734019\nhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4683206.

de Givry, S., M. Bouchez, P. Chabrier, D. Milan, and T. Schiex. 2005. CARTHAGENE: Multipopulation integrated genetic and radiated hybrid mapping. Bioinformatics 21: 1703–1704.

Griffiths, S., J. Simmonds, M. Leverington, Y. Wang, L. Fish, L. Sayers, L. Alibert, S. Orford, L. Wingen, L. Herry, S. Faure, D. Laurie, L. Bilham, and J. Snape. 2009. Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. Theor. Appl. Genet. 119(3): 383–395.

Guedira, M., M. Xiong, Y.F. Hao, J. Johnson, S. Harrison, D. Marshall, and G. Brown-Guedira. 2016. Heading date QTL in winter wheat (Triticum aestivum L.) coincide with major developmental genes VERNALIZATION1 and PHOTOPERIOD1. PLoS One 11(5): e0154242. Available at http://dx.plos.org/10.1371/journal.pone.0154242.

Hill, C.B., J.D. Taylor, J. Edwards, D. Mather, P. Langridge, A. Bacic, and U. Roessner. 2015. Detection of QTL for metabolic and agronomic traits in wheat with adjustments for variation at genetic loci that affect plant phenology. Plant Sci. 233: 143–154. Available at http://dx.doi.org/10.1016/j.plantsci.2015.01.008.

Huang, X.Q., S. Cloutier, L. Lycar, N. Radovanovic, D.G. Humphreys, J.S. Noll, D.J. Somers, and P.D. Brown. 2006. Molecular detection of QTLs for agronomic and quality traits in a

doubled haploid population derived from two Canadian wheats (Triticum aestivum L.). Theor. Appl. Genet. 113(4): 753–766.

Huang, B.E., A.W. George, K.L. Forrest, A. Kilian, M.J. Hayden, M.K. Morell, and C.R. Cavanagh. 2012. A multiparent advanced generation inter-cross population for genetic analysis in wheat. Plant Biotechnol. J. 10(7): 826–839.

Ibrahim, S.E., A. Schubert, K. Pillen, and J. Léon. 2012a. QTL analysis of drought tolerance for seedling root morphological traits in an advanced backcross population of spring wheat. 2(July): 619–629.

Ibrahim, S.E., A. Schubert, K. Pillen, and J. Léon. 2012b. Comparison of QTLs for drought tolerance traits between two advanced backcross populations of spring wheat. Int. J. AgriScience 2(3): 216–227.

Kamran, A., M. Iqbal, A. Navabi, H. Randhawa, C. Pozniak, and D. Spaner. 2013. Earliness per se QTLs and their interaction with the photoperiod insensitive allele Ppd-D1a in the Cutler × AC Barrie spring wheat population. Theor. Appl. Genet. 126(8): 1965–1976.

Kato, K., H. Miura, and S. Sawada. 1999. Detection of an earliness per se quantitative trait locus in the proximal region of wheat chromosome 5AL. Plant Breed. 118(5): 391–394.

Kirigwi, F.M., M. Van Ginkel, G. Brown-Guedira, B.S. Gill, G.M. Paulsen, and A.K. Fritz. 2007. Markers associated with a QTL for grain yield in wheat under drought. Mol. Breed. 20(4): 401–413.

Kosambi, D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12: 172–175.

Kuchel, H., K.J. Williams, P. Langridge, H.A. Eagles, and S.P. Jefferies. 2007. Genetic dissection of grain yield in bread wheat. I. QTL analysis. Theor. Appl. Genet. 115(8): 1029–1041.

Kumar, A., E.E. Mantovani, R. Seetan, A. Soltani, M. Echeverry-Solarte, S. Jain, S. Simsek, D. Doehlert, M.S. Alamri, E.M. Elias, S.F. Kianian, and M. Mergoum. 2016. Dissection of genetic factors underlying wheat kernel shape and size in an Elite × Nonadapted cross using a high density SNP linkage map. Plant Genome 9(1): 1-22. Available at https://dl.sciencesocieties.org/publications/tpg/abstracts/9/1/plantgenome2015.09.0081.

Kumar, S., S.K. Sehgal, U. Kumar, P.V.V. Prasad, A.K. Joshi, and B.S. Gill. 2012. Genomic characterization of drought tolerance-related traits in spring wheat. Euphytica 186(1): 265–276.

Lanceras, J., G. Pantuwan, B. Jongdee, and T. Toojinda. 2004. Quantitative trait loci associated with drought tolerance at reproductive stage in rice. Plant Physiology 135(May): 384–399. Available at http://www.plantphysiol.org/content/135/1/384.short.

Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199.

Lanning, S.P., G.R. Carlson, P.F. Lamb, D. Nash, D.M. Wichman, and K.D. Kephart. 2011. Registration of "Duclair" hard red spring wheat. J. Plant Regist. 5: 349–352.

Li, C., G. Bai, S. Chao, B. Carver, and Z. Wang. 2016. Single nucleotide polymorphisms linked to quantitative trait loci for grain quality traits in wheat. Cj 4: 1–11. Available at http://dx.doi.org/10.1016/j.cj.2015.10.002.

Li, S., J. Jia, X. Wei, X. Zhang, L. Li, H. Chen, Y. Fan, H. Sun, X. Zhao, T. Lei, Y. Xu, F. Jiang, H. Wang, and L. Li. 2007. An intervarietal genetic map and QTL analysis for yield traits in wheat. Mol. Breed. 20(2): 167–178.

Li, X., X. Xia, Y. Xiao, Z. He, D. Wang, R. Trethowan, H. Wang, and X. Chen. 2015. QTL mapping for plant height and yield components in common wheat under water limited and full irrigation environments. Crop Pasture Sci. 8: 660–670. Available at http://www.publish.csiro.au/view/journals/dsp_journals_pip_abstract_Scholar1.cfm?nid=40&pip=CP14236.

Liu, S., S.O. Assanga, S. Dhakal, X. Gu, C.T. Tan, Y. Yang, J. Rudd, D. Hays, A.M.H. Ibrahim, Q. Xue, S. Chao, R. Devkota, C. Shachter, T. Huggins, S. Mohammed, and M.P. Fuentealba. 2016. Validation of chromosomal locations of 90K array single nucleotide polymorphisms in US wheat. Crop Sci. 56: 364-373.

Liu, G., S.B. Xu, Z.F. Ni, C.J. Xie, D.D. Qin, J. Li, L.H. Lu, J.P. Zhang, H.R. Peng, and Q.X. Sun. 2011. Molecular dissection of plant height QTLs using recombinant inbred lines from hybrids between common wheat (Triticum aestivum L.) and spelt wheat (Triticum spelta L.). Chinese Sci. Bull. 56(18): 1897–1903.

Maccaferri, M., M.C. Sanguineti, S. Corneti, J.L.A. Ortega, M. Ben Salem, J. Bort, E. DeAmbrogio, L.F.G. Del Moral, A. Demontis, A. El-Ahmed, F. Maalouf, H. Machlab, V. Martos, M. Moragues, J. Motawaj, M. Nachit, N. Nserallah, H. Ouabbou, C. Royo, A. Slama, and R. Tuberosa. 2008. Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178(1): 489–511.

Malik, S., T.A. Malik, and G. Engineering. 2015. Genetic mapping of potential Qtls associated with drought tolerance in wheat. 25(4): 1032–1040.

McCartney, C.A., D.J. Somers, G. Fedak, R.M. DePauw, J. Thomas, S.L. Fox, D.G. Humphreys, O. Lukow, M.E. Savard, B.D. McCallum, J. Gilbert, and W. Cao. 2007. The evaluation of FHB resistance QTLs introgressed into elite Canadian spring wheat germplasm. Mol. Breed. 20(3): 209–221.

McCartney, C.A., D.J. Somers, D.G. Humphreys, O. Lukow, N. Ames, J. Noll, S. Cloutier, and B.D. McCallum. 2005. Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452x'AC Domain.' Genome 48(5): 870–883. Available at http://www.ncbi.nlm.nih.gov/pubmed/16391693.

Mergoum, M., R. Frohberg, J. Rasmussen, T.L. Friesen, G.A. Hareland, and S. Simsek. 2009. Breeding for CLEARFIELD herbicide tolerance: Registration of "ND901CL" spring wheat. J. Plant Regist. 3: 170–174.

Mergoum, M., R.C. Frohberg, R.W. Stack, T. Olson, T.L. Friesen, and J.B. Rasmussen. 2006. Registration of "Glenn" wheat. Crop Sci.: 46:473–474.

Mergoum, M., R.C. Frohberg, R.W. Stack, J.W. Rasmussen, and T.L. Friesen. 2008. Registration of "Faller" spring wheat. J. Plant Regist. 2(3): 224–229. Available at <Go to ISI>://CCC:000268932600013\nhttp://www.crops.org.

Mergoum, M., R.C. Frohberg, R.W. Stack, S. Simsek, T.B. Adhikari, J.W. Rasmussen, M.S. Alamri, P.K. Singh, and T.L. Friesen. 2013. "Prosper": A high-yielding hard red spring wheat cultivar adapted to the North Central Plains of the USA. J. Plant Regist. 7: 75–80.

Mergoum, M., R.C. Frohberg, J.D. Miller, T. Olson, and J.B. Rasmussen. 2005a. Registration of "Dapps" wheat. Crop Sci. 45: 420–421.

Mergoum, M., R.C. Frohberg, J.D. Miller, and R. Stack. 2005b. Registration of "Steele-ND" wheat. Crop Sci. 45: 1163–1164.

Mergoum, M., S. Simsek, S. Zhong, M. Acevedo, T.L. Friesen, P.K. Singh, T.B. Adhikari, M.S. Alamri, and R.C. Frohberg. 2014. "Velva" spring wheat: An adapted cultivar to North-Central Plains of the United States with high agronomic and quality performance. J. Plant Regist. 8(1): 32. Available at https://www.crops.org/publications/jpr/abstracts/8/1/32.

Milner, S.G., M. Maccaferri, B.E. Huang, P. Mantovani, A. Massi, E. Frascaroli, R. Tuberosa, and S. Salvi. 2016. A multiparental cross population for mapping QTL for agronomic traits in durum wheat (Triticum turgidum ssp. durum). Plant Biotechnol. J. 14(2): 735–48. Available at http://www.ncbi.nlm.nih.gov/pubmed/26132599.

Muchero, W., J.D. Ehlers, T.J. Close, and P.A. Roberts. 2009. Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [Vigna unguiculata (L.) Walp.]. Theor. Appl. Genet. 118(5): 849–863.

Narasimhamoorthy, B., B.S. Gill, A.K. Fritz, J.C. Nelson, and G.L. Brown-Guedira. 2006. Advanced backcross QTL analysis of a hard winter wheat x synthetic wheat population. Theor. Appl. Genet. 112(5): 787–796.

Narjesi, V., M. Mardi, E.M. Hervan, and A. Azadi. 2015. Analysis of quantitative trait loci (QTL) for grain yield and agronomic traits in wheat (*Triticum aestivum* L.) Under normal and salt- stress conditions. Plant Mol Biol 33:2030–2040.

Peleg, Z., T. Fahima, T. Krugman, S. Abbo, D. Yakir, A.B. Korol, and Y. Saranga. 2009. Genomic dissection of drought resistance in durum wheat × wild emmer wheat recombinant inbreed line population. Plant, Cell Environ. 32(7): 758–779.

Pfeiffer, W.H., R.M. Trethowan, M.V. Ginkel, M.I. Ortiz, and S. Rajaram. 2005. Breeding for abiotic stress tolerance in wheat. pp. 401–489. *In* Harris, M.A. and P.J.C. (eds.), Abiotic stresses: Plant resistance through breeding and molecular approaches. The Haworth Press, New York, NY.

Pinter, J.P.J., G. Zipoli, R.J. Reginato, R.D. Jackson, and S.B. Idso. 1990. Canopy temperature as an indicator of differential water use and yield performance among wheat cultivars. Agric. Water Manag: 18: 35–48.

Pushpendra, K.G., S.B. Harindra, L.K. Pawan, K. Neeraj, K. Ajay, R.M. Reyazul, M. Amita, and K. Jitendra. 2007. QTL analysis for some quantitative traits in bread wheat. J. Zhejiang Univ. Sci. B 8(11): 807–14. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2064952&tool=pmcentrez&rendertype=abstract.

Quarrie, S.A., S. Pekic Quarrie, R. Radosevic, D. Rancic, A. Kaminska, J.D. Barnes, M. Leverington, C. Ceoloni, and D. Dodig. 2006. Dissecting a wheat QTL for yield present in a range of environments: From the QTL to candidate genes. J. Exp. Bot. 57(11): 2627–2637.

Ramya, P., A. Chaubal, K. Kulkarni, L. Gupta, N. Kadoo, H.S. Dhaliwal, P. Chhuneja, M. Lagu, and V. Gupta. 2010. QTL mapping of 1000-kernel weight, kernel length, and kernel width in bread wheat (Triticum aestivum L.). J. Appl. Genet. 51(4): 421–429.

Russo, M.A., D.B.M. Ficco, G. Laidò, D. Marone, R. Papa, A. Blanco, A. Gadaleta, P. De Vita, and A.M. Mastrangelo. 2014. A dense durum wheat × T. dicoccum linkage map based on SNP markers for the study of seed morphology. Mol. Breed. 34(4): 1579–1597.

Rustgi, S., M.N. Shafqat, N. Kumar, P.S. Baenziger, M.L. Ali, I. Dweikat, B.T. Campbell, and K.S. Gill. 2013. Genetic dissection of yield and its component traits using high-density composite map of wheat chromosome 3A: Bridging gaps between QTLs and underlying genes. PLoS One 8(7).

Sayed, M.A.E.A.E. 2011. QTL analysis for drought tolerance related to root and shoot traits in barley (Hordeum vulgare L.). PhD Thesis Bonn Universitäts-und Landesbibliothek Bonn: 165.

Shindo, C., H. Tsujimoto, and T. Sasakuma. 2003. Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity (Edinb.). 90(1): 56–63.

Simmonds, J., P. Scott, M. Leverington-Waite, A.S. Turner, J. Brinton, V. Korzun, J. Snape, and C. Uauy. 2014. Identification and independent validation of a stable yield and thousand grain weight QTL on chromosome 6A of hexaploid wheat (Triticum aestivum L.). BMC Plant Biol. 14: 191. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4105860&tool=pmcentrez&rendertype=abstract.

Simmons, S.R., E.A. Oelke, and P.M. Anderson. 1914. Growth and development guide for spring wheat. Agricultral Extension Service University of Minnesota, AG-FO-2547.

Singh, A., R.E. Knox, R.M. DePauw, A.K. Singh, R.D. Cuthbert, S. Kumar, and H.L. Campbell. 2016. Genetic mapping of common bunt resistance and plant height QTL in wheat. Theor. Appl. Genet. 129(2): 243–256. Available at "http://dx.doi.org/10.1007/s00122-015-2624-8.

Sourdille, P., J.W. Snape, T. Cadalen, G. Charmet, N. Nakata, S. Bernard, and M. Bernard. 2000. Detection of QTLs for heading time and photoperiod response in wheat using a doubled-

haploid population. Genome 43(3): 487–494. Available at http://www.nrcresearchpress.com/doi/abs/10.1139/g00-013\nhttp://www.nrcresearchpress.com/doi/abs/10.1139/g00-013#.U80ianKYNko\nhttp://www.nrcresearchpress.com/doi/pdfplus/10.1139/g00-013.

Sun, X.Y., K. Wu, Y. Zhao, F.M. Kong, G.Z. Han, H.M. Jiang, X.J. Huang, R.J. Li, H.G. Wang, and S.S. Li. 2009. QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. Euphytica 165(3): 615–624.

Tadesse, W., F.C. Ogbonnaya, A. Jighly, M. Sanchez-Garcia, Q. Sohail, S. Rajaram, and M. Baum. 2015. Genome-wide association mapping of yield and grain quality traits in winter wheat genotypes. PLoS One 10(10): 1–18.

Turner, N.C. 1979. Drought resistance and adaptation to water deficits in crop plants. pp. 343–372. *In* H. Mussell, C.R.S. (ed.), Stress physiology in crop plants. John Wiley & Sons, New York, NY.

Turner, N.C., G.C. Wright, and K.H.M. Siddique. 2001. Adaptation of grain legumes (pulses) to water-limited environments. Adv. Agron. 71(September 2016): 193–231.

Vocke, G., and M. Ali. 2013. U.S. wheat production practices, costs, and yields: Variations across regions. Economic Information Bulletin(116). Economic Research Service, United States Department of Agriculture.

Voorrips, R. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered 93: 77–78.

Wang, S., C.J. Basten, and Z.B. Zeng. 2012. Windows QTL Cartographer 2.5_011.

Wang, S., D. Wong, K. Forrest, A. Allen, S. Chao, B.E. Huang, M. Maccaferri, S. Salvi, S.G. Milner, L. Cattivelli, A.M. Mastrangelo, A. Whan, S. Stephen, G. Barker, R. Wieseke, J.

Plieske, M. Lillemo, D. Mather, R. Appels, R. Dolferus, G. Brown-Guedira, A. Korol, A.R. Akhunova, C. Feuillet, J. Salse, M. Morgante, C. Pozniak, M.C. Luo, J. Dvorak, M. Morell, J. Dubcovsky, M. Ganal, R. Tuberosa, C. Lawley, I. Mikoulitch, C. Cavanagh, K.J. Edwards, M. Hayden, and E. Akhunov. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol. J. 12(6): 787–796.

Wei, L., S. Bai, J. Li, X. Hou, X. Wang, H. Li, B. Zhang, W. Chen, D. Liu, B. Liu, and H. Zhang. 2014. QTL positioning of thousand wheat grain weight in Qaidam Basin. (June): 239–244.

Wu, Q.H., Y.X. Chen, S.H. Zhou, L. Fu, J.J. Chen, Y. Xiao, D. Zhang, S.H. Ouyang, X.J. Zhao, Y. Cui, D.Y. Zhang, Y. Liang, Z.Z. Wang, J.Z. Xie, J.X. Qin, G.X. Wang, D.L. Li, Y.L. Huang, M.H. Yu, P. Lu, L.L. Wang, L. Wang, H. Wang, C. Dang, J. Li, Y. Zhang, H.R. Peng, C.G. Yuan, M.S. You, Q.X. Sun, J.R. Wang, L.X. Wang, M.C. Luo, J. Han, and Z.Y. Liu. 2015. High-density genetic linkage map construction and QTL mapping of grain shape and size in the wheat population Yanda1817 x Beinong6. PLoS One. 10 (2): 1-17.

Xu, X., G. Bai, B.F. Carver, and G.E. Shaner. 2005. A QTL for early heading in wheat cultivar Suwon 92. Euphytica 146(3): 233–237.

Zanke, C., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze, S. Beier, M.W. Ganal, and M.S. Röder. 2014a. Genetic architecture of main effect QTL for heading date in European winter wheat. Front. Plant Sci. 5(May): 217. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4033046&tool=pmcentrez&rendertype=abstract.

Zanke, C.D., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze, F. Neumann, A. Eichhorn, A. Polley, C. Jaenecke, M.W. Ganal, and M.S. Röder. 2015. Analysis of main effect QTL for thousand grain weight in European winter wheat (Triticum aestivum L.) by genome-wide association mapping. Front. Plant Sci. 6(September): 1–14. Available at http://journal.frontiersin.org/article/10.3389/fpls.2015.00644.

Zanke, C.D., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze, K. Neumann, M.W. Ganal, and M.S. Röder. 2014b. Whole genome association mapping of plant height in winter wheat (Triticum aestivum L). PLoS One 9(11).

Zhang, W., S. Chao, F. Manthey, O. Chicaiza, J.C. Brevis, V. Echenique, and J. Dubcovsky. 2008. QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. Theor. Appl. Genet. 117(8): 1361–1377.

CHAPTER 4. IDENTIFYING QTL FOR DROUGHT TOLERANCE IN SPRING WHEAT IN THE NORTHERN USA USING A GREENHOUSE EXPERIMENT

4.1. Abstract

Drought (water stress) is one of the major threats for wheat (*Triticum aestivum* L.) production in the northern USA, which necessitates the development and release of drought-tolerant cultivars. This study attempts to dissect the genetics of drought tolerance in spring wheat in the northern USA using QTL analysis. A population of 149 RILs was developed from a drought-tolerant cultivar, 'Reeder' (PI613586), and a drought-susceptible cultivar, 'Albany.' The RIL population was evaluated in the greenhouse with a randomized complete block design (RCBD) with factorial arrangements. Control and drought water regimes were maintained, and data were collected on days to heading (DH), plant height (PH), the number of tillers (TIL), the number of spikes (SPK), canopy dry weight (CDW), wilting score (WS), yield (YLD) and thousand kernel weight (TKW). The population was genotyped using Illumina's Infinium 90K SNP assay. The QTL analysis identified 38 QTL. Of these, eight QTL were specific to the drought water regime, six for the susceptibility index (DSI), and eight were constitutive QTL (identified under both water regimes). Among the QTL identified for the drought water regime, two had major effects (PV ≥ 10%), and explained 12.97 and 11.43% of phenotypic variation (PV). Both were associated with TKW and were found on chromosomes 5A and 5B. Three QTL identified for DSI had major effects which were located on 4D, 5D and 7B chromosomes. These QTL were associated with TKW and SPK. All the constitutive QTL had major effects. Three novel QTL were identified in this study, including two with major effects. The findings of this study can assist breeders in developing drought-tolerant cultivars using marker-assisted selection (MAS).

4.2. Introduction

The USA ranks fourth in world wheat production, producing 55.14 million metric tons of the total global wheat production of 729 million metric tons in 2014 (FAOSTAT, 2016). In terms of acreage, wheat surpasses all other crops worldwide, whereas in the USA, it ranks third after maize and soybeans (FAOSTAT, 2016). Hard red spring wheat (HRSW) is one of the five wheat classes grown in the Northern Plains (North Dakota (ND), Montana, South Dakota, and Minnesota). On average, ND produces over 50% of the total HRSW in the USA. It is considered to be the aristocratic class of wheat because of its high protein content (13 to 16%) and is used for making some of the world's best bread, hard rolls, and bagels. Due to its high quality, the HRSW grown in the USA is exported to over 70 countries, making it an economically important crop (North Dakota Wheat Commission, 2016).

The climate in ND can be severe, with periods of drought, especially in the semi-arid conditions of the western half of the state, which has a tendency to experience cyclical drought. Drought damages crops and causes immense economic losses, including statewide losses of $223 million in 2002, and $425 million in 2006 (Climate change and the economy, 2008). However, the drought of 2012 was the most serious agricultural disaster in the USA since the 1950s, costing many billions of dollars in losses across the country (Rippey, 2015). Hard red spring wheat production has been hindered in some years because of drought. Therefore, understanding the genetics of drought tolerance in spring wheat in the northern USA is a prerequisite for developing drought-tolerant HRSW cultivars. In this study, an attempt was made to identify drought-tolerant QTL in HRSW in the northern USA.

4. 3. Materials and Methods

4.3.1. Plant materials

A population consisting of 149 RILs was developed from a cross between cultivars 'Reeder' (PI613586) and 'Albany' by the HRSW and germplasm enhancement program at NDSU. Reeder, released by the North Dakota Agricultural Experiment Station at NDSU in 1999, is a semi-dwarf HRSW variety. It is best adapted to western ND, which is a semi-arid region requiring drought-tolerant cultivars. It has acceptable milling and baking qualities and possesses resistance to the Upper Midwest races of stem and leaf rust. Albany was developed by Trigen Seed LLC. It is a very high yielding, semi-dwarf HRSW cultivar adapted to intensive input management. This cultivar is susceptible to drought and better adapted to the eastern areas of the Northern Plains. A single seed descent method was used to advance the RIL population to the F_8 generation. The checks used in the study were 'Glenn' (Mergoum et al., 2006), 'Faller' (Mergoum et al., 2008), and 'Alsen' (Frohberg et al., 2006). Glenn and Alsen show moderate drought tolerance, whereas, Faller shows drought susceptibility.

4.3.2. Greenhouse experiment

The RIL population, their parents, and three checks were evaluated in the greenhouse in 2012. The experimental design was RCBD with three replicates. A factorial arrangement was followed using two factors. The first factor was water regimes (control and drought) and the second factor was the RILs under evaluation. Each 20.32-cm diameter pot containing five plants of each genotype was considered as the experimental unit. The planting soil consisted of Sunshine Mix #1 (Sun Gro Horticulture, Agawam, MA, USA) augmented with 20 g Osmocote® slow-release fertilizer (Scott's Company LLC, Marysville, OH). The control water regime was applied by maintaining the soil moisture at field capacity (about 50% moisture by volume). The

drought stress was imposed on the plants when the majority of the plants started to flower. The soil of the pots was allowed to dry up to about 15% moisture by volume, and then the pots were watered to saturate the soil. The stress was continued until the plants were harvested. The available soil moisture was measured using a soil-moisture meter (Spectrum technologies, Inc.).

4.3.3. Data collection

Data were collected on DH (days), PH (cm), TIL, SPK, CDW (g), WS, YLD (g/pot) and TKW (g). The DH of each genotype was collected when about 50% of the plants were heading. Plant height was measured from plant base to tip excluding the awn. The harvested plants from each pot were dried in the oven at 80^0 C for 48 hours and then CDW was measured. Wilting scores (1-9) were recorded at the end of the drought period, where 1 indicated no drought symptoms and 9 indicated all plants to be dry. A thousand kernels were counted using a seed counter (Model U, International Marketing and Design Co.) and were weighed to obtain TKW.

4.3.4. Phenotypic data analysis

Drought susceptibility for each trait was measured by a 'drought susceptibility index' (DSI) according to Fischer and Maurer(1978) as:

$$DSI = \frac{1 - (Y_{dry}/Y_{wet})}{1 - (X_{dry}/X_{wet})}$$

where Y_{dry} and Y_{wet} indicate mean performances of a specific genotype for a specific trait under respective water regimes, and X_{dry} and X_{wet} indicate mean performances of all genotypes for a specific trait under respective water regimes.

The proc anova procedure of the statistical analysis system was used to analyze the phenotypic data ("SAS Institute," 2004). Both factors were considered as fixed effects. The mean values were separated using the least significant difference (LSD) value at the $P \leq 0.05$ level of

significance. Pearson correlations between traits were calculated for a single water regime using the CORR procedure of SAS ("SAS Institute," 2004). Only the traits with a low coefficient of variation (CV) value and showing significant differences among the entries were reported in this study.

4.3.5. Genotyping

Young leaves were lyophilized and the genomic DNA of each genotype was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, cat. no. 69106). The quality of the DNA was checked using 0.8% agarose gel, and the DNA concentration was checked using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). The samples were genotyped using Illumina's Infinium 90K iSelect wheat SNP assay in the Small Grains Genotyping Lab, USDA-ARS, Fargo, ND, and the data were analyzed using the genotyping module GenomeStudio V2011.1 (www.illumina.com, verified 18 December 2015).

4.3.6. Map construction

A total of 81,587 SNP markers were produced using Illumina's Infinium iSelect 90K assay (Wang et al., 2014). Among those, 12,151 SNP markers were polymorphic between parental genotypes. The polymorphic markers showing 1) allele frequency <0.4 for any of the parental genotypes, 2) inconsistent results in five replicates of each parental genotype, 3) overlapping clusters for RILs, and 4) >20% missing data, were discarded. MapMaker 3.0 (Lander and Botstein, 1989) and CarthaGène v.1.2.3R (de Givry et al., 2005) software programs were used for constructing linkage maps using the remaining 10,760 polymorphic markers. Available map information from multiple populations (Wang et al., 2014) was used to select five to nine anchor markers from each chromosome. MapMaker 3.0 (Lander and Botstein, 1989) and the anchor markers were used to place 10,657 polymorphic markers onto 21 wheat chromosomes

using a minimum LOD score of 5.0 and a maximum distance of 40 cM. The linkage maps were then developed using CarthaGène V.1.2.3R (de Givry et al., 2005). Kosambi's mapping function (Kosambi, 1944) was used to determine the genetic distance among markers on the linkage groups.

4.3.7. QTL mapping

Composite interval mapping (CIM) was used to identify QTL for each trait in each water regime using QTL Cartographer V2.5_011 (Wang et al., 2012). In QTL Cartographer Model 6 (the standard model), forward and backward regression, five control markers (co-factors), a window size of 10 cM, and a walk speed of 1 cM were used. Significant QTL were identified by the LOD threshold determined by 1000 permutations. Confidence intervals for the QTL (CI) were estimated by the ± 2 LOD (from the peak) method. The QTL were considered to be the same if their CIs overlapped or they were located within 10 cM regions. Only significant QTL detected (above the threshold LOD score) were reported in this study. If any such QTL was identified with an LOD below the threshold, but >2.5 in other water regimes, the QTL were also included in the results as supporting information. The Mapchart 2.3 program (Voorrips, 2002) was used to draw the QTL regions. Map locations of the associated markers were used to determine if the identified QTL was novel.

4.4 Results

4.4.1. Phenotypic analyses

The genotypes (RILs and parents) had significant differences for all of the agronomic traits (Table 4.1). However, the CV of the WS was high (22.83), and therefore, was not included in this study. The RIL population showed continuous variation for all of the traits (Fig.4.1). Transgressive segregations were observed in both directions for all of the traits as well (Fig.

4.1), indicating that both parents had favorable alleles important for drought tolerance. The parent Reeder took more DH in the control water regime, whereas Albany took more DH in the drought water regime. Albany had a higher PH in both water regimes, while Reeder had a higher TIL and SPK in both. Albany had a greater CDW in the control water regime, while Reeder had a greater CDW in the drought water regime. Similar results were found for YLD and TKW (Table 4. 2).

Days to heading had a highly significant positive correlation with TIL, but a highly significant negative correlation with YLD. Late heading plants tended to be taller than early-heading plants in the drought water regime. Also, late-heading plants had a higher TKW in the control water regime, with the opposite effect in the drought water regime. The plants with more tillers also had more spikes in both water regimes. The plants with fewer tillers had a higher TKW in the drought water regime. The plants with more spikes had a lower CDW in the control water regime, but in drought conditions, they had a higher CDW. The plants with more spikes also had a higher YLD in the drought water regime. The plants with a greater CDW were taller and tended to give a higher YLD in both water regimes. Also, CDW was positively associated with TKW in the control conditions. The high-yielding plants also had a greater TKW in both water regimes. The taller plants were higher yielding, with a higher TKW in the control water regime (Table 4.3).

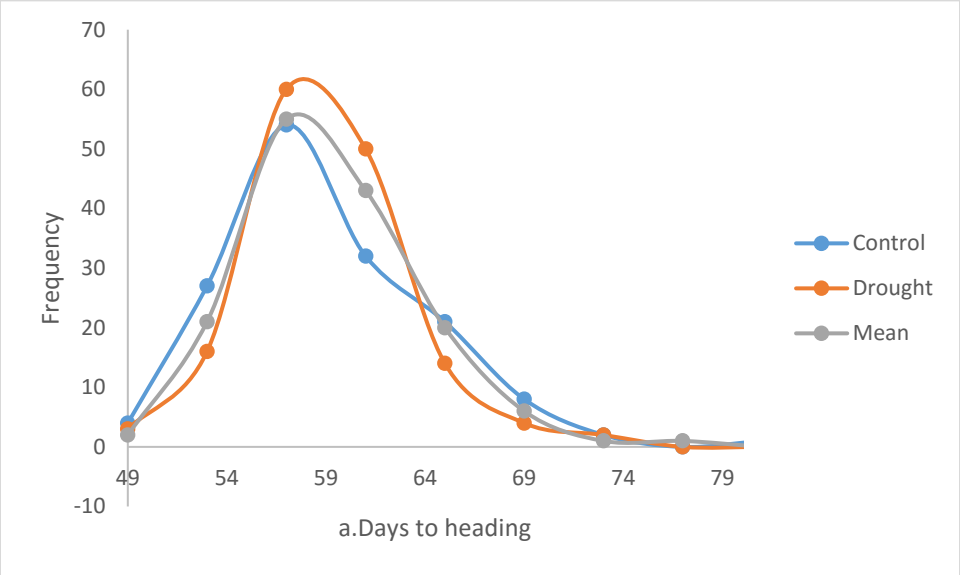
Table 4.1. Analysis of variance (ANOVA) for the agronomic traits

Sources[ɨ]	df	DH[ǂ]	PH[§]	TIL[¶]	SPK[#]	CDW[ǂǂ]	WS[ǂǂ]	YLD[§§]	TKW[¶¶]
A	1	1.79	495***	281.28***	478.12***	2103.45***	17731.6***	1180.5***	169.93***
B	153	11.67***	4.64***	9.19***	5.49***	5.22***	6.7***	5.58***	3.68***
A*B	153	1.31**	1	3.24***	2.57***	3.06***	6.7***	3.81***	2.18***
CV%		5.5	7.81	12.22	12.75	13.31	22.83	18.74	10.91

*Significant at 0.05, **Significant at 0.01, and ***Significant at 0.001 probablity level
[ɨ]A = water regime, B = RIL, [ǂ]DH = Days to heading, [§]PH= Plant height, [¶]TIL = Number of tillers, [#]SPK = Number of spikes, [ǂǂ]CDW = Carbon dry weight, [ǂǂ]WS = Wilting score, [§§]YLD = Yield, [¶¶]TKW = Thousand kernel weight

4.4.2. Genetic linkage map

A total of 10,657 markers, represented by 2,057 unique loci (19.3%), were mapped onto 28 linkage groups belonging to 21 wheat chromosomes (Table 4.4). The maximum number of markers were located on the B-genome, followed by the A-genome and the D-genome (Table 4.4). The linkage groups 1D1 and 5D2 contained the minimum number of markers (5), while 2B contained the maximum (1,221). Chromosome 3D contained the minimum number of markers (48), and chromosome 2B contained the maximum (Table 4.4). On average, 507.48 markers and 97.95 unique loci were mapped per chromosome. The average distance between two markers on the linkage map was 0.36 cM (Table 4.4). The total length of the A-genome was 1,542.2 cM, with an average distance of 0.37 cM between two markers, whereas the B-genome had a total map length of 1,259.1 cM, with an average distance of 0.35 cM between two markers. By comparison, the D-genome had a total map length of 991.8 cM, with an average distance of 1.52 cM between two markers. The longest chromosome was 5A, with a total map length of 299 cM, while the shortest chromosome was 6D, with a total map length of 51.5 cM (Table 4.4).



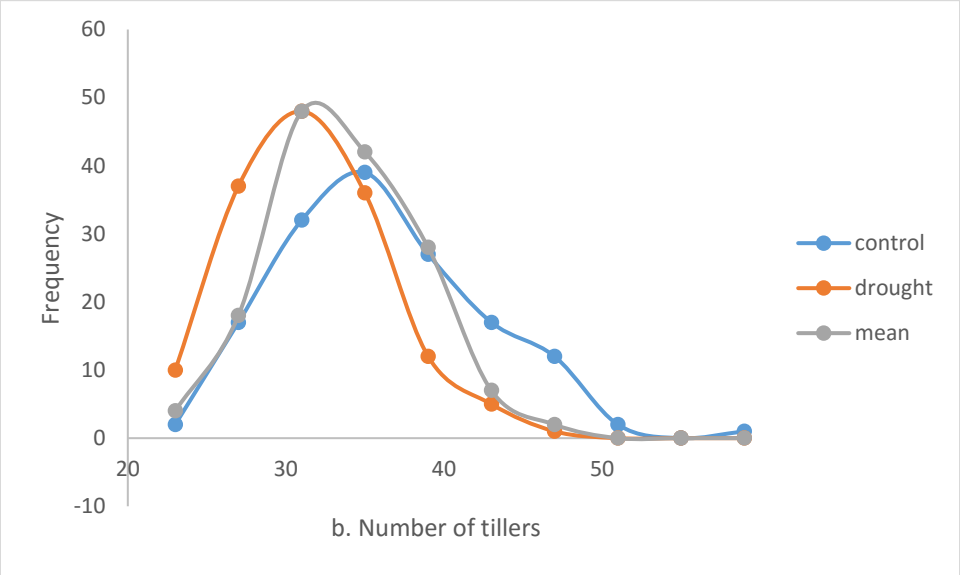


Fig. 4.1. Frequency distribution of the agronomic traits for 149 RILs of the Reeder and Albany mapping population (a. Days to heading, b. Number of tillers).

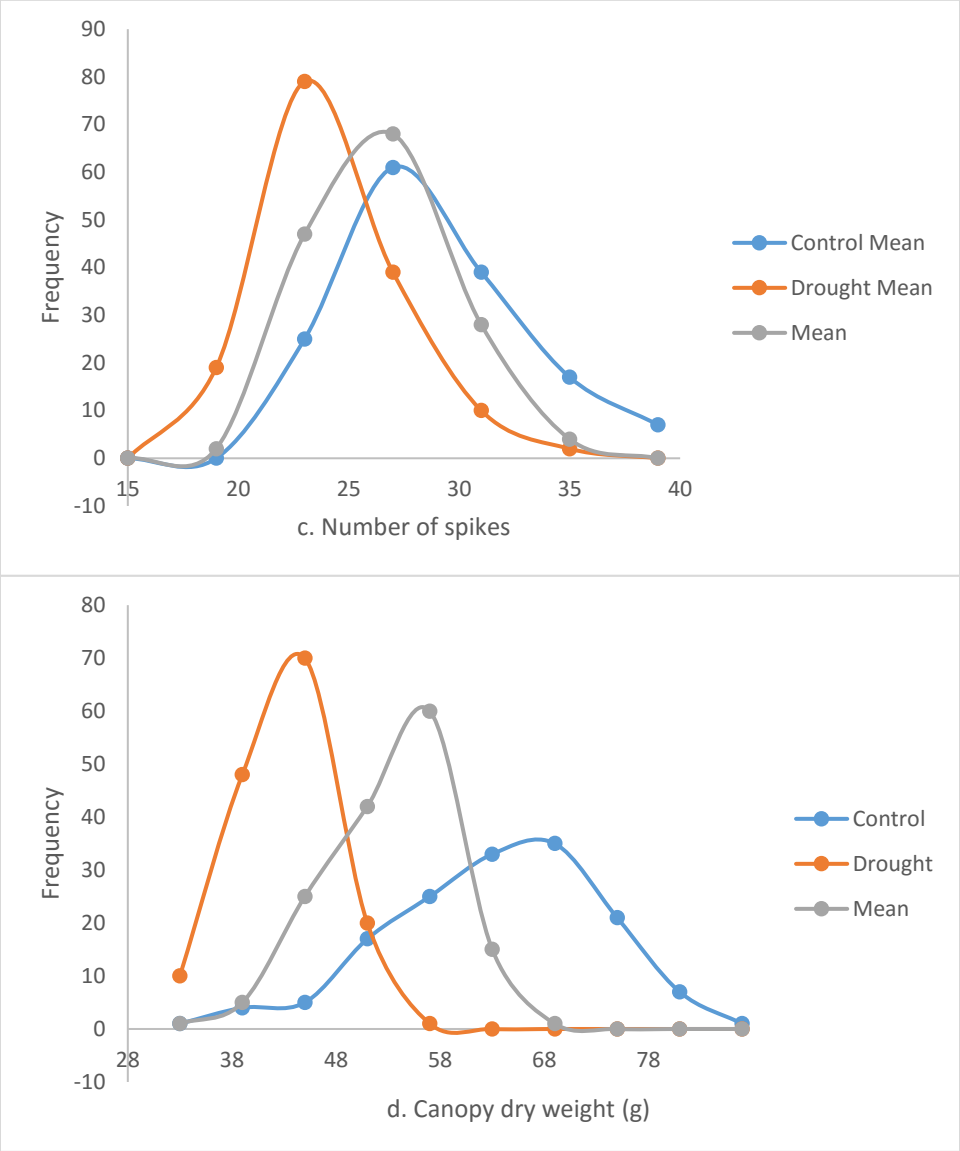
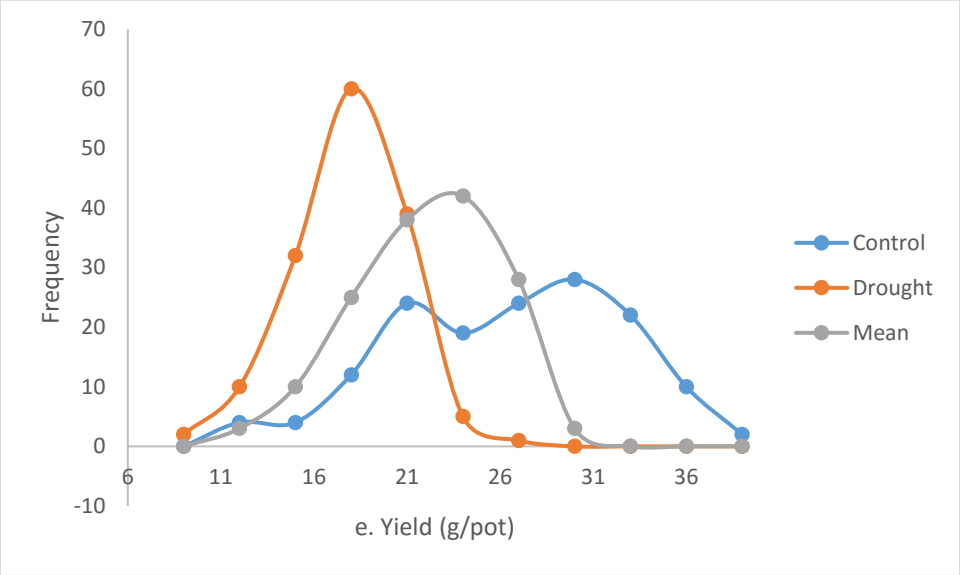


Fig. 4.1. Frequency distribution of the agronomic traits for 149 RILs of the Reeder and Albany mapping population (c. Number of spikes, d. Canopy dry weight) (continued).



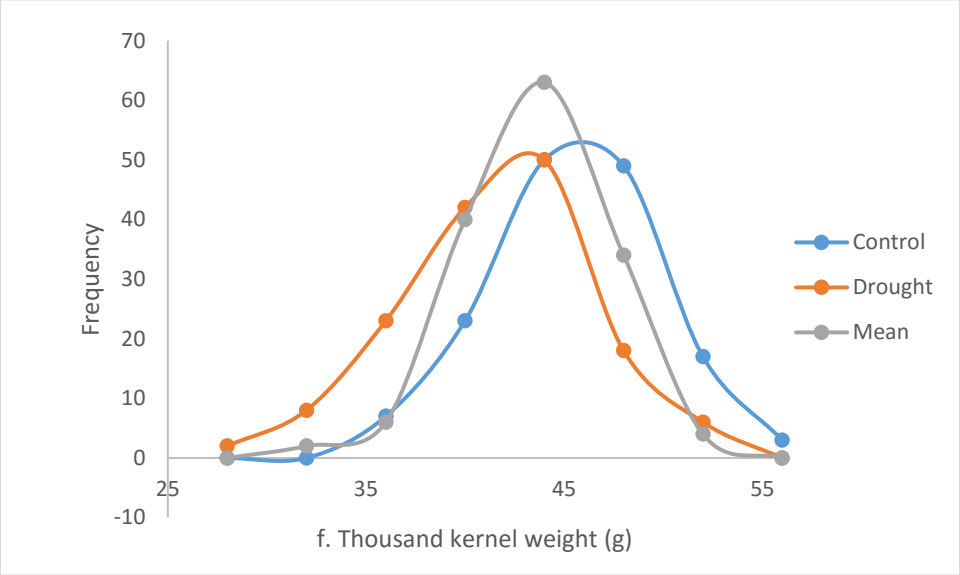


Fig. 4.1. Frequency distribution of the agronomic traits for 149 RILs of the Reeder and Albany mapping population (e. Yield, f. Thousand kernel weight) (continued).

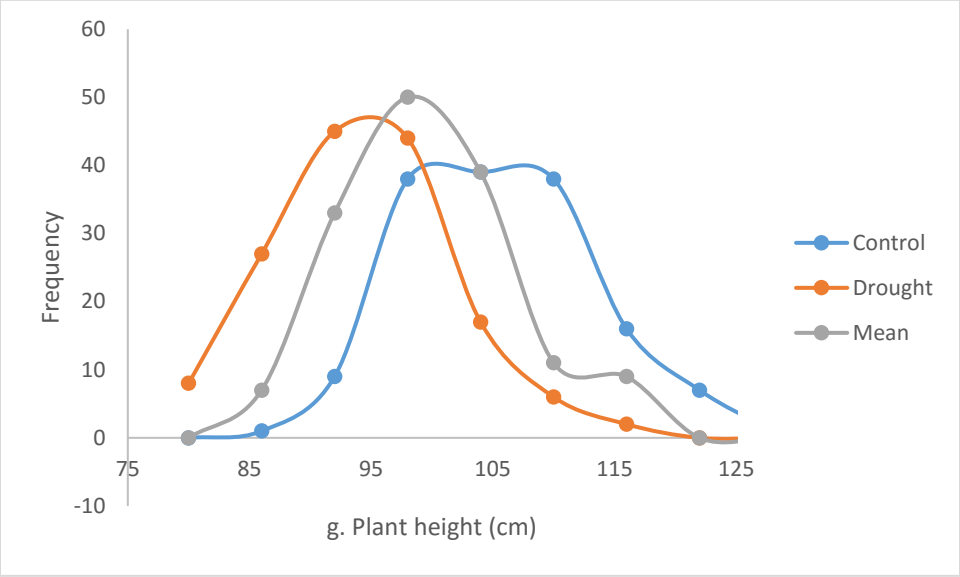


Fig. 4.1. Frequency distribution of the agronomic traits for 149 RILs of the Reeder and Albany mapping population (g. Plant height) (continued).

Table 4.2. Mean phenotypic performances of Reeder and Albany, their RIL population, and checks in different water regimes.

	Parental lines		RIL population			Checks			
Trait and Env.[ŧ]	Reeder	Albany	Min	Max	Mean	Glenn	Faller	Alsen	LSD (0.05)
DH, days									
Control	61.33	53.33	48	79	57.46	53.67	55.67	56	3.57
Drought	59	63.67	46.33	72	57.13	56	54.67	52.67	
PH, cm									
Control	91.44	105.83	83.82	122.77	102.2	115.15	100.75	105.83	8.55
Drought	82.13	83.82	76.2	110.91	91.28	91.44	91.44	89.75	
TIL									
Control	37	28.33	21.33	56.67	34.22	23.33	26.67	29.33	4.42
Drought	35.67	26	20.33	43.33	29.64	38.67	28.33	36.67	
SPK									
Control	28	24	19.33	38.67	27.02	20.67	22.67	20.33	3.56
Drought	25.33	22.33	15.33	32	22.3	28.67	20.67	25.67	
CDW									
Control	49.5	60.23	31.3	84.07	60.24	68.77	70.07	42.67	7.56
Drought	38.03	30.33	28.97	51.63	40.16	36.33	40.13	38.39	
YLD									
Control	21.9	30.13	10.8	37.43	24.89	30.63	36.37	15.37	4.36
Drought	15.7	12.67	6.47	24.5	16.25	11.77	18.77	14.5	
TKW									
Control	45.33	48	32.67	55.83	43.67	47.5	49.33	40.33	5.14
Drought	42.17	40.17	26.92	49.67	39.54	44.33	43.67	39	

[ŧ]DH = Days to heading, PH= Plant height, TIL = Number of tillers, SPK = Number of spikes, CDW = Canopy dry weight, WS = Wilting score, YLD = Yield, TKW = Thousand kernel weight

Table 4.3. Correlation coefficients between five agronomic traits in the RIL population (Reeder × Albany) in different environments (Env.) and overall mean across environments (M).

Trait and water regimes[ŧ]	DH	TIL	SPK	CDW	Yield	PH
TIL						
Control	0.45***					
Drought	0.27***					
M	0.44***					
SPK						
Control	0.15ns	0.8***				
Drought	-0.1ns	0.56***				
M	0.5ns	0.74***				
CDW						
Control	0.70ns	0.14ns	-0.17**			
Drought	0.40ns	0.13ns	0.29***			
M	0.11ns	0.14ns	0.11 ns			
YLD						
Control	-0.19*	0.04ns	-0.07ns	0.72***		
Drought	-0.37***	-0.08ns	0.2**	0.62***		
M	-0.28***	-0.1ns	-0.03ns	0.7***		
PH						
Control	0.05ns	-0.13ns	-0.19ns	0.41***	0.39***	
Drought	0.21***	0.03ns	-0.15ns	0.36***	0.06ns	
M	0.03ns	0.01ns	-0.05ns	0.09ns	0.16*	
TKW						
Control	0.21***	0.06ns	-0.04ns	0.18*	0.26***	0.17*
Drought	-0.46***	-0.17*	-0.09ns	0.11ns	0.55***	-0.02ns
M	-0.22***	-0.14ns	-0.13ns	0.12ns	0.4***	0.05ns

[ŧ]HD = Days to heading, PH= Plant height, TIL = Number of tillers, SPK = Number of spikes, CDW = Canopy dry weight, WS = Wilting score, YLD = Yield, TKW = Thousand kernel weight
*Significant at 0.05, **Significant at .01, ***Significant at 0.001 probability level

Table 4.4. Distribution of markers across linkage groups in the genetic map developed using the Reeder × Albany RIL population.

Linkage groups	No. of markers	No. of unique loci	Map length	Average map density	Average map density
				cM/marker	cM/locus
1A	567	126	174.90	0.31	1.39
2A	439	101	223.50	0.51	2.21
3A	659	123	213.90	0.32	1.74
4A	560	114	218.90	0.39	1.92
5A	605	163	299.00	0.49	1.83
6A	590	117	176.70	0.30	1.51
7A	905	168	235.30	0.26	1.40
1B	629	86	107.50	0.17	1.25
2B	1221	160	181.80	0.15	1.14
3B	1115	213	250.20	0.22	1.17
4B	244	78	120.90	0.50	1.55
5B1	565	125	209.40	0.37	1.68
5B2	25	8.00	18.00	0.72	2.25
6B	426	101	158.10	0.37	1.57
7B	723	134	213.20	0.29	1.59
1D1	5	2	0.30	0.06	0.15
1D2	254	40	87.80	0.35	2.20
1D3	91	26	126.10	1.39	4.85
2D	653	46	180.40	0.28	3.92
3D	48	18	162.90	3.39	9.05
4D	53	23	129.90	2.45	5.65
5D1	25	8	47.50	1.90	5.94
5D2	5	4	24.90	4.98	6.23
5D3	130	21	31.50	0.24	1.50
6D1	10	5	3.00	0.30	0.60
6D2	23	19	44.50	1.93	2.34
6D3	22	6	4.00	0.18	0.67
7D	65	22	149.00	2.29	6.77
A genome	4,325	912	1,542.20	0.37	1.72
B genome	4,948	905	1,259.10	0.35	1.52
D genome	1,384	240.00	991.80	1.52	3.84
Whole genome	**10,657**	**2,057**	**3,793.10**	**0.36**	**1.84**

4.4.3. QTL analysis

4.4.3.1. QTL for DH

Six QTL located on five different chromosomes were identified for DH using composite interval mapping (CIM). These QTL explained from 7.08 to 41.08% of phenotypic variation (PV) (Table 4.5; Fig.4.2). Only one QTL among them, *QDH.ndsu.2B,* was identified for the drought water regime along with the mean. This QTL (*QDH.ndsu.2B*) had a minor effect, and the desired allele was contributed by Albany. Three major constitutive QTL (present in both water regimes) were identified, where the QTL with the largest effect was on chromosome 5A and explained up to 41.08% of PV. The second major QTL was on chromosome 4A, explaining up to 18.85% of PV. The third major QTL was on chromosome 5D and explained 13.03% of PV. The desired alleles from the QTL on chromosomes 4A and 5A were contributed by Reeder, whereas the desired alleles from the QTL on chromosome 5D were contributed by Albany (Table 4.5).

4.4.3.2. QTL for PH

Four major QTL located on four different chromosomes were identified for PH (Table 4.5; Fig.4.2). Only one QTL among them, *QPH.ndsu.5A,* was identified for DSI, indicating stability of performance across water regimes. This QTL explained up to 9.41% of PV, with the desired allele (reduced PH) contributed by Albany. Two QTL were constitutive, with the QTL having the largest effect and explaining up to 11.99% of PV identified on chromosome 2B. The second major QTL was identified on chromosome 7B, explaining up to 10.56% of PV. The desired allele from the QTL on chromosome 2B was contributed by Reeder, and the desired alleles from the QTL on chromosome 7B were contributed by Albany (Table 4.5).

4.4.3.3. QTL for YLD

Four QTL located on four different chromosomes were identified for YLD (Table 4.5; Fig.4.2). Three minor QTL located on chromosomes 5A, 1A, and 4B were identified exclusively in the drought water regime. Reeder contributed the desirable alleles from all these loci (Table 4.5).

4.4.3.4. QTL for CDW

Two QTL from two different chromosomes were identified for CDW (Table 4.5; Fig.4.2). One of these QTL identified on chromosome 7B was constitutive and considered a major QTL, explaining up to 16.52% of PV. Albany contributed the desired allele for this QTL (Table 4.5).

4.4.3.5. QTL for TKW

Seven QTL located on seven different chromosomes were identified for TKW (Table 4.5; Fig.4.2). The QTL *QTKW.ndsu.5A* and *QTKW.ndsu.5B1*were identified exclusively in the drought water regime. Both QTL had a major effect, with Albany contributing the desirable alleles for both loci .Three QTL, *QTKW.ndsu.4D.2, QTKW.ndsu.5D2,* and *QTKW.ndsu.4A,* were identified for DSI, with the first two having major effects. The desirable alleles for *QTKW.ndsu.4D.2* and *QTKW.ndsu.4A* were contributed by Albany (Table 4.5).

4.4.3.6. QTL for SPK

Seven QTL were identified for SPK, but only one of these, *QSPK.ndsu.1A,* was identified in the drought water regime. It explained up to 9.6% of PV, with Reeder contributing the desirable allele. Also, two QTL, *QSPK.ndsu.7B* and *QSPK.ndsu.4A.2,* were identified for DSI. The first QTL explained up to 10.57% of PV, with Albany contributing the desirable allele,

whereas the second QTL explained up to 9.37% of PV, with Reeder contributing the desirable allele (Table 4.5).

4.4.3.7. QTL for TIL

Eight QTL located in eight different genomic regions were identified for TIL. Only one QTL among them, *QTL.ndsu.5A.3,* was identified as constitutive. The remaining QTL were identified in the control water regime. The constitutive QTL explained up to 20.68% of PV, where the desirable allele (more TIL) was contributed by Albany (Table 4.5).

4.4.4.8. Co-localized or pleiotropic QTL

Co-localized QTL enable simultaneous improvement for more than one trait when the desirable alleles are contributed by the same parent. A total of 38 QTL located on 15 different chromosomes were identified in this study (Table 4.5; Fig. 4.2). The QTL with overlapping CI or located within 10 cM of each other were considered as having the same QTL region. A total of 22 co-localized or pleiotropic QTL located on 11 QTL regions were identified. The individual genomic regions were associated with two to four traits. Genomic region 27 was associated with DH, PH, YLD, and CDW. The QTL for PH (*QPH.ndsu.7B*), YLD (*QYL.ndsu.7*), and CDW (*QCDW.ndsu.7B*) had major effects, with the desired alleles contributed by Reeder. Another QTL associated with this QTL region had a minor effect, with Albany contributing the desired allele. The genomic region 18 was associated with DH (*QDH.ndsu.5A*), YLD (*QYL.ndsu.5A*), and TIL (*QTL.ndsu.5A.3*), where the desired alleles for DH and YLD were contributed by Reeder. The genomic region 23 was associated with PH (*QPH.ndsu.6A*) and TKW (*QTKW.ndsu.6A*) where both QTL had major effects and the desired alleles were contributed from Reeder (Table 4.5; Fig.4.2).

Table 4.5. QTL identified for the agronomic traits in a RIL population derived from the cross between Reeder and Albany.

QTL and trait	QTL region	Other associated traits[ɾ]	Env.[ǂ]	Position[§]	LOD[¶]	Additive effect	R^2 (%)
Canopy dry weight							
QCDW.ndsu.7A	25	-	3	219.61	3.53	1.7	7.28
QCDW.ndsu.7B	27	DH, PH, YLD	1, 2, 3	27.41-35.11	7.2	4.21	16.52
Days to heading							
QDH.ndsu.2B	4	-	2,3	7.41-17.11	4.36	1.12	7.08
QDH.ndsu.4A	8	SPK	1, 2, 3	132.91-148.51	9.41	-1.84	18.85
QDH.ndsu.5A	18	YLD, TIL	1, 2, 3, 4	195.41-206.01	19.8	-3.54	41.08
QDH.ndsu.5D2	21	TKW	1, 2, 3	1.01-13.91	8.04	1.99	13.03
QDH.ndsu.5D3	22	-	1, 3	11.91	8.04	1.99	13.03
QDH.ndsu.7B	27	CDW, PH, YLD	1, 2*, 3	28.41-32.11	4.51	1.24	7.39
Plant height							
QPH.ndsu.2B	5	-	1*, 2*, 3	99.51-110.01	5.81	-2.16	11.99
QPH.ndsu.5A	17	-	4	106.01		0.19	9.41
QPH.ndsu.6A	23	TKW	1, 3	82.41	5.09	2.56	10.04
QPH.ndsu.7B	27	DH, CDW, YLD	1, 2*, 3	25.21-27.41	5.46	2.41	10.56
Number of spikes							
QSPK.ndsu.1A	2	TIL	2*, 3	55.11-63.11	4.2	0.95	9.6
QSPK.ndsu.2D	6	TIL	1, 4	112.21-122.11	7.23	-0.27	15.6
QSPK.ndsu.3B	7	-	1,3*	223.81-224.81	4	-1.37	10.21
QSPK.ndsu.4A.1	8	DH	2*, 3*	147.81	3.11	0.79	6.49
QSPK.ndsu.4A.2	10	-	4	198.51-211.21	4.2	0.23	9.37
QSPK.ndsu.5A.1	15	TIL	1	41.21-56.91	4.12	-1.28	8.53
QSPK.ndsu.7B	26	-	4	0.31	4.7	-0.25	10.57
Number of tillers							
QTL.ndsu.1A	2	SPK	3	57.81	5.04	1.46	8.84
QTL.ndsu.1D2	3	-	3	20.81	3.77	1.25	6.5
QTL.ndsu.2D	6	SPK	3	127.91	3.66	-1.31	7.08
QTL.ndsu.5A.1	15	SPK	1, 3*	59.91-61.91	4.76	-2.3	12.8

Table 4.5. QTL identified for the agronomic traits in an RIL population derived from the cross between Reeder and Albany (Continued).

QTL and trait	QTL region	Other associated traits[ɪ]	Env.[ǂ]	Position[§]	LOD[¶]	Additive effect	R^2 (%)
QTL.ndsu.5A.2	16	-	1, 3	74.71-77.81	3.8	-1.73	7.23
QTL.ndsu.5A.3	18	YLD, DH	1, 2, 3	198.61-206.01	10.76	-2.53	20.68
QTL.ndsu.5A.4	19	TIL	1	219.81	5.83	-2.51	15.71
QTL.ndsu.7A	24	YLD	1	132.31	3.35	-1.71	7.14
Thousand kernel weight							
QTKW.ndsu.4A	9	-	4	173.11	3.7	-0.43	8.95
QTKW.ndsu.4D.1	12	-	1	71.51	5.5	1.54	11.74
QTKW.ndsu.4D.2	13	-	4	126.81	6.71	-0.58	15.64
QTKW.ndsu.5A	14	-	2	9.61-18.71	4.06	-1.75	12.97
QTKW.ndsu.5B1	20	DH	2, 3	154.01-155.01	5.29	-1.39	11.43
QTKW.ndsu.5D2	21	DH	4	15.91	3.72	0.48	10.78
QTKW.ndsu.6A	23	PH	1, 3*	86.21-99.51	4.5	1.51	11.2
Yield							
QYL.ndsu.1A	1	-	2	14.41	3.5	0.48	8.21
QYL.ndsu.4B	11	-	2, 3*	81.41	3.49	0.9	7.69
QYL.ndsu.5A	18	TIL, DH	2	194.71	3.77	0.9	8.35
QYL.ndsu.7B	27	PH, CDW, DH	1, 3*	27.41	4.66	2.05	10.78

ɪDH = Days to heading, PH = Plant height, YLD = Yield, TW = Test weight, TKW = Thousand kernel weight, CDW =Canopy dry weight, SPK= Number of spikes, TIL = Number of tillers

[ǂ]Env. = environment, 1 = Control water regime, 2 = Drought water regime, 3 = Overll mean, 4 = DSI

[§]Position represents the peak point of the QTL interval

[¶]For lot of odds (LOD) score, additive effect, and R^2, the highest values across environments were reported in this table.

* The QTL in that environment was detected above 2.5 LOD score, but below the threshold score.

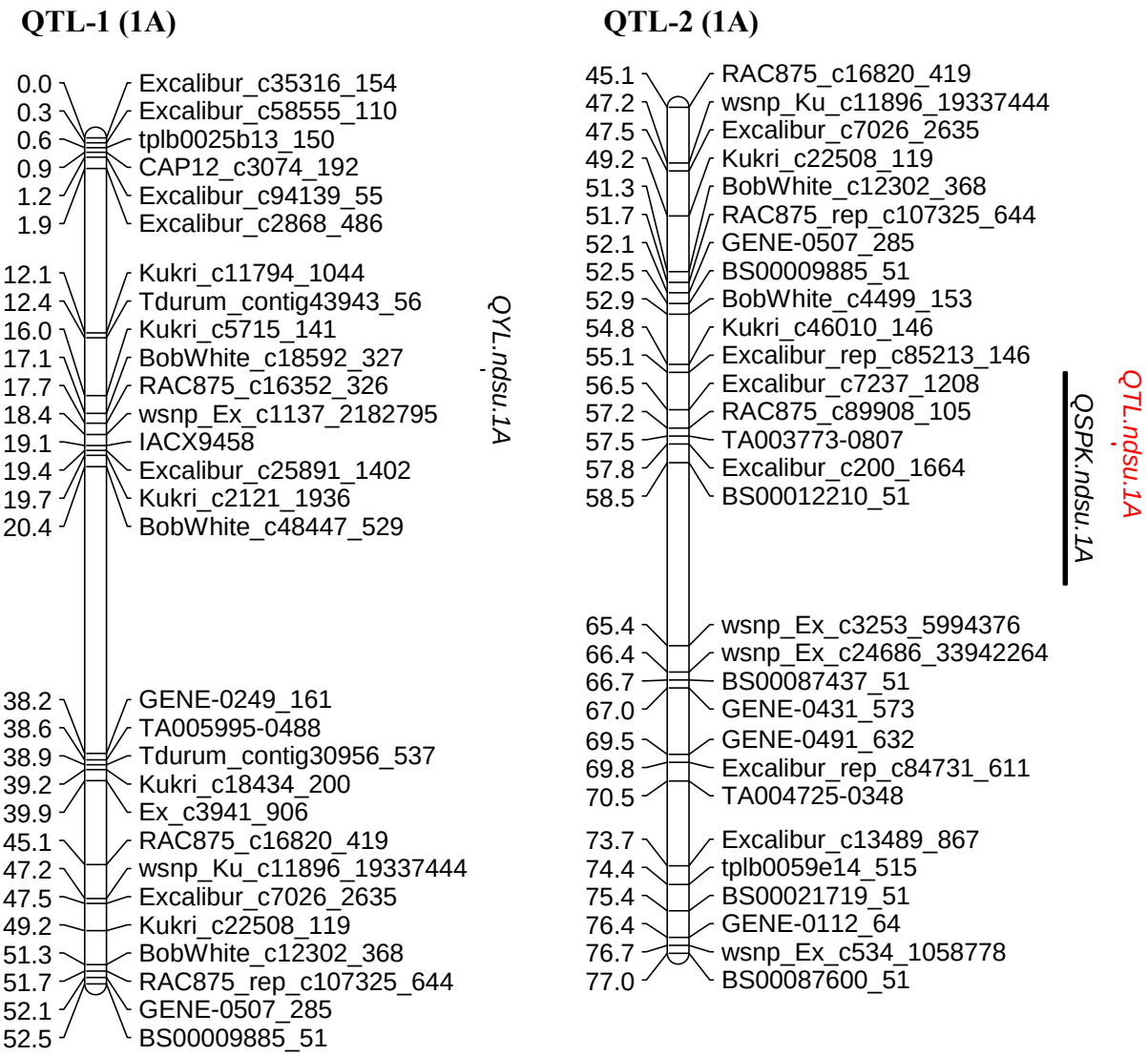


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany.

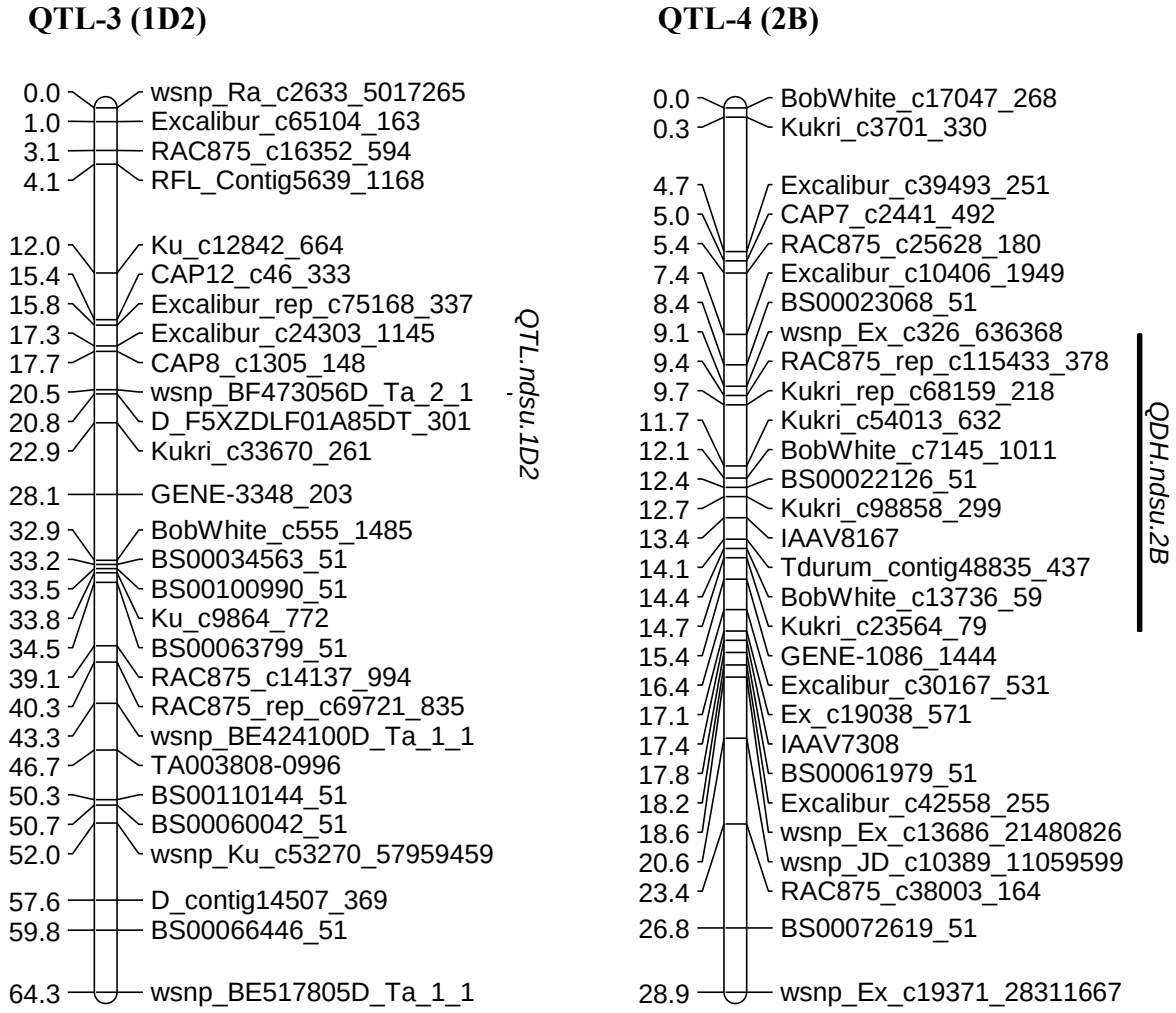


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

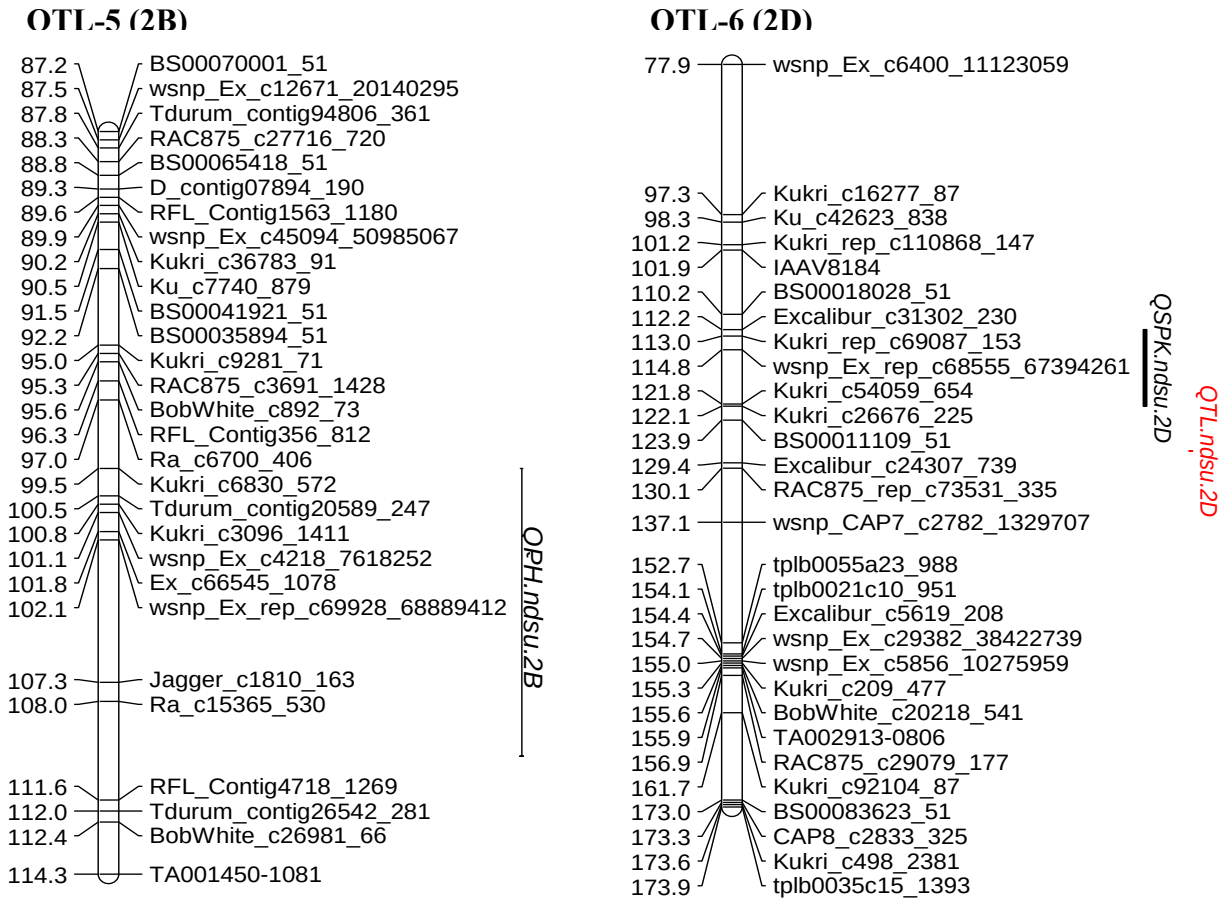


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

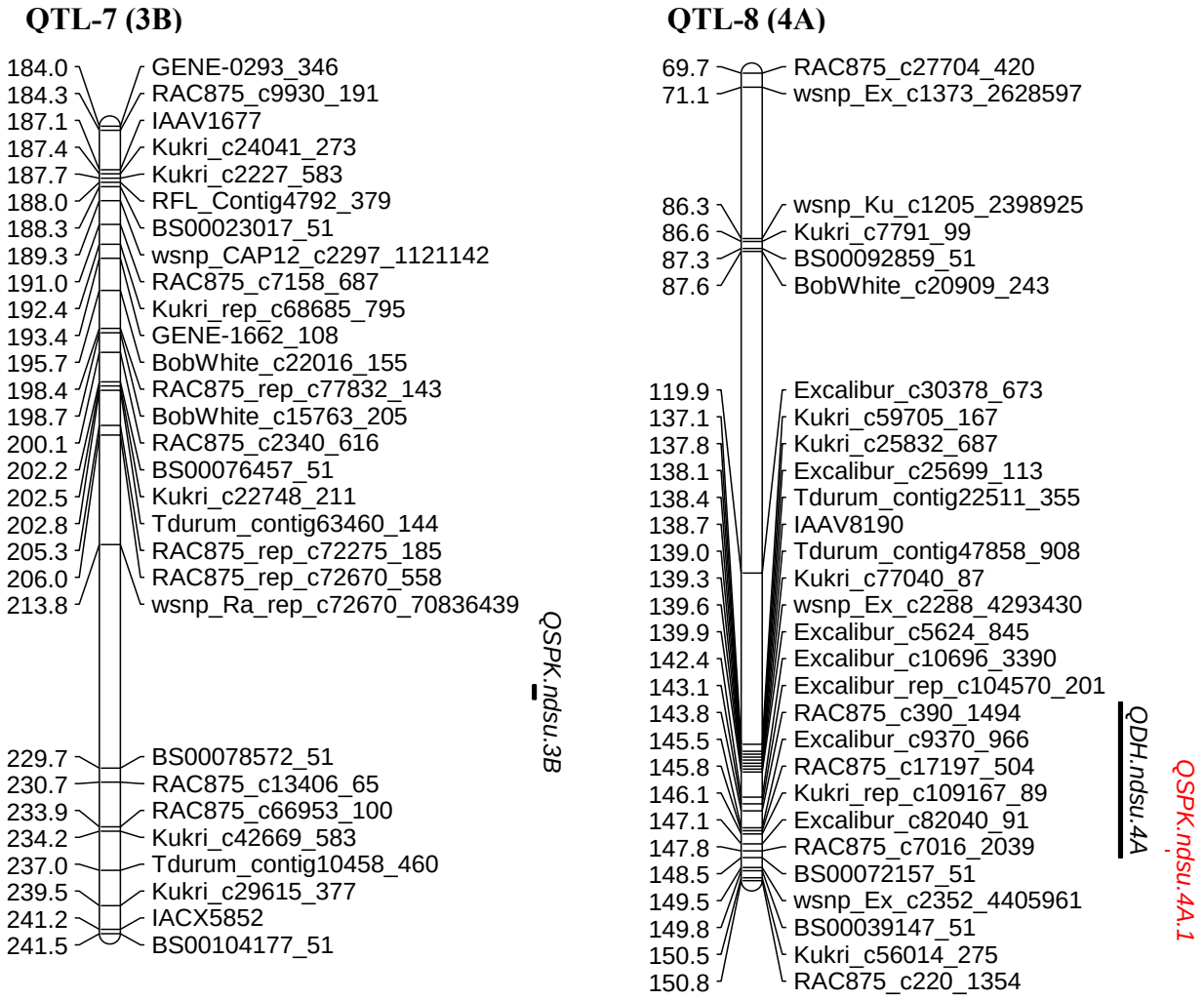


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

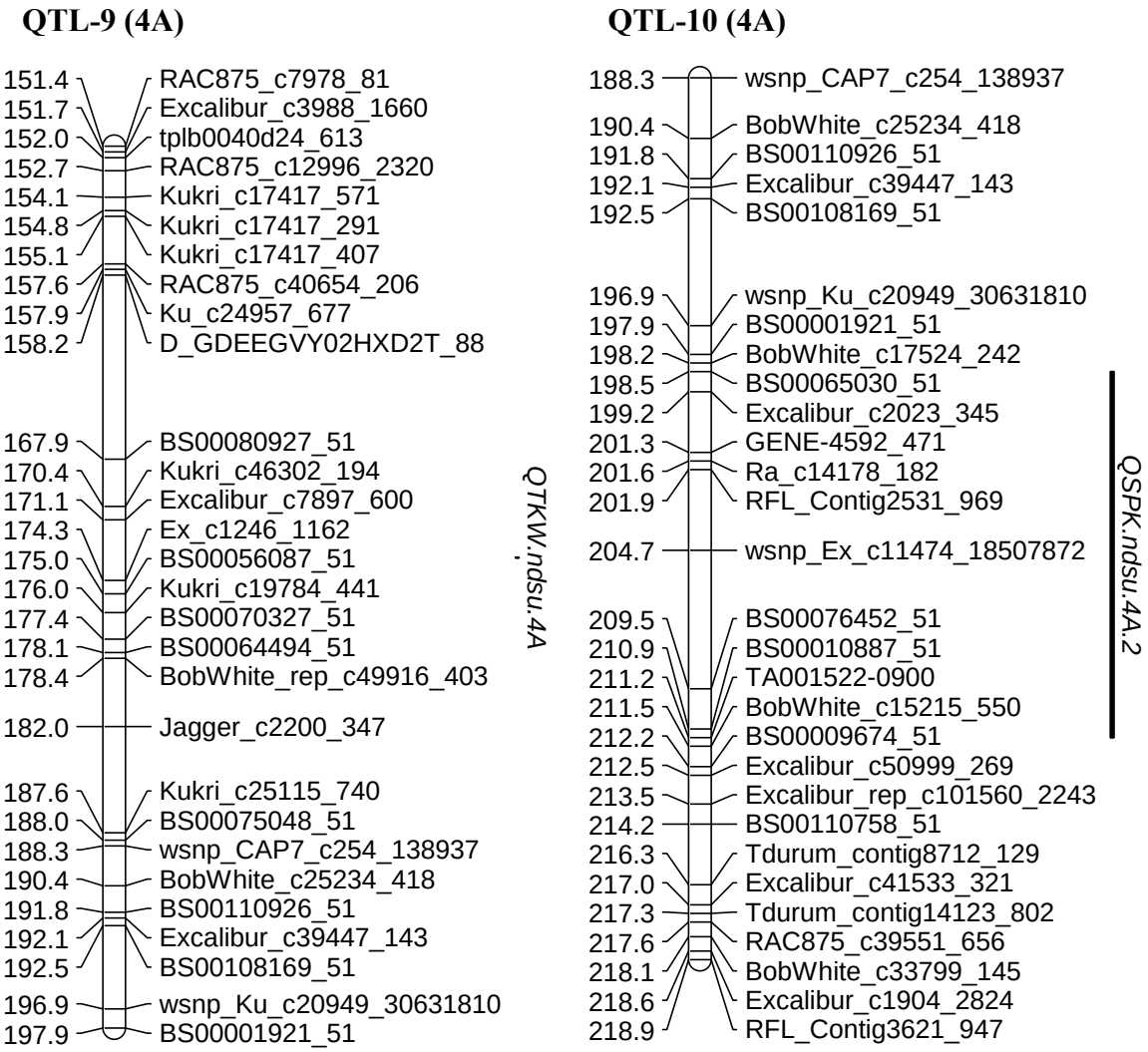


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

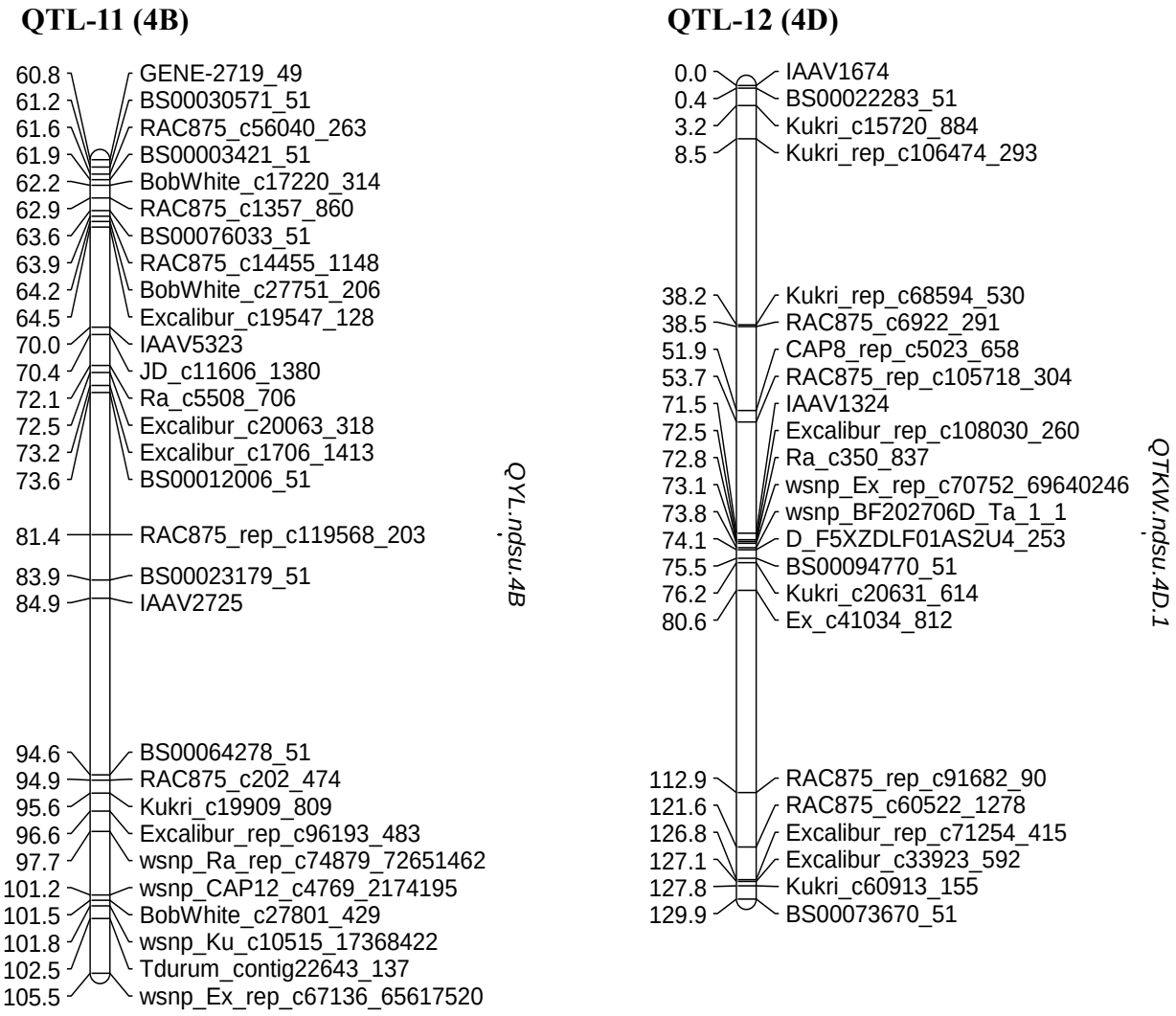


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

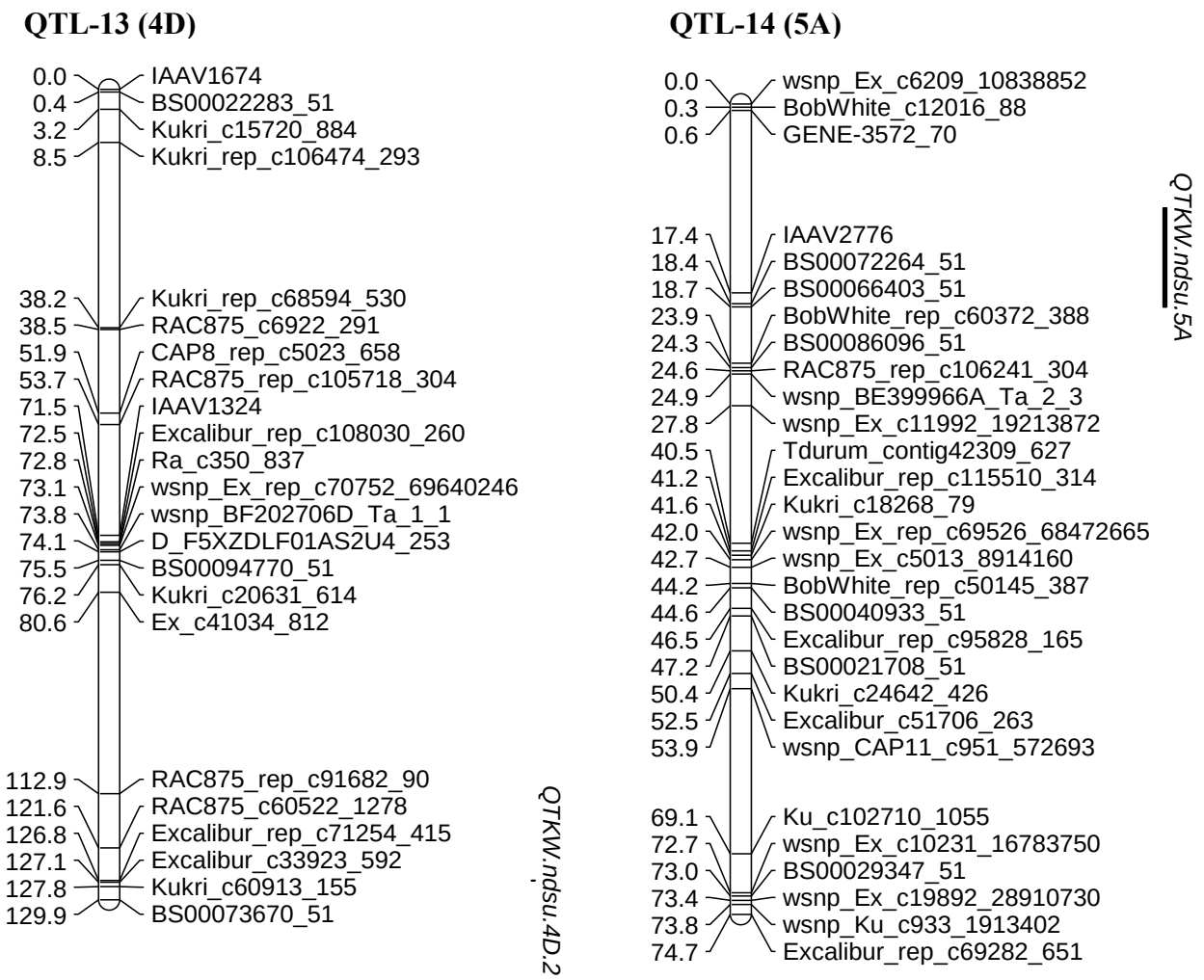


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

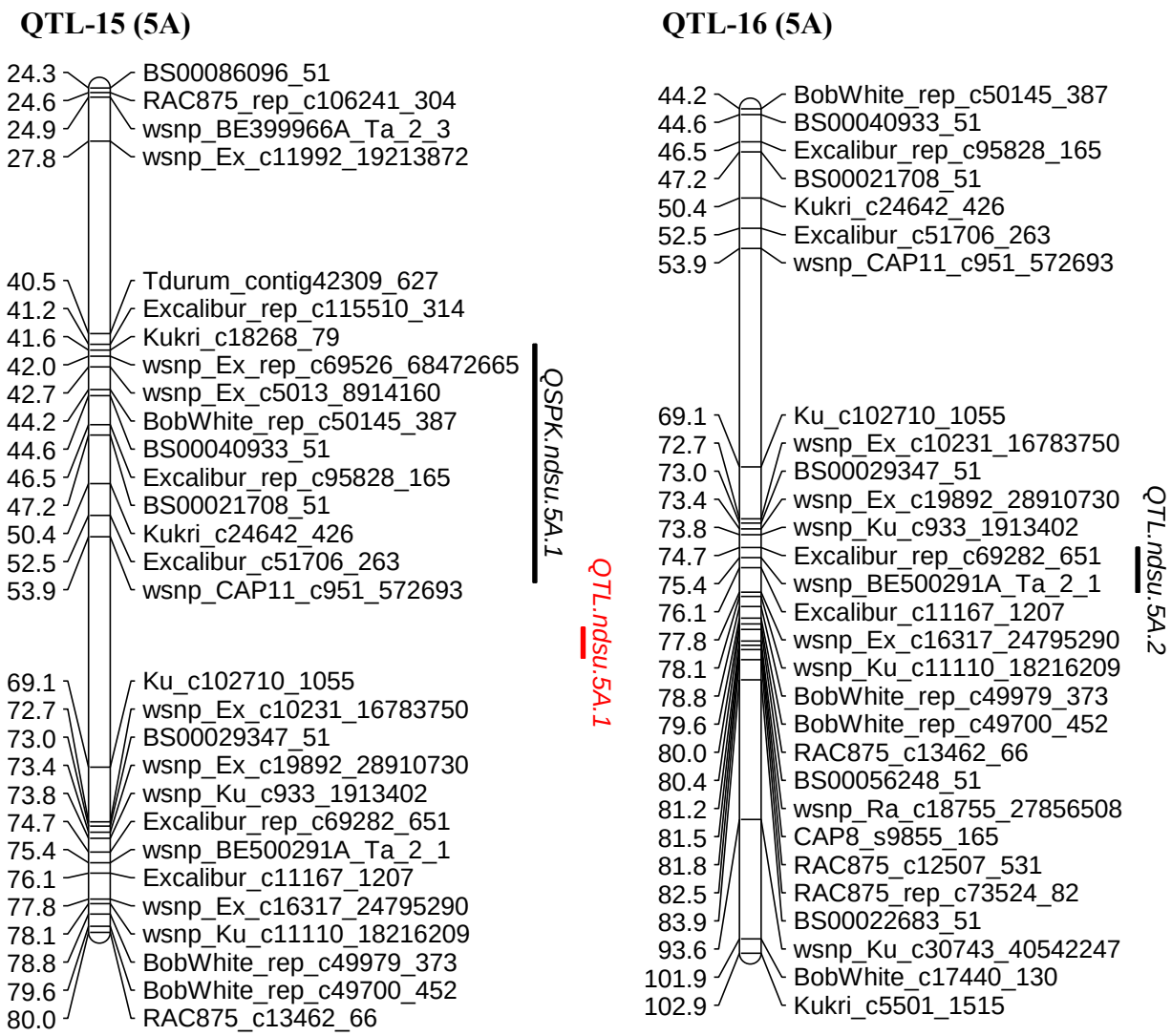


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

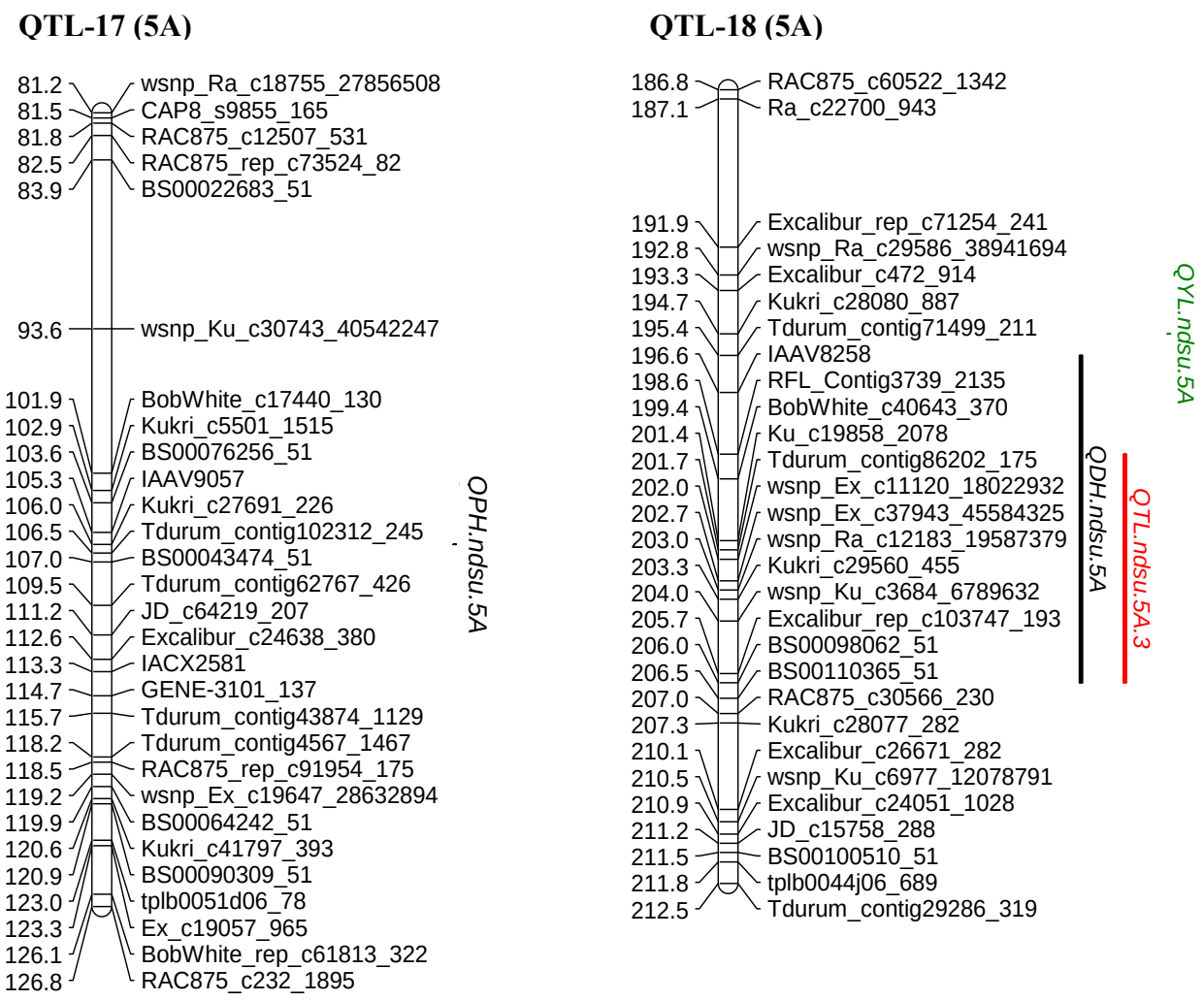


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

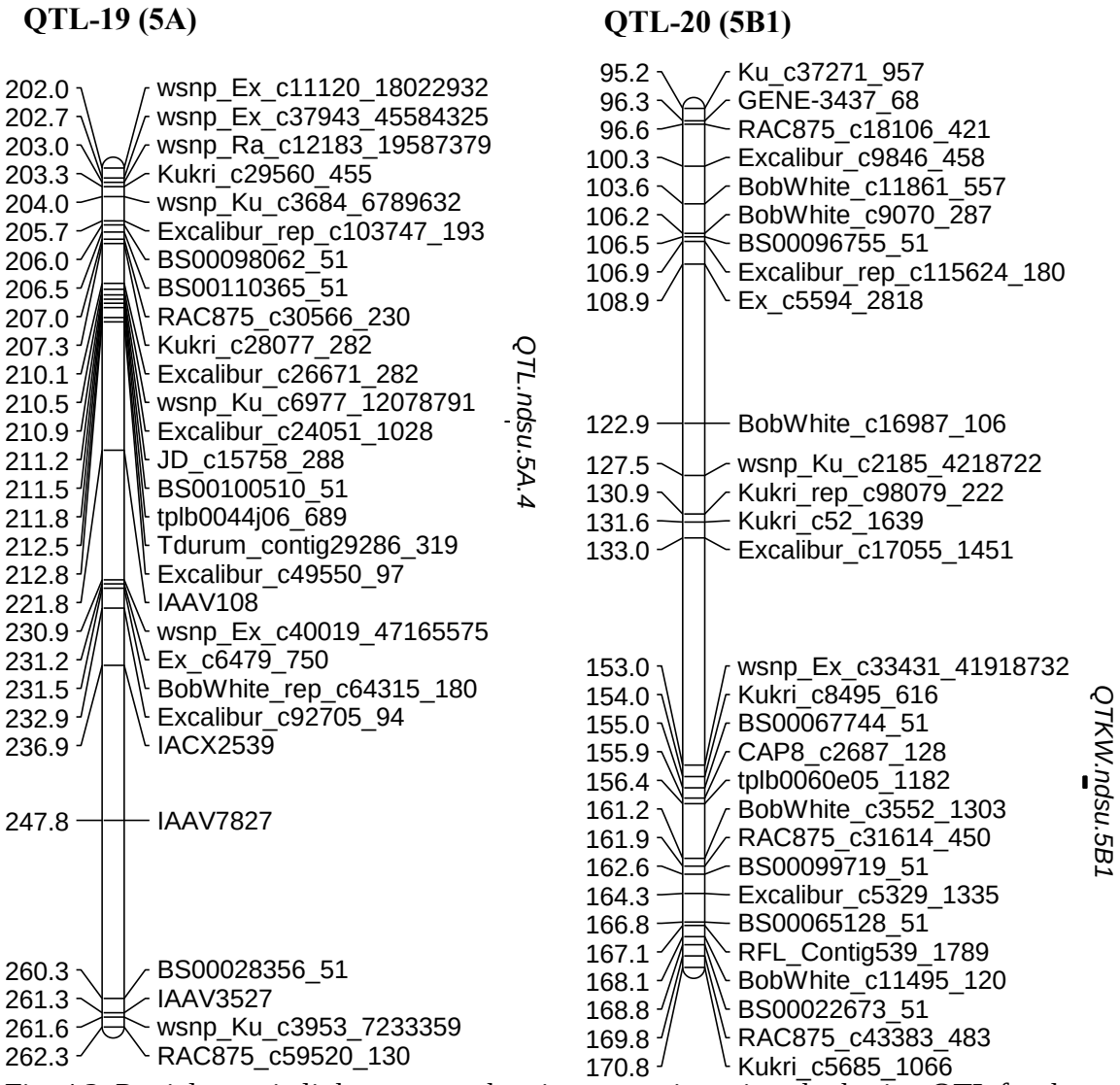


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

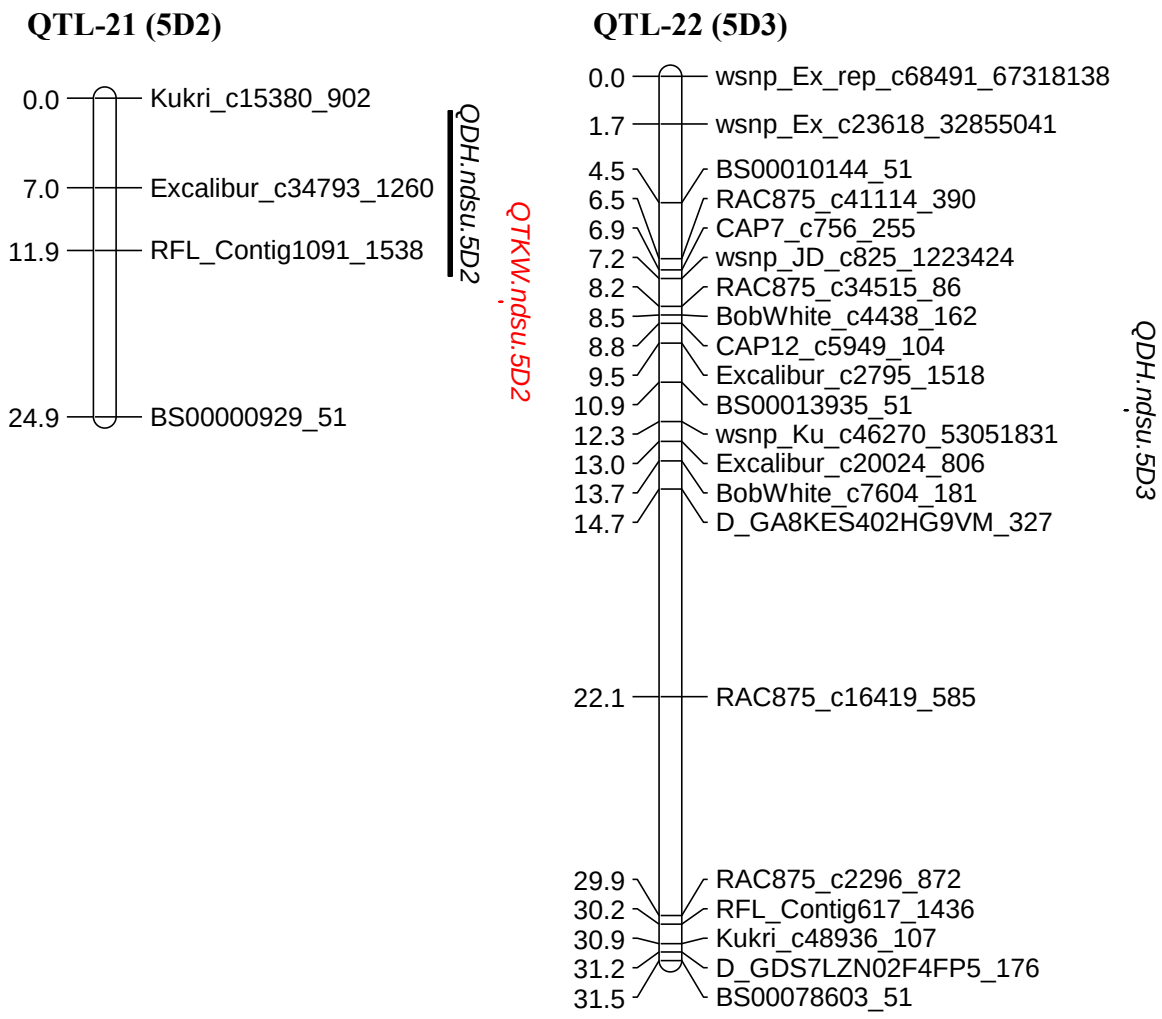


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

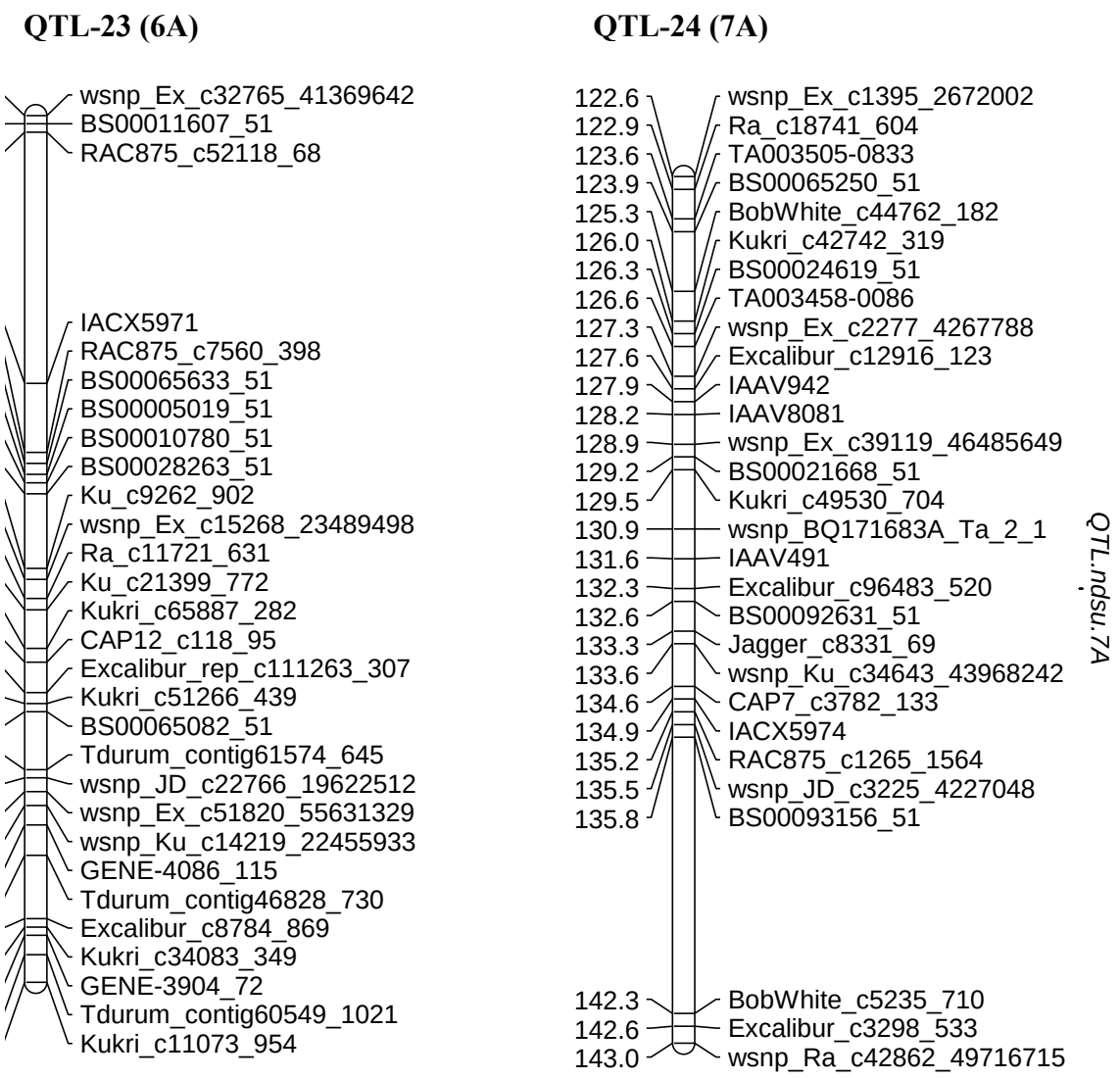


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

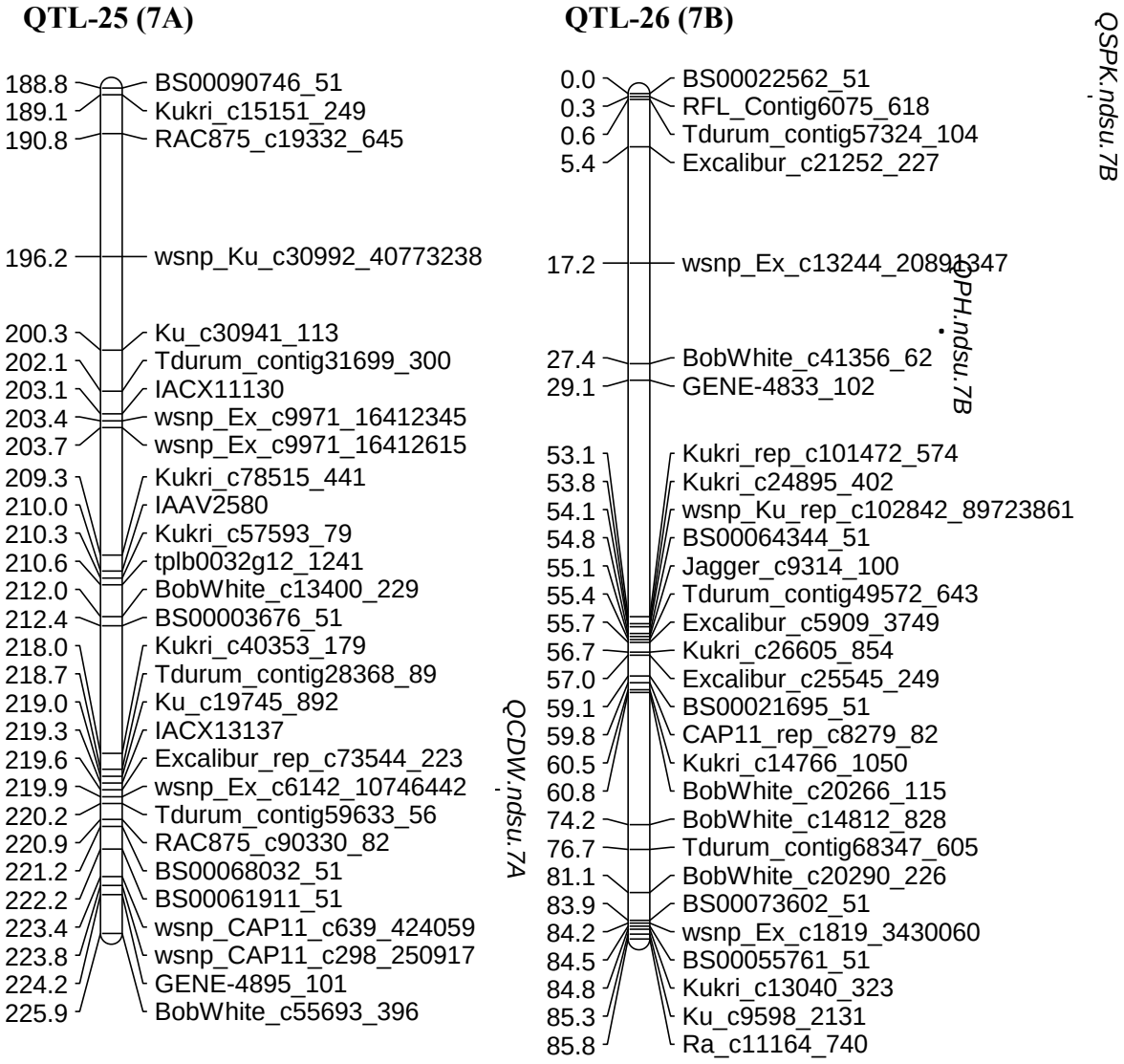


Fig. 4.2. Partial genetic linkage maps showing genomic regions harboring QTL for the agronomic traits in the RIL population derived from the cross of Reeder and Albany (continued).

4.5. Discussion

4.5.1. Linkage maps

The Infinium iSelect 90K assay (Wang et al., 2014) explored >81,000 gene-associated SNPs, revealing polymorphism in allohexaploid and allotetraploid wheat populations (Russo et al., 2014; Wang et al., 2014; Wu et al., 2015; Kumar et al., 2016; Liu et al., 2016). Higher genomic coverage and resolution in QTL mapping were achieved using this genotypic tool. The marker density (0.36 cM/marker), or unique locus density (1.84 cM/locus), and genetic map length (3793.1 cM) identified in this study corresponded other studies that used the 90K Infinium iSelect assay for genome mapping (Russo et al., 2014; Wang et al., 2014; Kumar et al., 2016). The A-genome was found to be the longest, while the D-genome was the shortest, which also corresponds with the results of previous studies. The marker orders identified in this study were also in harmony with several linkage maps developed using the Infinium iSelect 90K SNP assay (Cabral et al., 2014; Desiderio et al., 2014; Russo et al., 2014; Wang et al., 2014; Kumar et al., 2016).

Chromosome 5B had two linkage groups, whereas chromosomes 1D, 5D, and 6D had three linkage groups. The fragmentation could be the result of repeat elements located between gene-rich regions. Another reason could be the use of stringent mapping parameters (LOD score > 5 and distance < 40 cM) (Kumar et al., 2016). Most of the fragmentations were identified on the D-genome chromosomes and resulted from their very weak representation in the Infinium iSelect 90K assay (Wang et al., 2014).

4.5.2. QTL for DH

Quantitative trait loci mapping for DH has been done in many past studies (Kato et al., 1999; Sourdille et al., 2000; Bullrich et al., 2002; Shindo et al., 2003; Xu et al., 2005; Griffiths et

al., 2009; Alexander et al., 2012; Kamran et al., 2013; Bogard et al., 2014; Zanke et al., 2014a; Guedira et al., 2016; Milner et al., 2016). These studies indicated that the genes regulating DH can be divided into three major categories: photoperiod responsive genes, vernalization responsive genes, and 'earliness *per se*' genes. The photoperiod responsive genes regulate DH in response to day length for photosensitive wheat genotypes. The vernalization-responsive genes regulate DH in winter wheat, where the genes are activated under exposure to vernalization (cold temperatures). Finally, earliness *per se* stands for the only environment-independent genetic factor controlling earliness (Shindo et al., 2003).

The major QTL in this study *QDH.ndsu.5A* is associated with YLD and TIL and could correspond with the major earliness *per se* QTL*QEet.ocs.5A.2* (Kato et al., 1999). By reducing DH (additive value -3.54), this QTL saved the plant from the drought conditions. The second major constitutive QTL, *QDH.ndsu.4A,* occupied the same location as the early maturity QTL, *QMat.crc-4A* (McCartney et al., 2005). The third major constitutive QTL, *QDH.ndsu.5D2*, was also associated with TKW and corresponded with the QTL reported by Sourdille et al. (2000). The QTL *QDH.ndsu.2B,* identified under the drought condition, may be a possible locus controlling drought tolerance. This QTL corresponded with the QTL between the markers *wpt7200* and *wpt664520* reported earlier for DH (Narjesi et al., 2015). Also, a QTL for days from heading to maturity achieved under drought condition (Peleg et al., 2009) corresponded with the position of the QTL *QDH.ndsu.2B,* indicating the potential of this QTL for drought tolerance.

4.5.3. QTL for PH

Plant height is very important in wheat breeding as it is related to lodging resistance and a high harvest index. For example, the dwarfism gene from Nonglin-10 played a vital role in the

Green Revolution of the 1960s (Liu et al., 2011). In contrast, our study showed that PH had a positive correlation with DH in the drought water regime, while DH had a negative correlation with YLD. Therefore, it could be stated that reduced PH is desirable for higher YLD. Several studies on the QTL for PH have been conducted in the past (McCartney et al., 2005; Pushpendra et al., 2007; Liu et al., 2011; Huang et al., 2012; Zanke et al., 2014b; Gao et al., 2015; Li et al., 2015; Narjesi et al., 2015; Milner et al., 2016; Singh et al., 2016). The first major constitutive QTL, *QPH.ndsu.2B*, indicated that this locus provided a PH-reducing allele from Reeder that helped with drought tolerance. This locus corresponded to the SNP marker *BobWhite_rep_c64068*_241 associated with PH (Zanke et al., 2014b). Also, Peleg et al. (2009) identified a QTL at this locus for maturity under dry condition, which further indicated the association of this locus with drought tolerance. Likewise, the second constitutive QTL, *QPH.ndsu.7B*, contributing to drought tolerance corresponded with the QTL *QHt-7B-1* (Liu et al., 2011). The association of this locus with drought tolerance was further supported by the findings of Peleg et al.(2009), who identified a QTL for DH and maturity under dry conditions at 20 cM on chromosome 7B. Also, Alexander et al. (2012) identified a major QTL for drought tolerance (*QDt.ksu- 7B)* located at 34.7 cM on chromosome 7B. The QTL for stability (DSI) of PH, *QPH.ndsu.5A*, reduced the differences in PH across water regimes and hence improved drought tolerance. This locus occupied the same position as the SNP marker *wsnp_Ex_c23795_33033959*, which was reported earlier for PH (Zanke et al., 2014b). The same locus also corresponded with a QTL for the carbon isotope ratio achieved under dry condition, which verified that the locus was associated with drought tolerance (Peleg et al., 2009).

4.5.4. QTL for YLD

Grain YLD is the trait of ultimate interest to breeders as it reflects all of the plant processes, ranging from the vegetative to the reproductive stages, and hence, it possesses very complex genetic mechanisms (Quarrie et al., 2006). Yield QTL in wheat were reported in several studies (McCartney et al., 2005; Quarrie et al., 2006; Kirigwi et al., 2007; Li et al., 2007, 2015; Maccaferri et al., 2008; Azadi et al., 2014; Cui et al., 2014; Edae et al., 2014; Narjesi et al., 2015; Gao et al., 2015; Milner et al., 2016). In this study, the YLD QTL *QYL.ndsu.5A* was identified exclusively in the drought condition, indicating that it could augment drought tolerance, thereby allowing a better yield. The QTL *QYL.ndsu.1A* also could improve drought tolerance as it was identified exclusively in the drought conditions. This locus coincided with the QTL *QYld.abrii-1A1.2* (Azadi et al., 2014) and *QGY.caas-1A* (Li et al., 2015). Likewise, the QTL *QYL.ndsu.4B* was likely to improve drought tolerance, but it did not resemble any reported QTL for YLD, instead it occupied the same location as a QTL for total dry matter under dry conditions (Peleg et al., 2009). Ibrahim et al. (2012a) also found a QTL, *QAvd+*, around this locus that was important for drought tolerance. This locus was reported to control average root diameter (ARD) under both water regimes.

4.5.5. QTL for CDW

The QTL *QCDW.ndsu.7B* was a constitutive QTL for CDW and was also associated with DH, PH, and YLD; it improved drought tolerance through improving associated traits under the drought conditions. This locus is very important for drought tolerance as Peleg et al.(2009) also identified a QTL around this location for DH and maturity under dry treatment. Alexander et al. (2012), too, identified *QDt.ksu- 7B*, a major QTL for drought tolerance that spanned at 34.7 cM.

4.5.6. QTL for TKW

Thousand kernel weight is associated with yield and quality, as larger and uniformly-sized kernels are visually attractive, claim a higher market price, and indicate a higher yield (Ramya et al., 2010). Several studies on the QTL of wheat TKW have been reported in the past (Campbell et al., 1999; McCartney et al., 2005; Huang et al., 2006; Breseghello and Sorrells, 2007; Kuchel et al., 2007; Li et al., 2007, 2015; Zhang et al., 2008; Sun et al., 2009; Ramya et al., 2010; Azadi et al., 2014; Wei et al., 2014; Simmonds et al., 2014; Tadesse et al., 2015; Zanke et al., 2015). The QTL *QTKW.ndsu.4D.2*, identified for DSI stabilized TKW in drought conditions, rendering the plant drought tolerant. This locus did not correspond with any reported QTL for TKW or any other traits for drought tolerance, suggesting that this QTL could be novel. The QTL *QTKW.ndsu.5A* was important for drought tolerance as it was identified for TKW in both water regimes and corresponded with the QTL *QTgw.abrii- 5A* (Azadi et al., 2014). Another constitutive QTL, *QTKW.ndsu.5B1*, improved drought tolerance. No previously reported QTL was identified nearby, indicating its novelty. . *QTKW.ndsu.5D2* was another QTL giving stability across water regimes. It could correspond to the QTL*QRv+* and *Qsra+*, which improved root volume and surface root area, respectively, and were identified under both water regimes (Ibrahim et al., 2012a). The minor QTL, *QTKW.ndsu.4A* being identified for DSI, stabilized the change in TKW due to drought condition. This QTL was in the same location as *QTgw.abrii- 4A.2* for TKW reported earlier (Azadi et al., 2014).

4.5.7. QTL for SPK

The QTL *QSPK.ndsu.7B* was identified for DSI and hence stabilized the SPK due to the drought condition and improved drought tolerance. This locus was also reported for YLD under the dry treatment (Peleg et al., 2009). Another QTL, *QSPK.ndsu.1A*, identified under the drought

conditions, enhanced drought tolerance by increasing the number of spikes when experiencing drought. This locus could be the same QTL reported earlier for SPK (Li et al., 2007). Another QTL, *QSPK.ndsu.4A.2,* associated with stability in the number of SPK under drought conditions, seemed to be novel as it did not correspond with any reported QTL.

4.5.8. QTL for TIL

One major constitutive QTL, *QTL.ndsu.5A.3,* corresponded with the QTL reported by Kato et al. (1999).

4.6. Conclusions

Drought-tolerant wheat cultivars can strengthen food security as drought often poses a threat to wheat production in the northern USA and across the world. Understanding the genetic basis of drought tolerance in wheat is important for developing tolerant varieties. In this study, an attempt was made to elucidate the genetic factors of drought tolerance in HRSW in the northern USA. A high-density SNP-based genetic map was developed, and QTL analysis was carried out. Seven agronomic traits were evaluated in a greenhouse experiment under both control and drought water regimes.

A total of 22 QTL important for drought tolerance were identified. Among these QTL, eight were identified for the drought water regime, eight were constitutive, and six were identified for DSI. Besides those, 11 QTL were identified for the control conditions and four QTL for the mean. The QTL present on chromosomes 4D, 5D, 5A, 5B, 2B, and 4A had a maximum effect for drought tolerance.

The identified QTL could be very helpful in marker-assisted breeding programs aimed at improving drought tolerance. Also, the high-density maps could provide a better starting platform for the fine mapping and ultimately map-based cloning of major loci. More

interestingly, some of the desirable alleles were contributed by the parent Albany, which is apparently susceptible to drought. This resulted in the transgressive segregants combining desirable alleles from both parents, which could also be extremely useful for drought-tolerance breeding.

4.7. References

Alexander, L.M., F.M. Kirigwi, A.K. Fritz, and J.P. Fellers. 2012. Mapping and quantitative trait loci analysis of drought tolerance in a Spring wheat population using amplified fragment length polymorphism and diversity array technology markers. Crop Sci. 52(1): 253–261.

Azadi, A., M. Mardi, E.M. Hervan, S.A. Mohammadi, F. Moradi, M.T. Tabatabaee, S.M. Pirseyedi, M. Ebrahimi, F. Fayaz, M. Kazemi, S. Ashkani, B. Nakhoda, and G. Mohammadi-Nejad. 2014. QTL mapping of yield and yield components under normal and salt-stress conditions in bread wheat (Triticum aestivum L.). Plant Mol. Biol. Report. (April 2014).

Bogard, M., C. Ravel, E. Paux, J. Bordes, F. Balfourier, S.C. Chapman, J. Le Gouis, and V. Allard. 2014. Predictions of heading date in bread wheat (Triticum aestivum L.) using QTL-based parameters of an ecophysiological model. J. Exp. Bot. 65(20): 5849–5865.

Breseghello, F., and M.E. Sorrells. 2007. QTL analysis of kernel size and shape in two hexaploid wheat mapping populations. F. Crop. Res. 101(2): 172–179.

Bullrich, L., M.L. Appendino, G. Tranquilli, S. Lewis, and J. Dubcovsky. 2002. Mapping of a thermo-sensitive earliness per se gene on Triticum monococcum chromosome 1Am. Theor. Appl. Genet. 105(4): 585–593.

Cabral, A.L., M.C. Jordan, C. A. McCartney, F.M. You, D.G. Humphreys, R. MacLachlan, and C.J. Pozniak. 2014. Identification of candidate genes, regions and markers for pre-harvest

sprouting resistance in wheat (Triticum aestivum L.). BMC Plant Biol. 14: 340. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4253633&tool=pmcentrez&rendertype=abstract.

Campbell, K.G., C.J. Bergman, D.G. Gualberto, J.A. Anderson, M.J. Giroux, G. Hareland, R.G. Fulcher, M.E. Sorrells, and P.L. Finney. 1999. Quantitative trait loci associated with kernel traits in a soft x hard wheat cross. Crop Sci. 39(4): 1184–1195.

Climate change and the economy. 2008. Natl. Conf. State Legis. Available at http://www.ncsl.org/print/environ/ClimateChangeND.pdf (verified 15 December 2016).

Cui, F., X. Fan, C. Zhao, W. Zhang, M. Chen, and J. Li. 2014. A novel genetic map of wheat: utility for mapping QTL for yield under different nitrogen treatments. BMC Genet. 15: 57–73.

Desiderio, F., D. Guerra, D. Rubiales, L. Piarulli, M. Pasquini, A.M. Mastrangelo, R. Simeone, A. Blanco, L. Cattivelli, and G. Vale. 2014. Identification and mapping of quantitative trait loci for leaf rust resistance derived from a tetraploid wheat Triticum dicoccum accession. Mol. Breed. 34(4): 1659–1675.

Edae, E.A., P.F. Byrne, S.D. Haley, M.S. Lopes, and M.P. Reynolds. 2014. Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. Theor. Appl. Genet. 127: 791–807.

FAOSTAT. 2016. Available at http://faostat3.fao.org/faostat-gateway/go/to/home/E (verified 15 December 2016). FAO, Rome, Italy.

Fischer, R., and R. Maurer. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res. 29: 897. Available at http://www.publish.csiro.au/?paper=AR9780897.

Frohberg, R.C., R.W. Stack, T. Olson, J.D. Miller, and M. Mergoum. 2006. Registration of 'Alsen.' Crop Sci. 46: 2311–2312.

Gao, F., W. Wen, J. Liu, A. Rasheed, G. Yin, X. Xia, X. Wu, and Z. He. 2015. Genome-wide linkage mapping of QTL for yield components, plant height and yield-related physiological traits in the Chinese wheat cross Zhou 8425B/Chinese Spring. Front. Plant Sci. 6 (December): 1099. Available at http://www.ncbi.nlm.nih.gov/pubmed/26734019\nhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4683206.

de Givry, S., M. Bouchez, P. Chabrier, D. Milan, and T. Schiex. 2005. CARTHAGENE: Multipopulation integrated genetic and radiated hybrid mapping. Bioinformatics 21: 1703–1704.

Griffiths, S., J. Simmonds, M. Leverington, Y. Wang, L. Fish, L. Sayers, L. Alibert, S. Orford, L. Wingen, L. Herry, S. Faure, D. Laurie, L. Bilham, and J. Snape. 2009. Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. Theor. Appl. Genet. 119: 383–395.

Guedira, M., M. Xiong, Y.F. Hao, J. Johnson, S. Harrison, D. Marshall, and G. Brown-Guedira. 2016. Heading date QTL in winter wheat (Triticum aestivum L.) coincide with major developmental genes VERNALIZATION1 and PHOTOPERIOD1. PLoS One 11(5): e0154242. Available at http://dx.plos.org/10.1371/journal.pone.0154242.

Huang, X.Q., S. Cloutier, L. Lycar, N. Radovanovic, D.G. Humphreys, J.S. Noll, D.J. Somers, and P.D. Brown. 2006. Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (Triticum aestivum L.). Theor. Appl. Genet. 113: 753–766.

Huang, B.E., A.W. George, K.L. Forrest, A. Kilian, M.J. Hayden, M.K. Morell, and C.R. Cavanagh. 2012. A multiparent advanced generation inter-cross population for genetic analysis in wheat. Plant Biotechnol. J. 10: 826–839.

Ibrahim, S.E., A. Schubert, K. Pillen, and J. Léon. 2012. QTL analysis of drought tolerance for seedling root morphological traits in an advanced backcross population of spring wheat. 2: 619–629.

Kamran, A., M. Iqbal, A. Navabi, H. Randhawa, C. Pozniak, and D. Spaner. 2013. Earliness per se QTLs and their interaction with the photoperiod insensitive allele Ppd-D1a in the Cutler × AC Barrie spring wheat population. Theor. Appl. Genet. 126: 1965–1976.

Kato, K., H. Miura, and S. Sawada. 1999. Detection of an earliness per se quantitative trait locus in the proximal region of wheat chromosome 5AL. Plant Breed. 118(5): 391–394.

Kirigwi, F.M., M. Van Ginkel, G. Brown-Guedira, B.S. Gill, G.M. Paulsen, and A.K. Fritz. 2007. Markers associated with a QTL for grain yield in wheat under drought. Mol. Breed. 20: 401–413.

Kosambi, D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12: 172–175.

Kuchel, H., K.J. Williams, P. Langridge, H.A. Eagles, and S.P. Jefferies. 2007. Genetic dissection of grain yield in bread wheat. I. QTL analysis. Theor. Appl. Genet. 115(8): 1029–1041.

Kumar, A., E.E. Mantovani, R. Seetan, A. Soltani, M. Echeverry-Solarte, S. Jain, S. Simsek, D. Doehlert, M.S. Alamri, E.M. Elias, S.F. Kianian, and M. Mergoum. 2016. Dissection of genetic factors underlying wheat kernel shape and size in an Elite × Nonadapted cross using a high density SNP linkage map. Plant Genome 9: 1-22. Available at https://dl.sciencesocieties.org/publications/tpg/abstracts/9/1/plantgenome2015.09.0081.

Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199.

Li, S., J. Jia, X. Wei, X. Zhang, L. Li, H. Chen, Y. Fan, H. Sun, X. Zhao, T. Lei, Y. Xu, F. Jiang, H. Wang, and L. Li. 2007. An intervarietal genetic map and QTL analysis for yield traits in wheat. Mol. Breed. 20(2): 167–178.

Li, X., X. Xia, Y. Xiao, Z. He, D. Wang, R. Trethowan, H. Wang, and X. Chen. 2015. QTL mapping for plant height and yield components in common wheat under water limited and full irrigation environments. Crop Pasture Sci. 8: 660–670. Available at http://www.publish.csiro.au/view/journals/dsp_journals_pip_abstract_Scholar1.cfm?nid=40&pip=CP14236.

Liu, S., S.O. Assanga, S. Dhakal, X. Gu, C.T. Tan, Y. Yang, J. Rudd, D. Hays, A.M.H. Ibrahim, Q. Xue, S. Chao, R. Devkota, C. Shachter, T. Huggins, S. Mohammed, and M.P. Fuentealba. 2016. Validation of chromosomal locations of 90K array single nucleotide polymorphisms in US wheat. Crop Sci. 56: 364-373.

Liu, G., S.B. Xu, Z.F. Ni, C.J. Xie, D.D. Qin, J. Li, L.H. Lu, J.P. Zhang, H.R. Peng, and Q.X. Sun. 2011. Molecular dissection of plant height QTLs using recombinant inbred lines from hybrids between common wheat (Triticum aestivum L.) and spelt wheat (Triticum spelta L.). Chinese Sci. Bull. 56(18): 1897–1903.

Maccaferri, M., M.C. Sanguineti, S. Corneti, J.L.A. Ortega, M. Ben Salem, J. Bort, E. DeAmbrogio, L.F.G. Del Moral, A. Demontis, A. El-Ahmed, F. Maalouf, H. Machlab, V. Martos, M. Moragues, J. Motawaj, M. Nachit, N. Nserallah, H. Ouabbou, C. Royo, A. Slama, and R. Tuberosa. 2008. Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178(1): 489–511.

McCartney, C.A., D.J. Somers, D.G. Humphreys, O. Lukow, N. Ames, J. Noll, S. Cloutier, and B.D. McCallum. 2005. Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452x 'AC Domain.' Genome 48(5): 870–883. Available at http://www.ncbi.nlm.nih.gov/pubmed/16391693.

Mergoum, M., R.C. Frohberg, R.W. Stack, T. Olson, T.L. Friesen, and J.B. Rasmussen. 2006. Registration of “Glenn” wheat. Crop Sci.: 46:473–474.

Mergoum, M., R.C. Frohberg, R.W. Stack, J.W. Rasmussen, and T.L. Friesen. 2008. Registration of “Faller” Spring Wheat. J. Plant Regist. 2(3): 224–229. Available at <Go to ISI>://CCC:000268932600013\nhttp://www.crops.org.

Milner, S.G., M. Maccaferri, B.E. Huang, P. Mantovani, A. Massi, E. Frascaroli, R. Tuberosa, and S. Salvi. 2016. A multiparental cross population for mapping QTL for agronomic traits in durum wheat (Triticum turgidum ssp. durum). Plant Biotechnol. J. 14(2): 735–748. Available at http://www.ncbi.nlm.nih.gov/pubmed/26132599.

Narjesi, V., M. Mardi, E.M. Hervan, and A. Azadi. 2015. Analysis of quantitative trait loci (QTL) for grain yield and agronomic traits in wheat (Triticum aestivum L .) under normal and salt- stress conditions. Plant Mol Biol 33: 2030–2040.

North Dakota Wheat Commission. 2016. Building bigger better Mark. Available at http://www.ndwheat.com/buyers/default.asp?ID=294 (verified 15 December 2016).

Peleg, Z., T. Fahima, T. Krugman, S. Abbo, D. Yakir, A.B. Korol, and Y. Saranga. 2009. Genomic dissection of drought resistance in durum wheat × wild emmer wheat recombinant inbreed line population. Plant, Cell Environ. 32(7): 758–779.

Pushpendra, K.G., S.B. Harindra, L.K. Pawan, K. Neeraj, K. Ajay, R.M. Reyazul, M. Amita, and K. Jitendra. 2007. QTL analysis for some quantitative traits in bread wheat. J. Zhejiang Univ. Sci. B 8(11): 807–14. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2064952&tool=pmcentrez&rendertype=abstract.

Quarrie, S.A., S. Pekic Quarrie, R. Radosevic, D. Rancic, A. Kaminska, J.D. Barnes, M. Leverington, C. Ceoloni, and D. Dodig. 2006. Dissecting a wheat QTL for yield present in a range of environments: From the QTL to candidate genes. J. Exp. Bot. 57(11): 2627–2637.

Ramya, P., A. Chaubal, K. Kulkarni, L. Gupta, N. Kadoo, H.S. Dhaliwal, P. Chhuneja, M. Lagu, and V. Gupta. 2010. QTL mapping of 1000-kernel weight, kernel length, and kernel width in bread wheat (Triticum aestivum L.). J. Appl. Genet. 51(4): 421–429.

Rippey, B.R. 2015. The U.S. drought of 2012. Weather Clim. Extrem. 10: 57–64.

Russo, M.A., D.B.M. Ficco, G. Laidò, D. Marone, R. Papa, A. Blanco, A. Gadaleta, P. De Vita, and A.M. Mastrangelo. 2014. A dense durum wheat × T. dicoccum linkage map based on SNP markers for the study of seed morphology. Mol. Breed. 34(4): 1579–1597.

SAS Institute. 2004. SAS Online Doc, v. 9.1.2. SAS Inst., Cary, NC.

Shindo, C., H. Tsujimoto, and T. Sasakuma. 2003. Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity (Edinb.). 90(1): 56–63.

Simmonds, J., P. Scott, M. Leverington-Waite, A.S. Turner, J. Brinton, V. Korzun, J. Snape, and C. Uauy. 2014. Identification and independent validation of a stable yield and thousand grain weight QTL on chromosome 6A of hexaploid wheat (Triticum aestivum L.). BMC Plant Biol. 14: 191. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4105860&tool=pmcentrez&rendertype=abstract.

Singh, A., R.E. Knox, R.M. DePauw, A.K. Singh, R.D. Cuthbert, S. Kumar, and H.L. Campbell. 2016. Genetic mapping of common bunt resistance and plant height QTL in wheat. Theor. Appl. Genet. 129(2): 243–256. Available at "http://dx.doi.org/10.1007/s00122-015-2624-8.

Sourdille, P., J.W. Snape, T. Cadalen, G. Charmet, N. Nakata, S. Bernard, and M. Bernard. 2000. Detection of QTLs for heading time and photoperiod response in wheat using a doubled-haploid population. Genome 43(3): 487–494. Available at http://www.nrcresearchpress.com/doi/abs/10.1139/g00-013\nhttp://www.nrcresearchpress.com/doi/abs/10.1139/g00-013#.U80ianKYNko\nhttp://www.nrcresearchpress.com/doi/pdfplus/10.1139/g00-013.

Sun, X.Y., K. Wu, Y. Zhao, F.M. Kong, G.Z. Han, H.M. Jiang, X.J. Huang, R.J. Li, H.G. Wang, and S.S. Li. 2009. QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. Euphytica 165(3): 615–624.

Tadesse, W., F.C. Ogbonnaya, A. Jighly, M. Sanchez-Garcia, Q. Sohail, S. Rajaram, and M. Baum. 2015. Genome-wide association mapping of yield and grain quality traits in winter wheat genotypes. PLoS One 10(10): 1–18.

Voorrips, R.. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered 93: 77–78.

Wang, S., D. Wong, K. Forrest, A. Allen, S. Chao, B.E. Huang, M. Maccaferri, S. Salvi, S.G. Milner, L. Cattivelli, A.M. Mastrangelo, A. Whan, S. Stephen, G. Barker, R. Wieseke, J. Plieske, M. Lillemo, D. Mather, R. Appels, R. Dolferus, G. Brown-Guedira, A. Korol, A.R. Akhunova, C. Feuillet, J. Salse, M. Morgante, C. Pozniak, M.C. Luo, J. Dvorak, M. Morell, J. Dubcovsky, M. Ganal, R. Tuberosa, C. Lawley, I. Mikoulitch, C. Cavanagh, K.J. Edwards, M. Hayden, and E.

Akhunov. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol. J. 12(6): 787–796.

Wei, L., S. Bai, J. Li, X. Hou, X. Wang, H. Li, B. Zhang, W. Chen, D. Liu, B. Liu, and H. Zhang. 2014. QTL positioning of thousand wheat grain weight in Qaidam Basin. (June): 239–244.

Wu, Q.H., Y.X. Chen, S.H. Zhou, L. Fu, J.J. Chen, Y. Xiao, D. Zhang, S.H. Ouyang, X.J. Zhao, Y. Cui, D.Y. Zhang, Y. Liang, Z.Z. Wang, J.Z. Xie, J.X. Qin, G.X. Wang, D.L. Li, Y.L. Huang, M.H. Yu, P. Lu, L.L. Wang, L. Wang, H. Wang, C. Dang, J. Li, Y. Zhang, H.R. Peng, C.G. Yuan, M.S. You, Q.X. Sun, J.R. Wang, L.X. Wang, M.C. Luo, J. Han, and Z.Y. Liu. 2015. High-density genetic linkage map construction and QTL mapping of grain shape and size in the wheat population Yanda1817 x Beinong6. PLoS One. 10(2): 1-17.

Xu, X., G. Bai, B.F. Carver, and G.E. Shaner. 2005. A QTL for early heading in wheat cultivar Suwon 92. Euphytica 146(3): 233–237.

Zanke, C., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze, S. Beier, M.W. Ganal, and M.S. Röder. 2014a. Genetic architecture of main effect QTL for heading date in European winter wheat. Front. Plant Sci. 5(May): 217. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4033046&tool=pmcentrez&rendertype=abstract.

Zanke, C.D., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze, F. Neumann, A. Eichhorn, A. Polley, C. Jaenecke, M.W. Ganal, and M.S. Röder. 2015. Analysis of main effect QTL for thousand grain weight in European winter wheat (Triticum aestivum L.) by genome-wide association mapping. Front. Plant Sci. 6(September): 1–14. Available at http://journal.frontiersin.org/article/10.3389/fpls.2015.00644.

Zanke, C.D., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze, K. Neumann, M.W. Ganal, and M.S. Röder. 2014b. Whole genome association mapping of plant height in winter wheat (Triticum aestivum L). PLoS One 9(11).

Zhang, W., S. Chao, F. Manthey, O. Chicaiza, J.C. Brevis, V. Echenique, and J. Dubcovsky. 2008. QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. Theor. Appl. Genet. 117(8): 1361–1377.

CHAPTER 5. GENOME-WIDE ASSOCIATION MAPPING FOR DROUGHT TOLERANCE IN SPRING WHEAT IN THE NORTHERN USA

5.1. Abstract

Understanding the genetics of drought tolerance in hard red spring wheat (HRSW) in the northern USA is a prerequisite for developing drought-tolerant cultivars for this region. Association mapping (AM) could be a better option than QTL mapping to use the natural variations present in diverse germplasm panel and with a higher resolution. An AM study for drought tolerance in spring wheat in the northern USA was undertaken using ≥ 350 wheat genotypes. The genotypes were evaluated in different locations of North Dakota (ND) for plant height (PH), days to heading (DH), yield (YLD), test weight (TW), and thousand kernel weight (TKW) in rain-fed conditions. Rainfall data for the experimental sites were collected from the North Dakota Agricultural Weather Network (NDAWN) website to assess drought conditions. The AM panel was genotyped using Illumina's Infinium 90K SNP (Single Nucleotide polymorphism) assay. A total of 14,816 SNP markers were used for the association, employing a mixed linear model (MLM) with (PC + K). A total of 66 consistent QTL involved with drought tolerance were identified, with $p \leq 0.001$. The chromosomes 1A, 3A, 3B, 4B, 4D, 5B, 6A, and 6B were identified to harbor major QTL for drought tolerance. Six novel QTL were identified on chromosomes 3D, 4A, 5B, 7A, and 7B. The findings of this study can be used in marker-assisted selection (MAS) for drought-tolerant breeding in spring wheat.

5.2. Introduction

Drought poses a major threat for crop yield, highlighting the urgent need to develop drought-tolerant cultivars (Ergen and Budak, 2009). The majority of countries worldwide experience drought problems, even those in humid regions as they often have dry spells at some

point. Obviously, drought is more severe in arid areas with minimal rainfall (Sun et al., 2006). North Dakota is the biggest producer of HRSW in the USA (North Dakota Wheat Commission, 2016). The state, especially the semi-arid western half experiences frequent droughts (Climate change and the economy, 2008). Consequently, HRSW, a major cash crop for ND and the USA, is regularly affected by drought in this region. Developing and releasing drought-tolerant HRSW cultivars is critical to counter ND drought conditions, but this cannot be done without understanding the genetics of drought tolerance for HRSW in the northern USA.

Quantitative trait loci analysis allows genetic dissection, which can be a sound approach for understanding the molecular basis of drought tolerance in HRSW. In the past, several QTL mapping studies for drought tolerance in wheat were conducted (Kirigwi et al., 2007; Peleg et al., 2009; Sayed, 2011; Alexander et al., 2012; Ibrahim et al., 2012a; b; Kumar et al., 2012; Malik et al., 2015). These studies have used different types of markers, including SSRs, EST-STS, and DArTs. However, almost all of these studies were based on low-resolution molecular maps consisting of 102 to 690 markers. The number of markers in the previous studies seems insufficient to saturate the wheat genome due to its large size of 17 gigabase-pairs (Brenchley et al., 2012). Also, drought tolerance is a quantitative trait adopting different mechanisms (Blum, 1988) and should have a number of QTL distributed throughout the whole genome. A high resolution map can provide a more complete genetic dissection of drought tolerance and also a successful application of associated molecular markers through marker-assisted selection (MAS) programs. The Infinium iSelect 90K assay (Wang et al., 2014), with more than 81,000 gene-associated SNPs to assess polymorphism in bread wheat, provides a better means to identify SNPs tightly linked to drought tolerance.

Bi-parental QTL mapping, even when using high-density linkage maps, suffers some limitations. The bi-parental population has fewer recombination events, and therefore, has low resolution. By comparison, association mapping (AM) exploits a broader population and multiple alleles and has a better resolution of the QTL (Yu and Buckler, 2006). A few AM studies on drought tolerance conducted in the past have used a small number of markers (Dodig et al., 2012; Edae et al., 2013, 2014), which seems insufficient to explore the variation in wheat efficiently. Dodig et al. (2012) used 46 SSR markers, and Edae et al. (2013) used 78 DArT markers. Also, to date, no study of drought-tolerant QTL has been done for HRSW in the northern USA. Therefore, an AM study was carried out for drought tolerance in HRSW in the northern USA using the Infinium iSelect 90K assay to dissect the genetics of this important trait and identify closely-linked markers for marker-assisted breeding.

5. 3. Materials and Methods

5.3.1. Plant materials

In 2012, a panel of 350 germplasms composed of HRSW inbred lines developed by the HRSW breeding program at North Dakota State University (NDSU) and different cultivars with varying drought tolerance, was used for this study (Appendix Table A1). Eleven more accessions were added for the experiments conducted in 2013 and 2014 (Appendix Table A2). These lines were developed over time from different crosses and pedigree selections for different purposes, such as drought tolerance, disease resistance, quality, yield, etc. Therefore, the AM panel represented a wide range of diversity.

5.3.2. Field experiments

The evaluation of agronomic performances of the AM panel was carried out under non-irrigated field conditions at different locations of ND. In 2012, the plant material was evaluated

at Prosper, Casselton, and Minot. In 2013, the evaluation was carried out in Prosper, Minot, and Williston. And in 2014, the plant material was evaluated in Prosper, Minot, and Hettinger. Prosper and Casselton are located in eastern ND, at 46.9630° N, 97.0198° W and 46.9^0 N, 97.210556^0 W, respectively. Minot is located between western ND's semi-arid grassland and central ND's sub-humid grassland (48.2330° N, 101.2923° W). Williston is located in northwestern ND (48.1470° N, 103.6180° W), and Hettinger is in southwestern ND (46.0014° N, 102.6368° W). The total rainfall during the growing period (seed sowing to ripening) in 2012, 2013, and 2014 at Prosper was 119.6 mm, 269.7 mm, and 168.6 mm, respectively (Table 5.1). Minot had a total growing period rainfall of 168 mm in 2012, 159.8 mm in 2013, and 230.9 mm in 2014. And, Casselton, Williston, and Hettinger had a total rainfall of 122.8 mm (2012), 319.3 mm (2013), and 200.3 mm (2014), respectively (Table 5.1) during the growing season (NDAWN, 2015). The available soil moisture of the experimental sites (Table 5.1) was considered to assess the drought condition. The available soil moisture was achieved from the soil type of the experimental sites (Frazen, 2003). Each experiment was conducted in a randomized complete block design (RCBD) with two replicates in 2012, whereas a simple Lattice design was used in 2013 and 2014. The plots had an area of 2.44 m. × 1.22 m and seven rows with a 15.24cm gap between them in 2012 and 2013. The plot size of 2.44m × 1.42m was larger in 2014, but the number of rows was still seven with a bigger 17.78 cm gap between them.

Table 5.1. Soil types, plant-available water (water-holding capacity of soil), and total rainfall for eight environments

Environments	Soil type	Plant-available water (mm water/30.48 cm soil)	Rainfall (mm)
Casselton 2012	Fine silty loam	45.72-63.5	120.1
Prosper 2012	Fine silty loam	45.72-63.5	119.6
Minot 2012	Fine sandy loam	31.75-45.72	168
Prosper 2013	Fine silty loam	45.72-63.5	269.7
Minot 2013	Fine sandy loam	31.75-45.72	442.3
Williston 2013	Fine sandy loam	31.75-45.72	319.3
Minot 2014	Fine sandy loam	31.75-45.72	230.9
Prosper 2014	Fine silty loam	45.72-63.5	168.6
Hettinger 2014	Fine sandy loam	31.75-45.72	200.3

5.3.3. Data collection

The phenotypic data was collected on DH, PH, YLD, TW, and TKW. Heading date was recorded when more than 50% of the plants in the plot were starting to flower. Plant height was measured in the middle of the plot from plant base to tip excluding the awn. Yield per plot was converted to yield/ha for further analysis. Similarly, Kg/0.5 pint cup was converted to Kg/m^3 as the TW for further analysis. A thousand kernels were counted using a seed counter and were weighed for TKW.

5.3.4. Phenotypic data analysis

The ANOVA Proc MIXED procedure was used (SAS Institute, 2004) to analyze the phenotypic data from 2012, whereas for 2013 and 2014, the Proc LATTICE was used. The accessions of the AM panel were considered as fixed effects, and environments and blocks were considered as random effects in the ANOVA Proc MIXED procedure. The mean values were separated using the *F*-protected least significant difference (LSD) value at the $P \leq 0.05$ level of significance. The phenotypic data with a low coefficient of variance (CV) value and significant differences among entries were used for further analysis. The locations that did not show

significant differences for most of the traits and with a high CV were not included for further analysis and reporting.

5.3.5. Genotyping

Genomic DNA was isolated from lyophilized young leaves of each genotype using the DNeay Plant Mini Kit (Qiagen, Valencia, CA, cat. no. 69106). The quality of the DNA was checked on 0.8% agarose gel. The NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) was used to check the DNA concentration. The accessions of the AM panel were genotyped using the Illumina 90K iSelect wheat SNP assay in the Small Grains Genotyping Lab, USDA-ARS, Fargo, ND. The genotyping module GenomeStudio V2011.1 (www.illumina.com, verified 18 December 2015) was used to analyze the SNP data.

5.3.6. Association analysis

The Illumina iSelect 90K assay (Wang et al., 2014) produced data for 81,587 SNPs. The clustering of the SNP alleles and calling of the genotypes were performed with GS v2011.1 (www.illumina.com, verified 18 December 2015). The minimum number of points used in the cluster was 10 (Wang et al., 2014). Monomorphic SNPs and SNPs having more than 20% missing genotypic data and 10% heterozygosity were excluded. The best linear unbiased prediction (iBLUP) method (Yang et al., 2014a) was used to impute the missing genotypic data for the remaining SNPs. A total of 17, 900 polymorphic SNPs were screened for their positions on the chromosomes based on the wheat consensus genetic map (Wang et al., 2014). An additional 2,756 SNPs were excluded for lacking map positions on the consensus maps. The software TASSEL v.5.0 was used for the AM study. The mixed linear model (MLM) with PC + Kinship (K) was used for AM, where the genotypic data were filtered for minor allele (≤ 5%) frequency. A total of 14,816 filtered SNPs were used for further AM study. The association was

analyzed using five principal components (PC), which captured 25% of variation (Table 5.4). The initial cut-off point for marker trait association (MTA) was considered at $p \leq 0.001$. Then, this cut-off was subjected to Bonferroni-correction (Yang et al., 2014b) to get the threshold ($p \leq 3.4 * 10^{-6}$). Only the markers identified to be associated in at least two environments were reported. The sequences of the markers showing MTAs were obtained from the GrainGenes database, and the NCBI BLAST database was used to check if the markers represented any candidate genes.

5.4 Results

5.4.1. Phenotypic analyses

A significant difference among genotypes was found in the environments of Casselton 2012, Prosper 2012, Minot 2012, Prosper 2013, Prosper and Minot 2013 and 2014, and Hettinger 2014 (Table 5.2). Williston 2013 did not show significant variation among genotypes (except for TW and TKW). Also, it had a very high CV% for the trait YLD, indicating less precision in that location. Minot 2014 had similar issues to Willison (Table 5.2). Therefore, data from these two environments were not analyzed further. The seeds of Minot 2013 could not be cleaned due to Fusarium head blight infection, and hence, YLD, TW and TKW could not be reported for that environment.

Table 5.2. Analysis of variance for agronomic traits in nine environments

Environment and sources	df	HD[ı]	PH[ǂ]	YLD[§]	TW[¶]	TKW[#]
Casselton, 2012						
Treatment	349	12.35***	4.2***	2.51***	4.82***	4.08***
Error		0.48	18.69	155709	92.82	2.13
CV%		1.57	5.2	9.98	1.22	5.02
Prosper, 2012						
Treatment	349	11.23***	4.71***	2***	5.87***	4.49***
Error		0.42	16.87	158617	131.65	2.85
CV%		1.38	4.63	11.15	1.51	5.86
Minot, 2012						
Treatment		8.75***	4.44***	2.26***	3.95***	2.98***
Error		0.7	24.91	150677	264.68	3.37
CV%		1.36	5.34	11.73	2.13	6.75
Prosper, 2013						
Treatment	342	12.81***	4.94***	2.72***	7.42***	8.62***
Error		0.63	13.76	135437	31.2	1.56
CV%		1.65	4.33	7.78	0.69	3.57
Williston, 2013						
Treatment	342	1.13	1.18	0.84	2.67***	5.41***
Error		5.24	75.79	520718	77.83	2.25
CV%		3.8	14.3	31.1	1.09	4.81
Minot, 2013						
Treatment	342	1.21*	1.51***	.	.	.
Error		3.59	52.54	.	.	.
CV%		3.67	9.66	.	.	.
Minot, 2014						
Treatment	342	0.96	1.05	1.06	0.99	.
Error		4.01	53.81	422853	58417	.
CV%		3.78	7.21	13.18	29.17	.
Prosper, 2014						
Treatment	342	15.67***	1.34***	4.42***	7.96***	.
Error		0.53	106.97	116745	42.2	.
CV%		1.48	10.56	6.43	0.83	.
Hettinger, 2014						
Treatment	342	1.52***	2.55***	1.54***	11.49***	.
Error		1.99	6.6	517964	38.89	.
CV%		2.48	6.55	17.17	0.8	.

*Significant at 0.05, ***Significant at 0.001 probability level

[ı]HD = Days to heading, [ǂ]PH = Plant height, [§]YLD = Yield, [¶]TW = Test weight, [#]TKW = Thousand kernel weight

5.4.2. Analysis of SNP markers

Out of 14,816 SNP markers used in the association study, 7,848 were located on the B-genome, 5,503 on the A-genome, and 1,465 markers on the D-genome. The D-genome had the lowest density of markers, with an average distance of 0.87 cM between two markers (Table 5.4). The number of markers on individual chromosomes ranked from 56 (4D) to 1,433 (2B). The average number of markers per chromosome was 705.52 (Table 5.3).

5.4.3. Association analysis

5.4.3.1. QTL for DH

Twenty QTL were identified to be associated with DH. These QTL explained from 5.6 to 11.33% of phenotypic variation (PV) (Table 5.5). Five QTL were identified to explain >10% of PV, and therefore were considered major QTL. Twelve of the QTL were identified to be constitutive, and eight of the QTL were identified exclusively in drought-prone environments (Table 5.5).

5.4.3.2. QTL for PH

A total of 20 QTL were identified to have an association with PH. These QTL explained from 4.54 to 48.01% of PV (Table 5.5). Seven QTL explained >10% of PV and can be considered major QTL. Sixteen QTL were identified as constitutive, three were identified in the control environments and one was identified in the drought environment (Table 5.5).

Table 5.3. Distribution of markers in wheat chromosomes and genomes based on the 90k SNP consensus map (Wang et al., 2014).

Chromosome	No. of markers	Map length	Average map density cM/marker
1A	785	156.3	0.2
2A	861	185.47	0.22
3A	661	197.2	0.3
4A	663	166.71	0.25
5A	783	148.3	0.19
6A	852	175.32	0.21
7A	898	244.16	0.28
1B	1197	173.62	0.15
2B	1433	188.27	0.13
3B	1139	154.48	0.14
4B	635	118.91	0.19
5B	1348	219.77	0.16
6B	1216	122.92	0.1
7B	880	188.64	0.21
1D	261	199.86	0.77
2D	476	152.84	0.32
3D	207	152.84	0.74
4D	56	170.43	3.04
5D	147	207.33	1.41
6D	170	160.5	0.94
7D	148	226.87	1.53
A genome	5,503	1,273.46	0.23
B genome	7,848	1,166.61	0.15
D genome	1,465	1270.67	0.87
Whole genome	**14,816**	**3,710.74**	**0.25**

Table 5.4. Number of principal components (PC) with Eigen values and the proportion of variations they explained.

PC	Eigen value	Proportion of individual PC	Cumulative proportion
0	138.17	0.06	0.06
1	106.88	0.05	0.11
2	89.08	0.04	0.15
3	76.52	0.04	0.19
4	65.57	0.03	0.22
5	63.14	0.03	0.25
6	50.93	0.03	0.28
7	45.58	0.02	0.3
8	42.6	0.02	0.32
9	40.83	0.02	0.34
10	34.81	0.02	0.36
11	30.72	0.02	0.38
12	29.16	0.01	0.39
13	27.74	0.01	0.4
14	27.18	0.01	0.41
15	26.39	0.01	0.42
16	26.09	0.01	0.43
17	23.49	0.01	0.44
18	23.04	0.01	0.45
19	21.94	0.01	0.46
20	21.13	0.01	0.47
21	20.31	0.01	0.48
22	19.29	0.01	0.49
23	18.65	0.01	0.5

5.4.3.3. QTL for YLD

Seventeen QTL were identified to be associated with YLD. These QTL explained 4.11 to 12.04% of PV (Table 5.5). Only one QTL, located on chromosome 4B, had a major effect. Sixteen QTL were identified as constitutive, and the remaining QTL was identified in the drought-prone experimental sites (Table 5.5).

Table 5.5. Traits and associated QTL along with QTL region, chromosome number, position, associated traits, water regimes, and p and R2 values.

QTL and trait	QTL region	Other associated traits[ɨ]	Env.[ɨ]	Water regime	Position[§]	p[¶]	R^2 (%)
Days to heading							
QDH.ndsu.1B	5		1*, 2, 4*	Constitutive	90.26	$4.83*10^{-7}$	8.96
QDH.ndsu.2A.2	10		1*, 2, 4*	Constitutive	113.30	$1.92*10^{-6}$	8.07
QDH.ndsu.2B.2	14	PH, TKW	1*, 2, 4*, 6*, 7*	Constitutive	99.8-104.39	$4.86*10^{-8}$	10.44
QDH.ndsu.2D	16		2*, 4	Constitutive	19.03	$2.98*10^{-6}$	7.54
QDH.ndsu.3A.1	17	PH, TW	1*, 2, 6*, 7*	Constitutive	90.55	$1.25*10^{-8}$	11.33
QDH.ndsu.3B	21	TW, YLD	1*, 2, 3*, 4*, 5*	Constitutive	70.09	$1.44*10^{-8}$	11.24
QDH.ndsu.4A.1	25	TKW	1*, 2, 3*, 4*	Constitutive	51.97	$1.83*10^{-6}$	8.10
QDH.ndsu.4B	28	TW, PH, YLD	1*, 2*, 4*, 7*	Constitutive	64.03-75.64	$7.35*10^{-6}$	7.22
QDH.ndsu.4D	29		1*, 2, 4*	Constitutive	94.22	$3.11*10^{-8}$	10.74
QDH.ndsu.5B.2	35		1*, 2*, 3*, 4*, 5*	Constitutive	100.64-110.19	$7.67*10^{-6}$	7.19
QDH.ndsu.6B	39	TKW, PH, YLD	1*, 2*, 6*, 7*	Constitutive	63.14-71.76	$2.97*10^{-5}$	6.34
QDH.ndsu.7B	44	YLD	2*, 3*, 4*	Constitutive	98.30-101.18	$9.18*10^{-5}$	5.60
QDH.ndsu.2A.1	8	TW	1*, 2	Drought	25.02	$4.95*10^{-8}$	10.43
QDH.ndsu.2A.3	11	YLD	1*, 2	Drought	141.66	$1.81*10^{-6}$	8.11
QDH.ndsu.2B.1	13	YLD	1*, 2	Drought	83.80	$8.13*10^{-7}$	8.62
QDH.ndsu.3A.2	18	YLD	1*, 2	Drought	117.73	$1.58*10^{-7}$	9.68
QDH.ndsu.4A.2	26	TKW	1*, 2*, 3*	Drought	99.19-103.03	$3.69*10^{-5}$	6.21
QDH.ndsu.5A.1	30		1*, 2	Drought	55.01	$2.54*10^{-6}$	7.90
QDH.ndsu.5A.2	31		1*, 2*, 3*	Drought	84.13	$3.98*10^{-6}$	7.61
QDH.ndsu.5B.1	33	TKW	1*, 2	Drought	5.70	$8.35*10^{-7}$	8.61
Plant height							
QPH.ndsu.1A	3		1*, 5, 6*	Constitutive	105.74	$9.80*10^{-13}$	16.83
QPH.ndsu.1B	4	YLD	2*, 4*, 7	Constitutive	76.89	$3.2*10^{-7}$	7.73
QPH.ndsu.2A.1	9		2*, 3*, 4*, 7	Constitutive	98.43-101.97	$2.03*10^{-5}$	6.31
QPH.ndsu.2A.2	12		2*, 6*,7*	Constitutive	156.23-162.89	$2.4*10^{-5}$	6.25
QPH.ndsu.2B	14	TKW, DH	2*, 3*, 4*, 5, 7*	Constitutive	109.53	$2.24*10^{-7}$	8.99
QPH.ndsu.3A.1	17	TW, DH	2*, 3*, 4*	Constitutive	77.57	$1.68*10^{-4}$	5.13
QPH.ndsu.3A.2	19		2*, 6	Constitutive	128.64	$6.08*10^{-7}$	8.46
QPH.ndsu.3A.3	20		3*, 4*, 6*	Constitutive	180.33	$5.42*10^{-5}$	5.72

Table 5.5. Traits and associated QTL along with QTL region, chromosome number, position, associated traits, water regimes, and p and R2 values (continued).

QTL and trait	QTL region	Other associated traits[ǂ]	Env.[‡]	Water regime	Position[§]	p[¶]	R^2 (%)
QPH.ndsu.3B	22		2*, 3*, 5*	Constitutive	102.54-106.73	$5.29*10^{-4}$	4.54
QPH.ndsu.3D.1	23		2*, 5*, 7*	Constitutive	0-4.46	$3.29*10^{-4}$	4.67
QPH.ndsu.3D.2	24		2*, 3*, 4*	Constitutive	66.99	$1.62*10^{-4}$	5.27
QPH.ndsu.4B	28	TW, YLD, DH	1, 2, 3, 4, 5*, 6, 7*	Constitutive	56.19	$3.79*10^{-14}$	19.97
QPH.ndsu.5B	34	YLD	2*, 3*, 4*,5, 6, 7*	Constitutive	63.07	$6.51*10^{-31}$	48.01
QPH.ndsu.6A.2	38		1, 5*	Constitutive	133.74	$8.66*10^{-8}$	10.07
QPH.ndsu.6B.1	39	TKW, DH, YLD	3*, 4*, 5, 6*, 7	Constitutive	56.98	$1.8*10^{-30}$	47.15
QPH.ndsu.7A.2	43		2*, 3*, 4*	Constitutive	212.66	$4.3*10^{-6}$	7.55
QPH.ndsu.6A.1	37		4, 5	Control	82.38	$4.61*10^{-10}$	12.85
QPH.ndsu.6B.2	40	YLD	5, 7*	Control	108.86	$1.07*10^{-30}$	47.60
QPH.ndsu.6D	41		4*, 5, 6*, 7*	Control	22.92	$1.56*10^{-7}$	9.21
QPH.ndsu.7A.1	42		1*, 2 *, 3*	Drought	61.36	$3.38*10^{-4}$	4.83
Thousand kernel weight							
QTKW.ndsu.2B.1	14	PH, DH	1*, 2*, 3*	Drought	106.56-114.57	$7.44*10^{-5}$	5.64
QTKW.ndsu.2B.2	15	YLD	2*, 3	Drought	155.41	$9.33*10^{-7}$	8.55
QTKW.ndsu.4A.1	25	DH	1*, 2*, 3*	Drought	48.98-51.97	$1.74*10^{-4}$	5.22
QTKW.ndsu.4A.2	26	DH	1*, 2*, 3*	Drought	105.87-108.72	$1*10^{-4}$	5.59
QTKW.ndsu.4A.3	27		2, 3*	Drought	154.30	$2.44*10^{-7}$	9.20
QTKW.ndsu.6B	39	PH, DH, YLD	1*, 2*, 3*	Drought	56.64-64.82	$1.79*10^{-4}$	5.20
QTKW.ndsu.5B	33	DH	1*, 3, 4*	Constitutive	17.48	$1.98*10^{-6}$	6.92
Test weight							
QTW.ndsu.1A	1		1*, 2*, 6*	Constitutive	29.11-38.11	$4.39*10^{-4}$	4.63
QTW.ndsu.2A	8	DH	1*, 4*, 5*	Constitutive	20.26	$7.12*10^{-4}$	4.07
QTW.ndsu.3A	17	PH, DH	1*, 2*, 5*	Constitutive	85.73	$284*10^{-4}$	3.70
QTW.ndsu.3B	21	DH, YLD	1*, 3*, 4*, 5*	Constitutive	62.31-69.53	$3.83*10^{-6}$	7.58
QTW.ndsu.4B	28	PH, YLD, DH	1, 2*, 3, 4*, 5*, 6*	Constitutive	55.55	$4.66*10^{-7}$	7.66
Yield							
QYL.ndsu.1A	2		1*, 3*, 5*	Constitutive	48.45-56.81	$1.49*10^{-5}$	6.77
QYL.ndsu.1B.1	4	PH	1*, 2*, 3*, 4	Constitutive	70.08	$2.22*10^{-6}$	6.59
QYL.ndsu.1B.2	6		1*, 5*, 6*	Constitutive	112.07	$3.68*10^{-5}$	6.20
QYL.ndsu.1D	7		1, 5*	Constitutive	3.40	$2.89*10^{-6}$	7.80

Table 5.5. Traits and associated QTL along with QTL region, chromosome number, position, associated traits, water regimes, and p and R2 values (continued).

QTL and trait	QTL region	Other associated traits[ɪ]	Env.[ǂ]	Water regime	Position[§]	p[¶]	R^2 (%)
QYL.ndsu.2A	11	DH	1, 4*, 5*	Constitutive	144.41	$1.86*10^{-6}$	8.08
QYL.ndsu.2B.1	13	DH	1, 4*, 6*	Constitutive	88.93-90.971	$1.31*10^{-5}$	5.57
QYL.ndsu.2B.2	15	TKW	1, 3, 4, 5, 6	Constitutive	157.21	$1.79*10^{-6}$	8.11
QYL.ndsu.3B	21	TW, DH	1*, 4*, 5*, 6*	Constitutive	62.31-69.53	$5.81*10^{-6}$	7.36
QYL.ndsu.4B	28	PH, DH, TW	1, 4*, 5, 6*	Constitutive	56.19	$4.17*10^{-9}$	12.04
QYL.ndsu.5A	32		1*, 4*, 6*	Constitutive	116.35-117.67	$1.7*10^{-4}$	4.11
QYL.ndsu.5B	34	PH	1, 4*	Constitutive	68.36	$1.94*10^{-6}$	6.91
QYL.ndsu.6A	36		1, 3*, 4*	Constitutive	12.48	$1.52*10^{-6}$	8.21
QYL.ndsu.6B.1	39		1*, 4*, 5*	Constitutive	64.08-64.71	$6.08*10^{-6}$	7.33
QYL.ndsu.6B.2	40	PH	1, 3*, 4	Constitutive	115.25	$1.38*10^{-6}$	7.12
QYL.ndsu.7B	44	DH	1*, 3*, 4*	Constitutive	89.82-92.52	$6.64*10^{-4}$	4.26
QYL.ndsu.7D	45		1*, 4*, 5*	Constitutive	128.15-135.55	$3.19*10^{-5}$	5.24
QYL.ndsu.3A	18	DH	1, 2*, 3*	Drought	109.95	$1.52*10^{-6}$	8.21

ɪ DH = Days to heading, PH = Plant height, YLD = Yield, TW = Test weight, TKW = Thousand kernel weight.
[ǂ]1 = Casselton 2012, 2 = Prosper 2012, 3 = Minot 2012, 4 = Prosper 2013, 5 = Prosper 2014, 6 = Hettinger 2014, 7 = Minot 2013, 8 = Mean across environments
[§]Position represents the peak point of the QTL interval. The position is based on consensus map of Wang et al. (2014).
* p less than 0.001 but above the threshold level.

5.4.3.4. QTL for TW

Five QTL were identified to have an association with TW. All of these QTL had minor effects, explaining from 3.7 to7.66% of PV. All of the QTL identified were constitutive (Table 5.5).

5.4.3.5. QTL for TKW

Seven QTL were identified for TKW, all of which had minor effects, explaining from 5.2 to 9.2% of PV. One QTL among them was constitutive, and the remaining six were identified in the drought-prone environments (Table 5.5).

5.5. Discussion

5.5.1. Association analyses

In this study, the iBLUP method (Yang et al., 2014a) was used to impute missing genotypic data as it was reported to tolerate a high rate of missing data especially for rare alleles, compared to the common imputation methods. High-density single nucleotide polymorphism (SNP) genotyping arrays explore genomic diversity and MTAs very efficiently (Wang et al., 2014). Infinium iSelect 90K assay, uses more than 81,000 gene-associated SNPs to reveal polymorphism in allohexaploid and allotetraploid wheat populations (Wang et al., 2014; Wu et al., 2015; Kumar et al., 2016; Liu et al., 2016). Higher genome coverage and resolution in the dissection of wheat's agronomic traits are possible using this genotypic tool (Kirigwi et al., 2007; Muchero et al., 2009; Sayed, 2011; Alexander et al., 2012; Ibrahim et al., 2012a; Kumar et al., 2012; Milner et al., 2016). The marker density found in this study (0.49cM/marker) was in agreement with the previous studies using the 90K Infinium iSelet assay (Wang et al., 2014; Ain et al., 2015; Kumar et al., 2016).

The MLM model used in this association study is a newly-developed model that is proving to be very efficient for genome-wise association studies (GWAS) (Li and Zhu, 2013). The MLM can be used with either structured (R) or principal component (PC). The utilization of MLM with PC was considered a better option because the structured association is more likely to give a false positive association due to the historical relationship (Larsson et al., 2013). However,

the PCs are a smaller number of uncorrelated variables transformed from correlated variables. Among the PCs, the first PC captures the maximum variation, and the others follow in descending order of variation (Table 5.5). This study used five PCs, which captured 25% of the variation. Again, the MLM with PC can be without Kinship (K) or with K. The K is the "coefficient of relatedness," which minimizes spurious association (Khan, 2013). Therefore, The MLM (PC + K) was used for the association study.

Determining the threshold for the p-value is crucial. A liberal threshold will declare a false positive association (a type I error), whereas too stringent a threshold is likely to miss a true association (a type II error). Taking this into consideration, the initial cut-off was chosen as $p \leq 0.001$, which was not very stringent. Then, the threshold ($p \leq 3.4 * 10^{-6}$) was determined using the Bonferroni-correction (Yang et al., 2014b), which was very stringent. The MTAs identified at the initial cut-off and the threshold were reported if they were identified in at least at two environments. This repetition of the MTA further minimized any false associations.

5.5.2. Use of secondary data to assess drought conditions

Drought can be assessed by variable weather conditions, soil moisture, and crop conditions over a particular growing season (Lanceras et al., 2004). Therefore, rainfall data were collected, and the soil types of the experimental sites, which reflect soil moisture, were taken into consideration to assess drought conditions for this study. The total amount of rainfall was collected from planting date to plant physiological maturity. The dates for the physiological maturity of the plants were calculated by adding 30 days to DH (Simmons, 1914). Among the experimental locations, Casselton 2012, Prosper 2012, and Minot 2012 were considered to have drought conditions, whereas Prosper 2013, Minot 2013, Prosper 2014, and Hettinger 2014 were considered to have control conditions. Although Minot 2012 and Prosper 2014 had about the

same amount of rainfall, the soil in Prosper had a better water-holding capacity. Therefore, Minot 2012 was considered to have drought conditions.

5.5.3. Use of agronomic data to assess drought tolerance

Several studies suggested that drought tolerance can be incorporated into a breeding program most effectively by identifying QTL for YLD or YLD-related traits (Lanceras et al., 2004; Alexander et al., 2012; Dodig et al., 2012; Besufekad and Bantte, 2013; Edae et al., 2014; Ain et al., 2015). The agronomic traits used in this study for identifying SNP markers associated with drought are DH, PH, YLD, TW, and TKW. Among those, YLD is the trait of ultimate interest to breeders.

5.5.4. DH

Several major and minor QTL were revealed for DH, which indicated the quantitative nature of the trait. The eight QTL for DH, identified exclusively under drought conditions, could play a vital role in drought tolerance. Also, the constitutive QTL can be used for drought tolerance breeding in wheat. Some of these QTL (exclusively for drought or constitutive) identified in this study corresponded with some already reported QTL associated with drought tolerance. Malik et al. (2015) identified three adjacent QTL on chromosome 2A for drought tolerance related to the photosynthetic rate, cell membrane stability, and relative water content. The QTL *QDH.ndsu.2A.1* in this study could represent one of those QTL. Two QTL identified in this study on the chromosome 3A, which were important for drought tolerance, *QDH.ndsu.3A.1* and *QDH.ndsu.3A.2* could represent the QTL,*QHea.T84-3A* (Ibrahim et al., 2012a) which was found to increase DH under both drought and non-drought conditions. Chromosomal arm 3AL also harbors a gene for earliness *per se* (Edae et al., 2014), gene for enhanced response to abscisic acid (*ERA1)* which gives drought tolerance (Edae et al., 2014). The gene *ERA1*, also

located on chromosome 3B, could represent the QTL *QDH.ndsu.3B* identified in this study. Kamran et al. (2013) identified a QTL, *QFlt.dms-4A.1,* for reduced DH at 4A 61.2 cM on chromosome 4A, which may represent the constitutive QTL *QDH.ndsu.4A.1* identified in this study. The constitutive QTL *QDH.ndsu.2B.2* corresponded with the QTL *QCrs*- (Ibrahim et al., 2012b), which was reported to deteriorate the number of root crossing in both water regimes. A QTL for drought tolerance on 4AL reported by Alexander et al. (2012) may represent the QTL *QDH.ndsu.4A.2,* which was identified exclusively for drought-prone environments in this study. The constitutive QTL *QDH.ndsu.6B* was located in the same genomic location as the QTL *QHea+*, which was reported to reduce DH in both water conditions (Ibrahim et al., 2012a). Huang et al. (2006) reported a QTL for days to maturity, *QDtm.crc-2D,* that corresponded with the constitutive QTL in this study, *QDH.ndsu.2D*, according to the GrainGenes database. No reported QTL, however were identified that could correspond with the QTL *QDH.ndsu.5B.2* and *QDH.ndsu.7B* identified in this study.

5.5.5. PH

The QTL *QPH.ndsu.5B* could represent the orthologos gene to the GA-insensitive dwarf gene,*GID1L2* in rice, indicating the syngenic relationship of rice and wheat (Zanke et al., 2014). The major QTL for PH, *QPH.ndsu.6B.1* and *QPH.ndsu.6B.2,* were also reported by Zanke et al. (2014). The major QTL *QPH.ndsu.4B* could represent the reduced height gene *Rht-B1* (Wilhelm, 2011), which was reported to be on the short arm of chromosome 4B. This gene encodes the DELLA protein that reduces a plant's sensitivity to gibberellin (GA), thereby reducing stalk length and making the plant semi-dwarf. The QTL *QPH.ndsu.1A***,** *QPH.ndsu.2A.1, QPH.ndsu.6A.2,* and *QPH.ndsu.3A.3* could represent the QTL for PH reported b**y** Zanke et al. (2014**)**. The QTL *QPH.ndsu.3A.2* and *QPH.ndsu.3D.2* important for drought tolerance could be

the same as those reported by Ibrahim et al. (2012a). Liu et al. (2011) identified a QTL for PH, *QHt-3B,* which could occupy the same region as the QTL *QPH.ndsu.3B.* The QTL *QPH.ndsu.7A.1* coincided with the QTL *QHt.crc-7A* (McCartney et al., 2005). The QTL *QPH.ndsu.7A.2* and *QPH.ndsu.3D.1* in this study did not correspond with any reported QTL and hence could be novel.

5.5.6. YLD

In the past, Edae et al. (2014) reported a QTL for TKW on chromosome 1BL and a QTL for TW on chromosome 2BL that could correspond with the QTL *QYL.ndsu.1B.1* and *QYL.ndsu.2B.2,* respectively. Ibrahim et al. (2012a) identified a QTL, *QCrs.D84-2B,* on chromosome 2B at 93.4 cM that deteriorates the number of root crossings under both water regimes and could represent the QTL *QYL.ndsu.2B.1* found in this study. Ibrahim et al. (2012b) identified a YLD QTL, *QYld.T84-3Bat,* occupying the same location as the QTL *QYL.ndsu.3B* identified in this study. They identified another QTL, *QYld.T84-3Bat* 59.8, which deteriorated YLD under both water regimes and could coincide with the QTL *QYL.ndsu.4B* identified in this study. The QTL *QYL.ndsu.5B* and *QYL.ndsu.6B.2* corresponded with QTL for TW and TKW (Edae et al., 2014). Also, the QTL *QYL.ndsu.5B* corresponded with the QTL *QYld**, which was reported to improve YLD under drought stress (Ibrahim et al., 2012a). The QTL *QYL.ndsu.1B.2* had the same genomic location as the constitutive QTL for green leaf area reported by Edae et al. (2014). Ibrahim et al. (2012a) reported a QTL, *QTgw+*, which improved thousand grain weight under both water conditions and could represent the QTL *QYL.ndsu.1D.* The QTL *QYL.ndsu.2A* could coincide with the YLD QTL *QGY.caas-2A* (Li et al., 2015). Huang et al. (2006) identified the QTL *QTgw.crc-6A* for TKW that seemed to correspond with this study's QTL *QYL.ndsu.6A.*

The QTL *QYL.ndsu.7D* corresponded with the QTL *QHi+*, which was reported to improve the harvest index under both water conditions (Ibrahim et al., 2012a).

5.5.7. TW

The QTL *QTW.ndsu.4B* was reported by Li et al. (2016) as they identified QTL for TW in this region. The QTL *QTW.ndsu.1A* corresponded with two QTL for YLD, *QYld.abrii- 1A1.2* (Azadi et al., 2014) and *QGY.caas-1A* (Li et al., 2015). The constitutive QTL *QTW.ndsu.2A* occupied the same genomic region as the QTL for drought tolerance related to photosynthetic rate reported by Malik et al. (2015). The QTL *QTW.ndsu.3B* corresponded with the YLD QTL *QYld.T84-3Bat* reported by Ibrahim et al. (2012b).

5.5.8. TKW

The QTL *QTKW.ndsu.4A.2* had the same genomic location as the QTL reported by Kirigwi et al. (2007) for YLD and YLD-related traits under drought stress. Ibrahim et al. (2012a) identified the QTL *QTgw-* for thousand grain weight in both water conditions, which seemed to represent the QTL *QTKW.ndsu.6B* identified in this study. The QTL *QTKW.ndsu.2B.1*, *QTKW.ndsu.2B.*2, and *QTKW.ndsu.4A.3* could be the same QTL for thousand grain weight reported by Zanke et al. (2015). The QTL *QTKW.ndsu.4A.1* and *QTKW.ndsu.5B* seem to be novel QTL as they do not correspond with any reported QTL.

5.6. Conclusions

This study revealed 69 QTL, which included 50 constitutive QTL, three QTL identified for the control water regime, and 16 QTL exclusively under the drought conditions. Of those 16 QTL, several could be used for developing lines suitable for drought conditions. Chromosome 5B, 6B, and 4B seemed to be very important for drought tolerance by reducing PH and increasing YLD and YLD-related traits. Several identified QTL occupied genomic regions

reported for earliness *per se*, drought tolerance, and reduced height. The consistency of some QTL in the different environments indicated their validity. To conclude, this study could provide valuable information to breeders in their attempts to breed drought tolerant wheat cultivars.

5.7. References

Ain, Q.U., A. Rasheed, A. Anwar, T. Mahmood, M. Imtiaz, X. Xia, Z. He, and U.M. Quraishi. 2015. Genome-wide association for grain yield under rainfed conditions in historical wheat cultivars from Pakistan. Front. Plant Sci. 6(September): 743. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4585131&tool=pmcentrez&rendertype=abstract.

Alexander, L.M., F.M. Kirigwi, A.K. Fritz, and J.P. Fellers. 2012. Mapping and quantitative trait loci analysis of drought tolerance in a Spring wheat population using amplified fragment length polymorphism and diversity array technology markers. Crop Sci. 52(1): 253–261.

Azadi, A., M. Mardi, E.M. Hervan, S.A. Mohammadi, F. Moradi, M.T. Tabatabaee, S.M. Pirseyedi, M. Ebrahimi, F. Fayaz, M. Kazemi, S. Ashkani, B. Nakhoda, and G. Mohammadi-Nejad. 2014. QTL mapping of yield and yield components under normal and salt-stress conditions in bread wheat (Triticum aestivum L.). Plant Mol. Biol. Report. (April 2014).

Besufekad, Y., and K. Bantte. 2013. Evaluation and association mapping for drought tolerance in sorghum [Sorghum bicolor (L.) Moench]. Glob. J. Sci. Front. Res. Agric. Vet. 13(5).

Brenchley, R., M. Spannagl, M. Pfeifer, G.L.A. Barker, R. D'Amore, A.M. Allen, N. McKenzie, M. Kramer, A. Kerhornou, D. Bolser, S. Kay, D. Waite, M. Trick, I. Bancroft, Y. Gu, N. Huo, M.-C. Luo, S. Sehgal, B. Gill, S. Kianian, O. Anderson, P. Kersey, J. Dvorak, W.R. McCombie, A. Hall, K.F.X. Mayer, K.J. Edwards, M.W. Bevan, and N. Hall. 2012.

Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491(7426): 705–10. Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3510651&tool=pmcentrez&rendertype=abstract.

Climate change and the economy. 2008. Natl. Conf. State Legis. Available at http://www.ncsl.org/print/environ/ClimateChangeND.pdf (verified 15 December 2016).

Dodig, D., M. Zoric, B. Kobiljski, J. Savic, V. Kandic, S. Quarrie, and J. Barnes. 2012. Genetic and association mapping study of wheat agronomic traits under contrasting water regimes. Int. J. Mol. Sci. 13(5): 6167–6188.

Edae, E.A., P.F. Byrne, S.D. Haley, M.S. Lopes, and M.P. Reynolds. 2014. Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. Theor. Appl. Genet. 127(4): 791–807.

Edae, E. A., P.F. Byrne, H. Manmathan, S.D. Haley, M. Moragues, M.S. Lopes, and M.P. Reynolds. 2013. Association mapping and nucleotide sequence variation in five drought tolerance candidate genes in spring wheat. Plant Gen. 6: Available at https://dl.sciencesocieties.org/publications/tpg/abstracts/6/2/.

ErgenN, Z., and H. Budak. 2009. Sequencing over 13,000 expressed sequence tags from six subtractive cDNA libraries of wild and modern wheats following slow drought stress. Plant Cell Env. 32: 220–236.

Frazen, D.W. 2003. North Dakota soil and fertilizer handbook. NDSU Extension Service, North Dakota State University, Fargo, ND.

Huang, X.Q., S. Cloutier, L. Lycar, N. Radovanovic, D.G. Humphreys, J.S. Noll, D.J. Somers, and P.D. Brown. 2006. Molecular detection of QTLs for agronomic and quality traits in a

doubled haploid population derived from two Canadian wheats (Triticum aestivum L.). Theor. Appl. Genet. 113(4): 753–766.

Ibrahim, S.E., A. Schubert, K. Pillen, and J. Léon. 2012a. Comparison of QTLs for drought tolerance traits between two advanced backcross populations of spring wheat. Int. J. AgriScience 2(3): 216–227.

Ibrahim, S.E., A. Schubert, K. Pillen, and J. Léon. 2012b. QTL analysis of drought tolerance for seedling root morphological traits in an advanced backcross population of spring wheat. 2(July): 619–629.

Kamran, A., M. Iqbal, A. Navabi, H. Randhawa, C. Pozniak, and D. Spaner. 2013. Earliness per se QTLs and their interaction with the photoperiod insensitive allele Ppd-D1a in the Cutler × AC Barrie spring wheat population. Theor. Appl. Genet. 126(8): 1965–1976.

Khan, M. 2013. Association mapping using TASSEL software. Plant Breed. Genomics. Available at http://articles.extension.org/pages/62755/association-mapping-using-tassel-software (verified 7 January 2017).

Kirigwi, F.M., M. Van Ginkel, G. Brown-Guedira, B.S. Gill, G.M. Paulsen, and A.K. Fritz. 2007. Markers associated with a QTL for grain yield in wheat under drought. Mol. Breed. 20(4): 401–413.

Kumar, A., E.E. Mantovani, R. Seetan, A. Soltani, M. Echeverry-Solarte, S. Jain, S. Simsek, D. Doehlert, M.S. Alamri, E.M. Elias, S.F. Kianian, and M. Mergoum. 2016. Dissection of genetic factors underlying wheat kernel shape and size in an Elite × Nonadapted cross using a high density SNP linkage map. Plant Genome 9(1): 1-22. Available at https://dl.sciencesocieties.org/publications/tpg/abstracts/9/1/plantgenome2015.09.0081.

Kumar, S., S.K. Sehgal, U. Kumar, P.V.V. Prasad, A.K. Joshi, and B.S. Gill. 2012. Genomic characterization of drought tolerance-related traits in spring wheat. Euphytica 186(1): 265–276.

Lanceras, J., G. Pantuwan, B. Jongdee, and T. Toojinda. 2004. Quantitative trait loci associated with drought tolerance at reproductive stage in rice. Plant Physiology 135(May): 384–399. Available at http://www.plantphysiol.org/content/135/1/384.short.

Larsson, S.J., A.E. Lipka, and E.S. Buckler. 2013. Lessons from Dwarf8 on the strengths and weaknesses of structured association mapping. PLoS Genet. 9(2).

Li, C., G. Bai, S. Chao, B. Carver, and Z. Wang. 2016. Single nucleotide polymorphisms linked to quantitative trait loci for grain quality traits in wheat. Cj 4: 1–11. Available at http://dx.doi.org/10.1016/j.cj.2015.10.002.

Li, X., X. Xia, Y. Xiao, Z. He, D. Wang, R. Trethowan, H. Wang, and X. Chen. 2015. QTL mapping for plant height and yield components in common wheat under water limited and full irrigation environments. Crop Pasture Sci. 8: 660–670. Available at http://www.publish.csiro.au/view/journals/dsp_journals_pip_abstract_Scholar1.cfm?nid=40&pip=CP14236.

Li, G., and H. Zhu. 2013. Genetic studies : The linear mixed models in genome-wide association studies. Open Bioinforma. J. 7(Suppl 1, M2): 27–33.

Liu, S., S.O. Assanga, S. Dhakal, X. Gu, C.T. Tan, Y. Yang, J. Rudd, D. Hays, A.M.H. Ibrahim, Q. Xue, S. Chao, R. Devkota, C. Shachter, T. Huggins, S. Mohammed, and M.P. Fuentealba. 2016. Validation of chromosomal locations of 90K array single nucleotide polymorphisms in US wheat. Crop Sci. 56: 364-373.

Liu, G., S.B. Xu, Z.F. Ni, C.J. Xie, D.D. Qin, J. Li, L.H. Lu, J.P. Zhang, H.R. Peng, and Q.X. Sun. 2011. Molecular dissection of plant height QTLs using recombinant inbred lines from hybrids between common wheat (Triticum aestivum L.) and spelt wheat (Triticum spelta L.). Chinese Sci. Bull. 56(18): 1897–1903.

Malik, S., T.A. Malik, and G. Engineering. 2015. Genetic mapping of potential QTLs associated with drought tolerance in wheat. 25(4): 1032–1040.

McCartney, C.A., D.J. Somers, D.G. Humphreys, O. Lukow, N. Ames, J. Noll, S. Cloutier, and B.D. McCallum. 2005. Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452x'AC Domain.' Genome 48(5): 870–883. Available at http://www.ncbi.nlm.nih.gov/pubmed/16391693.

Milner, S.G., M. Maccaferri, B.E. Huang, P. Mantovani, A. Massi, E. Frascaroli, R. Tuberosa, and S. Salvi. 2016. A multiparental cross population for mapping QTL for agronomic traits in durum wheat (Triticum turgidum ssp. durum). Plant Biotechnol. J. 14(2): 735–748. Available at http://www.ncbi.nlm.nih.gov/pubmed/26132599.

Muchero, W., J.D. Ehlers, T.J. Close, and P.A. Roberts. 2009. Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [Vigna unguiculata (L.) Walp.]. Theor. Appl. Genet. 118(5): 849–863.

North Dakota Wheat Commission. 2016. Building bigger better Mark. Available at http://www.ndwheat.com/buyers/default.asp?ID=294 (verified 15 December 2016).

Peleg, Z., T. Fahima, T. Krugman, S. Abbo, D. Yakir, A.B. Korol, and Y. Saranga. 2009. Genomic dissection of drought resistance in durum wheat × wild emmer wheat recombinant inbreed line population. Plant, Cell Environ. 32(7): 758–779.

Sayed, M.A.E.A.E. 2011. QTL analysis for drought tolerance related to root and shoot traits in barley (Hordeum vulgare L.). PhD thesis Bonn Universitäts-und Landesbibliothek Bonn: 165.

Simmons, S.R., E.A. Oelke, and P.M. Anderson. 1914. Growth and development guide for spring wheat. Agricultural Extension Service University of Minnesota, AG-FO-2547.

Sun, Y., S. Solomon, A. Dai, and R.W. Portmann. 2006. How often does it rain? J. Clim. 19(6): 916–934.

Wang, S., D. Wong, K. Forrest, A. Allen, S. Chao, B.E. Huang, M. Maccaferri, S. Salvi, S.G. Milner, L. Cattivelli, A.M. Mastrangelo, A. Whan, S. Stephen, G. Barker, R. Wieseke, J. Plieske, M. Lillemo, D. Mather, R. Appels, R. Dolferus, G. Brown-Guedira, A. Korol, A.R. Akhunova, C. Feuillet, J. Salse, M. Morgante, C. Pozniak, M.C. Luo, J. Dvorak, M. Morell, J. Dubcovsky, M. Ganal, R. Tuberosa, C. Lawley, I. Mikoulitch, C. Cavanagh, K.J. Edwards, M. Hayden, and E. Akhunov. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol. J. 12(6): 787–796.

Wilhelm, E. 2011. Genetic analysis of the Group IV Rht LOCI in wheat. (June). Available at https://ueaeprints.uea.ac.uk/49761/1/THESIS_EdWilhelm.pdf.

Wu, Q.H., Y.X. Chen, S.H. Zhou, L. Fu, J.J. Chen, Y. Xiao, D. Zhang, S.H. Ouyang, X.J. Zhao, Y. Cui, D.Y. Zhang, Y. Liang, Z.Z. Wang, J.Z. Xie, J.X. Qin, G.X. Wang, D.L. Li, Y.L. Huang, M.H. Yu, P. Lu, L.L. Wang, L. Wang, H. Wang, C. Dang, J. Li, Y. Zhang, H.R. Peng, C.G. Yuan, M.S. You, Q.X. Sun, J.R. Wang, L.X. Wang, M.C. Luo, J. Han, and Z.Y. Liu. 2015. High-density genetic linkage map construction and QTL mapping of grain shape and size in the wheat population Yanda1817 x Beinong6. PLoS One. 10 (2): 1-17.

Yang, Y., Q. Wang, Q. Chen, R. Liao, X. Zhang, H. Yang, Y. Zheng, Z. Zhang, and Y. Pan. 2014a. A new genotype imputation method with tolerance to high missing rate and rare variants. PLoS One 9(6).

Yang, N., L. Yl, X. Yang, J. Huang, Y. Zhou, F. Ali, W. Wen, J. Liu, J. Li, and J. Yan. 2014b. Genome wide association studies using a new nonparametric model reveal the genetic architecture of 17 agronomic traits in an enlarged maize association panel. PLoS Genet 10(9): e1004573.

Yu, J., and E.S. Buckler. 2006. Genetic association mapping and genome organization of maize. Curr. Opin. Biotechnol. 17: 155–160.

Zanke, C.D., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze, F. Neumann, A. Eichhorn, A. Polley, C. Jaenecke, M.W. Ganal, and M.S. Röder. 2015. Analysis of main effect QTL for thousand grain weight in European winter wheat (Triticum aestivum L.) by genome-wide association mapping. Front. Plant Sci. 6(September): 1–14. Available at http://journal.frontiersin.org/article/10.3389/fpls.2015.00644.

Zanke, C.D., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze, K. Neumann, M.W. Ganal, and M.S. Röder. 2014. Whole genome association mapping of plant height in winter wheat (Triticum aestivum L). PLoS One 9(11).

CHAPTER 6. GENERAL CONCLUSIONS

Genetic dissection of drought tolerance in wheat is very important for developing drought-tolerant wheat cultivars. Genomic locations controlling drought tolerance in wheat can be identified by bi-parental QTL mapping or association mapping (AM). Bi-parental mapping offers the opportunity to discover rare alleles, whereas AM offers the opportunity to discover common variants. Association mapping exploits a broader population, allowing for a higher resolution. Combining these approaches to find the genomic location controlling drought tolerance in wheat can be complementary and overcome any shortcomings of using only one approach. Therefore, a combined approach using both bi-parental QTL mapping and AM was taken to study the genetics of drought tolerance in hard red spring wheat (HRSW) in the northern USA.

A total of 11 consistent QTL giving drought tolerance were revealed in the field experiments using the bi-parental population. Chromosomes 7B, 2B, 5A, 5D, and 6A were found to have a maximum effect for drought tolerance in these experiments. A total of 22 QTL with drought tolerance were identified in the greenhouse experiment using the bi-parental population. Chromosomes 4D, 5D, 5A, 5B, 2B, and 4A were identified to have the most vital effects for drought tolerance in this experiment. The experiments using the AM panel revealed 66 QTL associated with drought tolerance. The marker-trait associations (MTAs) for drought tolerance were higher in chromosomes 1A, 3A, 3B, 4B, 4D, 5B, 6A, and 6B.

Twelve genomic regions were repeatedly identified to be associated with drought tolerance across two-three studies described in this dissertation. The genomic regions within 14.41-38.11 cM and 48.45-63.11cM on chromosome 1A were found to be associated with drought tolerance in the bi-parental QTL mapping study (greenhouse) and the AM study. The

genomic region within 7.41-30.11 cM on chromosome 2B was identified to give drought tolerance in both bi-parental studies (greenhouse and field). The genomic region within 81.31-110.01 cM on chromosome 2B was identified to be involved in drought tolerance in all three studies. Quantitative trait loci for drought tolerance were identified on chromosome 4A within 132.91 -154.3 cM in all three studies. The genomic region within 55.55-81.41 cM on chromosome 4B was involved in drought tolerance in the bi-parental QTL mapping study (greenhouse) and the AM study. Also, these two studies shared a common genomic region (94.22-126.81 cM) on chromosome 4D and (84.13-106.01) on chromosome 5A cM for drought tolerance. The genomic region within 116.35-142.01 cM on chromosome 5A was involved in drought tolerance in the bi-parental study (field) and AM study. Quantitative trait loci were identified for drought tolerance in both bi-parental mapping studies on chromosome 5A at 194.71- 208.31 cM. The genomic region on the linkage group 5D2 at 1.01-20.91 cM was identified to be associated with drought tolerance in both bi-parental mapping studies. Also, chromosome 7B was involved in drought tolerance at 22.21-40.11 cM.

One novel QTL for drought tolerance was identified in the bi-parental mapping study (field), whereas the bi-parental mapping study (greenhouse) revealed three novel QTL for drought tolerance. Six novel QTL were identified for drought tolerance in the AM study. The QTL identified in these studies could be used in marker-assisted selections for developing drought-tolerant HRSW cultivars for the northern USA.

APPENDIX

Table A1. Germplasms used in the AM panel for 2012.

Serial number	Germplasms used in the AM panel
1	Glenn
2	Frontana/W9207//Alsen/3/ND752/4/ND2857/Dapps
3	ND708/ND706//Alsen
4	Polaris/Glenn
5	Oklee/Reeder
6	ND2902/Parshall//ND751
7	ND709-9/ND2902
8	W9207/Grandin//Alsen/3/ND752
9	Traverse/Glenn
10	SD3618/Howard
11	Arina/Steele-ND//ND806
12	Granger/ND806
13	Glenn/ND735
14	Howard/Glenn
15	ND706/Parshall//Howard/3/Faller
16	Briggs/Glenn
17	WCB703/Alsen//ND744/ND721
18	Glenn/Reeder
19	Parshall/Howard
20	Dapps/Briggs
21	SD3870/ND807
22	9950146ES/Alsen
23	ND721*2/Tokai66//Glenn
24	Frontana/W9207//Alsen/3/ND2849/ND721/4/Granite
25	Faller
26	ND744/ND721//ND744/ND721/3/Briggs
27	Frontana/W9207//Alsen/3/ND756/4/Freyr
28	ND810/Alsen
29	9950146ES/Glenn
30	SD3936/Steele-ND
31	MN01NIL84-5-5-15/Alsen
32	SD3901/Alsen
33	SD3635/Barlow
34	ND2902/Reeder//ND716-21/3/ND804
35	ND818'S'/ND810
36	ND810/ND819'S'

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
37	Velva/Bigg Red
38	ND802/Alsen//Dapps/3/Steele-ND/4/Glenn
39	Frontana/W9207//Alsen/3/ND721/Parshall/4/ND751
40	HJ98/Glenn
41	Frontana/W9207//ND748
42	ND822 'S'
43	K1157a1
44	PI350768/ND751//Parshall/6/Tam107/TA749//Wrangler/3/2*Reeder/4/Parshall/5/Dapps
45	ND807/Alsen
46	Buck Pronto/Glenn
47	Arina/Steele-ND//ND806
48	Ada/Alsen
49	ND816'S'/Faller
50	Barlow
51	Dapps/ND804
52	Briggs/Steele-ND
53	SD3618/Howard
54	Rush/Oklee
55	SD3936/MT0415
56	9950146ES/ND806
57	Barlow/Reeder
58	ND2709/3/Grandin*3//Ramsey/ND622/4/ND688/ND674/5/MT0415
59	Tom/ND806
60	SD3870/Glenn
61	ND2831/ND706sib//MT0416
62	Oklee/Howard
63	ND816'S'/Faller
64	ND802/MT0415//Steele-ND/3/ND812
65	Steele-ND/ND815
66	ND815/ND803
67	Samson/ND812
68	ND2879/ND721
69	ND819 'S'
70	ND726/ND2831
71	ND735/Steele-ND
72	Dapps/LDN(DIC3A)//ND2955/ND729
73	ND817 'S'
74	Alsen/Walworth//ND744
75	Prosper

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
76	Kuntz/Howard
77	Briggs//ND744/ND721
78	Frontana//W9207//2*Alsen/3/ND744
79	KS86WGRCO2//Len/Butte86/3/ND674/4/2*Parshall
80	ND2948/Parshall//Alsen
81	PI157593/Parshall//Alsen
82	Glenn/Briggs//ND753/3/Steele-ND
83	Granger/ND806
84	ND2902/Parshall//ND751
85	Glenn/Briggs//ND753/3/Steele-ND
86	Reeder*2/3/Altar84/AE.SQ//Opata
87	ND819'S'/3/SD8070/ND674//ND2831
88	ND721*2/Tokai66//ND803
89	Prosper/Ulen
90	Parshall/Howard
91	ND2891/ND721
92	Kadett/Bobwhitesib//Grandin/3/ND706/4/ND752
93	Frontana/W9207//2*Alsen/3/ND752/4/Steele-ND
94	ND2849/ND721//Goldfield/2*Alsen
95	McVey//Vance/MN2540W/4/ND2831//Parshall/ND706/3/ND721/5/Alsen
96	ND820 'S'
97	Frontana/W9207//2*Alsen/3/2*ND752
98	VERDE/3/BCN//DOY1/AE.SQUARROSA (447)
99	RB07/Faller
100	Velva
101	ND815/MULT757
102	Dapps/ND804
103	Granite/Faller
104	SS5/Alsen//XC03B-736/3/Alsen/4/Alsen
105	SS156/Alsen//Alsen
106	Glenn/ND740'S'
107	ND2849/ND721//ND735/3/Steele-ND
108	ND2849/ND721//ND735/3/Steele-ND
109	Walworth/Reeder//ND721
110	ND744/Glenn//Parshall
111	ND2948/Alsen//ND740
112	ND652/Parshall//Reeder/3/Steele-ND
113	ND740//ND2955/ND721
114	ND740//ND2955/ND721

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
115	ND734/Knudson
116	Dapps/LDN(DIC3A)//ND2955/ND729
117	ND735/Steele-ND
118	Grandin*4/FO.971(WRT238)//Parshall/3/Dapps/4/Steele-ND
119	Kadett/Bobwhitesib//Grandin/3/ND706/4/Dapps
120	ND744/ND721//Faller'S'
121	ND708/ND706//Steele-ND
122	Glenn/Steele-ND
123	ND726/ND2831
124	Knudson/Dapps
125	Reeder
126	Kuntz/Dapps
127	Alsen/ND803
128	Briggs/Glenn
129	ND737/Reeder
130	Knudson/ND721
131	ND753/Alsen
132	WCB713/Alsen
133	Granite/ND804
134	Frontana//W9207//2*Alsen/3/ND744
135	Faller/Howard
136	ND2709/ND688//Grandin
137	Howard/Alsen//ND749/3/Steele-ND
138	Faller/Howard
139	ND807/Alsen
140	Prosper/Ulen
141	ND2831/ND706//Dapps/3/Alsen
142	ND721*2/Tokai66
143	Parshall/ND803
144	WCB716/Reeder//Alsen
145	ND2879/ND721//Steele-ND
146	ND721*2/Tokai66//Steele-ND
147	Velva/Bigg Red
148	Howard/Faller
149	ND721*2/Tokai66
150	Traverse
151	Cltr9445/2*Alsen
152	ND739 'S'
153	ND823 'S'

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
154	ND810//ND2906/ND721
155	ND2902/Reeder//ND716-21/3/Glenn
156	Dapps/Briggs
157	Glenn/Howard
158	Arsenal//Kormorran/Rohau72-839/3/Parshall/4/ND706
159	W9207/Grandin//Alsen/3/ND752
160	MN00261-4/Prosper
161	Glenn/Howard
162	Reeder/SD3618
163	Granite/Faller
164	ND802/MT0415
165	ND804/SD3635
166	SD3635/Prosper
167	W9207/Grandin//Alsen/3/2*Howard/4/Dapps
168	Glenn/Briggs//ND753/3/Steele-ND
169	ND807/SD3635
170	Howard/Alsen
171	ND823 'S'
172	Tokai66/Parshall//Alsen
173	ND721*2/Tokai66
174	Howard/Alsen//ND749/3/Steele-ND/4/ND803
175	RB07
176	SD3635/Howard
177	Dapps/ND804
178	Glenn/ND810
179	Ember/Howard
180	Ember/Howard
181	Briggs/Glenn
182	Brick/Reeder
183	MN01333-A/ND744'S'
184	Dapps/2*Reeder/3/ND803
185	Tom/ND807//Glenn
186	Briggs/ND803//Granite
187	ND744'S'/MT0415//Steele-ND
188	Dapps/Prosper
189	ND802/Glenn
190	Glenn/Barlow
191	ND812/Steele-ND
192	ND815/Velva

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
193	ND2948/Parshall//Alsen/3/Faller
194	Goldfield/2*Alsen//Glenn
195	Dandy/Dapps
196	Duo/Howard//Faller/3/Granite
197	Granite/ND807
198	Freyr/Velva
199	Ada/Velva
200	Glenn
201	SD3901/Parshall
202	ND815/ND806
203	Goldfield/2*Alsen//Faller
204	Faller/Parshall//ND807
205	Goldfield/2*Alsen//Steele-ND//Parshall
206	Reeder/ND813
207	MN02252-A/Steele-ND
208	Kuntz/Glenn
209	Blade/Glenn
210	Glenn/ND740'S'//Howard
211	Barlow/Glenn
212	Barlow/Vantage
213	ND815/Faller
214	ND815/ND806
215	Goldfield/2*Alsen//Faller
216	Arina//Parshall/2817/3/ND716-21/4/Faller
217	Albany/Parshall
218	Albany/ND815
219	Dapps/ND804
220	ND802/Traverse//Granite
221	SD3618/ND803//Dapps
222	SD3868/MT0415//ND807
223	MN02252-A/Glenn
224	Faller/Gunner
225	Faller
226	N99-0241/Glenn
227	Steele-ND/Traverse
228	Barlow/Goldfield/2*Alsen
229	Barlow/MN02252-A
230	ND2879/ND721//Glenn
231	ND819'S'/Steele-ND

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
232	ND803/ND816'S'
233	Saturn/Alsen
234	Faller/ND803
235	ND810//ND2906/ND721
236	SD8070/ND674//ND2831/3/ND801
237	SD3635/Barlow
238	ND802/Faller
239	Faller/Brick
240	ND2849/ND721//ND807
241	Oklee/Reeder
242	ND819'S'/SD3635
243	Alsen/Briggs//Dapps/3/Steele-ND/4/ND751
244	Reeder/Glenn
245	ND752/Hanna//Dapps/3/ND744/4/Howard
246	ND721*2/Tokai66
247	PF9293/2*Alsen
248	Stoa//Gemini/ND658/3/Keene/4/Parshall/5/ND752
249	Walworth/Dapps//ND725/3/ND751
250	Barlow
251	ND706/Parshall//ND744/ND721
252	ND751/Alsen//ND744/ND721/3/ND755
253	ND726/ND2831
254	Fujian5114-1/MN2538//Alsen
255	Tokai66/Parshall//Alsen
256	ND2887/ND721
257	ND2849/ND721
258	ND735/Steele-ND
259	ND2902/Parshall
260	ND673-D//M3*2/ND673/3/Steele-ND
261	ND818 'S'
262	Howard/Alsen
263	ND752/Hanna//Dapps/3/ND744/4/ND751
264	Briggs/Alsen
265	Briggs/Glenn
266	PI350768/ND751//Parshall
267	PF9293/Alsen//ND752
268	Cltr9445/2*Alsen
269	ND825 'S'
270	ND820 'S'

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
271	Kadett/Bobwhitesib//Grandin/3/ND706
272	Goldfield/ND2902//Alsen'S'
273	Kadett/Bobwhitesib//Grandin/3/ND706/4/ND752
274	KS86WGRCO2//Len/Butte86/3/ND674/4/2*Parshall
275	Howard
276	Howard//ND2831/ND706Sib
277	ND2709/ND688//Grandin
278	ND819 'S'
279	ND2849/ND721//ND740'S'
280	ND709-9/ND2902
281	ND2902/Parshall//ND751
282	ND744/Parshall
283	Glenn/Steele-ND
284	PrairieRed/Keene//ND721
285	Spelt#20/98W1147*3
286	Parshall'S'/4/Stoa*2//Butte*3/CI9321/3/Trenton/5/Steele-ND
287	SD3618/ND806
288	9950146ES/Alsen
289	ND810/ND744'S'
290	Glenn/Briggs//ND753/3/Steele-ND
291	Granger/ND804
292	Kuntz/Steele-ND
293	ND803/Glenn
294	Dapps/Granite
295	ND802//Goldfield/2*Alsen
296	ND2891/ND721
297	Kadett/Bobwhitesib//Grandin/3/ND706
298	ND2948/Alsen//ND721
299	PF9293/Alsen//ND721/Parshall
300	Briggs
301	SD3618/ND740'S'
302	ND2891/ND721
303	ND2849/ND721//ND810
304	Frontana/W9207//Alsen/3/ND740'S'
305	Ember/Glenn
306	Dapps/ND804
307	Faller/Brick
308	ND2902/2*Parshall
309	Glenn/ND810

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
310	Ember/Howard
311	Ember/Howard
312	Keene/ND803
313	Stoa/ND803
314	Parshall/Prosper
315	Alsen/ND807
316	ND744'S'/Parshall
317	ND721*2/Tokai66//Velva
318	PF9293/2*Alsen//Faller
319	ND744'S'/Kuntz
320	ND816'S'/Parshall
321	ND816'S'/Faller
322	ND814/ND804
323	ND815/Faller
324	ND2902/2*Parshall
325	Kelby
326	Goldfield/2*Alsen//Faller
327	Goldfield/2*Alsen//Velva
328	ND2709/3/Grandin*3//Ramsey/ND622/4/ND688/ND674/5/Velva
329	ND2831/ND706sib//Granite
330	ND721*2/Tokai66//Parshall
331	ND721/Parshall//Parshall/ND706/3/Dapps
332	SD3942/Howard
333	Albany/Parshall
334	Frontana/W9207//Alsen/3/ND2849/ND721/4/Freyr/5/Faller
335	Steele-ND/Prosper
336	Dapps/Faller
337	Granite/Faller
338	ND721*2/Tokai66//ND804/3/Granite
339	SD3870/Glenn
340	ND815/MULT757//ND807
341	ND807/Briggs
342	Duo/Glenn
343	Fiorina/ND802
344	MT0416/Howard
345	ND2902/2*Parshall
346	Rush/Parshall//Faller
347	Howard/ND814
348	PF9293/2*Alsen//Faller

Table A1. Germplasms used in the AM panel for 2012 (continued).

Serial number	Germplasms used in the AM panel
349	ND2902/2*Parshall
350	Albany

Table A2. Additional germplasms used in the AM panel for 2013 and 2014.

Serial number	Germplasms used in the AM panel
1	Elgin
2	Rowyn
3	Linkert
4	Norden
5	Advance
6	Forefront
7	Mott
8	Brick
9	Steele-ND
10	Vantage
11	Brennan